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Original Article

Eight Probiotics Are Effective in Lowering Serum Lipids

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Many types of lactobacilli (LBs) can be used to decrease serum lipid levels, and multi-strain LB cultures are beneficial for health. In the present study, eight probiotics including four LBs (*L. acidophilus, L. casei, L. paracasei,* and *L. rhamnosus*) were selected to test their combined ability to decrease the serum lipid levels of male hamsters on a high-fat (12%) and high-cholesterol (0.2%) diet. This experiment involved the oral injection of a set ratio of probiotics into hamsters that were given high-lipid and high-cholesterol diets. The levels of serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerols (TGs), and total cholesterol (TC) increased in the group given high-cholesterol and high-fat diets (110 ± 12, 241 ± 97, 586 ± 218, and 546 ± 303 mg/dl, respectively) compared with those of the control group (51 ± 8, 19 ± 5, 75 ± 15 and 104 ± 42 mg/dl, respectively). When treated with various doses of probiotics (200, 400, and 800 mg/kg), similar to the rosuvastatin-cotreated group, the hamsters presented different levels of recovery of serum LDL-C, TG, and TC levels in a dose-dependent manner. Therefore, the eight probiotics have the potential to decrease serum lipid levels in hamsters.

Key Words: cholesterol, probiotics, triglycerols

Introduction

Probiotics, or simply beneficial bacteria, are popular bacteria that are conducive to human health (33). The cholesterol-lowering effect of probiotic bacteria was discovered by Mann and Spoerry (19). They found that high consumption of fermented milk reduces serum cholesterol levels in Maasai tribesmen in Africa. Several possible reasons for this result were suggested (26). For example, some lactic acid bacterial strains and propionic acid bacterial strains can eliminate cholesterol from their growth media (17, 27, 32). Bile salt hydrolase, which is produced from several strains of lactobacilli *in vitro,* is an enzyme responsible for the de-conjugation of bile acids (12, 28). When the bile salt hydrolysis in the terminal ileum increases, the uptake of bile salt decreases (30). Furthermore, the short-chain fatty acids produced by bacterial fermentation (*e.g.*, propionate) may limit fatty acid and cholesterol synthesis in the liver (26).

Animal studies showed a hypocholesterolemic effect after the administration of probiotic bacteria (6, 8, 38). However, the results in humans are controversial (26, 34). Many human studies indicated that the daily consumption of fermented milk products decreases serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (1, 2, 5-7, 15), whereas several other studies did not show any hypocholesterolemic effect of fermented milk products or other fermented food products (3, 11, 13, 20, 21). Specifically, *Lactobacillus rhamnosus* LC705 is able to assimilate cholesterol *in vitro*, but a combination of *L. rhamnosus* LC705 and *Propionibacterium freudenreichii* JS did not affect serum lipids in human studies (14). Therefore, further studies are needed on the new combinations of probiotics to obtain an effect on serum lipids.

Recently, reports have indicated that a multi-species probiotic mix culture can potentially generate complimentary beneficial effects and surpass the healing properties of the sum of individuals, particularly in terms of improving the balance of gut germs. Numerous *in vitro*, animal, and clinical tests have confirmed

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the presence of this complimentary effect. An *in vitro* cell test conducted by Collado *et al.* (9) indicated that a multi-germ culture treatment increases the effectiveness in preventing bad germ from attaching to the intestinal mucus and the high replacement factor of bad germs that have already attached. Paubert-Braquet *et al.* (25) conducted a 14-day test where mono-species probiotic culture and multi-species culture were fed to rats infected with salmonella. They confirmed that the survivability of the rats that were given a mixed culture is significantly higher (87.5%) than the rats fed with a mono-species culture, non-fermented milk, or nothing at all (50%, 12.5%, and 0% respectively). This result demonstrates the ability of a mixed culture to counter salmonella in the intestines of rats. These types of results have also been supported by human tests. Williams *et al.* (37) used a product of lactobacillus mixed with bifidobacterium on patients with irritable bowel syndrome (IBS) and found that mixed culture affirmatively and safely decreases the symptoms of IBS on patients.

