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Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content

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Abstract

Background: Lactic acid bacteria (LAB) are beneficial probiotic organisms that contribute to improved nutrition, microbial balance, and immuno-enhancement of the intestinal tract, as well as lower cholesterol. Although present in many foods, most trials have been in spreads or dairy products. Here we tested whether *Bifidobacteria* isolates could lower cholesterol, inhibit harmful enzyme activities, and control fecal water content.

Methods: *In vitro* culture experiments were performed to evaluate the ability of *Bifidobacterium* spp. isolated from healthy Koreans (20~30 years old) to reduce cholesterol-levels in MRS broth containing polyoxyethanylcholesterol sebacate. Animal experiments were performed to investigate the effects on lowering cholesterol, inhibiting harmful enzyme activities, and controlling fecal water content. For animal studies, 0.2 ml of the selected strain cultures (10^8 ~ 10^9 CFU/ml) were orally administered to SD rats (fed a high-cholesterol diet) every day for 2 weeks.

Results: *B. longum* SPM1207 reduced serum total cholesterol and LDL levels significantly ($p < 0.05$), and slightly increased serum HDL. *B. longum* SPM1207 also increased fecal LAB levels and fecal water content, and reduced body weight and harmful intestinal enzyme activities.

Conclusion: Daily consumption of *B. longum* SPM1207 can help in managing mild to moderate hypercholesterolemia, with potential to improve human health by helping to prevent colon cancer and constipation.

Background

Probiotic bacteria have multiple potential health effects, including blocking gastroenteric pathogens [1-4], neutralizing food mutagens produced in the colon [1,5-10], enhancing the immune response [6,9,11-14], lowering serum cholesterol, and stopping intestinal dysfunction [15-21]. In general, probiotic bacteria must colonize the gastrointestinal tract (GIT) of the host, have acid- and bile salt-tolerance, and block putrefactive bacteria in the GIT. Lactic acid bacteria (LAB), especially *Lactobacillus* spp. and *Bifidobacterium* spp. are important GIT residents and are used as probiotic strains to improve health [22-24]. *Lactobacillus* and *Bifidobacterium* have been used in fermented foods for several centuries without adverse effects [25,26] and are classified as Generally Recognized as Safe (GRAS) because of their long history of safe use, particularly in dairy foods [27,28].

Here, we evaluated the ability of *Bifidobacteria* spp. isolated from healthy Koreans (20~30 years old) to lower cholesterol, inhibit harmful enzyme activities, and control the fecal water content.

Materials and methods

Bacterial strains

The origins of the strains used in this study are shown in Table 1. Isolation of *Bifidobacteria* was performed from fecal samples of healthy Koreans (20~30 years old) collected by BBL's anaerobic sample collection and transport system to maintain anaerobic conditions, and were used within 24 h. Fecal samples were serially diluted 10-fold from 10^{-1} to 10^{-8} , and 100 μ l was spread onto selective BL agar containing 5% sheep blood. After 48 h of incubation in anaerobic conditions (90% N₂, 5% H₂, 5% CO₂) (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc., USA) at 37°C, brown or reddish-brown colonies 2~3 mm in diameter were selected for further identification [29].

A fructose-6-phosphate phosphoketolase (F6PPK) test was performed [30] to ensure that the colonies selected

were *Bifidobacteria*. To identify the isolated *Bifidobacterium* spp. at the species level, 16s rRNA sequencing was performed by Bioleaders (Daejeon, Korea).

In vitro cholesterol-lowering test

MRS broth (pH7.0) (Difco, USA) containing 0.05% L-cysteine·HCl·H₂O (w/v) was prepared and autoclaved at 121°C for 15 min. Soluble cholesterol (polyoxyethan-yl-cholesterol sebacate, Sigma, USA) was added to the prepared MRS broth and filtered through a 0.45 μ m Millipore filter. The inoculation volume was 15 μ l of provisional probiotic bacterial culture (10^8 ~ 10^9 CFU/ml) solution per 1 ml cholesterol-MRS broth, and that was anaerobically incubated at 37°C for 24 h. Uninoculated MRS broth was also incubated at 37°C for 24 h for the control.

Following incubation, bacterial cells were removed by centrifugation (3,000 rpm, 10 min), and the spent broth and uninoculated control broth were then assayed for their cholesterol content. The remaining volume of cholesterol in the cholesterol-MRS broth was determined by the method reported by Rudel and Morris with a small modification [31]. To measure the amount of cholesterol, the dye layer is observed at 560 nm.

