Effect of cholesterol lowering multiplex lactic acid bacteria on lipid metabolism in a hamster model

Cheng-Chih Tsai¹,²*, Lan-Chun Chou², Shu-E Lai³ and Chung-Chih Huang²

¹Department of Applied Cosmetology, Master Program of Cosmetic Science, Hung Kuang University, Taichung City 43302, Taiwan, R.O.C.
²Department of Food Science and Technology, Hung Kuang University, Taichung City 43302, Taiwan, R.O.C.
³Department of Nutrition, Chung-Kang Branch, Cheng Ching Hospital, Taichung City 40764, Taiwan, R.O.C.

Received 16 August 2015; Accepted 12 February, 2016

This study aimed to investigate the effects of a probiotic complex product including Pediococcus, Lactobacillus, and Bifidobacteria on the lipid metabolism of hamsters fed a high-fat and high-cholesterol diet. Fifty male Syrian hamsters were assigned to five experimental groups: control, high-fat plus high-cholesterol diet (HFC), and HFC supplemented with low-, medium-, and high-dose of probiotic product. The hamsters in the control group were fed an AIN-76 basal diet. A high-cholesterol diet was based on the AIN-76 basal diet, supplemented with 0.5% (w/w) cholesterol, 12% corn oil, and 3% (w/w) lard to adjust the fat content. After a one-week adaptation period, the experimental period started, during which the animals were fed for 10 weeks, and food intake and body weight were recorded periodically. Blood samples were obtained for the analysis of serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and thiobarbituric acid reactive substances (TBARS). The results show that feeding animals with the low, medium, and high doses of probiotic complex product had significantly lowered serum LDL-C, and TBARS levels, as well as LDL-C/HDL-C ratio (P < 0.05), while we observed a significant increase in HDL-C levels (P < 0.05). These results indicate that the probiotic complex product could reduce obesity, dyslipidemia, and lipid peroxidation.

Key words: Probiotics, cholesterol, LDL-C/HDL-C ratio, thiobarbituric acid reactive substances, hamster.

INTRODUCTION

In 2013, the World Health Organization (WHO) reported that cardiovascular diseases (CVDs) were responsible for 30% of deaths worldwide, and by 2030, CVDs would affect approximately 23.3 million people around the world (WHO, 2013). According to Hjermann et al. (1981), every 1% reduction in serum cholesterol lowers the risk of coronary heart diseases by 2%. Although drug therapies effectively decrease cholesterol levels, they are expensive and have some side effects. For example, myopathy was found to occur in 10% of statin-treated patients.
Moosmann and Behl, 2004; Eckel, 2010), and gastrointestinal discomfort such as constipation was described in patients consuming bile-salt sequestrants (Davidson et al., 1999). Moreover, cognitive impairment was reported in patients administered with lovastatin or simvastatin (Muloon et al., 2000, 2004).

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). Probiotics were reported to have cholesterol-lowering effects in vitro or in vivo, especially the strains of genera Lactobacillus and Bifidobacterium (Pan et al., 2011; Wang et al., 2012; Huang et al., 2013; Hu et al., 2013; Tsai et al., 2014). In recent years, several mechanisms for cholesterol removal by probiotics have been proposed, such as the cholesterol assimilation into bacterial cell membranes (Kimoto et al., 2002), deconjugation of bile salts by bile-salt hydrolase (BSH) (Ahn et al., 2003; Tsai et al., 2014), and production of short-chain fatty acids (SCFAs) during growth of probiotics (Tabuchi et al., 2004).