In this study on multi-species probiotic cultures, we investigated the cholesterol-lowering effect of the symbiosis between eight probiotics including three strains of bifidobacterium and five strains of lactobacillus. Food companies regularly add these strains into various foods to promote beneficial bodily effects. In the test, hamsters were fed with 12% high-fat diet containing 0.2% cholesterol. After eight weeks, their blood serum biochemical indexes were determined and the combination of probiotics was evaluated for its potential as a cholesterol-lowering mixture.

Materials and Methods

Bacterial Strains

This study selected two out of many types of probiotics for the experiment: lactobacilli and bifidobacteria. These probiotics have a great capacity for surviving in and colonizing the human gut and improving the ratio of beneficial human intestinal microflora (22). Eight probiotics including three strains of bifidobacterium and five strains of lactobacillus (*L. acidophilus, B. bifidum, L. casei, B. infantis, B. longum, L. lactis, L. paracasei, L. rhamnosus*) were chosen. These bacteria were obtained from Tensall Bio-Tech Co. Ltd. (Yilan, Taiwan).

Preparation of Probiotics (Fermented Milk)

Fermented milk: skim powder was weighed and dissolved in water to constitute 4% skim milk (wt/vol), which was then sterilized using an autoclave at a temperature of 121°C and pressure of 1.2 kg/cm^2 for 15 min and then cooled to room temperature. The milk was inoculated by adding a 1% bacterial solution (vol/vol) in a lamina flow cabinet and by incubation at 37°C for 18 h. After adding 0.2% carboxylmethyl cellulose as a stabilizer, the fermented milk was homogenized by a blender and fed to the experiment hamsters.

Animal Experiment

A total of 56 experiment animals (hamsters) were purchased from the National Animal Center and were divided into 7 groups (8 hamsters each). In the process of this study, the animals were fed with a 12% high-lipid diet including 0.2% cholesterol and given different doses of probiotics. The control group was given a normal diet (ND). All other groups were given a high-fat and high-cholesterol diet (HFCD), except 1 group which was given a high-fat diet (HFD). In addition to the diet, all groups were orally injected with liquids proportional to their weight (2 ml/kg) at 3 p.m. every day of the experiment. The ND, HFD, and HFCD groups were given oral injections of water. The HFCD+R group was given 1 mg/kg of rosurastatin mixed with water. Three of the groups given with HFCDs were respectively given 800 mg/kg (HFCD+P8), 400 mg/kg (HFCD+P4), and 200 mg/kg (HFCD+P2) of probiotics mixed with water. At the end of 8 weeks, blood serum samples were obtained from all animals at 12 h after the last feeding for serum biochemical index screening.

Serum Lipid Profile Estimation

All biochemical assays were conducted in the clinical laboratories of the Kuang Tien General Hospital, Taichung, Taiwan. The biochemical parameters were measured on a Beckman Coulter Synchron Clinical Systems Analyzer (LX20PRO Autoanalyzer, Beckman Coulter Inc. Taipei, Taiwan). To determine the serum lipid profile, TC, total triglyceride (TG), LDL-C, and high-density lipoprotein cholesterol (HDL-C) were measured by Hitachi 7170 autoanalyzer (Hitachi, Japan).

Statistical Analysis

The experimental results are expressed as mean ± SD. Data were assessed using ANOVA. Student's *t*-test was used in the comparison between groups. A P value less than 0.05 was considered statistically different.

Results

Body Weight Gain, Liver Weight Gain, Food Consumption, and Clinical Observations of Hamsters

The changes in body weight and food consumption of the hamsters influence their growth rate and physiolog-

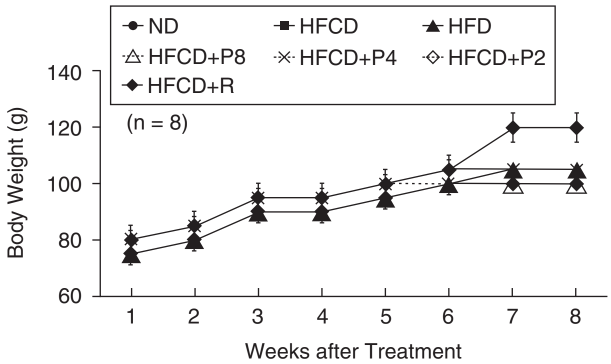

Fig. 1. Change of body weight in hamsters fed with HFCDs and co-treated with different doses of eight probiotics in eight weeks.

