

# Reduction of Cholesterol by *Lactobacillus acidophilus* in Culture Broth

MEEI-YN LIN\* AND TSENG-WEI CHEN

Department of Food Science, National Chung-Hsing University, 250 Kuokuang Rd., Taichung, Taiwan, R.O.C.

## ABSTRACT

Cholesterol reducing abilities of six strains of *Lactobacillus acidophilus* including ATCC 4356, B, E, Farr, LA-1 and N-1 were investigated *in vitro*. All these strains were able to reduce cholesterol at various levels in the broth system used in this study. Among the strains tested, *L. acidophilus* ATCC 4356 demonstrated the best cholesterol reducing ability at 57 and 71% in the presence of oxgall and cholic acid, respectively, in the broth. Results from the acid tolerance and growth in oxgall studies demonstrated that these *L. acidophilus* strains would likely survive in the human gastrointestinal tract, where acid and oxgall are present in the stomach and small intestine. Coprecipitation, which was observed in the presence of cholic acid, of cholesterol with deconjugated bile at acidic pH contributed to the reduction of cholesterol *in vitro*. However, coprecipitation is not likely to take place *in vivo* since the pH in the small intestine is higher than neutral. Results from this study indicate that the *in vivo* hypocholesteremic ability is likely due to the assimilation of cholesterol by *L. acidophilus* cells or/and attachment of cholesterol to the surface of *L. acidophilus* cells.

**Key words:** *Lactobacillus acidophilus*, cholesterol reduction, bile, assimilation.

## INTRODUCTION

Information published by the Lipid Research Clinic Program<sup>(1)</sup> indicates that the higher the total serum cholesterol is in humans, the greater is the risk for the emergence of coronary heart disease, a major cause of death. Reduction of serum cholesterol can lower the incidence of coronary thrombosis in hypercholesteremic individuals<sup>(1)</sup>. Several investigators have suggested that certain lactic acid bacteria including strains of *L. acidophilus* are capable of reducing cholesterol<sup>(2-12)</sup>.

Among lactic acid bacteria, *L. acidophilus* has

attracted much attention for its potential role in promoting human health. Therefore, there is increasing interest in the incorporation of *L. acidophilus* in fermented dairy products.

The objectives of this study were to quantify the *in vitro* cholesterol reducing ability of six strains of *L. acidophilus* in broth media containing cholesterol and bile and to propose possible hypotheses for cholesterol reduction *in vivo* with *L. acidophilus*.

## MATERIALS AND METHODS

Journal of Food and Drug Analysis, 2000, 8(2)

### I. Bacterial Strains

Six strains of *Lactobacillus acidophilus*, including ATCC 4356, B, E, Farr, LA-1 and N-1, were obtained from our stock culture collection. All these strains were grown in MRS broth at 37°C. All strains were serially transferred at least three times prior to use in these studies.

### II. Acid Tolerance of *L. acidophilus* Strains

Acid tolerances were evaluated by growing *L. acidophilus* strains in MRS broth adjusted to acidic pH 3 and 2 by adding HCl. Viable cell counts were determined by plating on agar plates following anaerobic incubation (BBL Anaerobic Systems, Cockeysville, Maryland, USA) at 37°C for 2 to 3 days.

### III. Growth Studies of *L. acidophilus* Strains in Oxgall

Growth studies were performed for *L. acidophilus* strains in the absence and presence of 0.5% oxgall. Aliquots were removed and optical density and pH were monitored every hour for 20 hours. Optical density was measured spectrophotometrically (absorbance at 600 nm) with a Spectronic 20 colorimeter (Bausch and Lomb, Rochester, NY, USA). The pH was measured using an Orion model 601 digital ionalyzer (Orion Research Inc., Boston, MA, USA).