Experimental animals and diets

A total of 24 Sprague-Dawley (SD) male rats (5-week-old) were purchased from Central Lab Animal Inc. (Korea), and were housed in a temperature-controlled animal room ($22 \pm 2^\circ\text{C}$) with a 12 h light/dark cycle and humidity $55 \pm 5\%$. Food and water were freely supplied. The animals were randomly selected and assigned to three groups (8 rats per group) according to the type of diet. Group 1 was fed a normal diet. Group 2 was fed a high-cholesterol diet and saline (as control). Group 3 was fed a high-cholesterol diet and *B. longum* SPM1207 (the best strain at lowering cholesterol *in vitro*). The composition of high-cholesterol feed is shown in Table 2. All the rats were acclimatized to the respective diets for a week before the experiment started. Rats in groups 2 or 3 received daily administrations of 0.2 ml of saline or *B. longum* SPM1207 (10^8 ~ 10^9 CFU/ml), respectively, for 2 weeks. Body weight was monitored weekly and food consumption was monitored daily.

Analysis of blood serum

At the end of the experimental period of 3 weeks, blood samples from each animal were collected into tubes by cardiac puncture to determine the serum cholesterol level. Serum was separated from the blood by centrifugation at 3,500 rpm for 10 min. The total cholesterol, HDL-cholesterol, and LDL-cholesterol were analyzed by Samkwang Lab (Korea).

Table 1: List of lactic acid bacteria used in this study

Bacterial strains	Source	Origin
<i>Bifidobacterium adolescentis</i> SPM1005	Isolate ^a	Human feces
<i>Bifidobacterium longum</i> SPM1207	Isolate	Human feces
<i>Bifidobacterium adolescentis</i> SPM1601	Isolate	Human feces
<i>Bifidobacterium adolescentis</i> KCTC3352	Commercial ^b	Intestine of adult
<i>Bifidobacterium longum</i> KCTC3128	Commercial	Intestine of adult
<i>Lactobacillus acidophilus</i> (LH) CBT	Commercial	NA ^c
<i>Lactobacillus plantarum</i> KCTC1048	Commercial	NA
<i>Lactobacillus plantarum</i> (LP) CBT	Commercial	NA
<i>Enterococcus faecium</i> SPM1206	Isolate	Human feces

^aIsolated from healthy Korean.

^bPurchased from Korean Collection for Type Culture (KCTC).

^cNot available.

Table 2: Compositions of high-cholesterol diets for SD rats

Ingredients	Compositions	
	g	kcal
Casein (from milk)	200	800
Corn Starch	155.036	620
Sucrose	50	200
Dextrose	132	528
Cellulose	50	0
Soybean Oil	25	225
Lard	175	1575
Mineral Mixture	35	0
Vitamin Mixture	10	40
TBHQ	0.014	0
DL-Methionine	0	0
L-Cystine	3	12
Choline Bitartrate	2.5	0
Total	838	4,000
Cholesterol	20 g/kg	(2%)
Cholic acid	5 g/kg	(0.5%)

Fecal sampling and bacteriological analysis

Fecal samples were collected weekly to determine the number of LAB, harmful enzyme activity, and water content. Fecal samples were taken directly from the rectum by rectal stimulation and immediately transferred into sterile tubes and kept at 4°C. Total LAB counts was performed on MRS-agar and incubated at 37°C for 48 h under anaerobic conditions (90% N₂, 5% H₂, 5% CO₂). The numbers of colony forming units (CFU) are expressed as log₁₀ CFU per gram.

Harmful enzyme activity of rat intestinal microflora

Enzyme activities related to colon cancer were tested in fecal samples of rats as previously described [32-34].

Tryptophanase activity assay

Tryptophanase activity was assayed using 2.5 ml of a reaction mixture consisting of 0.2 ml of complete reagent solution (2.75 mg pyridoxal phosphate, 19.6 mg disodium EDTA dihydrate, and 10 mg bovine serum albumin in 100 ml of 0.05 M potassium phosphate buffer, pH 7.5), 0.2 ml of 20 mM tryptophan, and 0.1 ml of the enzyme solution (suspended fecal sample), incubated for 1 h at 37°C, and then stopped by adding 2 ml of color reagent solution (14.7 g p-dimethylaminobenzaldehyde in 52 ml H₂SO₄ and 948 ml 95% ethanol). The stopped reaction mixture was centrifuged at 3,000 rpm for 10 min and enzyme activity was measured by monitoring absorbance at 550 nm.