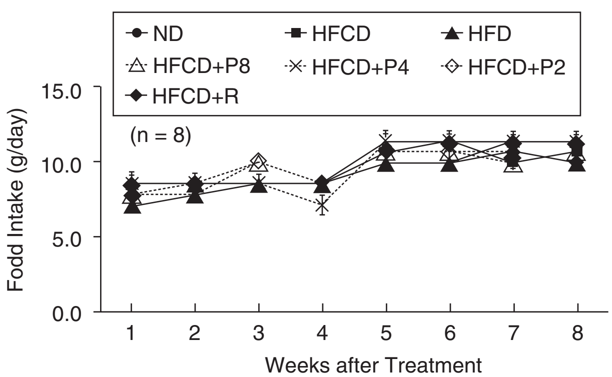

Fig. 2. Change of food intake in hamsters fed with HFCDs and co-treated with different doses of eight probiotics in eight weeks.

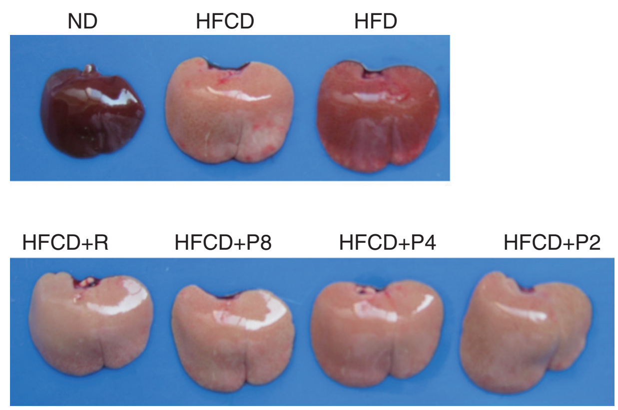

Fig. 3. Liver images of hamsters fed with HFCDs and co-treated with different doses of eight probiotics at the end of eight weeks.

Table 1. The effect of eight probiotics on the change of relative liver weight in hamsters treated with high cholesterol diet

Group	No	Liver Related Weight (%, liver weight/body weight)
ND	8	2.691 ± 0.224
HFCD	8	4.122 ± 0.507*
HFD	8	3.295 ± 0.283*, #
HFCD+R	8	3.986 ± 0.314*
HFCD+P8	8	3.812 ± 0.310*
HFCD+P4	8	3.678 ± 0.423*
HFCD+P2	8	4.034 ± 0.420*

*$P < 0.05$ versus control group; #$P < 0.05$ versus cholesterol and high fat-treated group.

ical conditions. The mean body weight (g) and daily food intake (g/hamster) were increased by adequate feeding during the experiment period (Figs. 1 and 2). All hamsters in each group survived for the entire length of the study. However, in the HFCD group, the hamsters were visually lethargic at seven or eight weeks.

No differences were observed in body weight between 100.1 and 120.3 g (Fig. 1) and in food intake between 10.0 and 11.4 g/day (Fig. 2) among the experimental groups during the eight weeks of the experiment. At eight weeks, liver images showed that all HFCD groups have significantly reduced redness compared with the ND and HFD group (Fig. 3). The relative liver weight of the HFCD groups was also significantly higher than the other groups (Table 1).

Induction of Hyperlipidaemia with High-Cholesterol Diet

The levels of plasma cholesterol and triglyceride of all the groups eight weeks after treatment are reported in Table 2. The levels of serum HDL-C, LDL-C, TC, and TG increased in the HFCD group (110 ± 12, 241 ± 97, 586 ± 218, and 546 ± 303 mg/dl, respectively) compared with the ND group (51 ± 8, 19 ± 5, 75 ± 15, and 104 ± 42 mg/dl, respectively). Compared with the ND group, HFCD caused a marked increase in plasma concentrations of TC and TG by 781% and 525%, respectively. Both HDL-C and LDL-C also increased by more than 2.2 and 12.1 times, respectively. No differences were observed between the HDL-C level in the HFD and HFCD groups, whereas the LDL-C was higher in the HFCD group than in the HFD group. Moreover, HFCD caused a marked increase in the plasma concentrations of TC and TG by 386% and 317% compared with the HFD group.

