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Influence of Functional Food Containing *Bacillus polyfermenticus* SCD on Lipid and Antioxidant Metabolisms in Rats Fed a High-Fat and High-Cholesterol Diet

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Abstract We evaluated the effects of newly developed functional food containing *Bacillus polyfermenticus* SCD as the main material on the lipid and antioxidant metabolisms of hypercholesterolemic rats. Thirty male SD rats were divided into three groups after a 1-week adaptation period and were fed with a high fat-cholesterol diet (control), or with a high fat-cholesterol diet supplemented with low or high doses (3.1×10^6 cfu/day or 3.1×10^8 cfu/day) of *B. polyfermenticus* SCD and other physiological active materials for 6 weeks. Both doses of *B. polyfermenticus* SCD significantly reduced hepatic total cholesterol and triglycerides, while increasing the fecal excretion rates of total lipid, total cholesterol and triglycerides. *B. polyfermenticus* SCD increased the total radical trapping antioxidant potential (TRAP). The erythrocytic glutathione peroxidase activity in the *B. polyfermenticus* groups was significantly lower than that in the control group. Plasma TRAP levels exhibited a highly significant negative correlation with hepatic total cholesterol while a significant positive correlation was detected between fecal total cholesterol and plasma TRAP. This hypolipidemic and antioxidative effect of *B. polyfermenticus* SCD seemed to be unrelated to its dosage. These results suggest that functional food containing *B. polyfermenticus* SCD can improve oxidative stress and hepatic lipid profiles by enhancing the excretion of cholesterol and triglycerides in feces of rats fed with high fat-high cholesterol diet.

Key words: functional food, *Bacillus polyfermenticus*, cholesterol-lowering activity, antioxidative activity

Introduction

Strains of *Bacillus polyfermenticus* SCD, commonly known as Bispan strains commercially, have been used in the treatment of long-term intestinal disorders, as the live strains are able to access the intestines in the form of active endospores (1). *B. polyfermenticus* strain was first isolated from an air sample by Dr. Terakado in 1933. It produces a variety of enzymes, most of which can lyse pathogenic strains such as typhoid bacillus, paratyphoid bacillus, shigella, and cholera. Moreover, the uptake of *B. polyfermenticus* strains promotes human digestion, serving as a source of vitamins B_1 and B_2 and bolstering protection against nonoral infections and oral immunization (2).

In a recent *in vitro* experiment, we have determined that *B. polyfermenticus* has cholesterol-reducing activity, and exerts antioxidative and antimutagenic effects (3, 4).

Hypercholesterolemia is a major risk factor for cardiovascular disease such as atherosclerosis, myocardial infarction, heart attacks, and cerebrovascular diseases (5). Recently, hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation (6). Increased generation of oxidized low-density-lipoprotein (LDL) is a major factor in the vascular damage associated with high cholesterol levels (7). Therefore the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach, and many efforts have been made to identify the antioxidative functions of various materials, including medicinal plants as dietary additives. Numerous drugs that lower cholesterol, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitors like lovastatin and simvastatin, which inhibit cholesterol biosynthesis, have been used to treat hypercholesterolemia (8). However, the undesirable side effects of these compounds have raised concerns regarding their therapeutic use (9).

Recently, we have developed functional food containing *Bacillus polyfermenticus* SCD as the main material with other physiological active materials such as chitosan, chicory, a-tocopherol and flavonoids. Therefore, the objective of this study was to test the newly developed functional food with regard to its use as an agent for improving plasma, hepatic, and fecal lipid profiles and antioxidant.

Materials and Methods

Bacterial strains and media The producer strain of *B. polyfermenticus* SCD was maintained at -70°C in a tryptic soy broth (TSB, Difco) to which 20% (v/v) glycerol was added. Working cultures were propagated in TSB, with shaking, at 37°C.

Production of *B. polyfermenticus* SCD *B. polyferxmenticus* SCD production was performed as previously described

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(2). Briefly, *B. polyfermenticus* SCD was inoculated into 60 mL of sterile TSB (Difco), and the seed culture (2%, v/v) was then transferred to a jar fermenter (3-L working volume; Korea Fermenter Co., Korea). The temperature was maintained at 37°C and the pH at 7.0 ± 0.1. *B. polyfermenticus* SCD was obtained by centrifugation (10,000 rpm, 30 min) of the resulting stationary-phase cells after 72 hr of incubation. The pellets were then freeze-dried and stored at 4°C.