### IV. Quantitative Assay of Cholesterol

A modification of the broth system utilized by Gilliland *et al.*<sup>(14)</sup> was used for this study. Sterile MRS broth was supplemented with 2% pleuropneumonia-like organism (PPLO) serum fraction and 0.5% oxgall, cholic acid, or taurocholic acid. PPLO served as the source of cholesterol. Oxgall, which is the dehydrated fresh bile, was added to the media in order to mimic conditions that would be encountered in the human gastrointestinal tract. Cholic acid was added as the source of a deconjugated bile and taurocholic acid was added as the source of a conjugated bile. Aliquots were removed prior to inoculation to determine the initial cholesterol content of the media. Strains of *L.*

*acidophilus* were grown in the absence or presence of 0.5% oxgall in MRS broth containing 2% PPLO under anaerobic conditions at 37°C. Following incubation for 24 hours, cells were centrifuged at 6000 xg for 10 min and the residual cholesterol in the supernatant was determined using the method described below.

The cholesterol levels were determined by a modification of the method of Rudel and Morris<sup>(15)</sup>. Added to 1 mL samples were 3 mL of 95% ethanol and 2 mL of 50% potassium hydroxide. The contents of the tubes were mixed after the addition of each component and then heated for 10 min in a 60°C water bath. After cooling, 5 mL of hexane was dispensed into each tube and mixed thoroughly. A sample of 1 mL aliquot of distilled water was added, mixed, and tubes were allowed to stand at room temperature to permit phase separation. A sample of 3 mL aliquot of hexane layer was transferred to a clean tube and the hexane evaporated under the flow of nitrogen gas. A 4 mL sample of o-phthalaldehyde reagent was added to each tube and tubes were allowed to stand at room temperature for 10 min. Following the addition of 2 mL of concentrated sulfuric acid and standing for an additional 10 min, the absorbance at 550 nm was read against a reagent blank. Absorbance values were compared to those obtained with cholesterol standards.

### V. Coprecipitation of Cholesterol with Deconjugated Bile in Different Acids

To evaluate the effect of different acids on the coprecipitation of cholesterol with deconjugated bile, broth media were adjusted to pH values ranging from 6 to 3-2 by adding lactic acid, acetic acid, or HCl. As mentioned previously, 2% PPLO was added as the source of cholesterol and cholic acid was added as the source of deconjugated bile. Coprecipitation was observed and the cholesterol was determined using the method described above.

### VI. Statistical Analysis

Cholesterol reduction was analyzed using the ANOVA, analysis of variance of Statistical

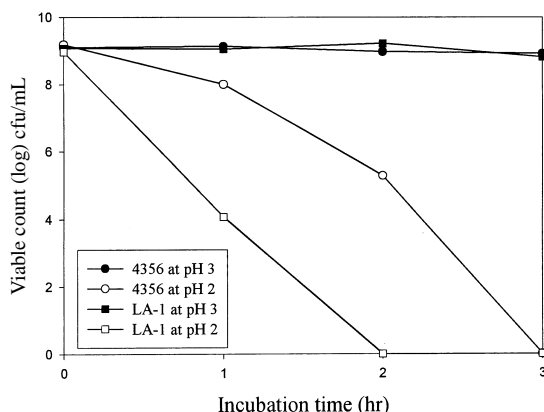
Journal of Food and Drug Analysis, 2000, 8(2)

Analysis System<sup>(16)</sup>. The least significant difference was used to compare means.

