Incorporation of Cholesterol into the Cellular Membrane of *Lactobacillus acidophilus* ATCC 43121[1]

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ABSTRACT

Cholesterol that was assimilated by *Lactobacillus acidophilus* ATCC 43121 was not metabolically degraded; most of it was recovered with the cells. Cells that were grown in the presence of cholesterol micelles and bile salts were more resistant to lysis by sonication than were those grown in their absence, suggesting a possible alteration of the cell wall or membrane. Cholesterol assimilation occurred during growth at pH 6.0 as well as during growth without pH control. Part of the cholesterol that was assimilated by cells was recovered in the membrane fractions of cells grown under both conditions. There was no difference in the amount taken up from cholesterol micelles that were prepared using dioleoyl L-α-phosphatidylcholine or distearoyl L-α-phosphatidylcholine. Thus, the type of fatty acid (unsaturated or saturated) in the phospholipid did not influence the assimilation. As the amount of Tween 80 in the growth media increased beyond 0.05%, cholesterol uptake decreased, and the amount of growth remained the same. The higher concentrations of Tween 80 may have adversely affected the permeability of the cells. (**Key words**: cholesterol, cell membrane, *Lactobacillus acidophilus*)

INTRODUCTION

The growth of certain lactic acid bacteria having the ability to take up cholesterol in the small intestine has the potential to aid in the control of serum cholesterol concentrations in humans because the small intestine is the primary site of cholesterol absorption in the human body (2, 5). Mann and Spoerry (12) reported that the consumption of milk fermented with strains of *Lactobacillus* sp. reduced serum cholesterol levels in Maasai warriors. Since then, the potential hypocholesterolemic effect of products containing *Lactobacillus acidophilus* has been shown in feeding studies using humans or animals (3, 7, 9, 10, 30).

Harrison and Peat (10) reported that serum concentrations of cholesterol in infants fed formula containing *L. acidophilus* was lower than in those receiving formula without *L. acidophilus*. Two studies using swine fed high cholesterol diets revealed significantly lower serum cholesterol concentrations in groups receiving diets that had been supplemented with a strain of *L. acidophilus* that assimilated cholesterol during growth in laboratory media (3, 7). Zacconi et al. (30) observed reduced serum cholesterol concentrations in axenic mice contaminated with *L. acidophilus*. Grunewald (9) reported that serum cholesterol concentrations were lower in rats fed milk that had been fermented with *L. acidophilus* than in those fed unfermented milk.

Gilliland et al. (7) reported the assimilation of cholesterol by *L. acidophilus* during growth in laboratory media. The assimilation required growth under anaerobic conditions and the presence of bile acids. The ability to assimilate cholesterol varied significantly among strains. A strain that did not assimilate cholesterol during growth in laboratory media had no effect on serum cholesterol in pigs on a high cholesterol diet; a strain that actively assimilated cholesterol had a significant effect.

In studies with *Mycoplasma* sp., which require exogenous cholesterol to grow, cholesterol uptake by the cells was closely associated with the membrane (4, 13). Razin (16) reported that cholesterol increased the tensile strength of the mycoplasma membrane and permitted the survival and growth of these organisms without the protection of cell walls. Some bacteria, including *Micrococcus lysodeikticus*, *Bacillus megaterium*, and *Proteus mirabilis*, also can incorporate cholesterol into their membranes (17, 27). We have not found reports showing that the membranes of lactobacilli contain cholesterol, regardless of the composition of the growth medium.

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Safonova et al. (22) reported that the presence of saturated fatty acids inhibited cholesterol uptake by epithelial cells in the small intestine of rats and also observed increased cholesterol uptake in the presence of oleic acid, a monounsaturated fatty acid. Razin et al. (19) reported a higher rate constant for cholesterol uptake in oleate-enriched cells than in palmitate-enriched cells of *Acholeplasma laidlawii.* Any impact of saturated or unsaturated fatty acids on the uptake of cholesterol by lactobacilli has not been reported.

The objectives of this study were 1) to measure the incorporation of cholesterol into the cell membrane fraction of *L. acidophilus* ATCC 43121, 2) to measure the effect of phospholipids having different fatty acid components on the cholesterol uptake by *L. acidophilus* ATCC 43121, and 3) to measure the effect of Tween 80 on the cholesterol uptake by *L. acidophilus* ATCC 43121.

