How Much In Vitro Cholesterol Reducing Activity of Lactobacilli Predicts Their In Vivo Cholesterol Function?

Golnoush Madani¹, Maryam Mirlohi¹², Mahmoud Yahay¹², Akbar Hassanzadeh¹

ABSTRACT

Background: Based on literature, in vitro cholesterol removal of lactic acid bacteria has been accounted for their in vivo cholesterol reduction. But recently it has been proposed that such in vitro characteristic may not be directly relevant to their in vivo activity. The objective of this study was to find how much in vitro cholesterol reducing potential of Lactobacillus plantarum A7 (LA7), a native strain isolated from an infant fecal flora, reflects its in vivo efficiency. LA7 previously showed serum cholesterol reducing capability in mice subjected to fatty diet. Here, we investigate whether the given strain is capable of in vitro cholesterol assimilation or consumption.

Method: LA7 was cultured in whole milk and de-Man–Rogosa–Sharpe (MRS) added with water-soluble cholesterol. Colorimetric method was adopted for cholesterol determination in both cultured media during incubation period.

Results: No cholesterol assimilation was detected by growth and incubation of the active culture in either of the medium. Thus, in vivo cholesterol function of LA7 was not caused by cholesterol consumption. A comprehensive review of literature on the related studies also showed that there are other documented studies which evidenced the uncertainty of the direct relation between in vitro and in vivo studies.

Conclusion: Cholesterol removal from the cultured media may not be considered as an appropriate integral index for selection of Lactobacillus strains with cholesterol-lowering activity.

Keywords: Cholesterol, in vitro, in vivo, Lactobacillus plantarum, milk

INTRODUCTION

Alleviation of hypercholesterolemia by introduction of high population of lactic acid bacteria in the diet has remained a controversial subject.¹³ Some strains of Lactobacillus genera have been identified to exhibit cholesterol-reducing capability through in vitro or/and in vivo examinations.⁴⁻¹¹ The mechanisms underlying this activity have been proposed to involve assimilation of cholesterol, cholesterol adherence to the bacterial cell wall or its incorporation into bacterial cells, physiological action
of the end products of short chain fatty acids by fermentation, destabilization and co-precipitation of the cholesterol micelles, bile salt hydrolase activity of the lactobacilli,\textsuperscript{[8,11]} cholesterol oxidize activity,\textsuperscript{[12]} and finally production of some functional peptides.\textsuperscript{[4]}

Since lowering the serum cholesterol is a health promoting characteristic, the idea of selection of microbial strains with cholesterol-reducing effect has been developed as a tool in order to introduce new probiotic microorganisms.\textsuperscript{[9]} Some studies looked to the cholesterol-reducing activity of some \textit{Lactobacillus} strains for providing healthier cholesterol-reduced fermented products.\textsuperscript{[10,12]} Probiotics are viable microbial supplements that beneficially affect the host through their effects in the intestinal tract.\textsuperscript{[8]} Despite many mechanisms involved, strain dependency is the general concept emphasized in most of the related studies.

To find new probiotics with cholesterol-reducing capability, many \textit{Lactobacillus} strains have been examined using \textit{in vivo} and/or \textit{in vitro} tests. Generally, application of \textit{in vitro} tests precedes the \textit{in vivo} trials. However, some positive \textit{in vivo} studies used \textit{Lactobacillus} strains lacking any history of \textit{in vitro} cholesterol activity. Considering the \textit{in vitro} studies, most of them proposed one or more mechanisms mentioned above to be responsible for their observed results. In Table 1, 16 \textit{in vitro} studies are summarized.\textsuperscript{[4,8,10,13-25]} Cholesterol assimilation or reduction in the culture media is one of the most referred mechanisms among the \textit{in vitro} experiments, which is measured by determination of cholesterol in the cholesterol added de-Man–Rogosa–Sharpe (MRS) medium before and after the complete growth of the examined \textit{Lactobacillus} strains.\textsuperscript{[8-11]} In addition, full fat milk is occasionally used as the culture media.\textsuperscript{[10]} The more assimilated cholesterol in the spent culture broth, the more cholesterol-reducing activity is attributed to the examined strain. The bacterial strains with the potential cholesterol assimilation or cholesterol adherence display a positive reaction in the test tube.

