Serum Cholesterol-lowering Effect of Fermented Milk with *Streptococcus thermophilus* TMC 1543

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Abstract The serum cholesterol lowering action of fermented milk with *Streptococcus thermophilus* TMC 1543 (1543 FM), the mechanism behind the cholesterol lowering activity and the active factor in animal experiments were investigated using rats. The serum total cholesterol level of the group fed 1543 FM was significantly lower than that of the control group ($P < 0.05$). However, there were no serum cholesterol lowering activities in fermented milks with the other two lactic acid bacteria. The serum total cholesterol concentration of the group fed low-molecular-weight fraction (LMF) from 1543 FM was significantly lower than that of the control group ($P < 0.05$). High-molecular-weight fraction (HMF) also tended to lower serum cholesterol level in the present animal study. The amounts of excretion of both total bile acid and neutral sterol in feces of the 1543 FM group increased significantly in comparison with the control group ($P < 0.05$). These results suggest that the mechanism behind serum cholesterol lowering activity of 1543 FM would be attributable to the promotion of excretion of bile acid and neutral steroid through feces, and probably the low-molecular material in LMF.

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Mann and Spoerry¹⁴⁾ first reported serum cholesterol-lowering activity of fermented milk. Afterwards, the potential cholesterol-lowering activity of fermented milk on humans have been reported, and some studies have observed significant reduction in serum cholesterol level^{1, 2, 9, 11)}. It has also been reported that fermented milk decrease serum cholesterol level in rats^{11, 18, 20, 26)}. Although there are many reports on the mechanism behind cholesterol-lowering activity of fermented milk *in vitro* and *in vivo*, there are only a few reports on the mechanism and active factor behind fermented milk. Rao *et al.*²⁰⁾ reported that the milk fermented by *Streptococcus thermophilus* and methanol solubles from the fermented milk decreased plasma cholesterol levels in rats and that the

methanol solubles inhibited hepatic cholesterologenesis *in vitro*. However, the cholesterol lowering factor has been not identified yet. Nakajima *et al.*^{17, 18)} reported that milk fermented by *Lactococcus lactis* subsp. *cremoris* SBT 0495 isolated from viili (Viili is Finnish traditional fermented milk) has cholesterol lowering activity and that the active factor might be slime materials, which contained phosphopolysaccharide produced by the lactic acid bacteria. Although it is difficult to characterize the active factors from a fermented milk with serum cholesterol lowering activity, the characterization is very important for development of functional foods (medical foods).

Our previous report showed that a fermented milk containing whey protein concentrate (WPC) with

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Table 1. Lactic acid bacterial numbers and acidities (lactic acid) in fermented milks

Lactic acid bacteria		Lactic acid bacterial number (log cfu/ml)	Acidity (Lactic acid)
109 FM ¹⁾	<i>L. casei</i> TMC 109	1.4×10^9	1.34
114 FM	<i>L. casei</i> TMC 114	1.0×10^9	1.12
1543 FM	<i>S. thermophilus</i> TMC 1543	1.0×10^7	1.24

¹⁾ 109 FM=Fermented milk with *L. casei* TMC 109, 114 FM=Fermented milk with *L. casei* TMC 114, 1543 FM=Fermented milk with *S. thermophilus* TMC 1543.

both *S. thermophilus* TMC 1543 and *Lactobacillus casei* TMC 0409 lowered the serum total cholesterol level in rat induced by high-cholesterol diet¹¹⁾. Furthermore, the fermented milk lowered the serum triglyceride level and the atherogenic index in healthy men¹¹⁾. However, it is necessary to investigate serum cholesterol lowering activity of fermented milk (not contained WPC) with *S. thermophilus* TMC 1543 in animals.

The objectives of this study were to investigate serum cholesterol lowering action of fermented milk with *S. thermophilus* TMC 1543 (1543 FM), the mechanism behind the cholesterol lowering activity and the active factor in animals using rats.

Materials and Methods

Bacterial strains

L. casei TMC 109 and *S. thermophilus* TMC 1543 were isolated from fermented milk products. *L. casei* TMC 114 was isolated from human feces. All bacterial strains were maintained in Technical Research Laboratory, Takanashi Milk Products. Co., Ltd.