Urease activity assay

Urease activity was assayed using 0.5 ml of a reaction mixture consisting of 0.3 ml of urea substrate solution (4 mM urea in 20 mM sodium phosphate buffer, pH 7.0) and

0.1 ml of the enzyme solution (suspended fecal sample) incubated for 30 min at 37°C and then stopped by adding 0.1 ml of 1 N (NH₄)₂SO₄. Phenolnitroprusside reagent (1 ml) and alkaline hypochlorite reagent (NaClO, 1 ml) were added to the stopped reaction mixture and incubated for 20 min at 65°C. The reaction mixture was centrifuged at 3,000 rpm for 10 min. Enzyme activity was measured by monitoring absorbance at 630 nm.

β-glucosidase activity assay

β-glucosidase activity was assayed using 2 ml of a reaction mixture consisting of 0.8 ml of 2 mM p-nitrophenyl-β-D-glucopyranoside and 0.2 ml of the enzyme solution (suspended fecal sample), incubated for 30 min at 37°C, and then stopped by adding 1 ml of 0.5 N NaOH. The stopped reaction mixture was centrifuged at 3,000 rpm for 10 min. Enzyme activity was measured by monitoring absorbance at 405 nm.

β-glucuronidase activity assay

β-glucuronidase activity was assayed using 2 ml of a reaction mixture consisting of 0.8 ml of 2 mM p-nitrophenyl-β-D-glucuronide and 0.2 ml of the enzyme solution (suspended fecal sample), incubated for 30 min at 37°C, and then stopped by adding 1 ml of 0.5 N NaOH. The stopped reaction mixture was centrifuged at 3,000 rpm for 10 min. Enzyme activity was measured by monitoring absorbance at 405 nm.

Measurement of fecal water content

The water content of fecal samples was measured using a drying oven (105°C, 24 h). Fecal water content (%) is calculated by:

$$\text{Fecal water content(\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100$$

where W_{wet} and W_{dry} are the weight of the fecal sample before and after drying in the oven.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). For statistical evaluation of data, one-way ANOVA was applied using SPSS 13.0 for Windows followed by post hoc comparisons using the Tukey's test. Differences were considered significant at $p < 0.05$.

Results**Isolation and characterization of B. longum SPM1207**

B. longum SPM1207 isolated from healthy Korean faeces was Gram-positive rods, with a translucent glossy colony on general anaerobic medium (GAM, Nissui Pharm. Co. Ltd., Japan) under anaerobic conditions (90% N₂, 5% H₂, 5% CO₂). Sequence analysis (Figure 1) and BLAST

Table 4: Effects of the *B. longum* SPM1207 on serum total-, HDL-, and LDL-cholesterol of SD rats fed on a high-cholesterol diet

Index	Basal diet	High-cholesterol diet		p value
		Control	SPM1207 ^b	
Total cholesterol (mg/dl)	55.0 ± 5.5 ^a	111.3 ± 25.2	84.8 ± 4.3	p < 0.05 ^c
HDL ^e -cholesterol (mg/dl)	23.9 ± 1.4	30.0 ± 2.6	30.6 ± 1.5	NS ^d
LDL ^f -cholesterol (mg/dl)	11.6 ± 1.1	33.3 ± 8.5	23.5 ± 2.3	p < 0.05

^aEach value provided is the mean ± SD for 8 rats.
^b*Bifidobacterium longum* SPM1207 isolated from healthy Korean.
^cp < 0.05 significantly different compared with control.
^dNS: not significant.
^eHDL: high density lipoprotein.
^fLDL: low density lipoprotein.