Recently, in an animal study in Isfahan University of Medical Science, \textit{Lactobacillus plantarum} A7 (LA7) effectively changed the serum lipid profile of the tested animal. LA7 is a native strain, isolated from an infant fecal flora and was characterized as probiotic potential.\textsuperscript{[26,27]} Administration of $10^8$ cells of LA7 through milk-based formula led to lower plasma cholesterol in a group of mice, under fatty diet.\textsuperscript{[27]} At the end of the study, low-density lipoprotein (LDL) cholesterol and total triglycerides in the treated group of mice were shown to be level off by 1.73% and 7.77% than that of the original concentration, respectively, demonstrating 28-30% reduction in both parameters in the treated group. Here in the present study, the objective was to investigate the reaction of LA7 in the \textit{Lactobacillus}-specific media enriched with cholesterol and milk. The expectation was to observe a noticeable \textit{in vitro} cholesterol-reducing activity.

**METHODS**

**Bacterial strain and culture media**

LA7 was provided by microbial collection of Food Microbiology and Biotechnology of Isfahan University of Technology, Isfahan, Iran. Water-soluble cholesterol; polyoxyethanyl cholesteryl sebacate (Sigma Chemical Co., St. Louis, MO, USA) was used as a source of cholesterol to be added (100 mg/l) into the sterilized de-Mans–Rogosa broth (Merck-Germany). One hundred milliliter of commercial ultra high temperature (UHT) sterilized milk (Mihan, Iran) 30 g/l fat and MRS added cholesterol were inoculated with the 1% overnight culture of LA7 and incubated at 37°C aerobically for 24 and 72 hours, respectively. Viable plate count and change in pH was performed for bacterial growth monitoring in both media.

**Cholesterol determination**

Five milliliter samples of the cultured media were removed at the time intervals of 3, 5, 7, 12, and 24 hours incubation of both media and continued up to 72 hours for cultured UHT milk. Samples were centrifuged and spent broth as well as the cell pellet fractions were used individually for cholesterol measurement. Lipid extract from milk and cultured milk was prepared using the Folch method; briefly 5 ml of milk were mixed by 50 ml chloroform–methanol solvent (2:1, v/v) and lipid extract was washed by 20% of its volume ratio with distilled water, chloroform layer was dried under nitrogen.\textsuperscript{[28]} The modified colorimetric method\textsuperscript{[18,29]} was adopted in order to determine the water-soluble cholesterol and natural milk cholesterol. One milliliter of the tested solution was added to 1 ml
of 33% w/v potassium hydroxide and 2 ml of absolute ethanol, mixed for 1 min and incubated at 37°C for 15 min. After cooling, 2 ml of distilled water and 3 ml of hexane layer were removed and transferred into a test tube and evaporated under nitrogen. The dried material was dissolved in 2 ml of o-phthalaldehyed reagent and mixed thoroughly. Then, 0.5 ml of sulfuric acid (12 N, Merck), was added and the mixture was mixed for 1 min. After 10 min, absorbance was read at 550 nm (Genway–model 6800). Cell pellet fraction was resolved in 1 ml of phosphate buffer (pH = 7) and processed as mentioned above. Determination of cholesterol in milk and cultured milk were carried out in the same manner as cultured media with the exception of applying 1 ml lipid extract of milk instead of 1 ml MRS as the sample. The milk lipid extract was obtained as described elsewhere. [28,30]