MATERIALS AND METHODS

Source and Maintenance of Culture

Lactobacillus acidophilus ATCC 43121 (formerly strain RP32) was from our laboratory stock culture collection. The strain was originally isolated from intestinal contents of a pig (7). The culture was maintained by subculture in lactobacilli MRS broth (Difco Laboratories, Detroit, MI) using 1% inocula and incubation at 37°C for 18 h. The culture was stored at 5°C between transfers and was subcultured at least three times prior to experimental use.

Preparation of Broth

The MRS-THIO broth was prepared the day of experimental use by supplementing lactobacilli MRS broth (Difco Laboratories) with 0.2% sodium thioglycolate (Sigma Chemical Co., St. Louis, MO). The broth was further supplemented, when desired, with 0.004 *M* sodium taurocholate (Sigma Chemical Co.) or 0.3% oxgall (Difco Laboratories). The broth media were autoclaved for 15 min at 121°C.

Measurement of Cholesterol Assimilation

One milliliter of cholesterol-phosphatidylcholine micelles prepared according to Razin et al. (18) was added to the tubes containing 9 ml of MRS-THIO broth. Egg yolk-lecithin (Type III-E: Sigma Chemical Co.) was used to prepare the micelles. Following mixing, 2 ml were transferred to a clean test tube and stored in the refrigerator at 5°C. This mixture was used as an uninoculated control. The remaining broth was inoculated (1%) with a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121 and incubated at 37°C for the desired time. After incubation, cells were removed by centrifugation at 12,000 × *g* and 4°C for 10 min. (Centrifugation of uninoculated broth at 12,000 × *g* and 4°C for 10 min did not result in pelleted micelles.)

The *o*-phthalaldehyde method described by Rudel and Morris (21) was used to determine the amount of cholesterol in the spent broth and the uninoculated control. In most experiments, the amount that was assimilated (micrograms per milliliter) by the cells was calculated by subtracting the amount in the spent broth from that in the uninoculated control. However, in initial experiments, the cells were resuspended in distilled water to the original volume of the culture and were assayed for cholesterol to determine the amount assimilated.

For experiments in which cellular membranes were isolated, cells were grown and harvested as just described. The cell pellets were washed with distilled water, and membranes were isolated according to the method of Throne and Barker (27). The washed cells and membrane fractions were assayed for cholesterol (21), ATPase activity, and protein.

Measurement of ATPase Activity and Protein Content

The ATPase activity was assayed by measuring the amount of inorganic phosphate released from ATP during incubation at 37°C for 30 min according to the methods of Rottem and Razin (20). The amount of inorganic phosphate that was released was measured by the method of Fiske and Subbarow (6). The specific ATPase activity was expressed as micromoles of inorganic phosphate released per milligram of protein per minute. The protein content was measured by the method of Bradford (1) using human albumin (Sigma Chemical Co.) as a standard.

Resistance of Cells to Lysis by Sonication

A freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121 was inoculated (1%) into MRS-THIO broth and into MRS-THIO broth containing 0.3% oxgall (Difco Laboratories) and cholesterol-phosphatidylcholine micelles (100 μg of cholesterol/ml). The inoculated media were incubated at 37°C for 18 h. Cells from each medium were recovered by centrifugation at 12,000 × *g* and 4°C for 10 min and resuspended in distilled water to a population of ap-

proximately 4×10^9/ml. Ten-milliliter aliquots of each cell suspension were transferred to small beakers and were sonicated for 15 min (sonic dismembrator; Fisher Scientific, Pittsburgh, PA) adjusted to a maximum output. The beakers containing the cell suspensions were held in an ice-water mixture during sonication to prevent heating. The numbers of intact cells per milliliter from each medium before and after sonication were determined by direct microscopic count (15).