However, the mechanism underlying the hypocholesterolemic effect of probiotics might be strain-specific. Lactobacillus fermentum normally adheres to epithelial cells in the human gastrointestinal tract and promotes the survival of healthy intestinal microflora (Wickström et al., 2013). Several studies have reported beneficial effects of exo-polysaccharides on host health, including cholesterol-lowering effect. For example, the exo-polysaccharide produced by L. kefiranofaciens, kefiran, was reported to reduce serum cholesterol levels as well as suppress the blood pressure increase in SHRSP/Hos rats consuming excessive amounts of cholesterol (Maeda et al., 2004). Pigeon et al. (2002) suggested that cholesterol removal by L. delbrueckii and Streptococcus thermophilus strains was due to the binding of free bile acids to their cell membranes through extracellular polysaccharides. Oral administration of probiotics was shown to significantly reduce cholesterol levels by as much as 22 to 33% (Pereira and Gibson, 2002), or prevent elevated cholesterol levels in mice that had been fed a fat-enriched diet (Taranto et al., 2000).

Previously, we isolated different probiotic isolates from animal and plant sources to evaluate their bile acid deconjugation abilities of Pediococcus acidilactici NBHK002, B. adolescentis NBHK006, L. rhamnosus NBHK007, and L. acidophilus NBHK008 were higher than those of the other probiotic strains. NBHK002, NBHK006 and NBHK007 reduced apo B secretion by 33, 38 and 39%, respectively, after 24 h of incubation. The product PROBIO S-23 caused a greater decrease in the total concentration of cholesterol, low-density lipoprotein, TG and thioctetic acid reactive substances in the serum or livers of hamsters with hypercholesterolemia in the pre-induced high blood lipid animal model (fed a high-fat and high-cholesterol diet ten days before the experimental period) (Tsai et al., 2014).

Based on these results, the present study was conducted to determine the effect of PROBIO S-23 on cholesterol-lowering in hamsters fed a high-fat and high-cholesterol diet simultaneously with PROBIO S-23 during the experimental period (non-pre-induced high blood lipid animal model).

MATERIALS AND METHODS

Bacterial strains, culture medium and growth conditions

Each lactic acid bacteria (LAB) stock culture was maintained in 20% glycerol at -80°C. Bacterial cells were propagated twice in lactobacilli Man, Rogosa, Sharpe (MRS) broth (DIFCO, Detroit, Michigan, USA), supplemented with 0.05% L-cysteine and incubated at 37°C for 20 h. The freeze dried powder of a novel multispecies probiotic mixture (PROBIO S-23) including Lactobacillus rhamnosus NBHK007 (strain LCR177), Bifidobacterium adolescentis NBHK006 (strain BA286), and Pediococcus acidilactici NBHK002 (strain PA318), were isolated from pickled vegetables and human feces, respectively.

Animals and experimental groups

This experimental protocol (No. 10105) was approved by the Institutional Animal Care and Use Committee of HungKuang University, Taichung, Taiwan. Fifty 3-week-old male hamsters were purchased from the National Laboratory Animal Center (Taipei, Taiwan). They were housed individually in a controlled environment with 20 ± 2°C temperature, 55 ± 5% humidity, and a 12 h light-dark cycle with the light period from 8 AM to 8 PM. During the first week of the acclimatization period, the animals were fed chow pellets (AIN-76; Jin Long Technology Co. Ltd, Taichung, Taiwan) and water ad libitum. They were then randomly divided into one control group and four experimental groups, namely, high-fat and high-cholesterol diet (HFC group), and HFC + low- (78 mg/kg BW/day), HFC + medium-(390 mg/kg BW/day), and HFC + high-dose PROBIO S-23 powder (1950 mg/kg BW/day) groups. Hamsters in the four experimental groups were fed a basal AIN-76 diet supplemented with 12% corn oil, 3% lard and 0.5% cholesterol. Simultaneously, during the experimental period (10 weeks), different doses of LAB were orally administered with a sterile orogastric tube once a day to the animals in the three PROBIO S-23 groups, in addition to the HFC diet. PROBIO S-23 powder with high viable counts of LAB (1 × 10^9 to 1 × 10^10 CFU/mg) was produced by freeze-drying (New Bellus Enterprise Co., Ltd, Tainan, Taiwan). Weights of the animals and food intake were recorded. Serum was collected to measure the concentrations of total cholesterol (TC), triglycerol (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), as well as the lipid peroxidation index (thiobarbituric acid reactive substances, TBARS).