**Preparation of standard curve**

Several preparations of the standard solutions

### Table 1: In vitro studies on the cholesterol reduction capability of Lactobacillus strains in MRS broth or in milk

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of study</th>
<th>Lactobacillus strains</th>
<th>Used culture media</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L.D. Guo</em></td>
<td>2011</td>
<td><em>L. plantarum</em> KLD S 1 0.344</td>
<td>MRS-Thio-oxgall</td>
<td>+</td>
<td>[14]</td>
</tr>
<tr>
<td>S.M. Lim</td>
<td>2011</td>
<td>lactic acid bacteria MLK11, MLK22, MLK27, MLK41, and MLK67</td>
<td>MRS supplemented with 0.3% oxgall, cholic acid, and taurocholic acid</td>
<td>+</td>
<td>[15]</td>
</tr>
<tr>
<td>X.Q. Zeng</td>
<td>2011</td>
<td><em>L. fermentum</em> F1</td>
<td>MRS</td>
<td>+</td>
<td>[16]</td>
</tr>
<tr>
<td>C.F. Hwang</td>
<td>2011</td>
<td><em>L. plantarum</em> PL02</td>
<td>MRS-Thio-oxgall</td>
<td>+</td>
<td>[17]</td>
</tr>
<tr>
<td>H.S. Lye</td>
<td>2010</td>
<td><em>L. acidophilus</em> ATCC 314, <em>L. acidophilus</em> FTCC 0291, <em>L. bulgaricus</em> FTCC 0411, <em>L. bulgaricus</em> FTDC 1311 and <em>Lactobacillus casei</em> ATCC 393</td>
<td>MRS/oxgall/+taurocholic acid/d/+cholic acid</td>
<td>+ for all examined strains</td>
<td>[18]</td>
</tr>
<tr>
<td>S. Belviso</td>
<td>2009</td>
<td>Eight <em>L. plantarum</em> and five <em>L. paracasei</em> strains</td>
<td>MRS and Milk</td>
<td>Two <em>L. plantarum</em> and three <em>L. paracasei</em> strains were+</td>
<td>[10]</td>
</tr>
<tr>
<td>Y. Kim</td>
<td>2008</td>
<td><em>L. acidophilus</em> ATCC 43121</td>
<td>MRS-Thio</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. plantarum</em> KUO71</td>
<td>MRS-Thio</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
<td>M.T. Liong</td>
<td>2005</td>
<td>Eleven strains of lactobacilli</td>
<td>MRS-oxgall</td>
<td>+</td>
<td>[8]</td>
</tr>
<tr>
<td>M.T. Liong</td>
<td>2005</td>
<td><em>Lactobacillus casei</em> ASCC 292</td>
<td>MRS-oxgall</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
<td>H. Kimoto</td>
<td>2002</td>
<td>Seven strains of the genus <em>Lactococcus</em></td>
<td>*</td>
<td>+</td>
<td>[22]</td>
</tr>
<tr>
<td>M.Y. Lin</td>
<td>2000</td>
<td><em>Lactobacillus acidophilus</em> including ATCC 4356, B, E, Farr, LA-1 and N-1</td>
<td>*</td>
<td>+</td>
<td>[23]</td>
</tr>
<tr>
<td>P.C. Dambekodi</td>
<td>1998</td>
<td><em>Bifidobacterium longum</em></td>
<td>*</td>
<td>+</td>
<td>[24]</td>
</tr>
<tr>
<td>D.O. Noh</td>
<td>1997</td>
<td><em>Lactobacillus acidophilus</em> ATCC 43121</td>
<td>*</td>
<td>+</td>
<td>[25]</td>
</tr>
</tbody>
</table>

*Full text was not available MRS=de-Man–Rogosa–Sharpe
at the concentrations of 1, 2, 5, 7, 10, 50, 100, and 200 mg/l of polyoxyethanol cholesteryl sebacate in sterilized MRS (Merck) were prepared. Each standard solution was treated as a sample and underwent the whole procedure of the experiment.

**Evaluation of reproducibility**

For the evaluation of reproducibility of the method, inter assay coefficient of variation (Cv) for the measurement of 50 mg/l solution of polyoxyethanol cholesteryl sebacate in MRS during four consequent days (n = 2 × 4) was determined. Intra assay Cv for the measurement of the given concentration of polyoxyethanol cholesteryl sebacate was investigated by ten 6 replicate of the measurement through (n = 6 × 1) a day.

**Statistical analysis**

Data was analyzed using Minitab statistical software version 16 (Minitab Inc, State College, PA, USA), diagrams were drawn using Excel 2007.

**RESULTS**

The growth of LA7 was not restricted or enhanced by the presence of water-soluble cholesterol in MRS. In MRS containing 100 mg/l water-soluble cholesterol, LA7 showed a typical growth pattern of *L. plantarum*, producing the maximum optical density of 3.5 at 620 nm corresponding to 10^{10} colony forming unit (CFU/ml) after 20 hours of incubation. Cells of LA7 showed slow growth in milk, however, after 72 hours, total count reached at 10^8 CFU/ml.

**Quality parameters**

Standard curve for water-soluble cholesterol showed linearity over the range of tested concentrations (R^2 = 0.985, a = 1748, b = −3.8). Inter and intra assay Cv for cholesterol determination experiment were obtained as 26% and 11.21%, respectively, and the analytical recovery was determined as 86% using 50 mg/l standard sample as spiked.