Fermented milk

L. casei TMC 109, *L. casei* TMC 114 and *S. thermophilus* TMC 1543 were used as starters of fermented milk. The composition of pasteurized milk base was 10.5% skim milk powder, 0.35% soybean peptide, 0.87% sucrose, 0.87% fructose and 87.4% water. Three fermented milks were made by fermenting pasteurized milk base with these lactic acid bacteria. The lactic acid bacterial numbers and acidity (lactic acid) in the fermented milks are shown in Table 1. All fermented milks were lyophilized for experimental

diets. The lyophilized fermented milks per 100 g of dry weight contained 30.0–32.6 g of protein, 0.5–0.8 g of fat, 55.7–57.4 g of sugar, 6.7–7.1 g of ash, and 4.2–4.8 g of water. Skim milk per 100 g dry weight for the control diet contained 33.9 g of protein, 0.7 g of fat, 53.2 g of sugar, 7.9 g of ash, and 4.1 g of water.

Preparation of fractions from fermented milk

Fermented milk with *S. thermophilus* TMC 1543 was centrifuged at $10,000 \times g$ for 10 min, and the supernatant and precipitate were defined as whey fraction and casein fraction, respectively. Whey fraction was heated at 65°C for 30 min, and cooled, and was ultrafiltrated by a ceramic membrane filter (MW cut-off 10,000, Cefilt; Nihon Gaishi corp., Japan), and then the low-molecular-weight fraction (LMF) from the filtrate was obtained after freeze-drying. Also, whey fraction was heated at 65°C for 30 min, and cooled, and was hydrolyzed by addition of 0.07% acidic protease (Orientase 20 A, from *Aspergillus niger*; Hankyu-Bio-Industry, Osaka, Japan) at 50°C for 17 h. After that, the digest was heated at 80 °C for 30 min. An 80 l distilled water was added to 20 l of the digest, and the whole solution was concentrated to 20 l by a ceramic membrane filter (MW cut-off 10,000, Cefilt; Nihon Gaishi corp., Japan), and then the high-molecular-weight fraction (HMF) from the concentrate was obtained after freeze-drying. The weight of each fraction was measured. The yields and chemical compositions of each fraction prepared from 1543 FM are shown in Table 2.

Animals

Male Sprague-Dawley rats, 4 week old, were purchased from Clea Japan, Inc. Tokyo, Japan. All rats were initially given a standard diet (MF, Oriental

Table 2. Yield of each fraction from fermented milk with *S. thermophilus* TMC 1543

	Total contents (g)	Yield (%)	Sample composition (g/100 g of dried sample)				
			Protein	Lipid	Sugar	Ash	Water
1543 FM ¹⁾	2920	100	32.6	0.8	55.7	6.7	4.2
Casein fraction	1138	39.0	60.0	0.0	31.0	4.0	5.0
Whey fraction	1766	60.5	6.6	1.6	76.8	8.6	6.4
LMF	230	7.9	3.7	0.0	74.6	11.6	10.1
HMF	320	11	7.0	1.8	74.2	7.2	9.8

¹⁾ 1543 FM=Fermented milk with *S. thermophilus* TMC 1543, LMF=low-molecular-weight fraction, HMF=high-molecular-weight fraction.

Table 3. Composition of experimental diets (Animal experiment 1)

	Diets (g/100 g)	
	Control group	Experimental diet group
Casein	15.0	15.0
Skim milk	20.0	0.0
Fermented milk	0.0	20.0
Lard	9.0	9.0
Corn oil	1.0	1.0
Vitamin mixture ¹⁾	0.85	0.85
Mineral mixture ²⁾	4.0	4.0
Choline chloride	0.15	0.15
Cellulose powder	2.0	2.0
Cholesterol	0.5	0.5
Sodium cholate	0.125	0.125
Sucrose	47.375	47.375
Total	100.0	100.0

^{1,2)} ; AIN-76.