The animals were sacrificed after 10 weeks. Livers of the animals from all experimental groups were removed and fixed in 10% neutral formalin for preservation. After fixation in 10% neutral formalin solution, sections were cut from the livers of animals from all groups and examined for histopathology. The formalin-solution-fixed livers were coarsely repaired and processed via dehydration, clarification, paraffin infusion, and embedding steps to prepare paraffin tissue blocks. The slices were stained with hematoxylin & eosin (H&E) and observed by microscopy.

Statistical analysis

Statistical analyses were performed using the SPSS 17.0 software.
(SPSS Inc., Chicago, IL, USA). The data of body weight, TC, TG, LDL-C, HDL-C, and TBARS were analyzed by one-way analysis of variance (one-way ANOVA), followed by the Duncan’s multiple range test to determine significant differences among groups (P < 0.05).

RESULTS AND DISCUSSION

Body weight, the weight percentage gain, and food intake

The mean body weight values of hamsters in the three PROBIO S-23 dosage groups, the HFC group, and the control group are shown in Figure 1. The results of the ANOVA analysis indicated that the final body weight values of the hamsters from the PROBIO S-23 dosage groups and the HFC group were not significantly different from the control group (P > 0.05).

On calculating the percentage weight gain during the experiment, we found that the weight percentage gain of the HFC group was approximately 28.5% (Table 1). For hamsters fed high-fat and high-cholesterol diet and simultaneously administered different (low-, medium-, or high-) doses of LAB, the weight percentage gains were 25.4, 23.8, and 21.7%, respectively (Table 1). We noted that, with the increasing dose of LAB the weight percentage gain decreased. Although the HFC diets resulted in overall increase of the body weight in all four experimental groups, we demonstrated that the HFC group without the LAB supplement showed the highest increase in weight percentage gain. Therefore, administering lactic acid bacteria product reduced the body weight gain in the animals that were fed the high-cholesterol and high-fat diet.

The mean food-intake values for the hamsters in different groups during the experimental period are listed in Table 1. No significant differences were observed throughout the study among the three PROBIO S-23 dosage groups, the HFC group and the control group (P >0.05). Therefore, we conclude that feeding the mixture of multiple strains of the LAB product did not affect the appetite or digestive function of hamsters. The results obtained here are in concordance with the findings of Huang et al. (2013), who used L. plantarum Lp09, an isolate from kefir grains, as supplementation of a high-cholesterol diet. In their study, it was found that there were no significant differences in the total food intake between the groups with or without the supplement; however, they noted lower weight gain and food efficiency in the group administered L. plantarum Lp09 than in the high-cholesterol diet group.

Figure 1. Body weight with high fat plus high cholesterol diet for 10 weeks with different concentrations of probiotics complex product.
Table 1. Body weight gain and food intake of hamsters fed a high-fat plus high-cholesterol diet for 10 weeks, supplemented or not with different concentrations of probiotics complex product, PROBIO S-23.

| Group                | Body weight (g) | Weight percentage gain (%) | Daily weight gain (g) | Food intake (g/day) |
|----------------------|----------------|----------------------------|----------------------|--------------------|
|                      | Initial        | Final                      |                      |                    |
| Control              | 91.48±4.35a    | 124.59±9.56a               | 26.6                 | 0.43±0.06          | 10.95±2.41a |
| HFC                  | 84.93±4.87a    | 118.85±10.04a              | 28.5                 | 0.44±0.06          | 10.16±2.35a |
| Low dose LAB         | 91.72±6.29a    | 122.92±6.33a               | 25.4                 | 0.41±0.08          | 10.36±2.35a |
| Medium dose LAB      | 89.93±3.90a    | 118.08±9.43a               | 23.8                 | 0.37±0.05          | 9.90±2.4a  |
| High dose LAB        | 93.10±7.20a    | 118.84±10.02a              | 21.7                 | 0.33±0.10          | 9.81±2.50a |

Data are expressed as means ± SD (n=10). a,b:Values in the same column with different superscripts mean significant difference (P < 0.05). Weight percentage gain= (final weight-initial weight) / final weight *100. Daily weight gain = (final weight- initial weight) / days.