Animals and diets Eight-week-old male Sprague Dawley rats (SD, n=30) were purchased from Samtako Inc. (Osan, Korea) and housed and cared for in accordance with the Guide for Care and Use of Laboratory Animals (Department of Health, Education, and Welfare, 1985). The rats were allowed free access to water and fed for the first week with a commercially prepared pelleted diet for adjustment. The rats were then randomly divided into three groups of 10 animals each and fed on either a high-fat and high-cholesterol diet (control group), or a high-fat and high-cholesterol diet supplemented with either a low (3.1×10^6 cfu/day) (LBP group) or high (3.1×10^8 cfu/day) (HBP group) dose of *B. polyfermenticus* SCD for 6 weeks. Both *B. polyfermenticus* SCD products contained additionally the same amount of chitosan (0.25 g/day), chicory (0.3 g/day), α-tocopherol (0.007 g/day) and flavonoids (0.02 g/day). The composition of the fat and cholesterol-enriched diet was (g/100 g): casein, 20; corn starch, 42.949; sucrose, 10; corn oil, 5; lard, 12; cholesterol, 1; cellulose, 4; vitamin mixture (AIN-93; American Institute of Nutrition, 1993), 1; mineral mixture (AIN-93; American Institute of Nutrition, 1993), 3.5; choline bitartrate, 0.25; DL-methionine, 0.3; and butylated hydroxy toluene, 0.001. Animals were monitored daily for general health, and body weights were recorded every week for the duration of the study. Feces were collected during the final 3 days and were used to determine fecal lipid profiles. At the end of the experimental period, the rats were anesthetized with ethyl ether, and blood was collected from the abdominal artery in a heparinated sterile tube. Plasma was obtained from the blood samples by centrifugation (1500 rpm for 30 min) and stored at -80°C until required for further analysis. Erythrocytes were washed three times with iso-osmotic phosphate-buffered saline (PBS) at a pH of 7.4 and resuspended to the original volume. The erythrocyte suspensions were frozen at -80°C until required for final analysis. Livers were removed from the rats washed with ice-cold saline and stored at -80°C before analysis.

Plasma lipid profiles Plasma lipid profiles [total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides] were measured using enzymatic colorimetry methods with a commercial kit (Sigma Chemical Co.) and a photometric autoanalyzer (SEAC, CH-100 plus, Italy).

Liver and fecal lipid profiles Hepatic and fecal lipids were extracted according to the procedure developed by Folch *et al.* (10). Liver or fecal lipid extract was prepared by extracting 1-2 g liver or feces with a chloroform: methanol mixture (2:1, v/v). The total lipids in the liver and feces were then quantified gravimetrically by evaporating the solvent in the liver lipid extract. Cholesterol and triglycerides in the liver and feces were analyzed with the same enzymatic kit as used in the plasma analysis.

Erythrocytic antioxidant enzyme activities Erythrocytic hemolysates were prepared by the dilution of erythrocytes to 1:20 (GSH-Px) and 1:500 (catalase) with distilled H_2O. The protein contents of the erythrocytic hemolysates were determined using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA, USA) with bovine serum albumin used as a reference protein. GSH-Px was determined according to the method described by Beutler (11). Erythrocytic hemolysate 10 μL or liver tissue preparation was added to 100 μL of 1 M Tris-HCI 5 mM EDTA buffer (pH 8.0), 20 μL of glutathione 0.1 M, 100 mL of glutathione reductase 10 U/mL, and 100 μL of NADPH 2 mM, and filled with H_2O to a final volume of 1 mL. After 10 min of incubation at 37°C, the reaction was initiated by the addition of 10 mL of t-butyl hydroperoxide, and the absorbance was measured at 340 nm. The reaction was run for 90 sec, and the loss of NADPH was monitored by the change in A_{340}/min. Catalase activity was measured according to the method developed by Aebi (12). Erythrocytic hemolysate 100 μL or liver tissue preparation was dissolved in 50 mL of phosphate buffer 50 mM (pH 7), and 2 mL of the mixture was added to a cuvette. The reaction was initiated by the addition of 1 mL of H_2O_2 30 nM at 20°C. The H_2O_2 decomposition rate was measured at 240 nm for 30 sec using a spectrophotometer.