Yeast Co., Ltd., Tokyo, Japan) for 7 days. The rats were divided on the basis of their mean body weight and housed in an air-conditioned room at $25 \pm 2^\circ\text{C}$ with $55 \pm 5\%$ humidity on a 12 h light-dark cycle. After 7 days, each group was given the experimental diets for 14 days shown in the following paragraphs and allowed tap water *ad libitum*. The overnight (15 h) fasted rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight, Nenbutal ; Abbott Laboratories, North Chicago, IL, USA) at 14 day for serum lipid analysis, and

Table 4. Composition of experimental diets (Animal experiment 2)

	Diets (g/100 g)	
	Control group	Experimental diet group
Casein	15.0	15.0
Skim milk	20.0	20.0
Lard	9.0	9.0
Corn oil	1.0	1.0
Vitamin mixture ¹⁾	0.85	0.85
Mineral mixture ²⁾	4.0	4.0
Choline chloride	0.15	0.15
Cellulose powder	2.0	2.0
Cholesterol	0.5	0.5
Sodium cholate	0.125	0.125
Test sample	0.0	5.0
Sucrose	47.375	42.375
Total	100.0	100.0

^{1,2)} ; AIN-76.

blood samples were taken from the abdominal aorta. All experiments using animals were performed in accordance with the Guideline for the Use and Care of Laboratory Animals (The Prime Minister's Office announcement No. 6, Japan, 1980).

Animal experiment 1

Rats were divided into three groups of seven on the basis of their mean body weight. Each group was given experimental diets for 14 days shown in Table 3.

Animal experiment 2

The rats were divided five groups of seven rats on

the basis of their mean body weight. They were allowed experimental diets with paired feeding for 14 days shown in Table 4.

Animal experiment 3

The rats were divided into two groups of twelve on the basis of their mean body weight. Each group was given either control diet or experimental diet of 1543 FM for 14 days shown in Table 3. After blood collection, livers were excised, and were stored in a similar method as above. All feces during 14 days of test period were collected, and weights were measured. The feces were freeze-dried, were powdered by a mixer.

Serum lipid analysis

Serum total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol, and phospholipid were measured using Cholesterol E Test Waco, Triglyceride G Test Waco, HDL-cholesterol Test Waco and Phospholipid C Test Waco (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively.

Liver lipid analysis

Liver lipids were extracted by the modified method of Kovacs *et al.*¹²⁾. One gram of homogenized aliquot was saponified in a tightly capped tube containing 10 ml of 99.5% ethanol and 5 ml of 50% KOH at 80°C for 1 h. After cooling, 10 ml distilled water was added and unsaponifiables were extracted 3 times with hexane (each 15 ml). The combined extracts were dried under nitrogen gas. Lipids in the dried residue were analyzed according to Danno *et al.*⁵⁾. Thus, the dried residues were dissolved in 10 ml hexane, 1 ml of the aliquots were dried and were redissolved in 600 μ l *tert*-butyl alcohol and 400 μ l Triton X-100/methanol (1 : 1) mixture. These redissolved materials were mixed, and Cholesterol and Triglyceride in the mixtures were measured using Cholesterol E Test Waco and Triglyceride G Test Waco (Wako Pure Chemical Industries, Ltd.), respectively.

Fecal steroid analysis

The analyses of total bile acid in feces were examined by the method of Sautier *et al.*²²⁾. Thus, 0.1 g freeze-dried fecal samples were suspended in 2.5 ml hot ethanol in the test tube with the screw cap and extracted at 80°C for 1 h. The supernatant was collected by centrifugation (1,690 g, 15 min). This ex-

traction procedure was conducted three times, and the supernatants were evaporated under nitrogen gas at 50°C. The residues were redissolved in 8.0 ml ethanol. Fecal total bile acid was measured by commercial test kit (Enzabile II, Daiichi Kagaku Yakuhin, Tokyo, Japan).

The extraction of cholesterol and coprostanol from feces was conducted by the modified method for liver lipid analysis. A 0.1 g of homogenized aliquot was saponified in a tightly capped tube containing 4 ml of 99.5% ethanol and 1 ml of 50% KOH at 80°C for 1 h. After the saponification, the solution in tubes were added with 1 ml 5 α -cholestan solution (25 mg/100 ml) as internal standard. The solution in tubes were added with 1.0 ml distilled water and unsaponifiables were extracted 3 times with hexane (each 2.5 ml). The combined extracts were dried under nitrogen gas at 50°C. The residues were redissolved in 4.0 ml hexane and were filtered. Cholesterol and coprostanol in feces were measured by gas liquid chromatography (GLC). GLC analysis was performed on GLC (GC-353 B, GL science, Tokyo, Japan) with FID detector. The column was used capillary column (DV-17, 0.25 $\times$ 30 m, film thickness 0.15 μ m, J & W Scientific, CA, USA). The conditions of GLC were column temperature : 260°C, injection temperature : 250°C, detector : 300°C, carrier gas : He (1 ml/min), make up gas : N₂ (30 ml/min) and split ratio : 1/50.