Effect of Probiotics and Rosurastatin on Plasma Lipid Profile

At eight weeks after the administration of probiotics to hamsters fed with fat- and cholesterol-enriched diets, both plasma TC and TG of the HFCD+P8 group decreased by 56.5% and 50.2%, respectively, compared with HCFD (Table 2); the plasma TC and TG of the

Table 2. The effect of eight probiotics on the change of serum lipid in hamster treated high cholesterol diet

Group	HDL-C (mg/dl)	LDL-C (mg/dl)	TC (mg/dl)	TG (mg/dl)
ND	50 ± 8*	20 ± 5*	75 ± 15*	104 ± 42*
HFCD	110 ± 12$^{\#}$	241 ± 97$^{\#}$	586 ± 218$^{\#}$	546 ± 303$^{\#}$
HFD	95 ± 15$^{\#}$	27 ± 12*	152 ± 25*	172 ± 61*
HFCD+R	116 ± 14$^{\#}$	107 ± 48*	318 ± 70$^{\#,*}$	158 ± 54*
HFCD+P8	87 ± 26$^{\#}$	93 ± 31*	255 ± 89*	272 ± 264
HFCD+P4	80 ± 14$^{\#}$	117 ± 79*	302 ± 196$^{\#,*}$	247 ± 228*
HFCD+P2	105 ± 19$^{\#}$	220 ± 57$^{\#}$	503 ± 123$^{\#}$	474 ± 207$^{\#}$

In protective testing in hamsters (8 weeks application period), a maximum daily dose of 800 mg probiotics per kg body weight was orally administered to hamsters (W.S.). The data were expressed as mean ± SD. n = 8. $^{\#}P < 0.05$ *vs.* control group; $*P < 0.05$ *vs.* High fat and high choesterol group.

HFCD+P4 group decreased by 48.5% and 54.7%, respectively. Rosuvastatin, which is an HMG-CoA reductase inhibitor, was used as the reference drug (1 mg/kg body weight) to compare with probiotics. The hypolipidaemic effect of rosuvastatin in plasma was effective in TC and TG levels, which were respectively decreased by 45.7% and 71.1% in the HFCD group (Table 2). In HFCD+P8 and HFCD+P4 hamsters, no significant differences were observed in the HDL-C level in hamsters given with HFCD, but LDL-C decreased by 61.4% and 51.4%, respectively, compared with HCFD (Table 2). The reduction induced by rosuvastatin in LDL-C was 55.6%.

Discussion

Multi-species probiotics are superior to mono-strain probiotics (35). Probiotics in a multi-species environment compete with each other and form their own mini-ecosystem. This phenomenon forces antagonistic species to strengthen their defense mechanisms and create their own niche within the culture until equilibrium is reached and the overall effects of antagonistic activity is reduced. Moreover, within the mini-ecosystem, metabolites are exchanged between resident species, thus helping enhance their biological activity for the benefit of the host. Our results show that at the end of the experiment, the serum TC of the HFCD plus probiotics groups, as well as the HFCD plus rosuvastatin group, were 50% lower than that of the HFCD group. However, the mechanism of exchanges between probiotics that contribute to the enhanced benefit over their individual traits is intrinsically complex to investigate.

In this study, eight probiotics (three strains of bifidobacterium and five strains of lactobacillus) were selected and tested. Six types of bacteria, namely, *L. acidophilus, L. casei, L. paracasei, L. rhamnosus, B. longum,* and *B. bifidum*, have been reported to be effective in the reduction of cholesterol in animals (8, 10, 16, 23, 36). Furthermore, *B. infantis* and *L. lactis* were chosen to aid the growth of the six bacteria in the culture and gut environment. *L. lactis* produces extracellular polysaccharides (EPS), which may protect microorganisms against anti-microbial factors (18). EPS can also serve as a bifidogenic growth factor for the bifidobacteria (4). *B. infantis* exerts immunomodulatory effects on intestinal immune cells and the maintenance of gut homeostasis (24). Given their theoretical stability and complimentary properties, these eight probiotics were chosen for this study with the original intention of improving the cholesterol reduction effect inside human intestines. The intention of this study is to provide a method of cholesterol intake reduction alternative to taking drugs. The test has successively shown that the effectiveness of the probiotics is relatively similar to that of taking drugs such as rosuvastatin. Therefore, probiotics are suitable supplements for cholesterol reduction because of the lack of drug-like side effects.