**The results of cholesterol measurement**

Figure 1 illustrates the cholesterol concentration measured in the spent MRS broth and in the corresponding precipitate fraction, a variation of about 10 mg/l cholesterol from that of the adjusted concentration was seen in the supernatant during the incubation period. However, the final cholesterol concentration remained stable during the growth of LA7 strain in MRS. In addition, a slight increase in the cholesterol content of the precipitate fraction was observed at the end stage of incubation. Cholesterol concentration of the whole milk during 72 hours of LA7 growth was not reduced [Figure 2]. The synthetic water-soluble cholesterol was not assimilated by the given strain nor the milk lipoprotein-bounded cholesterol concentration was affected during the growth in milk; therefore the serum cholesterol reduction capability, which was observed in mice, was not associated with the *in vitro* cholesterol assimilation and consumption.

**DISCUSSION**

The *in vitro* study of cholesterol removal of *Lactobacilli* has been consistently used as a screening tool for selection of probiotic stains with diverse

![Figure 1: Cholesterol in the cell free supernatant of MRS broth and in corresponding precipitate fraction measured during the growth of *L. plantarum* A7](image1)

![Figure 2: Measured cholesterol in whole milk during the growth of *L. plantarum* A7](image2)
health promoting characteristics. Gilliland et al. were the first to show that in vivo efficiency of Lactobacilli could be directly associated with the strains' cholesterol-removal capability in the cholesterol-enriched media. In their studies, only 2 µg/ml difference in cholesterol removal between Lactobacillus strains resulted in significant different responses in reducing the pig's plasma cholesterol, they emphasized on the impact of anaerobic condition as well as presence of bile in the medium, as the intransitive factors, affecting the results of in vitro cholesterol removal or uptake by Lactobacilli as if, in the absence of bile salts, no cholesterol reduction has taken place. Cholesterol removal from the culture was attributed to the following mechanisms: Cholesterol assimilation, incorporation to cell membrane or attachment to the bacterial cell surfaces, and destabilization of cholesterol micelles resulting in the co-precipitation of cholesterol with bile salts. Presence of bile salts in the cholesterol containing test media is needed to mimic the human gut, however, the role of the bile in the uptake of cholesterol by Lactobacilli is explained by the co-precipitation of bile acid with cholesterol in low pH environment and more likely to occur at the end of fermentation process. Moreover, it was stated that due to the impact of bacterial bile salt hydrolysis, conjugated bile acids turn to be less soluble de-conjugated counterparts leading to more precipitating of cholesterol and bile acids. Accordingly, lack of cholesterol removal capability of LA7 in the present study may be due to the absence of bile in the medium. Note that, reviewing the literature showed that having a media free of bile could not be very restrictive; in Table 1, in vitro studies on the cholesterol removal properties of lactic strains are summarized; based on the result of the studies presented in this table, positive in vitro studies without using bile salts are evidenced. The highest in vitro cholesterol reduction of nearly 310-490 mg/l was reported for L. plantarum strains in the absence of bile. When milk was used as the culture media in some studies, again, bile component was not added to the milk. Moreover, it was shown that cheese isolated L. plantarum strains in an aerobic condition can remove off 8% of the whole homogenized milk cholesterol after complete growth. Cell wall binding capacity was stated to be the major mechanism involved in the latter study. In another study, 100% of milk cholesterol reduction of L. helveticus cultured in aerobic, bile free condition was attributed to the production of cholesterol oxidase. Cholesterol reducing capability of 3-100 mg/l from the media or milk is reported by other studies as presented in Table 1. In this study, slight increase in the cholesterol content of growing cell precipitates unlikely explains the L. plantarum in vivo cholesterol activity and our final conclusion was that, despite the effectiveness of LA7 for reduction of lipid parameters in mice, it was unable to remove cholesterol from culture media or milk. Since this result sound not to be in accordance with the general belief that “in vitro cholesterol removal of a lactobacillus strain predicts it is in vivo action”; a comprehensive review literature was carried out in order to explain to what extent this relation exists. The results are shown in Tables 2 and 3. Table 2 presents the summary of 11 rat or human studies in which different lactic strains administered to the subjects to investigate the serum cholesterol properties of the strains. Some of these studies lacked information on the in vitro cholesterol removal of the used bacterial strains. Others pointed to this property but they did not mention how much the tested strains were capable of cholesterol removal in the primary in vitro experiment. Among these studies, four studies relied on cholesterol-reducing activity of the tested strains that had been observed primarily in their in vitro test. However, there was a great difference in the efficacy of the lactic strains between animal and human studies. Among the animal studies, 12-92.5% reduction in total cholesterol was observed, as if, the reduction rate of 20-40% was prevalent. While in the human studies, reduction rate of serum cholesterol did not exceeds 4.4%. Reduction rate of LDL cholesterol and serum triglyceride was 17-47% and 15.7-51% among the animal studies but 3.4% and 5.7% for the human trials. Considering the vast number of positive in vivo studies, general agreement on probiotic Lactobacillus intervention for serum cholesterol reduction exists; however, there is controversy over the effectiveness of such intervention particularly in human studies. It was known that the outcomes of in vivo experiments can be affected by some identified reasons. One of the most important challenges is the absence of proper placebo. Milk was used in many of these studies as placebo, whereas it has
been identified to pose hypocholesterolemic effect and not to be a proper placebo. In some studies, yogurt was considered as placebo. Traditional yogurt starter culture is composed of *L. bulgaricus*