Statistical analysis

Statistical significance of the differences between control and diet groups in animal experiments was calculated using unpaired Student's *t*-test with Microsoft Excel, Version 5.0.

Results

Animal experiment 1

Table 5 shows the food intake, body weight, serum lipids and liver lipids in rats. There were no significant differences in food intake between the control group and the three experimental groups. The body weight and the body weight gain for the group (109 FM) fed fermented milk with *L. casei* TMC 109 were significantly lower than those of the control group, respectively ($P < 0.05$). The serum total cholesterol

Table 5. Food intake, body weight, serum lipid concentrations in Rat (Animal experiment 1)

	Groups ²⁾			
	Control	109 FM	114 FM	1543 FM
Food intake (g/14 days)	232.4±4.7 ¹⁾	230.4±5.5	238.6±6.6	229.8±7.6
Body weight at 0 day (g)	143.7±2.2	143.1±1.6	140.7±1.4	143.0±1.4
Body weight at 14 day (g)	240.0±3.7	229.0±2.2*	229.1±4.3	231.6±4.6
Body weight gain (g/14 days)	96.3±2.8	85.9±2.8*	88.4±4.1	88.6±3.4
Serum lipids				
Total cholesterol (mg/dl)	175.0±12.5	152.3±12.2	169.3±18.6	128.1±8.8**
Triglyceride (mg/dl)	88.5±11.7	101.5±13.7	104.2±14.5	95.9±8.0
HDL-cholesterol (mg/dl)	15.2±1.8	15.0±1.0	18.5±2.1	16.1±8.1
Phospholipid (mg/dl)	121.2±3.3	110.7±6.7	117.2±7.9	103.8±4.7*

¹⁾ Values are means±SEM (n=7).

²⁾ 109 FM= Fermented milk with *L. casei* TMC 109, 114 FM= Fermented milk with *L. casei* TMC 114, 1543 FM= Fermented milk with *S. thermophilus* TMC 1543.

Asterisks are significantly different from control (*P<0.05, **P<0.01).

Table 6. Food intake, body weight, serum lipid concentrations in Rat (Animal experiment 2)

	Groups				
	Control	Casein fraction	Whey fraction	LMF ²⁾	HMF ²⁾
Food intake (g/14 days)	222.5±8.0 ¹⁾	233.4±8.6	235.2±6.5	238.7±5.8	230.3±6.1
Body weight at 0 day (g)	144.3±1.5	143.0±2.3	142.3±1.7	144.4±1.6	144.1±1.9
Body weight at 14 day (g)	233.4±5.5	240.3±5.9	236.3±4.1	243.1±4.7	236.9±4.9
Body weight gain (g/14 days)	89.1±4.1	97.3±3.7	94.0±3.2	98.7±4.0	92.7±3.5
Serum lipids					
Total cholesterol (mg/dl)	222.6±22.3	179.9±16.5	176.1±18.8	143.5±16.1*	167.4±14.4
HDL-cholesterol (mg/dl)	20.4±1.7	19.6±1.5	20.4±1.8	19.4±0.9	17.8±1.6
Triglyceride (mg/dl)	130.5±16.9	101.8±18.9	91.2±17.7	67.2±10.8**	83.1±8.3*
Phospholipid (mg/dl)	144.7±12.0	126.8±8.4	122.4±7.8	113.4±2.6*	117.5±4.0*

¹⁾ Values are means±SEM (n=7).

²⁾ LMF=low-molecular-weight fraction, HMF=high-molecular-weight fraction.

Asterisks are significantly different from control (*P<0.05, **P<0.01).

concentration for the group fed 1543 FM was significantly lower than that of the control group (P<0.05). However, the serum triglyceride concentration and HDL-cholesterol concentration did not show statistical significance between the control group and the other three groups.