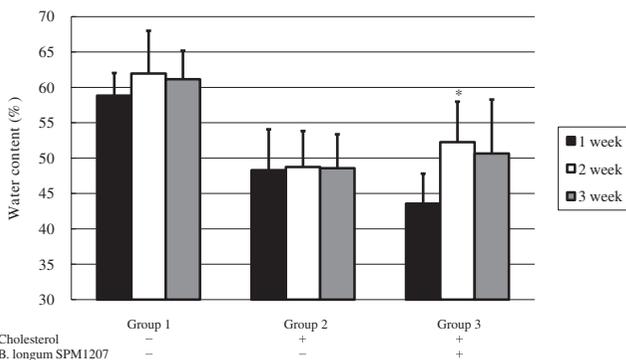


Figure 2
Effect of *B. longum* SPM1207 on fecal water content. All rats were acclimatized to the respective diets for a week. Then the rats in group 2 or 3 received daily 0.2 ml saline or *B. longum* SPM1207 (10⁸~10⁹ CFU/ml), respectively, for 2 weeks. Data are presented as means and standard deviation. *p < 0.05 significantly different compared with 1 week.

containing lactic acid bacteria, can reduce cholesterol. Since the early studies of Mann and Spoerry [41], the cholesterol-lowering potential of lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium* is commonly studied *in vitro* or *in vivo* (experimental animals and human subjects) [21,23,36,42-46].

Here, *B. longum* SPM1207 isolated from healthy Korean feces had hypocholesterolemic effects *in vitro* and in experimental animals (from 345.0 mg/dl to 262.8 mg/dl, and 111.3 mg/dl to 84.8 mg/dl, respectively). *B. longum* SPM1207 also slightly increased HDL-cholesterol levels, in agreement with other findings that decreased total cholesterol was accompanied by simultaneous increases of HDL-cholesterol [47,48].

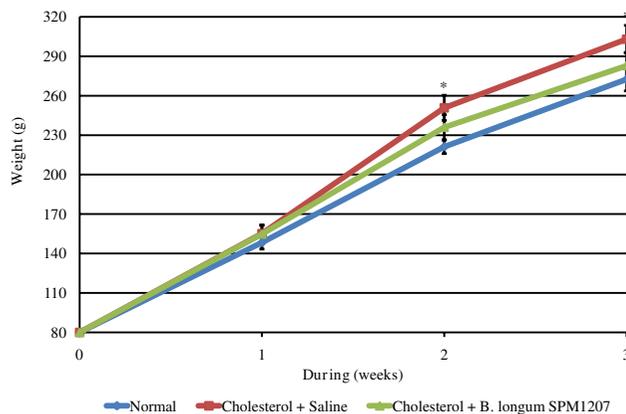


Figure 3
Changes in body weight fed experimental diets for 3 weeks. All rats were acclimatized to the respective diets for a week. Then the rats in group 2 or 3 received daily 0.2 ml saline or *B. longum* SPM1207 (10⁸~10⁹ CFU/ml), respectively, for 2 weeks. Data are presented as means and standard deviation. *p < 0.05 significantly different compared with control (Cholesterol + Saline).

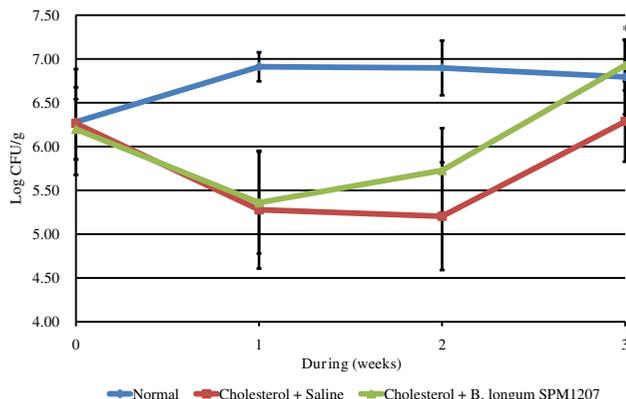


Figure 4
Changes in total LAB counts in rats fed experimental diets for 3 weeks. All rats were acclimatized to the respective diets for a week. Then the rats in group 2 or 3 received daily 0.2 ml saline or *B. longum* SPM1207 (10⁸~10⁹ CFU/ml), respectively, for 2 weeks. Data are presented as means and standard deviation. *p < 0.05 significantly different compared with control (Cholesterol + Saline).