Plasma total radical trapping antioxidant potential (TRAP) The plasma total radical trapping antioxidant potential (TRAP) was measured using a modification of the photometric method developed by Rice-Evans and Miller (13). The method for measuring antioxidant activity is based on the antioxidant-induced inhibition of the absorbance of the radical cation of 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) ($ABTS^+$). The $ABTS^+$ radical cation is formed by the interaction of $ABTS^+$ (150 μM) with the ferrylmyoglobin radical species, which is, in turn, generated by the activation of metmyoglobin (2.5 μM) by H_2O_2 (75 μM). Ten microliters of sample/buffer/Trolox-standard was added to tubes containing 400 μL of PBS buffer, 20 μL of metmyoglobin, and 400 μL of ABTS and mixed by vortexing. The reaction was initiated by the addition of 170 μL of H_2O_2. After 6 min of incubation, the absorbance was measured at 734 nm using a spectrophotometer. Values were expressed as Trolox equivalent antioxidant capacity (TEAC) and defined as the millimolar concentration of the Trolox antioxidant capacity of a calibration curve.

Statistical analysis Data were analyzed using the SPSS package for Windows (Version 10). Values were expressed as mean ± standard error (S.E.). The data was evaluated by one-way ANOVA and the differences between the means were assessed using *Duncan's* multiple-range test. The differences were considered significant at $P<0.05$. Evaluation of the associations between parameters was carried out using Pearson's correlation.

Results and Discussion

Food intake, weight gain, and organ weight Body weight gain, food intake, and the food efficiency ratio (FER) were not influenced by *B. polyfermenticus* supplementation (Table 1). Organ weights did not differ between the groups (data not shown).

Plasma, hepatic, and fecal lipid profiles Plasma, hepatic, and fecal lipid profiles are summarized in Table 2 and Figs. 1 and 2, respectively. Plasma lipid profiles were not significantly affected by *B. polyfermenticus* supplementation (Table 2). However, hepatic total cholesterol and triglyceride levels were significantly lower in both of the *B. polyfermenticus* groups than in the control group, by 23% and 26% in the LBP group and by 19% and 24% in the HBP group, respectively (Fig. 1). These significant reductions in hepatic total cholesterol and triglyceride levels were correlated with higher fecal excretion levels of total cholesterol and triglycerides. Fecal total cholesterol was increased significantly by 27% in the LBP group compared to the control group. In the HBP group, fecal total cholesterol and triglyceride levels were increased significantly by 28% and 19%, respectively, compared to the control group (Fig. 2). Both of *B. polyfermenticus* groups exhibited a non-significant decrease in hepatic total lipid levels and a significant increase in fecal total lipids (Fig. 1 & 2).

A great deal of research has been carried out, both in animal experiments with rats and human clinical trials, on the hypocholesterolemic activity of lactic acid bacteria (LAB) and yogurt fermented with LAB, including *Lactobacillus* spp. and *Bifidobacterium* spp. (14, 15, 16). Usman and Hosono (14) reported that *Lactobacillus gasseri* SBT0270 exerted its hypocholesterolemic effects by suppressing the reabsorption of bile acids into the enterohepatic circulation system and by enhancing the excretion of acidic steroids in the feces of hypercholesterolemic rats. In a placebo-controlled study, fermented milk with *Lactobacillus acidophilus* L1 resulted in a 2.9% reduction in serum cholesterol concentrations in hypercholesterolemic individuals (15). Several *in vitro* and *in vivo* studies have proposed a number of mechanisms for the observed cholesterol-lowering actions of probiotic bacteria. These include the inhibition of cholesterol absorption by the binding of cholesterol to the bacterial cell wall (17), assimilation (uptake) of cholesterol by LAB (18, 19), promotion of bile acid excretion from the deconjugation of bile acid by the bacteria (20, 21), and inhibition of hepatic cholesterol synthesis and/or the