Animal experiment 2

Table 6 shows the food intake, body weight and

serum lipids in rats. There were no significant differences in both body weight gain and food intake between the control group and the four experimental groups. The serum total cholesterol concentration of the LMF group was significantly lower than that of the control group (P<0.05). The High-molecular-weight fraction (HMF) also tended to have low serum cholesterol level (P=0.08). The serum triglyceride

Table 7. Food intake, body weight, serum lipids, liver lipids and fecal steroids in Rats (Animal experiment 3)

	Control	1543 FM ²⁾
Food intake (g/14 days)	243.3 ± 6.6 ¹⁾	255.8 ± 3.8
Body weight at 0 day (g)	144.5 ± 1.9	145.1 ± 1.3
Body weight at 14 day (g)	228.8 ± 5.5	232.6 ± 2.3
Body weight gain (g/14 days)	84.3 ± 4.4	87.5 ± 2.4
Serum lipids		
Total cholesterol (mg/dl)	186.1 ± 15.9	130.9 ± 13.3*
Triglyceride (mg/dl)	98.9 ± 12.9	77.2 ± 6.8
HDL-cholesterol (mg/dl)	20.7 ± 0.7	21.3 ± 0.9
Phospholipid (mg/dl)	124.6 ± 6.3	106.2 ± 4.7*
Liver weight (g wet)		
Cholesterol (mg/g of Liver)	58.8 ± 0.8	59.8 ± 1.4
Triglyceride (mg/g of Liver)	15.5 ± 1.7	19.9 ± 1.4
Fecal weight (g wet/14 days)		
Cholesterol (mg/14 days)	321.2 ± 14.4	343.7 ± 13.3
Coprostanol (mg/14 days)	36.3 ± 2.2	51.7 ± 3.5**
Neutral sterol (mg/14 days)	357.4 ± 15.1	395.4 ± 11.8*
Total bile acid (μmol/14 days)	337.3 ± 23.1	390.3 ± 13.4*

¹⁾ Values are means ± SEM (n = 12).

²⁾ 1543 FM = Fermented milk with *S. thermophilus* TMC 1543.

Asterisks are significantly different from control (*P < 0.05, **P < 0.01).

concentration of the HMF group was significantly lower than that of the control group (P < 0.05), and that of the LMF group showed a much lower level (P < 0.01). The serum phospholipid concentrations for both the LMF group and the HMF group were significantly lower than those of control group (P < 0.05). However, the serum HDL-cholesterol concentration was not statistically significant between the control group and the other four groups.

Animal experiment 3

Table 7 shows the food intake, body weight, serum lipids and liver lipids in rats. There were no significant differences in food intake between the control group and the 1543 FM group. The serum total cholesterol and phospholipid concentrations of the 1543 FM group were significantly lower than those of the control group (P < 0.05). However, the serum triglyceride concentration and HDL-cholesterol concentration did not show any statistical significance be-

tween the control group and the 1543 FM group. The liver triglyceride and cholesterol concentrations of the 1543 FM group did not show statistical significance between the control group and the 1543 FM group (P < 0.05). Fecal steroid analyses were shown as total amounts of fecal steroids during 14 days. The amounts of excretion of both total bile acid and neutral sterol in feces of 1543 FM group increased significantly in comparison with the control group (P < 0.05).

Discussion

It was observed that fermented milk with *S. thermophilus* TMC 1543 containing whey protein concentrate (WPC) decreased the serum cholesterol level in rats more than the pasteurized milk base containing WPC (unpublished data). This result suggested that fermented milk with *S. thermophilus* TMC 1543 (1543 FM) may have cholesterol-lowering activity. It was

found that 1543 FM had serum cholesterol lowering activity in the present animal experiments. It is suggested that one of mechanisms behind serum cholesterol lowering action of this fermented milk was the inhibited absorption of bile acid and neutral steroid in the intestine, because the excretion of bile acid, neutral steroid and coprostanol through the feces significantly increased. All feces during experimental period were collected, because the analysis of the steroid in feces was important for the elucidation of the mechanism of serum cholesterol lowering activity. It has been known that inhibiting the absorption of bile acid in the intestine promotes the dissimulation from cholesterol to bile acid, which results in a decrease of serum cholesterol level¹⁹⁾. And, absorption of cholesterol in the intestine also may have been inhibited by feeding 1543 FM, because it has been known that coprostanol is produced from cholesterol by intestinal bacteria^{13,21)}. Suzuki *et al.*²⁶⁾ also reported that milk fermented by *Lactobacillus acidophilus* SBT 2056 inhibited the increase of serum cholesterol level in rats fed high cholesterol diets and resulted in the increase of bile acid and cholesterol in feces.