Cholesterol reduction by lactic acid bacteria can be explained by five mechanisms [49-54]: (a) fermentation products of lactic acid bacteria inhibit cholesterol synthesis enzymes and thus reduce cholesterol production; (b) the bacteria facilitate the elimination of cholesterol in feces; (c) the bacteria inhibit the absorption of cholesterol back into the body by binding with

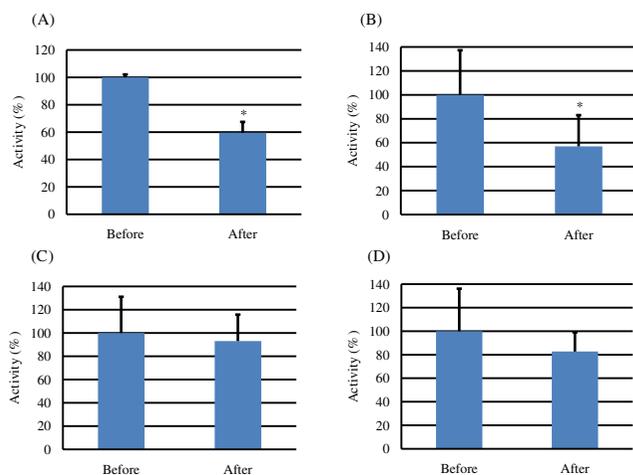


Figure 5
In vivo inhibitory effects of *B. longum* SPM1207 on fecal harmful enzymes in rats fed on high-cholesterol diet. (A) Tryptophanase activity, (B) Urease activity, (C) β -glucosidase activity, (D) β -glucuronidase activity; Before: before the experiment started, After: at the end of experiment.

cholesterol; (d) the bacteria interfere with the recycling of bile salt (a metabolic product of cholesterol) and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption; and, (e) due to the assimilation of lactic acid.

Lactic acid bacteria have anti-tumor effects [6,8,9,55,56] and block harmful intestinal enzyme activities, a recognized risk factor for colon cancer [8,57,58]. Consumption of *L. rhamnosus* GG decreased the activity of β -glucuronidase [59,60], nitroreductase [60], and cholyglycine hydrolase [60,61]. Consumption of milk enriched with *L. casei* for 4 weeks temporarily decreased β -glucuronidase activity in 10 healthy men but not in 10 healthy control subjects [62]. Consumption of milk fermented with a *Bifidobacterium* species decreased β -glucuronidase activity compared with baseline but did not affect fecal pH or the activity of nitrate reductase, nitroreductase, and azoreductase [63]. Consumption of fermented milk with *L. acidophilus*, *B. bifidum*, *Streptococcus lactis*, and *Streptococcus cremoris* for 3 weeks decreased nitroreductase activity but not β -glucuronidase and azoreductase [64].

Here, *B. longum* SPM1207 decreased tryptophanase, urease, β -glucosidase, and β -glucuronidase in rats. Fecal LAB counts in the *B. longum* SPM1207 feeding group was about 10 times greater than that in the control group, indicating bacterial survival through the gastrointestinal tract.

Conclusion

In conclusion, the incorporation of *B. longum* SPM1207 in the diet suppressed serum cholesterol levels on a cholesterol-enriched diet. This LAB also improved the balance of the intestinal flora, improved tryptophanase, urease, β -glucosidase, and β -glucuronidase, and increased fecal LAB levels and fecal water content. Therefore, *B. longum* SPM1207 may be a functional probiotic to treat hypercholesterolemia, help prevent colon cancer, and constipation. Studies in humans, however, could be resulted in contradictory outcomes. So, further clinical trials to confirm these effects must be conducted.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

This study was conceived by NJH and designed by NJH, KOL, KSL, and HSS. NJH, KOL, MJC, and JEK were responsible for obtaining funding and sample collection. The in vitro cholesterol-lowering test and animal experiments were done by DKL, SJ, EHB, and MJK. DKL performed data analysis and wrote the draft of the manuscript. All authors read and approved the final manuscript.