Measurement of Cholesterol Assimilation During Growth at pH 6.0

The MRS-THIO broth (500 ml) that was supplemented with 0.004 *M* sodium taurocholate and 0.5 ml of 0.2% aqueous methylene blue (as an oxidation-reduction indicator) was prepared and placed into a small fermentor made from a Pyrex jar (8.5 cm diameter) of about 1-L capacity and equipped with an autoclavable combination pH electrode. The fermentor also was equipped with a magnetic spin bar, a port for the addition of neutralizer, and a line (2 mm internal diameter) to permit continuous sparging with nitrogen gas. The entire fermentor containing the broth was autoclaved at 121°C for 15 min. After cooling, 50 ml of cholesterol-phosphatidylcholine micelles prepared (18) using egg yolk-lecithin (Type III-E) were added. The fermentor was placed in a 37°C water bath. A flask containing the neutralizer, 5% sodium carbonate in 5% ammonium hydroxide (8), was connected to the fermentor. The automatic pH controller (model 5997; Horizon Ecology Co., Chicago, IL) was adjusted to maintain the pH of the broth at 6.0. After 2 min of mixing, 10 ml were withdrawn aseptically from the fermentor and placed into a sterile test tube to serve as the uninoculated control. Then, the medium in the fermentor was inoculated with 5 ml of a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121. Nitrogen gas was sparged through the broth (from bottom to top) continuously at about 11 ml/min throughout the incubation period. After the incubation, 10 ml of culture were withdrawn aseptically from the fermentor and centrifuged; the spent broth was assayed for cholesterol (21).

Preparation of Water-Soluble Cholesterol

The stock solution of water-soluble cholesterol was prepared by dissolving polyoxyethanyl-cholesteryl sebacate (Sigma Chemical Co.) in distilled water to a concentration of 20 mg/ml. The solution was passed through a sterile 0.45-μm membrane filter into a sterile test tube and stored at 5°C. The stock solution was diluted as necessary with sterile distilled water and then added to the growth medium for the assay of cholesterol uptake.

Effect of Degree of Phospholipid Saturation on Cholesterol Uptake

In order to measure the influence of the source of phospholipids and of the degree phospholipid saturation on cholesterol assimilation, four different phospholipids were used. They were egg yolk-lecithin (Type III-E), soybean lecithin (Type III-S), and dioleoyl L-α-phosphatidylcholine, and distearoyl L-α-phosphatidylcholine (all from Sigma Chemical Co.). Cholesterol-phospholipid micelles were prepared (18) using each of the four phospholipids. The micelles were used as cholesterol sources to compare cholesterol uptake by *L. acidophilus* ATCC 43121 as described in the section on measurement of cholesterol uptake.

Influence of Tween 80 on Cholesterol Assimilation

Lactobacilli MRS broth was prepared from individual ingredients according to the formulation of the manufacturer (Difco Laboratories) without Tween 80 (polyoxyethylene sorbitan monooleate). The broth was supplemented with 0.2% sodium thioglycollate and 0.004 *M* sodium taurocholate. Tween 80 was added to aliquots of the broth to make MRS-THIO broth containing 0.05, 0.1, 0.15, or 0.2% Tween 80. Following autoclaving (121°C for 15 min) and cooling, 1-ml portions of cholesterol micelles (18) were added to 9-ml portions of MRS-THIO broth containing the different concentrations of Tween 80. Two-milliliter aliquots were taken from each to serve as uninoculated controls. The remaining broth in each tube was inoculated (1%) with a freshly prepared MRS broth culture of *L. acidophilus* ATCC 43121. After the incubation at 37°C for 18 h, 1-ml portions of the cultures were diluted with 9 ml of distilled water, and the absorbance was measured at 620 nm against a water blank with a Spectronic colorimeter (model 21D; Milton Roy, Rochester, NY) to compare relative amounts of growth. Cells from the remainders of the cultures were removed by centrifugation at $12{,}000 \times g$ and 4°C for 10 min. Then, the cholesterol contents of spent broths and uninoculated controls were assayed (21).

Statistical Analyses

Analysis of variance was performed on each set of data to determine whether significant differences ex-

isted among the samples (23). Least significant difference analysis was used to separate means.

RESULTS

Cholesterol Assimilation by *L. acidophilus* ATCC 43121

In static cultures of *L. acidophilus* ATCC 43121 grown in MRS-THIO broth and supplemented with 92 μg/ml of cholesterol and 0.3% oxgall, 48 μg/ml of the cholesterol were recovered with the cells (Table 1). The cholesterol content in resuspended cells plus that in the spent broth was approximately equal to that in the uninoculated control broth; thus, little, if any, of the cholesterol was degraded by the culture during growth.

Resistance of Cells to Lysis by Sonication

Cells of *L. acidophilus* ATCC 43121 that were grown in broth containing oxgall and cholesterol micelles were more resistant to lysis by sonication than were cells grown in broth without them (Table 2). When cells were grown in MRS-THIO broth, 95% of cells were lysed in 15 min. However, when cells were grown in MRS broth containing 0.3% oxgall and cholesterol micelles, only 17% of cells were lysed during the same time period.