### Table 2: *In vivo* studies on the cholesterol reduction capability of *Lactobacillus* strains in MRS broth or in milk without history of *in vitro* cholesterol reduction capability

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of study</th>
<th>Bacterial strain</th>
<th>History of <em>in vitro</em> test for cholesterol activity</th>
<th><em>In vivo</em> result</th>
<th>Tested population</th>
<th>Type of diet</th>
<th>Duration of the study</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Xie</td>
<td>2011</td>
<td><em>L. plantarum</em></td>
<td>Cholesterol removing activity in the growth media but data range was not addressed in the <em>in vivo</em> study</td>
<td>Compared with the control group, 25.5% reduction in total cholesterol, 32.9% reduction in LDL and 16% reduction in TG Compared with the control group, 12.5% reduction in total cholesterol, 17.3% reduction in LDL, and 15.7% reduction in TG</td>
<td>Rat</td>
<td>High fat diet</td>
<td>6 weeks</td>
<td>[33]</td>
</tr>
<tr>
<td>Y. Huang</td>
<td>2010</td>
<td><em>L. acidophilus</em></td>
<td>Down-regulating the expression of NPC1L1</td>
<td>Compared with control group, 92% reduction in total cholesterol, 35% reduction in LDL cholesterol, and 51% reduction in TG</td>
<td>Rat</td>
<td>High cholesterol diet</td>
<td>28 days</td>
<td>[34]</td>
</tr>
<tr>
<td>J. Jeun</td>
<td>2010</td>
<td><em>L. plantarum</em></td>
<td>-</td>
<td>Compared with control group, 33% reduction in the total cholesterol by live cells, 42% and 20% reduction in LDL by live and dead cell, respectively, and 32% reduction in TG</td>
<td>Mice</td>
<td>High cholesterol diet</td>
<td>4 weeks</td>
<td>[35]</td>
</tr>
<tr>
<td>Y. Wang</td>
<td>2009</td>
<td><em>L. plantarum</em></td>
<td>Positive for cholesterol removal but no publishing data</td>
<td>Compared with control group, 31% reduction in total serum cholesterol, 20% reduction in LDL and 31.18% reduction TG</td>
<td>Rat</td>
<td>High cholesterol diet</td>
<td>5 weeks</td>
<td>[36]</td>
</tr>
<tr>
<td>CH. Chiu</td>
<td>2006</td>
<td><em>L. paracasei</em></td>
<td>-</td>
<td>Compared with control group, about 16% reduction in total serum cholesterol, 27.8-47.4% reduction in LDL cholesterol</td>
<td>Rat</td>
<td>High cholesterol diet</td>
<td>8 weeks</td>
<td>[37]</td>
</tr>
<tr>
<td>J.R. Liu</td>
<td>2006</td>
<td>Kefir</td>
<td>-</td>
<td>30-34% reduction in non HDL cholesterol and 13-24% reduction in total TG</td>
<td>Hamester</td>
<td>Cholesterol free or Cholesterol enriched diet</td>
<td>8 weeks</td>
<td>[38]</td>
</tr>
</tbody>
</table>

*Contd..*
Table 2: Contd...