Table 1. Effect of functional food containing *Bacillus polyfermenticus* SCD on weight gains and food efficacy ratio of rats fed high fat-cholesterol diet

	Control	LBP	HBP
Daily weight gain (g)	4.0 ± 0.3^{ns}	3.7 ± 0.1	3.7 ± 0.2
Daily Food intakes (g)	14.8 ± 0.2^{ns}	14.6 ± 0.2	14.2 ± 0.2
FER (%)	27.0 ± 3.2^{ns}	25.7 ± 2.4	27.0 ± 2.5

Values are the mean ± SEM for 10 animals in each group. ns: not significant. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

Table 2. Effect of functional food containing *Bacillus polyfermenticus* SCD supplementation on plasma lipid profiles in SD male rats fed high fat-cholesterol diet

	Control	LBP	HBP
Total-cholesterol (mg/dL)	169.4 ± 10.3^{ns}	143.2 ± 11.0	169.0 ± 8.8
Triglycerides (mg/dL)	54.7 ± 5.7^{ns}	44.6 ± 3.1	43.8 ± 2.9
HDL-cholesterol (mg/dL)	21.4 ± 1.8^{ns}	22.7 ± 2.2	26.5 ± 1.8
HDL-C/Total-C	12.8 ± 3.1^{ns}	17.8 ± 2.6	15.9 ± 1.1
Atherogenic index	7.3 ± 0.8^{ns}	5.6 ± 0.9	5.6 ± 0.5

Values are the mean ± SEM for 10 animals in each group. ns: not significant. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

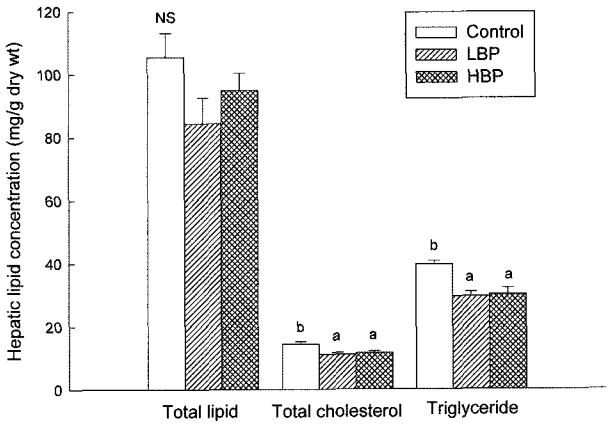

Fig. 1. Effect of functional food containing *Bacillus polyfermenticus* SCD supplementation on hepatic lipid concentration in SD male rats fed high fat-cholesterol diet. Each bar represents the mean ± SEM for 10 animals in each group. Bars with different superscript letters are significantly different at $p < 0.05$ by Duncan's test. ns: not significant. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

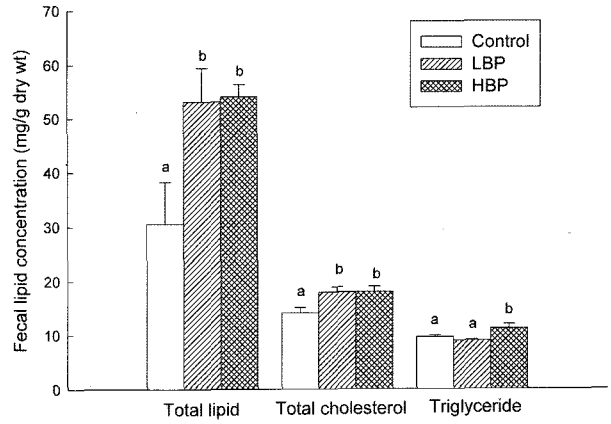

Fig. 2. Effect of functional food containing *Bacillus polyfermenticus* SCD supplementation on fecal lipid concentration in SD male rats fed high fat-cholesterol diet. Each bar represents the mean ± SEM for 10 animals in each group. Bars with different superscript letters are significantly different at $p < 0.05$ by Duncan's test. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