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References

- Gilliland SE: **Acidophilus milk products: a review of potential benefits to consumers.** *J Dairy Sci* 1989, **72**:2483–2494.
- Havenaar R and Huis in't Veld JHJ: **Probiotics a general view.** *The Lactic Acid Bacteria in Health And Disease* The Lactic Acid Bacteria, Elsevier Applied Science, London: Wood BJB 1992, **1**:271–281.
- Hudault S, Lieven B, Bernet-Camard M-F and Servin AL: **Antagonistic activity exerted in vitro and in vivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection.** *Appl Environ Microbiol* 1997, **63**:513–518.
- Salminen S, Deighton M and Gorbach S: **Lactic acid bacteria in health and disease.** *Lactic Acid Bacteria* Marcel Dekker Inc., New York: Salminen S, von Wright A 1993, 199–225.
- Fernandes CF, Shahani KM and Amer MA: **Therapeutic role of dietary lactobacilli and lactobacillus fermented dairy products.** *FEMS Microbiol Lett* 1987, **46**:343–356.
- Fernandes CF and Shahani KM: **Anticarcinogenic and immunological properties of dietary lactobacilli.** *J Food Prot* 1990, **53**:704–710.
- Fuller R: **Probiotics in man and animals.** *J Appl Bacteriol* 1989, **66**:365–378.
- Kim YA, Lee DK, Kim DH, Cho JY, Yang JW, Chung MJ, Kim KJ and Ha NJ: **Inhibition of proliferation in colon cancer cell lines and harmful enzyme activity of colon bacteria by *Bifidobacterium adolescentis* SPM0212.** *Arch Pharm Res* 2008, **31**:468–473.
- Lee DK, Jang S, Kim MJ, Kim JH, Chung MJ, Kim KJ and Ha NJ: **Anti-proliferative effects of *Bifidobacterium adolescentis* SPM0212 extract on human colon cancer cell lines.** *BMC Cancer* 2008, **8**:310.
- Pool-Zobel BL, Bertram B, Knoll M, Lambertz R, Neudecker C, Schillinger U, Schmezer P and Holzapfel WH: **Antigenotoxic properties of lactic acid bacteria in vivo in the gastrointestinal tract of rats.** *Nutr Cancer* 1993, **20**:271–281.