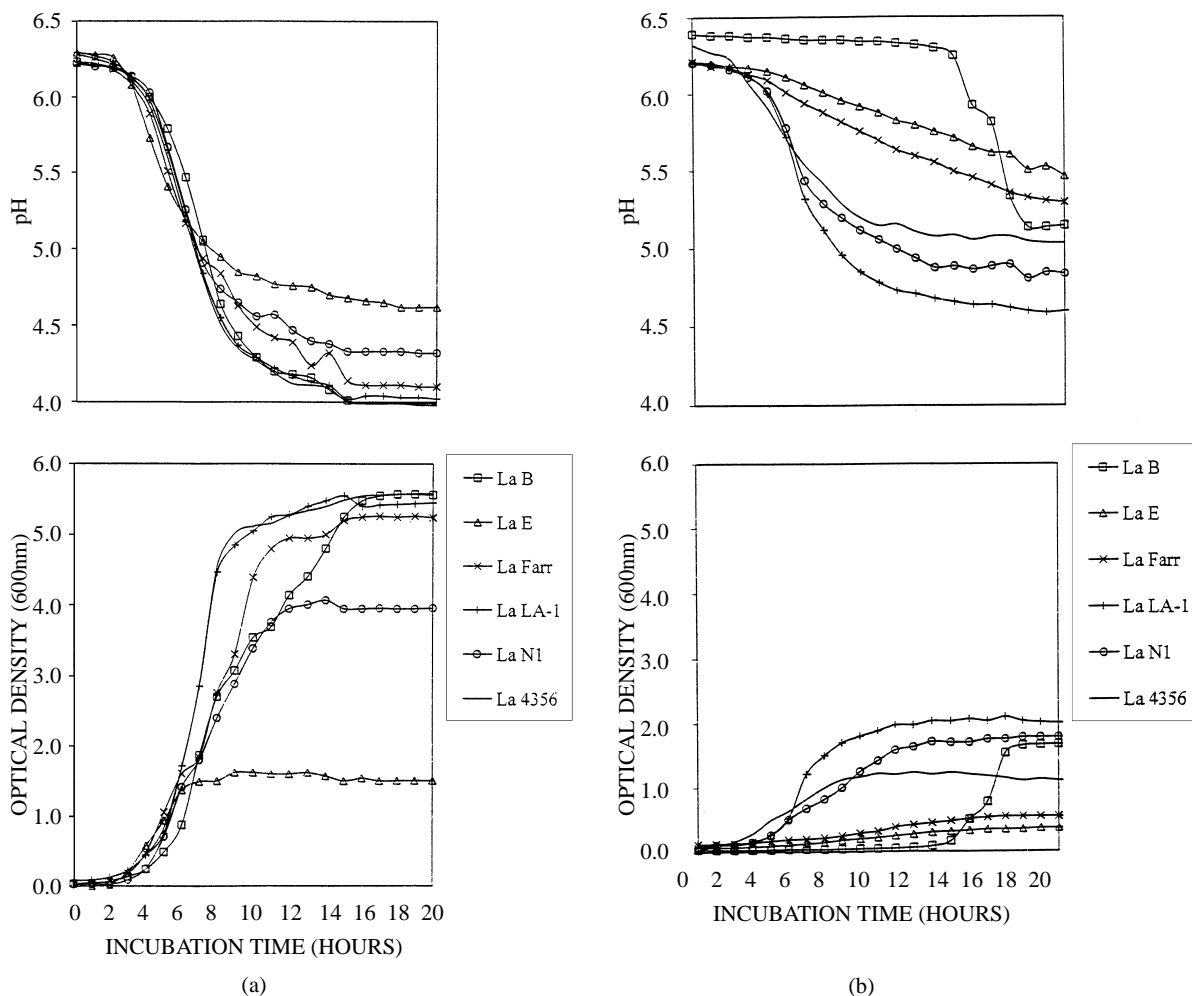
## RESULTS AND DISCUSSION

In the laboratory, acids and biles are frequently added to the media in order to mimic conditions encountered in human gastrointestinal tract. Therefore, the acid tolerances of *L. acidophilus* strains and growth in oxgall were studied. Acid tolerances of *L. acidophilus* strains are illustrated in Figure 1 (data not shown for strains B, E, Farr and N-1). Strains B, E, Farr and LA-1 had similar patterns for acid tolerances (only the curves of LA-1 shown in Figure 1). Strains 4356 and N-1

also demonstrated similar acid tolerances (only the curves of 4356 shown in Figure 1). Although