redistribution of cholesterol from plasma to the liver by the cholesterol-metabolizing enzyme systems in the liver, such as HMG-CoA reductase (22). The cholesterol-lowering action of *B. polyfermenticus* SCD in the present study might be attributable to the binding of dietary cholesterol by *B. polyfermenticus* SCD before cholesterol can be absorbed into the body, thus increasing the excretion of unabsorbed cholesterol in the feces. This possibility was raised in our *in vitro* experiment (3), which indicated that *B. polyfermenticus* SCD reduced cholesterol by 60% in the culture medium during 24 hr at 37°C, and that the removal of cholesterol was independent of bile acids. We also determined that cholesterol was in the cell extracts of the bacteria after cultivation, suggesting that the cholesterol-lowering action of *B. polyfermenticus* SCD might be due largely to the binding or assimilation of cholesterol.

The beneficial effect of this functional food on lipid metabolism could be ascribed not only to *B. polyfermenticus* SCD but also to other components of the functional food, such as chitosan and chicory. Maezake *et al.* (23) reported increased fecal excretion of bile acid in human male subjects consuming 3 to 6 g/day of chitosan. In rats, chitosan increased fecal neutral sterol excretion and reduced liver cholesterol (24). Chitosan is believed to affect cholesterol because it has positively charged amino acid, which binds to negatively charged molecules, such as lipids and bile, preventing their absorption and storage by the body (25). Chicory is known to be prebiotic and is composed mainly of inulin, which is a polymer of fructose with β-(2-1) glycosidic linkages (26). Kim and Shin have found that chicory extract supplementation in rats improved lipid metabolisms by altering the absorption and/or synthesis of cholesterol, which might result from the changes in cecal fermentation and from the increased fecal excretion of lipid, cholesterol and bile acid (27). Chicory inulin is expected to behave like a soluble fiber and to have hypolipidemic effect, because it is soluble in water and not hydrolyzed by human digestive enzymes (27).

Plasma TRAP and erythrocytic antioxidant enzymes

As shown in Fig. 3, the TRAP value, an indicator of total antioxidant defense, was significantly increased by *B. polyfermenticus* SCD supplementation by 24% and 21% in the LBP and HBP groups, respectively, above control levels ($P < 0.001$). The erythrocytic GSH-Px activity in the LBP was significantly lower by 23% than that in the control group. Catalase activity in the erythrocytes was not affected by *B. polyfermenticus* SCD supplementation (Fig. 4).

Plasma TRAP levels exhibited a highly significant negative correlation with hepatic total cholesterol levels (Fig. 5A, $r = -0.642$, $P < 0.001$) while there was a significant positive correlation between fecal total cholesterol and plasma TRAP levels (Fig. 5B, $r = 0.463$, $P < 0.05$).

Oxidative stress, defined as a disruption of the balance between oxidative and antioxidative processes, plays an important role in the pathogenesis of atherosclerosis (28). A cholesterol-rich diet increases lipid peroxidation by the induction of free radical production, followed by hypercholesterolemia, a major risk factor for atherosclerosis (29). The relationship between oxidative stress and cholesterol levels was confirmed in the present study,

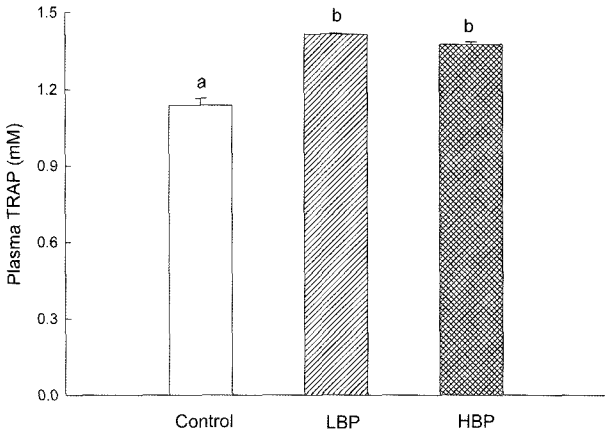

Fig. 3. Effect of functional food containing *Bacillus polyfermenticus* SCD supplementation on Plasma TRAP in SD male rats fed high fat-cholesterol diet. Each bar represents the mean ± SEM for 10 animals in each group. Bars with different superscript letters are significantly different at $p < 0.05$ by Duncan's test. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