Cholesterol Uptake During Growth at pH 6.0

The effect of maintaining the pH during growth at a level to prevent the precipitation of any free cholic acid (11) on the cholesterol uptake by *L. acidophilus* ATCC 43121 was tested by growing the culture in medium without pH control and in the medium maintained at pH 6.0 during growth. The culture took up cholesterol during growth at pH 6.0 (39 μg/ml) and during growth without pH control (28 μg/ml). The broth remained anaerobic during incubation (as indicated by the reduced methylene blue indicator).

TABLE 1. Assimilation of cholesterol by *Lactobacillus acidophilus* 43121.[1]

Sample	Cholesterol[2]
	(μg/ml)
Uninoculated control broth	92[a]
Spent broth	43[b]
Resuspended cells	48[b]

[a,b]Means without a common superscript differ ($P < 0.05$).

[1]Cells were incubated for 10 h at 37°C in MRS broth supplemented with 0.2% sodium thioglycolate, 0.3% oxgall, and 10% cholesterol micelles (prepared using Type III-E egg yolk lecithin).

[2]All values are the means (SE = 4.47) of 10 trials. 27 df.

TABLE 2. Comparison of lysis by sonication of cells of *Lactobacillus acidophilus* ATCC 43121 grown in the presence and absence of cholesterol and bile salts.[1]

Growth medium and sample	Number of cells	Disruption of cells
Medium A[2]	(DMC[3]/ml)	(%)
Control	4.0×10^9	
Sonicated	2.0×10^8	95
Medium B[4]		
Control	4.7×10^9	
Sonicated	3.9×10^9	17

[1]Cells were grown in medium A or medium B for 18 h at 37°C and sonicated for 15 min. All values are the means of two trials.

[2]Medium A: MRS broth supplemented with 0.2% sodium thioglycolate.

[3]Direct microscopic cell counts.

[4]Medium B: MRS broth supplemented with 0.2% sodium thioglycolate, 0.3% oxgall, and cholesterol micelles.

Cholesterol in the Membrane Fraction of Cells Grown Statically and at pH 6.0

Cholesterol was recovered in the membrane fractions of cells grown with and without pH control at pH 6.0 (Table 3). The specific ATPase activities were significantly higher ($P < 0.05$) in the membrane fractions than in the whole cells in cultures grown under either condition. The whole cells of both cultures (i.e., grown without pH control and at pH 6.0) showed the same level of enzyme activity. The ATPase activity for the whole cells that were grown under either condition was higher than expected. The specific activity of ATPase was higher ($P < 0.05$) in the membrane fraction of the cells grown without pH control than in the fraction from the cells grown at pH 6.0. This result suggests a greater degree of purification of the membrane from the cells grown without pH control because ATPase activity is normally associated with bacterial cellular membranes.

The amounts of cholesterol that were assimilated into cells and membranes were expressed as micrograms per milligram of protein. Cell membranes from the culture grown without pH control included more ($P < 0.05$) cholesterol than did those from cells grown at pH 6.0. Cell membranes from the culture grown without pH control contained more ($P < 0.05$)

TABLE 3. Cholesterol in cells and membranes of cultures of *Lactobacillus acidophilus* ATCC 43121 grown at pH 6.0 and without pH control.[1]

Growth conditions	Fraction	Cholesterol[2]	Specific ATPase activity
		(μg/mg of protein)	
Without pH control	Washed whole cells	188[b]	0.17[c]
	Membranes	353[a]	0.67[b]
Controlled at pH 6.0	Washed whole cells	110[b]	0.17[c]
	Membranes	122[b]	0.41[a]

[a,b,c]Means within a column without common superscript differ ($P < 0.05$).

[1]Cells were grown at 37°C for 18 h statically and at pH 6.0 in MRS broth supplemented with 0.2% sodium thioglycolate, 0.004 *M* sodium taurocholate, and cholesterol micelles (prepared with Type III-E egg yolk-lecithin).

[2]All cholesterol values are the means (SE = 0.16) of three trials. 8 df.

[3]The specific ATPase activity is expressed as micromoles per minute per milligram of protein. All activity values are the means (SE = 0.035) of three trials. 8 df.

TABLE 4. Influence of phospholipids containing different degrees of unsaturation on growth and cholesterol uptake by *Lactobacillus acidophilus* ATCC 43121.[1]

Phospholipid[2]	Growth[3]	Cholesterol uptake[4]
	(A_{620nm})	(μg/ml)
III-E	0.168[b]	21[b]
III-S	0.160[b]	33[ab]
Dioleoyl	0.224[a]	47[a]
Distearoyl	0.235[a]	41[a]

[a,b]Means within a column without a common superscript differ ($P < 0.05$).