11. Malin M, Suomalainen H, Saxelin M and Isolauri E: **Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus GG*.** *Ann Nutr Metab.* 1996, **40(3)**:137-145.
12. Mikes A, Ferencik M, Jahnová E, Ebringer L and Ciznár I: **Hypocholesterolemic and immunostimulatory effects of orally applied *Enterococcus faecium M-74* in man.** *Folia Microbiol (Praha).* 1995, **40(6)**:639-646.
13. Perdigon G, Alvarez S, Nader de Macias ME, Roux ME and Pesce de Ruiz Holgado A: **The oral administration of lactic acid bacteria increase the mucosal intestinal immunity in response to enteropathogens.** *J Food Prot* 1990, **53**:404-410.
14. Tortuero F, Fernandez E, Ruperez P and Moreno M: **Raffinose and lactic acid bacteria influence caecal fermentation and serum cholesterol in rats.** *Nutr Res* 1997, **17(1)**:41-49.
15. Chiu CH, Lu TY, Tseng YY and Pan TM: **The effects of *Lactobacillus*-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet.** *Appl Microbiol Biotechnol* 2006, **71**:238-245.
16. Foo HL, Loh TC, Law FL, Lim YZ, Kuffli CN and Rusul G: **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* 2003, **12**:403-408 http://fsnb.or.kr/publication/popup_paper_view.php?no=336.
17. Grill JP, Cayuela C, Antoine JM and Schneider F: **Effects of *Lactobacillus amylovorus* and *Bifidobacterium breve* on cholesterol.** *Lett Appl Microbiol* 2000, **31**:154-156.
18. Kim Y, Whang JY, Whang KY, Oh S and Kim SH: **Characterization of the cholesterol-reducing activity in a cell-free supernatant of *Lactobacillus acidophilus ATCC 43121*.** *Biosci Biotechnol Biochem.* 2008, **72(6)**:1483-1490, 70802-1-8..
19. Lim HJ, Kim SY and Lee WK: **Isolation of cholesterol-lowering lactic acid bacteria from human intestine for probiotic use.** *J Vet Sci* 2004, **5**:391-395.
20. Park YH, Kim JG, Shin YW, Kim HS, Kim YJ, Chun T, Kim SH and Whang KY: **Effects of *Lactobacillus acidophilus 43121* and a mixture of *Lactobacillus casei* and *Bifidobacterium longum* on the serum cholesterol level and fecal sterol excretion in hypercholesterolemia-induced pigs.** *Biosci Biotechnol Biochem* 2008, **72**:595-600.
21. Xiao JZ, Kondo S, Takahashi N, Miyaji K, Oshida K, Hiramatsu A, Iwatsuki K, Kokubo S and Hosono A: **Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers.** *J Dairy Sci* 2003, **86**:2452-2461.
22. Du Toit M, Franz CM, Dicks LM, Schillinger U, Haberer P, Warlies B, Ahrens F and Holzapfel WH: **Characterization and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content.** *Int J Food Microbiol* 1998, **40**:93-104.
23. Gilliland SE, Nelson CR and Maxwell C: **Assimilation of cholesterol by *Lactobacillus acidophilus*.** *Appl Environ Microbiol* 1985, **49**:377-381.
24. Mitsuoka T: **The human gastrointestinal tract.** *The Lactic Acid Bacteria In Health and Disease* The Lactic Acid Bacteria, vol. 1, Elsevier Applied Science, London: Wood BFB 1992, 69-114.
25. Fuller R: **History and development of probiotics.** *Probiotics-The Scientific Basis* Chapman and Hall, London: Fuller R 1992, 1-18.
26. Yoon KY, Woodams EE and Hang YD: **Probiotication of tomato juice by lactic acid bacteria.** *J Microbiol* 2004, **42**:315-318.
27. Donohue DC and Salminen S: **Safety of probiotic bacteria.** *Asia Pac J Clin Nutr* 1996, **5**:25-28 http://apjcn.nhri.org.tw/server/APJCN/Volume5/vol5_1/donohue.htm.
28. Donohue DC: **Safety of probiotics.** *Asia Pac J Clin Nutr* 2006, **15**:563-569 http://apjcn.nhri.org.tw/server/APJCN/Volume15/vol15_4/Finished/Donohue.pdf, Review..
29. Scardovi V: **Genus *Bifidobacterium*.** *Bergey's Manual of Systemic Bacteriology* Williams & Willikins, MD: Krieg NR, Holt JG 1986, **2**:1418-1434.
30. Ahn JB: **Isolation and characterization of *Bifidobacterium* producing exopolysaccharide.** *Food Eng Prog* 2005, **9**:291-296.
31. Rudel LL and Morris MD: **Determination of cholesterol using o-phthalaldehyde.** *J Lipid Res* 1973, **14**:364-366.
32. Gutmann I and Bergmeyer HU: **Urea.** *Methods of enzymatic analysis* Academic Press, New York: Bergmeyer HU 1974, **4**:1791-1794.
33. Kim DH, Kang HJ, Kim SW and Kobayashi K: **pH-inducible β -glucuronidase and β -glucosidase of intestinal bacteria.** *Chem Pharm Bull (Tokyo)* 1992, **40(6)**:1667-1669.
34. Kim DH, Lee JH, Bae EA and Han MJ: **Induction and inhibition of indole of intestinal bacteria.** *Arch Pharm Res* 1995, **18**:351-533.
35. Lee YW, Roh WS and Kim JG: **Benefits of fermented milk in rats fed by hypercholesterolemic diet (II).** *Kor J Food Hyg* 1992, **7**:123-135, http://ocean.kisti.re.kr/IS_mvpopo213L.do?ResultTotalCNT=12&pageNo=1&pageSize=10&method=view&poId=ksfhs&kojic=SPOHBV&vnc=v7n2&id=2&setId=36430&iTableId=4&iDocId=186290&sFree.
36. Anderson JW and Gilliland SE: **Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus LI* on serum cholesterol in hypercholesterolemic humans.** *J Am Coll Nutr* 1999, **18**:43-50.
37. Law MR, Wald NJ, Wu T, Hackshaw A and Bailey A: **Systematic underestimation of association between serum cholesterol concentration and ischaemic heart disease in observational studies: data from the BUPA study.** *BMJ.* 1994, **308(6925)**:363-366.
38. Ross R: **The pathogenesis of atherosclerosis: a perspective for 1990s.** *Nature* 1993, **362**:801-809.
39. Lee YW: **Effect of fermented milk on the blood cholesterol level of Korean.** *J Fd Hyg Safety* 1997, **12**:83-95, http://ocean.kisti.re.kr/IS_mvpopo213L.do?ResultTotalCNT=12&pageNo=1&pageSize=10&method=view&poId=ksfhs&kojic=SPOHBV&vnc=v12n1&id=0&setId=77327&iTableId=4&iDocId=216346&sFree.
40. Frick M, Elo O and Haapa K: **Helsinki heart study: primary prevention trial with gemfibrozil in middle-age men with dyslipemia.** *N Engl J Med* 1987, **317**:1237-1245.
41. Mann GV and Spoerry A: **Studies of a surfactant and cholesterolemia in the Massai.** *Am J Clin Nutr* 1974, **27**:464-469.
42. Harrison VC and Peat G: **Serum cholesterol and bowel flora in the newborn.** *Am J Clin Nutr* 1975, **28**:1351-1355.
43. Hepner G, Fried R, Jeor S, Fusetti St L and Morin R: **Hypocholesterolemic effect of yogurt and milk.** *Am J Clin Nutr* 1979, **32**:19-24.
44. Klaver FAM and Meer Van der R: **The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity.** *Appl Environ Microbiol* 1993, **59**:1120-1124.
45. Mann GV: **A factor in yogurt which lowers cholesteremia in man.** *Atherosclerosis* 1977, **26**:335-340.
46. Tahri K, Grill JP and Schneider F: ***Bifidobacteria* strain behavior toward cholesterol: coprecipitation with bile salts and assimilation.** *Curr Microbiol* 1996, **33**:187-193.
47. Hashimoto H, Yamazaki K, He F, Kawase M, Hosoda M and Hosono A: **Hypocholesterolemic effects of *Lactobacillus casei* subsp. *casei TMC 0409* strain observed in the rats fed cholesterol contained diets.** *Anim Sci J* 1999, **72**:90-97.
48. Taranto MP, Medici M, Perdigon G, Ruiz Holgado AP and Valdez GF: **Evidence for hypocholesterolemic effect of *Lactobacillus reuteri* in hypercholesterolemic mice.** *J Dairy Sci* 1998, **81**:2336-2340.
49. Beena A and Prasad V: **Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and lactosehydrolysed condensed whey on serum cholesterol and triacylglycerol levels in rats.** *J Dairy Res* 1997, **64**:453-457.
50. Fukushima M and Nakao M: **The effect of a probiotic on faecal and liver lipid classes in rats.** *Br J Nutr* 1995, **73**:701-710.
51. Grunewald KK: **Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*.** *J Food Sci* 1982, **47**:2078-2079.
52. Hashimoto H, Yamazaki K, Arai Y, Kawase M, He F, Hosoda M and Hosono A: **Effect of lactic acid bacteria on serum cholesterol level in rats fed cholesterol diet.** *Anim Sci Technol* 1998, **69**:702-707.
53. Rao DR, Chawan CB and Pulusani SR: **Influence of milk and Thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats.** *J Food Sci* 1981, **46**:1339-1341.
54. Suzuki Y, Kaizu H and Yamauchi Y: **Effect of cultured milk on serum cholesterol concentrations in rats which fed high-cholesterol diets.** *Animal Sci Technol* 1991, **62**:565-571.
55. Goldin BR and Gorbach SL: **Effect of *Lactobacillus acidophilus* dietary supplements in 1, 2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats.** *J Natl Cancer Inst* 1980, **64**:263-265.
56. Goldin BR, Gualtieri LJ and Moore RP: **The effect of *Lactobacillus GG* on the initiation and promotion of DMH-induced intestinal tumors in the rat.** *Nutr Cancer* 1996, **25**:197-204.
57. Reddy BS and Wynder E: **Metabolic epidemiology of colorectal cancer: fecal bile acids and neutral steroids in colon cancer patients with adenomatous polyps.** *Cancer* 1997, **39**:2533-2539.