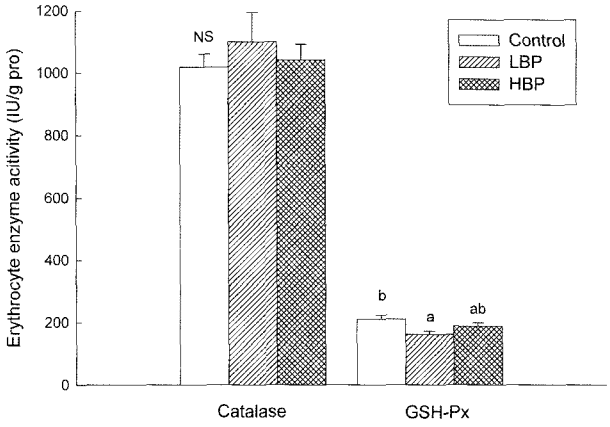

Fig. 4. Effect of functional food containing *Bacillus polyfermenticus* SCD supplementation on erythrocytic antioxidant enzymes (catalase and glutathione peroxidase) activities in SD male rats fed high fat-cholesterol diet. Each bar represents the mean ± SEM for 10 animals in each group. Bars with different superscript letters are significantly different at $p < 0.05$ by Duncan's test. ns: not significant. Control: high fat-cholesterol diet fed group, LBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^6 cfu/day), HBP: high fat-cholesterol diet + *B. polyfermenticus* SCD (3.1×10^8 cfu/day).

showing that plasma TRAP, an indicator of total antioxidant defense, exhibited a negative correlation with both plasma and hepatic total cholesterol levels, and a positive correlation with the fecal excretion levels of total cholesterol. There are two possible explanations for this relationship between plasma antioxidant defense and cholesterol levels mediated by *B. polyfermenticus* SCD. First, the oxidative stress due to hypercholesterolemia could be reduced by decreased cholesterol levels in plasma after *B. polyfermenticus* SCD supplementation. Second, although most of *B. polyfermenticus* SCD taken orally is known to reach the intestines in endospore form (1), some of the bacteria could be expected to lyse during transit

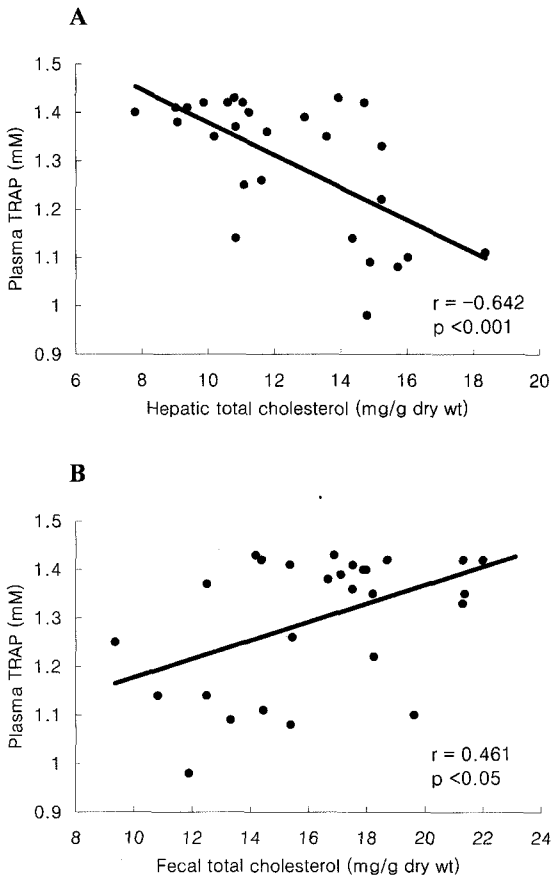

Fig. 5. Relationship between plasma TRAP and hepatic- (A), fecal- (B) total cholesterol. *r* = Pearson's correlation coefficient. TRAP: total radical trapping antioxidant potential.