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References

1. Agerbjk, M., Gerdes, L.U. and Richelsen, B. Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. *Eur. J. Clin. Nutr.* 49: 346-352, 1995.

2. Agerholm-Larsen, L., Raben, A., Haulrik, N., Hansen, A.S., Manders, M. and Astrup, A. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. *Eur. J. Clin. Nutr.* 54: 288-297, 2000.
3. Andersson, H., Bosaeus, I., Ellegård, L., Grahn, E., Tidehag, P., Hallmans, G., Holm, S. and Sandberg, A.S. Effects of low-fat milk and fermented low-fat milk on cholesterol absorption and excretion in ileostomy subjects. *Eur. J. Clin. Nutr.* 49: 274-281, 1995.
4. Bello, F.D., Walter, J., Hertel, C. and Hammes, W.P. *In vitro* study of prebiotic properties of levan-type exopolysaccharides from Lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *Syst. Appl. Microbiol.* 24: 232-237, 2001.
5. Bertolami, M.C., Faludi, A.A. and Batlouni, M. Evaluation of the effects of a new fermented milk product (Gaio) on primary hypercholesterolemia. *Eur. J. Clin. Nutr.* 53: 97-101, 1999.
6. Broccali, G., Berti, M., Pistolesi, E. and Cestaro, B. Study of the effect of lactobacillus GG supplementation in combination with and without arginine aspartate on lipoproteins and liver peroxidation in cholesterol-fed rats. *Intern. J. Food Sci. Nutr.* 51: 475-482, 2000.
7. Bukowska, H., Pieczul-Mróz, J., Jastrzebska, M., Chelstowski, K. and Naruszewicz, M. Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with *Lactobacillus plantarum* in subjects with moderately elevated cholesterol. *Atherosclerosis* 137: 437-438, 1998.
8. Chiu, C.H., Lu, T.Y., Tseng, Y.Y. and Pan, T.M. The effects of *Lactobacillus*-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Appl. Microbiol. Biotechnol.* 71: 238-245, 2006.
9. Collado, M.C., Grześkowiak, Ł. and Salminen, S. Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. *Curr. Microbiol.* 55: 260-265, 2007.
10. Corzo, G. and Gilliland, S.E. Bile salt hydrolase activity of three strains of Lactobacillus acidophilus. *J. Dairy Sci.* 82: 472-480, 1999.
11. de Roos, N.M., Schouten, G. and Katan, M.B. Yoghurt enriched with *Lactobacillus acidophilus* does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. *Eur. J. Clin. Nutr.* 53: 277-280, 1999.
12. du Toit, M., Franz, C.M.A.P., Dicks, L.M.T., Schillinger, U., Haberer, P., Warlies, B., Ahrens, F. and Holzapfel, W.H. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *Intern. J. Food Microbio.* l40: 93-104, 1998.
13. Fabian, E. and Elmadfa, I. Influence of daily consumption of probiotic and conventional yoghurt on the plasma lipid profile in young healthy women. *Ann. Nutr. Metab.* 50: 387-393, 2006.
14. Hatakka, K., Mutanen, M., Holma, R., Saxelin, M. and Korpela, R. Lactobacillus rhamnosus LC705 together with Propionibacterium freudenreichii ssp shermanii JS administered in capsules is ineffective in lowering serum lipids. *J. Am. Coll. Nutr.* 27: 441-447, 2008.
15. Hepner, G., Fried, R., St. Jeor, S., Fusetti, L. and Morin, R. Hypocholesterolemic effect of yogurt and milk. *Am. J. Clin. Nutr.* 32: 19-24, 1979.
16. Klaver, F.A. and 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.
17. Liong, M.T. and Shah, N.P. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J. Dairy Sci.* 88: 55-66, 2005.
18. Looijesteijn, P.J., Trapet, L., de Vries, E., Abee, T. and Hugenholtz, J. Physiological function of exopolysaccharides produced by Lactococcus lactis. *Int. J. Food Microbiol.* 64: 71-80, 2001.
19. Mann, G.V. and Spoerry, A. Studies of a surfactant and cholesteremia in the Maasai. *Am. J. Clin. Nutr.* 27: 464-469, 1974.