58. Williams RT: **Toxicological implications of biotransformation by intestinal microflora.** *Toxicol Appl Pharmacol* 1972, **23**:769–781.
59. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtiere L and Salminen S: **Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract.** *Dig Dis Sci* 1992, **37**:121–128.
60. Ling WH, Korpela R, Mykkanen H, Salminen S and Hanninen O: ***Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults.** *J Nutr* 1994, **124**:18–23.
61. Ling WH, Hänninen O, Mykkanen H, Heikura M, Salminen S and von Wright A: **Colonization and fecal enzyme activities after oral *Lactobacillus* GG administration in elderly nursing home residents.** *Ann Nutr Met* 1992, **36**:162–166.
62. Spanhaak S, Havenaar R and Schaafsma G: **The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans.** *Eur J Clin Nutr* 1998, **52**:1–9.
63. Bouhnik Y, Flourie B, Andrieux C, Bisetti N, Briet F and Rambaud J-C: **Effects of *Bifidobacterium* sp fermented milk ingested with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans.** *Eur J Clin Nutr* 1996, **50**:269–273.
64. Marteau P, Pochart P, Flourie B, Pellier P, Santos L, Desjeux JF and Rambaud JC: **Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic microflora.** *Am J Clin Nutr* 1990, **52**:685–688.

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