Total cholesterol and triglyceride levels in serum

Throughout the 10-week experimental period, the levels of total cholesterol were significantly lower (P < 0.05) in the medium- and high dose PROBIO S-23 groups compared with those in the HFC group (Figure 2). Compared with the HFC group, hamsters fed low-, medium-, and high doses of LAB showed total cholesterol levels in serum reduced by 3.30% (P > 0.05), 10.55% (P < 0.05) and 15.13% (P < 0.05), respectively, at the end of the trial (Figure 2A).

These results suggest that the higher doses of LAB reduce the serum total cholesterol levels more effectively compared to HFC group. There were no significant differences in triglyceride levels among the three PROBIO S-23 dosage groups and the HFC group (Figure 2B). The base levels of TG at week 0 were significantly different among groups, with the highest level in high-dose LAB group. Probably this is the reason why there were no significant differences in TG levels among the three PROBIO S-23 dosage groups and the HFC group.

High-density lipoprotein and low-density lipoprotein levels in serum, and LDL-C/HDL-C ratio

The levels of high-density lipoprotein cholesterol (HDL-C) were significantly higher (P < 0.05) in the medium- and high dose PROBIO S-23 groups compared with the HFC group throughout the 10-week experimental period (Figure 3A). Hamsters fed the high dose of LAB showed HDL-C levels increased by 31.1% (P < 0.05) compared with the HFC group at the end of the trial (Figure 3A). All three PROBIO S-23 dosage groups showed reduced LDL-C levels and LDL-C/HDL-C ratio at 6, 8, and 10 weeks compared with the HFC group (Figure 3B and C).

Compared with the HFC group, hamsters fed low, medium, and high doses of LAB showed LDL-C levels (LDL-C/HDL-C ratio) in serum reduced by 10.14% (7.64%) (P < 0.05), 34.29% (42.64%) (P < 0.05) and 42.89% (56.44%) (P < 0.05), respectively, at the end of the experimental period (Figure 3B and C). The high-dose PROBIO S-23 group showed the greatest reduction in LDL-C levels at 6, 8 and 10 weeks (by 27.62, 32.79, and 42.89%, respectively) compared with the HFC group (Figure 3B).

The present animal studies indicated not only a significant increase in the concentration of HDL-C but also a significant decrease in the concentrations of LDL-C in the groups administered the PROBIO S-23 product in comparison with the HFC group. The results related to HDL-C and LDL-C are in concordance with previous studies (Abd El-Gawad et al., 2005; Klein et al., 2008), which demonstrated that animals fed a cholesterol- and fat-rich diet showed reduced total cholesterol levels, LDL-C and increased the HDL-C fraction when supplemented with a daily intake of LAB or yogurt. Some studies have shown that animals fed cholesterol and fat showed a reduction in total cholesterol levels and LDL-C, but HDL-C concentrations did not increase significantly (Jones et al., 2012; Wang et al., 2009; Pan et al., 2010; Huang et al., 2013), while other studies did not observe cholesterol lowering effect following lactic acid bacteria consumption (de Roos, 1999; St-Onge et al., 2002). These conflicting results may be due to the different properties of the LAB strains used such as acid resistance, bile tolerance, or different mechanisms of lowering cholesterol in vitro (Akalin et al., 1997; Taranto et al., 1998). Other factors may be involved, e.g., the cholesterol content in diet, LAB ingestion dosage, LAB combinations and their different ratios, animals used, and length of the feeding period (Wang et al., 2009; Starovoitova et al., 2012).