20. Massey, L.K. Effect of changing milk and yogurt consumption on human nutrient intake and serum lipoproteins. *J. Dairy Sci.* 67: 255-262, 1984.
21. Naruszewicz, M., Johansson, M.L., Zapolska-Downar, D. and Bukowska, H. Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. *Am. J. Clin. Nutr.* 76: 1249-1255, 2002.
22. Nishida, S., Michinaka, A., Nakashima, K., Iino, H. and Fujii, T. Evaluation of the probiotic potential of Lactobacillus paracasei KW3110 based on *in vitro* tests and oral administration tests in healthy adults. *J. Gen. Appl. Microbiol.* 54: 267-276, 2008.
23. Noh, D.O., Kim, S.H. and Gilliland, S.E. Incorporation of cholesterol into the cellular membrane of Lactobacillus acidophilus ATCC 43121. *J. Dairy Sci.* 80: 3107-3113, 1997.
24. O'Hara, A.M., O'Regan, P., Fanning, A., O'Mahony, C., Macsharry, J., Lyons, A., Bienenstock, J., O'Mahony, L. and Shanahan, F. Functional modulation of human intestinal epithelial cell responses by Bifidobacterium infantis and Lactobacillus salivarius. *Immunology* 118: 202-215, 2006.
25. Paubert-Braquet, M., Gan, X.H., Gaudichon, C., Hedef, N., Serikoff, A., Bouley, C., Bonavida, B. and Braquet, P. Enhancement of host resistance against Salmonella Typhimurium in mice fed a diet supplemented with yoghurt or milks fermented with various Lactobacillus casei strains. *Int. J. Immunol.* 11: 153-161, 1995.
26. Pereira, D.I.A. and Gibson, G.R. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Crit. Rev. Biochem. Mol. Biol.* 37: 259-281, 2002.
27. Pereira, D.I.A. and Gibson, G.R. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Appl. Environ. Microbiol.* 68: 4689-4693, 2002.
28. Pereira, D.I.A., McCartney, A.L. and, Gibson, G.R. An *in vitro* study of the probiotic potential of a bile-salt-hydrolyzing Lactobacillus fermentum strain, and determination of its cholesterol-lowering properties. *Appl. Environ. Microbiol.* 69: 4743-4752, 2003.
29. Richelsen, B., Kristensen, K. and Pedersen, S.B. Long-term (6 months) effect of a new fermented milk product on the level of plasma lipoproteins - a placebo-controlled and double blind study. *Eur. J. Clin. Nutr.* 50: 811-815, 1996.
30. Ridlon, J.M., Kang, D.J. and Hylemon, P.B. Bile salt biotransformations by human intestinal bacteria. *J. Lipid. Res.* 47: 241-259, 2006.
31. Schaafsma, G., Meuling, W.J.A., van Dokkum, W. and Bouley, C. Effects of a milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in male volunteers. *Eur. J. Clin. Nutr.* 52: 436-440, 1998.
32. Somkuti, G.A. and Johnson, T.L. Cholesterol uptake by Propionibacterium freudenreichii. *Curr. Microbiol.* 20: 305-309, 1990.
33. Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P.B. and Ross, R.P. Am J Market potential for probiotics. *Clin. Nutr.* 73: 476S-483S, 2001.
34. St-Onge, M.P., Farnworth, E.R. and Jones, P.J.H. Consumption of fermented and nonfermented dairy products: effects on cholesterol concentrations and metabolism. *Am. J. Clin. Nutr.* 71: 674-681, 2000.
35. Timmerman, H.M., Koning, C.J., Mulder, L., Rombouts, F.M. and Beynen, A.C. Monostrain, multistrain and multispecies probiotics--A comparison of functionality and efficacy. *Int. J. Food Microbiol.* 96: 219-233, 2004.
36. Umeki, M., Oue, K., Mochizuki, S., Shirai, Y. and Sakai, K. Effect of Lactobacillus rhamnosus KY-3 and cellobiose as synbiotics on lipid metabolism in rats. *J. Nutr. Sci. Vitaminol.* 50: 330-334, 2004.
37. Williams, E., Stimpson, J., Wang, D., Plummer, S., Garaiova, I., Barker, M.E, and Corfe, B.M. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Aliment. Pharmacol. Ther.* 29: 97-103, 2009.
38. Xiao, J.Z., 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.* 86: 2452-2461, 2003.