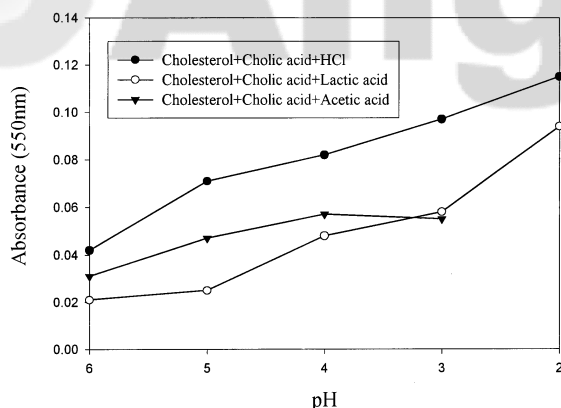


**Figure 1.** Acid tolerances of *L. acidophilus* strains.



**Figure 2.** The growth and pH changes of *L. acidophilus* strains in the absence (a) and presence (b) of 0.5% oxgall in MRS broth containing 2% PPLO. Growth was monitored by determining the optical density (bottom panel), and pH (top panel) of the cultures every hour.

Journal of Food and Drug Analysis, 2000, 8(2)



**Figure 3.** The effect of different acids on the coprecipitation of cholesterol with cholic acid.

most strains did not survive at pH 2, all of these strains tolerated pH 3 well. Therefore, these strains tolerated acidity well in general. The growth curves of *L. acidophilus* strains grown in the absence and presence of 0.5% oxgall are illustrated in Figure 2. Optical density and acid production, both used as the indication of bacterial growth, were monitored every hour for 20 hours. Growth in oxgall resulted in various levels of growth inhibition. This was consistently observed in all strains tested. Results from acid tolerance and growth in oxgall studies demonstrated that these *L. acidophilus* strains would likely survive in the human gastrointestinal tract, where acid and oxgall are present in the stomach and small intestine.

Gilliland *et al.*<sup>(14)</sup> demonstrated that certain strains of *L. acidophilus* were able to assimilate cholesterol only when *L. acidophilus* cells were grown in the presence of bile under anaerobic conditions. *In vitro* cholesterol reducing abilities of six *L. acidophilus* strains used in this study are shown in Table 1. These strains were grown in broth containing 2% PPLO as the source of cholesterol and 0.5% bile and cells were incubated anaerobically. Different bile including oxgall, cholic acid, or taurocholic acid was added to the broth. Cholic acid was used as the source of deconjugated bile and taurocholic acid was added as the source of conjugated bile. Oxgall is a mixture of conjugated and deconjugated bile. It is

**Table 1.** Cholesterol reduction by strains of *L. acidophilus* in the presence of different bile salts

Strain	Cholesterol Reduction (%) <sup>1,2</sup>		
	Oxgall	Cholic Acid	Taurocholic Acid
<i>L. acidophilus</i>			
B	49 <sup>b</sup>	70 <sup>a</sup>	43 <sup>c</sup>
E	20 <sup>d</sup>	43 <sup>c</sup>	12 <sup>e</sup>
Farr	25 <sup>d</sup>	60 <sup>a</sup>	11 <sup>e</sup>
LA-1	34 <sup>c</sup>	65 <sup>a</sup>	33 <sup>d</sup>
N-1	38 <sup>c</sup>	66 <sup>a</sup>	21 <sup>e</sup>
4356	57 <sup>a</sup>	71 <sup>a</sup>	52 <sup>b</sup>

<sup>1</sup>. All media contained 2% PPLO as the source of cholesterol and 0.5% oxgall, cholic acid, or taurocholic acid. The cholesterol contents of media ranged from 52 to 57 µg/mL.