It was speculated that there were not only one but also several cholesterol lowering factors in 1543 FM as the results in the present study. However, it was thought that special cholesterol lowering factors of 1543 FM existed except for the lactic acid, because there was no serum cholesterol lowering activity in fermented milk with *L. casei* TMC 109, despite that the acidity of *L. casei* TMC 109 was higher than that of *S. thermophilus* TMC 1543.

The serum cholesterol lowering activities of fractions isolated from 1543 FM were examined. HMF was hydrolyzed with a protease, because it has been already recognized that whey protein has a high serum cholesterol lowering action in rats^{15,16)}. There was a statistically significant serum cholesterol lowering activity in only LMF in these fractions ($P < 0.05$). It is considered that there are peptides, amino acids, sugars, organic acids, and so on in LMF. In the future, it is necessary to clarify in which component of LMF the activity exists. Zommara *et al.*³⁰⁾ reported that whey from cultured skim milk with *S. thermo-*

philus decreased the serum cholesterol level in rats, whereas cultured skim milks with *L. acidophilus* or *Bifidobacterium longum* had no effect. Cholesterol lowering factors, which Rao *et al.*²⁰⁾ suggested, may exist in whey from fermented milk with *S. thermophilus*. Nakajima *et al.*^{17,18)} reported that slime materials containing polysaccharide produced by *Lactococcus lactis* subsp. *cremoris* SBT 0495 has serum cholesterol lowering effect in rats. *S. thermophilus* TMC 1543 was selected on the grounds that it produces fermented milk with a high viscosity. High-molecular-weight fraction (HMF), which probably contains polysaccharides tended to lower serum cholesterol level in the present animal study, but the decrease was not statistically significant ($P = 0.08$). Unlike our prediction, the serum cholesterol level of casein fraction was almost the same as that of whey fraction. Casein fraction may have cholesterol lowering activity, because casein fraction contains cells of *S. thermophilus* TMC 1543, denatured whey protein, and casein protein. Hashimoto *et al.*⁷⁾ reported that freeze-dried cells of lactobacilli decreased the serum cholesterol levels in rats fed high cholesterol diet, and the amounts of bile acid in feces increased. There may be some insoluble peptides from milk protein produced by protease of *S. thermophilus* TMC 1543 in 1543 FM. Sugano *et al.*^{24,25)} reported that the hydrophobic peptide of soybean protein prepared after exhaustive digestion by microbial proteases, which bound conjugated bile salt, decreased the serum cholesterol level in rats. Nagaoka¹⁶⁾ reported that the micellar solubility of cholesterol was decreased by β -lactoglobulin tryptic hydrolysate compared with casein tryptic hydrolysate. It is necessary to investigate the soluble and insoluble peptides in 1543 FM.

Gilliland and Speck⁶⁾ and Usman and Hosono²⁷⁾ reported the deconjugation of bile acids by *Lactobacillus* spp. The deconjugation of bile acids trigger reduction of serum cholesterol level because free bile acids resulting from the deconjugation are excreted more rapidly than conjugated bile acids⁴⁾. However, *S. thermophilus* TMC 1543 is no resistant to gastric acid and bile acid, so that, deconjugating activity may not be one of the mechanisms behind the serum cholesterol lowering activity. Some reports^{3,10,28)}

described the binding of cholesterol with lactic acid bacterial cells. Moreover, Usman and Hosono²⁹⁾ reported the binding of cholesterol to the peptidoglycan of *Lactobacillus gasseri*. Hashimoto *et al.*⁸⁾ reported the effect of lactobacilli on bile acid, showing that lactobacilli had the binding ability of bile acid even in the dead condition. *S. thermophilus* TMC 1543 killed by gastric acid and bile acid also may bind bile acid and induce to excrete the bile acid through feces.

It is concluded that the mechanisms behind serum cholesterol lowering activity of fermented milk with *S. thermophilus* TMC 1543 would be attributable to promotion of excretion of bile acid and neutral steroid, and one of the cholesterol lowering factor may be a low-molecular material in LMF. However, the data gathered in the present study does not suffice to clarify the cholesterol lowering factors, and further investigations involving purification of the factors are needed.

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