Thiobarbituric acid reactive substances (TBARS) levels in serum

The highest TBARS levels in serum were detected in the HFC group of hamsters from the sixth week to the tenth week of the study. These values were significantly
different from the control and the three PROBIO S-23 dosage groups (P < 0.05). The high-dose PROBIO S-23 group showed the greatest reduction of TBARS levels at 6, 8, and 10 weeks (by 9.86, 43.07 and 49.09%, respectively) compared with the HFC group (Figure 4). Moreover, the malondialdehyde (MDA) levels in hamsters of the high-dose PROBIO S-23 group decreased and were similar to the MDA values in the control group, indicating that the probiotics reduced the effect of lipid peroxidation in serum in the high-fat and high-cholesterol diet.

Being a biomarker of lipid peroxidation, the plasma MDA level is considered a marker of the oxidative stress caused by cholesterol (Nagao et al., 2005). Holvoet et al. (1995) measured plasma MDA-LDL in humans and suggested that an increase in plasma MDA-LDL can be used as a marker of unstable atherosclerotic cardiovascular disease and that blood MDA-LDL is an independent factor not correlated with LDL cholesterol. Previously, MDA-LDL was measured indirectly by using TBARS, and many reports described that catechins prevent serum TBARS increase.

Malondialdehyde is the principal and the most studied product of polyunsaturated fatty acid peroxidation. This aldehyde is a highly toxic molecule and should be considered as more than just a marker of lipid peroxidation. Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic (Del Rio et al., 2005). Most assays to determine MDA are based on its derivatization with thiobarbituric acid.

Figure 2. Serum (A) total cholesterol and (B) triglyceride of hamsters fed a high-fat plus high-cholesterol diet and supplemented or not with different concentrations of probiotics complex product, PROBIO S-23, during 10 weeks of the study. Data are expressed as means ± SD (n=8). a,b,c Values in the same column with different superscripts mean significant difference (P < 0.05).
Figure 3. Serum (A) HDL-C, (B) LDL-C and (C) LDL-C/HDL-C ratio of hamsters fed a high-fat plus high-cholesterol diet and supplemented or not with different concentrations of probiotics complex product, PROBIO S-23, during 10 weeks of the study. Data are expressed as means ± SD (n=8). Values in the same column with different superscripts mean significant difference (P < 0.05).
Yogurt was shown to have similar effects as we observed after administering PROBIO S-23. In the study conducted by Al-Sheraji et al. (2012), rats in the positive control group fed a cholesterol-enriched diet showed significant increase in MDA after 8 weeks. However, groups fed a cholesterol-enriched diet and supplemented with a yogurt containing B. pseudocatenulatum G4 or B. longum BB536 had significantly lower MDA levels than the positive control group after 8 weeks of treatment (P < 0.05).

Histopathological examination

After ten weeks of the experiment, the liver cells of hamsters in the HFC group, fed a high-cholesterol and high-fat diet, appeared empty, swollen, and even exhibited necrosis in some cases. In the high dose PROBIO S-23 group, although the tumescence of liver cells was not improved, the cavities and necroses observed were relatively few (Figure 5). Similar results were obtained by Hu et al. (2013), where histopathological examinations suggested severe injuries in liver tissues of rats fed high-cholesterol diet, while L. plantarum NS5 and NS12 strains partially ameliorated these injuries.

Conclusion

The results of our present and previous study (Tsai et al., 2014) indicate that PROBIO S-23 is a potential multiplex-strain probiotic product with the bile-salt hydrolase activity, and reduces the serum cholesterol, low-density lipoprotein cholesterol, malondialdehyde, and increases high-density lipoprotein cholesterol levels for the host.

Conflict of Interests

The authors have not declared any conflict of interests.
Figure 5. Histopathological changes in livers of hamsters fed a high-fat plus high-cholesterol diet and supplemented or not with different concentrations of probiotics complex product, PROBIO S-23, during 10 weeks of the study. (A) Control, (B) HFC, (C) Low dose LAB, (D) Medium dose LAB, and (E) High dose LAB (magnification 100×).