<sup>2</sup>. The percentage of cholesterol reduction was defined as follows: [1-(residual cholesterol in 24-h culture)/(initial cholesterol content of medium with 2% PPLO and 0.5% oxgall, cholic acid, or taurocholic acid)] X 100%. Means with different superscript letters are significantly different (P < 0.05). Each value represents the average of triplication.

important to note that biles such as oxgall, cholic acid, and taurocholic acid contain cholesterol; therefore, un-inoculated controls were measured for initial cholesterol content of the media. After 24 hours of growth in the media, 11 to 71% of the cholesterol was reduced. As listed in Table 1, maximum levels of cholesterol were reduced when cholic acid was used as the source of bile. On the other hand, minimum levels of cholesterol decreased when taurocholic acid was used as the source of bile. Coprecipitation of cholesterol with deconjugated bile at acidic pH was observed in this study and also in the study of Tahri *et al.*<sup>(17)</sup>. However, precipitation was not observed in the presence of taurocholic acid. Nevertheless, cholesterol reduction was also observed in the presence of taurocholic acid, which would not coprecipitate with cholesterol. This indicates that cholesterol reduction is likely due to the assimilation by *L. acidophilus* cells. Another possibility for cholesterol reduction is due to the attachment of

Journal of Food and Drug Analysis. 2000. 8(2)

cholesterol to the surface of *L. acidophilus* cells. The excretion of *L. acidophilus* cells with cholesterol in feces can also decrease the cholesterol levels *in vivo*.

To evaluate the effect of different acids on the coprecipitation of cholesterol with deconjugated bile, broth media were adjusted to acidic pH values by adding lactic acid, acetic acid or HCl. As shown in Figure 3, higher absorbance indicated more coprecipitation. Higher levels of coprecipitation were observed as pH dropped. However, it was not just the acidity itself causing the coprecipitation, as different acids had different effects on the level of coprecipitation.

Several *in vivo* studies, including results from animal and human models, have reported that lactic acid bacteria decreased serum cholesterol<sup>(2-12)</sup>. Different hypotheses have been proposed to explain how the hypocholesteremic effect of lactic acid bacteria including *L. acidophilus* is possible. Results from this study indicate that the *in vivo* hypocholesteremic ability is likely due to the assimilation of cholesterol by *L. acidophilus* cells or/and attachment of cholesterol to the surface of *L. acidophilus* cells. Coprecipitation of cholesterol with deconjugated bile, which happens *in vitro*, is not likely to take place *in vivo* since the pH in small intestine is higher than neutral.

*L. acidophilus* and other lactic acid bacteria have been used as probiotics in various products. This study shows the potential of using *L. acidophilus* as an adjunct to reduce serum cholesterol levels. Further *in vivo* study is necessary to prove the hypocholesteremic effect of these *L. acidophilus* strains in humans. Lactic acid bacteria including *L. acidophilus* are frequently associated with probiotic effects in humans<sup>(18-20)</sup>. These health-promoting effects make *L. acidophilus* and other lactic acid bacteria desirable to be incorporated into dairy products or other functional foods designed to meet the demands of today's health-conscious public.