through the gastrointestinal tract and to release their intracellular antioxidative constituents. The absorbed antioxidants from the small intestine flow to the circulation system and may act as antioxidants in blood to increase plasma TRAP and decrease erythrocytic antioxidant enzyme activity (GSH-Px) in this study. As a decrease in antioxidant enzyme activity in general was most pronounced for the stronger antioxidants tested (30), it can be hypothesized that the antioxidant enzyme in erythrocytes is downregulated by the supplementation of *B. polyfermenticus* SCD in response to an improved antioxidant status of erythrocytes due to the presence of antioxidants in *B. polyfermenticus* SCD. Recently, we have reported the *in vitro* antioxidative ability of the culture supernatant of *B. polyfermenticus* SCD (3). We are currently attempting to identify the chemical nature of the bacterium to elucidate the antioxidative effects exerted by this strain.

This functional food contains other well known antioxidant sources, such as α-tocopherol and flavonoids (31, 32). In addition, the antioxidant activity of chitosan has attracted the most attention. Xie *et al.* (33) and Xue *et al.* (34) have shown that the scavenging effect of chitosan on hydroxy radicals inhibits lipid peroxidation of phosphatidylcholine and linoleate liposomes *in vitro*. A recent study has demonstrated that chicory is a good source of health-promoting antioxidant polyphenols, like caffeic acid, flavones and flavonoids (35).

Overall, we couldn't find any dose dependent effect of *B. polyfermenticus* SCD on either lipid metabolism or antioxidant metabolism. Maybe the 100-fold increase in the supplementation of *B. polyfermenticus* SCD in rats was not enough to show any progressed effects or possibly the concentration of 3.1×10^6 cfu/day of *B. polyfermenticus* SCD could be a threshold limit to obtain the beneficial effect. To our knowledge, however, there is no report which supports our unexpected result.

Our results suggest that the consumption of functional food containing *B. polyfermenticus* SCD as the main material has significant health benefits, occurring *via* the modulation of physiologic functions including various artherogenic lipid profiles and antioxidants in hypercholesterolemia. These hypolipidemic and antioxidative effects of *B. polyfermenticus* SCD seemed to be unrelated to its dosage. Human clinical trials with this functional food are currently in progress in an attempt to provide more information on this possibility.

Acknowledgments

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References

1. Jun KD, Lee KH, Kim WS, Paik HD. Microbiological identification of medical probiotic bispan strain. J. Kor. Soc. Food Sci. Nutr. 28: 124-127 (2000)
2. Lee KH, Jun KD, Kim WS, Paik HD. Partial characterization of polyfermenticin SCD, a newly identified bacteriocin of *Bacillus polyfermenticus*. Lett. Appl. Microbiol. 32: 146-151 (2001)
3. Jeong HY, Kim TH, Park JS, Kim KT, Paik HD. Antioxidative and cholesterol-reducing activity of *Bacillus polyfermenticus* SCD. Korean J. Biotechnol. Bioeng. 18: 371-376 (2003)
4. Park EJ, Park JS, Paik HD. Effect of *Bacillus polyfermenticus* SCD and its bacteriocin on MNNG-induced DNA damage. Food Sci. Biotechnol. 13: 684-688 (2004)
5. Wald NJ, Law MR. Serum cholesterol and ischaemic heart disease. Atherosclerosis 118: S1-S5 (1995)
6. Cox DA, Cohen ML. Effects of oxidized low-density lipoprotein on vascular contraction and relaxation: clinical and pharmacological implications in atherosclerosis. Pharmacol. Rev. 48: 3-19 (1996)
7. Pritchard KA Jr., Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P, Wolin MS, Stemerman MB. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. Circ. Res. 77: 510-518 (1995)
8. Pazzucconi F, Dorigotti F, Gianfranceschi G, Campagnoli G, Sirtori M, Franceschini G, Sirtori CR. Therapy with HMG CoA reductase inhibitors: characteristics of the long-term permanence of hypocholesterolemic activity. Atherosclerosis 117: 189-198 (1995)
9. Erkelens DW, Baggen MG, Van Doormaal JJ, Kettner M, Koningsberger JC. Clinical experience with simvastatin compared with cholestyramine Mol. M. J. Drugs 36 Suppl: 87-92 (1988)
10. Folch J, Lees M, Sloan-Stanley GH. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509 (1956).
11. Beutler E. Glutathione peroxidase. In: Red cell metabolism: A manual for biochemical methods. Verlag Grune and Straton, New York, pp. 71-73 (1984)
12. Aebi H. Catalase. In: Methods of enzymatic analysis. Verlag Chemie GmbH, Weinheim, pp. 673-684 (1974)
13. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. In: Methods in Enzymology. Academic Press, Inc., New York, pp. 279-293 (1994)
14. Usman T, Hosono A. Effect of administration of *Lactobacillus*