ACKNOWLEDGMENTS

This study was supported by HK-CCGH-101-07 and HK 98-039 projects from Hung Kuang University and Cheng Ching Hospital.

REFERENCES

Abd El-Gawad IA, El-Sayed EM, Hafez SA, El-Zeini HM, Saleh FA (2005). The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int. Dairy J. 15(1):37-44.
Ahn YT, Kim GB, Lim KS, Baek YT, Kim HU (2003). Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. Int. Dairy J. 13(4):303-311.

Akalin AS, Gönç S, Düzel S (1997). Influence of yogurt and acidophilus yogurts on serum cholesterol levels in mice. J. Dairy Sci. 80(11): 2721-2725.

Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM, Hassan FA (2012). Hypocholesterolemic effect of yohurt containing *Bifidobacterium pseudocatenulatum* G4 or *Bifidobacterium longum* BB536. Food Chem. 135(2):356-361.

Davidson MH, Dillon MA, Gordon B, Jones P, Samuels J, Weiss S, Isaacsohn J, Toth P, Burke SK (1999). Colessevelam hydrochloride (cholestagel): A new, potent bile acid sequestrant associated with a low incidence of gastrointestinal side effects. Arch. Int. Med. 159(16):1893-1900.

de Roos NM, Schouten G, Katan MB (1999). 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(4):275-277.

Del Rio D, Stewart AJ, Pellegrini N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metab Cardiovasc Dis. 15(4):316-328.

Eckel RH (2010). Approach to the patient who is intolerant of statin therapy. J. Clin. Endocrinol. Metab. 95(5):2015-2022.

FAO/WHO (2002). “Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food,” ftp://ftp.fao.org/es/en/food/wgreport2.pdf.

Hjermann I, Byre K, Holme I (1981). Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo Study Group of a randomised trial in healthy men. Laceret. 2(8259):1303-1310.

Hoelen PC, Perez G, Zhao Z, Brouwers E, BernarH, Collen D (1995). Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. J. Clin. Invest. 95(6):2611-2619.

Hu X, Wang T, Li W, Jin F, Wang L (2013). Effects of NS *Lactobacillus* strains on lipid metabolism of rats fed a high-cholesterol diet. Lipids Health Dis. 12:67.

Huang Y, Wang X, Wang J, Wu F, Sui Y, Yang L, Wang Z (2013). *Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol-lowering activity. J. Dairy Sci. 96(5):2746-2753.

Jones ML, Martoni CJ, Parent M, Prakash S (2012). Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. Br. J. Nutr. 107(10):1505-1513.

Kimoto H, Ohmomo S, Okamoto T (2002). Cholesterol removal from media by lacticocci. J. Dairy Sci. 85(12):3182-3188.

Klein A, Friedrich U, Vogelsang H, Jahreis G (2008). *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. lactis DGCC 420 modulate unspecific cellular immune response in healthy adults. Eur. J. Clin. Nutr. 62(5):584-593.

Maeda H, Zhu X, Omura K, Suzuki S, Kitamura S (2004). Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. Biofactors 22(4-6):197-200.

Moosmann B, Behl C (2004). Selenoprotein synthesis and side-effects of statins. Lancet 363(9412):892-894.

Muldoon MF, Barger SD, Ryan CM, Flory JD, Lehoczky JP, Matthews KA, Manuck SB (2000). Effects of Lovastatin on cognitive function and psychological well-being. Am. J. Med. 108(7):538-546.

Muldoon MF, Ryan CM, Sereika SM, Flory JD, Manuck SB (2004). Effects of simvastatin on cognitive functioning in hypercholesterolemia adults. Am. J. Med. 117(11):823-829.

Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, Tokimitsu I (2005). Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. Am. J. Clin. Nutr. 81(1):122-129.

Pan DD, Zeng XQ, Yan YT (2011). Characterisation of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. J. Sci. Food Agric. 91(3):512-518.