## REFERENCES

1. Lipid Research Clinics Program. 1984. The

- lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. J. Am. Med. Assoc. 251: 351-363.
2. Agerbak, M., Gerdes, L. U. and Richelsen, B. 1995. Hypocholesteremic effect of a new fermented milk product in healthy middle-aged men. Europ. J. Clin. Nutr. 49: 346-352.
3. Danielson, A. D., Peo, E. R., Shahani, K. M., Lewis, A. J., Whalen, P. J. and Amer, M. A. 1989. Anticholesteremic properties of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67: 966-974.
4. Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47: 2078-2079.
5. Harrison, B. C. and Peat, G. 1975. Serum cholesterol and bowel flora in the newborn. Am. J. Clin. Nutr. 28: 1351-1355.
6. Hepner, G., Fried, R., Jeor, S. S., Fusetti, L. and Morin, R. 1979. Hypocholesteremic effect of yogurt and milk. Am. J. Clin. Nutr. 32: 19-24.
7. Jaspers, D. A., Massey, L. K. and Luedecke, L. O. 1984. Effect of consuming yogurts prepared with three culture strains on human serum lipoproteins. J. Food Sci. 49: 1178-1181.
8. Nakajima, H., Suzuki, Y., Kaizu, H. and Hirota, T. 1992. Cholesterol lowering activity of ropy fermented milk. J. Food Sci. 57: 1327-1329.
9. Pulusani, S. R. and Rao, D. R. 1983. Whole body, liver and plasma cholesterol levels in rats fed thermophilus, bulgaricus and acidophilus milks. J. Food Sci. 48: 280-281.
10. Rao, D. R., Chawan, C. B. and Pulusani, S. R. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterologenesis in rats. J. Food Sci. 46: 1339-1341.
11. Smet, I., Boever, P. and Verstraete, W. 1998. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. Br. J. Nutr. 79: 185-194.

Journal of Food and Drug Analysis. 2000. 8(2)

12. Thompson, L. U., Jenkins, D. J. A., Amer, M. A.V., Reichert, R., Jenkins, A. and Kamulsky, J. 1982. The effect of fermented and unfermented milk on serum cholesterol. Am. J. Clin. Nutr. 36: 1106-1111.
13. Tahri, K., Crociani, J., Ballongue, J. and Schneider, F. 1995. Effects of three strains of bifidobacteria on cholesterol. Lett. Appl. Microbiol. 21: 149-151.
14. Gilliland, S. E., Nelson, C. R., Maxwell, C. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49: 377-381.
15. Rudel, L. L. and Morris, M. D. 1973. Determination of cholesterol using o-phthaladehyde. J. Lipid. Res. 14: 364-366.
16. SAS Guide for Personal Computers. Version 6.12. 1989. SAS Inst. Inc., N.C., USA.
17. Tahri, K., Grill, J. P. and Schneider, F. 1996. Bifidobacteria strain behavior toward cholesterol: Coprecipitation with bile salts and assimilation. Current Microb. 33: 187-193.
18. Gilliland, S. E. 1979. Beneficial interrelationships between certain microorganisms and humans. J. Food Prot. 42: 164-167.
19. Lin, M. Y. 1995. The beneficial effects of lactic acid bacteria on human health. J. Chinese Nutr. Soc. 20: 367-380.
20. Sandine, W. E. 1979. Role of *Lactobacillus* in the intestinal tract. J. Food Prot. 42: 259-262.

## 嗜酸乳桿菌在培養基中降低膽固醇之能力

林美吟\* 陳增蔚

國立中興大學食品科學系  
台中市國光路250號

### 摘 要

以六株嗜酸乳桿菌包括ATCC 4356、B、E、Farr、LA-1及N-1探討在試管試驗中降低膽固醇之能力。實驗結果發現，雖然降低膽固醇之程度不同，但所有的測試菌株在液體培養基系統中皆表現出降低膽固醇之能力，在這些測試菌株中，ATCC 4356表現出最好的膽固醇降低能力，可降低57-71%之膽固醇。在對酸及膽鹽的耐受測試中發現，嗜酸乳桿菌可耐受酸及膽鹽之存在。而在酸性的條件下，膽固醇雖會與去結型膽鹽產生共同沉澱而導致試管試驗中膽固醇之降低，然而由於人體小腸環境為偏微鹼性，此共同沉澱不會發生。而本實驗中發現，以不會與膽固醇發生沉澱的牛磺膽酸作為膽鹽來源時，嗜酸乳桿菌仍可降低膽固醇，因此在其他活體實驗中所發現之嗜酸乳桿菌所具之降低膽固醇能力，推論是由於菌體可同化膽固醇或膽固醇可附著於菌體表面隨著糞便排出之故。

關鍵詞：嗜酸乳桿菌，膽固醇之降低，膽鹽，同化作用。