gasseri on serum lipids and fecal steroids in hypercholesterolemic rats. J. Dairy Sci. 83: 1705-1711 (2000)
15. Anderson JW, Gilliland SE. Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans. J. Am. Coll. Nutr. 18: 43-50 (1999)
16. Foo HL, Loh TC, Law FL, Lim YZ. Kufli CN, Rusul Gulam. Effects of feeding *Lactobacillus plantarum* I-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. Food Sci. Biotechnol. 12: 403-408 (2003)
17. Hosono A, Tono-oka T. Binding of cholesterol with lactic acid bacterial cells. Milchwissenschaft 50: 556-560 (1995)
18. Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49: 377-381 (1985)
19. Pereira DIA, Gibson GR. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl. Environ. Microbiol. 68: 4689-4693 (2002)
20. Gilliland SE, Speck ML. Deconjugation of bile acids by intestinal lactobacilli. Appl. Environ. Microbiol. 33: 15-18 (1977)
21. Klaver FA, van der Meer R. The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59: 1120-1124 (1993)
22. Fukushima M, Nakano M. Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. Brit. J. Nutr. 76: 857-867 (1996)
23. Maezaki Y, Tsuji K, Nakagawa Y, Kawai Y, Akimoto M. Hypocholesterolemic effect of chitosan in adult males. Biosci. Biotech. Biochem. 57: 1439-1444 (1993)
24. Sugano M, Fujikawa T, Hiratsuji Y, Nakashima K, Fukuda N, Hasegawa Y. A novel use of chitosan as a hypocholesterolemic agent in rats. Am. J. Clin. Nutr. 33: 787-793 (1980)
25. Shields KM, Smock N, McQueen CE, Bryant PJ. Chitosan for weight loss and cholesterol management. Am. J. Health Syst. Pharm. 60: 1310-1312, 1315-6 (2003)
26. Flamm G, Glinsmann W, Kritchevsky D, Prosky L, Roberfroid M. Inulin and oligofructose as dietary fiber: a review of the evidence. Crit. Rev. Food Sci. Nutr. 41: 353-362 (2001)
27. Kim M, Shin HK. The water-soluble extract of chicory influences serum and liver lipid concentrations, cecal short-chain fatty acid concentrations and fecal lipid excretion in rats. J. Nutr. 128: 1731-1736 (1998)
28. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. New Engl. J. Med. 320: 915-92 (1989)
29. Wissler RW. Theories and new horizons in the pathogenesis of atherosclerosis and the mechanisms of clinical effects. Arch. Pathol. Lab. Med. 116: 1281-1291 (1992)
30. Klurfeld DM. Dietary fiber-mediated mechanisms in carcinogenesis. Cancer Res. 52: 2055S-2059S (1992)
31. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. J. Physiol. Biochem. 57: 43-56 (2001)
32. Rice-Evans C. Flavonoid antioxidants. Curr. Med. Chem. 8: 797-807 (2001)
33. Xie W, Xu P, Liu Q. Antioxidant activity of water-soluble chitosan derivatives. Bioorg. Med. Chem. Lett. 11: 1699-1701 (2001)
34. Xue C, Yu G, Hirata T, Terao J, Lin H. Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents. Biosci. Biotechnol. Biochem. 62: 206-209 (1998)
35. Llorach R, Tomas-Barberan FA, Ferreres F. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. J. Agric. Food Chem. 52: 5109-5116 (2004)