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year of study</th>
<th>Bacterial strain</th>
<th>History of in vitro test for cholesterol activity</th>
<th>In vivo result</th>
<th>Tested population</th>
<th>Type of diet</th>
<th>Duration of the study</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.Z. Xiao</td>
<td>2003</td>
<td><em>Bifidobacterium longum</em> BL1</td>
<td>Bile salt hydrolase activity</td>
<td>Compared with control group, 21%, 41%, and 13% reduction in total cholesterol, LDL cholesterol and TG in rat and 2.4%, 3.2% and 5.7% reduction in total cholesterol, LDL cholesterol, and TG in human</td>
<td>Rat-</td>
<td>Cholesterol containing diet for rat study</td>
<td>3 weeks rat study</td>
<td>[39]</td>
</tr>
<tr>
<td>M. P. Taranto</td>
<td>2000</td>
<td><em>Lactobacillus reuteri</em> CRL 1098</td>
<td>Bile salt hydrolase activity</td>
<td>Compared with control group 22% reduction LDL cholesterol, and 33% reduction TG</td>
<td>Mice</td>
<td>Fat enriched diet</td>
<td>1 week</td>
<td>[40]</td>
</tr>
<tr>
<td>J. W. Anderson</td>
<td>1999</td>
<td><em>L. acidophilus</em> L1 Human isolate</td>
<td>+</td>
<td>From the base line, 2.4% reduction in total cholesterol, 2.6% to 4.22% reduction in LDL cholesterol and 2.1% to 3.2% reduction in TG</td>
<td>Human</td>
<td>Normal diet</td>
<td>3-4 weeks</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. acidophilus</em> strain ATCC 43121 pig Isolates</td>
<td>+</td>
<td>From the base line, 0.9% reduction in total cholesterol, 1.1% reduction in LDL and 16.5% increase in TG</td>
<td>Human</td>
<td>Normal diet</td>
<td>3-4 weeks</td>
<td></td>
</tr>
<tr>
<td>M. P. Taranto</td>
<td>1998</td>
<td><em>L. reuteri</em> CRL 1098</td>
<td>Bile salt hydrolase activity</td>
<td>Compared with control group 38% and 43% reduction in total cholesterol and TG respectively</td>
<td>Mice</td>
<td>Fat enriched diet</td>
<td>1 week</td>
<td>[7]</td>
</tr>
<tr>
<td>G. Schaafsma</td>
<td>1998</td>
<td><em>L. acidophilus</em> DN.112.053</td>
<td>+</td>
<td>From the baseline, 4.4% reduction in total cholesterol, 5.4% reduction in total LDL cholesterol but increase in TG</td>
<td>Human</td>
<td>Normal diet</td>
<td>7 weeks</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. acidophilus</em> DN.112.096</td>
<td>NO exact data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MRS=de-Man–Rogosa–Sharpe, LDL=Low-density lipoprotein

and *Streptococcus thermophilus*. Alkalin[^43] and Schaafsma[^42] in two individual studies concluded that probiotic *L. acidophilus* strain could be more effective in reducing human serum cholesterol concentrations than that of normal yogurt. It is interesting to know that in the recent *in vitro* study performed by Ooi *et al.*[^3] *L. bulgaricus* strains showed more cholesterol-reducing activity from the culture medium than that of *Lactobacillus* strains belonging to the common probiotic species like *L. casei*. Therefore, the latter study can be regarded as a complete controversy if *in vitro* cholesterol reduction is believed to be a reliable index for *in situ* condition.

Table 3 presents the results of the studies including both *in vitro* and *in vivo* animal experiments[^31,32,41,42,44,45]. *In vitro* experiments on the
tested strains resulted in 5.8–71 mg/l cholesterol removal and subsequent animal studies using the given strains resulted in 1.2–24% reduction in the total serum cholesterol in the examined animals.

Comparing the results of the studies which are presented in Tables 2 and Table 3 regarding cholesterol reduction efficacy, it was revealed that the diversity of serum cholesterol reduction resulted by the in vivo studies without any in vitro background (Table 2) was in the range of 16–33%, not much different than that of the studies presented in Table 3 (1.2–24%). While the latter studies used bacterial
CONCLUSIONS

The capability of LA7 in serum lipid cholesterol reduction was not associated by cholesterol assimilation in either Lactobacilli specific medium or whole milk, at least in aerobic and bile free condition. Other mechanisms, for example, production of active metabolites may be involved in bioactivity of this strain. Evaluation of short chain fatty acids production is a characteristic which is overlooked when selecting a probiotic culture for cholesterol-reducing ability. Thus, cholesterol consumption or its removal from the cultured media may not be an integral, reliable index for selection of Lactobacillus strains with cholesterol-lowering activity.

ACKNOWLEDGMENTS

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