[1]Cells were incubated at 37°C for 12 h in MRS broth supplemented with 0.2% sodium thioglycolate, 0.004 *M* sodium taurocholate, and cholesterol micelles (prepared using the indicated phospholipid).

[2]III-E = Egg yolk-lecithin, III-S = soybean lecithin, dioleoyl = L-α-phosphatidylcholine containing oleic acid, distearoyl = L-α-phosphatidylcholine containing stearic acid.

[3]Absorbance at 620 nm (A_{620nm}) of 1:10 dilution of each sample; all values are the means (SE = 0.019) of three trials. 8 df.

[4]All values are the means (SE = 7.34) of three trials. 8 df.

cholesterol than did the whole cells grown under the same conditions. The cell membranes of the cultures grown at pH 6.0 did not contain more ($P > 0.05$) cholesterol than did the whole cells grown at pH 6.0.

Influence of Degree of Phospholipid Saturation on Cholesterol Uptake

Whether the phosphatidylcholine that was used to prepare the cholesterol-phosphophatidylcholine micelles contained saturated or unsaturated fatty acids had no influence on the amount of cholesterol that was assimilated by *L. acidophilus* ATCC 43121 (Table 4). More ($P < 0.05$) cholesterol was assimilated from the micelles prepared using dioleoyl L-α-phosphatidylcholine and distearoyl L-α-phosphatidylcholine than from those prepared using egg yolk-lecithin. However, the amount that was assimilated from micelles prepared using type III-S lecithin was not different from that assimilated from the dioleoyl and distearoyl phosphatidylcholines. There were no significant differences ($P > 0.05$) between the amounts taken up from micelles prepared using the dioleoyl and distearoyl phosphatidylcholines or between those micelles prepared using type III-E and type III-S lecithins. More ($P < 0.05$) growth occurred in the broths containing the micelles that were prepared using dioleoyl L-α-phosphatidylcholine and distearoyl L-α-phosphatidylcholine than in the broths containing micelles prepared using the egg yolk lecithin or the soybean lecithin.

Influence of Tween 80 on Cholesterol Uptake

More cholesterol was assimilated by *L. acidophilus* 43121 from water-soluble cholesterol than from the cholesterol-phosphatidylcholine micelles (Table 5). Without Tween 80, little, if any, cholesterol from either source was assimilated by *L. acidophilus* ATCC 43121, and little growth was observed. Growth was not significantly different ($P > 0.05$) among the media containing 0.05, 0.10, 0.15, or 0.20 Tween 80. However, in the presence of 0.05% Tween 80, more cholesterol was taken up ($P < 0.05$) for both cholesterol sources than in the presence of any of the other concentrations of Tween 80. As the amount of Tween 80 increased beyond 0.05%, the amount of uptake decreased for both sources of cholesterol.

DISCUSSION

Hypocholesterolemic activity of *L. acidophilus* has been reported in several studies (3, 7, 9, 10, 30). According to Gilliland et al. (7), cholesterol that was removed from laboratory media during the growth of *L. acidophilus* was assimilated by the cells. Klaver and Van der Meer (11) reported that the presumed assimilation of cholesterol by *L. acidophilus* was due to the coprecipitation of cholesterol along with free bile acids resulting from deconjugation of the bile acids by the lactobacilli during growth. Those researchers (11) based that conclusion largely on the fact that, in their experiments, no cholesterol was removed from the broth medium when cells were

TABLE 5. Influence of Tween 80 on growth and uptake of cholesterol from two sources by *Lactobacillus acidophilus* ATCC 43121.[1]

Cholesterol source[2]	Tween 80	A_{620nm}[3]	Cholesterol uptake[4]
	(%)		(μg/ml)
Cholesterol-phosphatidylcholine micelles	0	0.087[b]	8[f]
	0.05	0.155[a]	55[cd]
	0.10	0.160[a]	42[de]
	0.15	0.160[a]	32[e]
	0.20	0.155[a]	22[e]
Water-soluble cholesterol	0	0.021[c]	7[f]
	0.05	0.151[a]	117[a]
	0.10	0.158[a]	82[bc]
	0.15	0.151[a]	72[c]
	0.20	0.155[a]	66[c]

[a,b,c,d,e,f]Means within a column without common superscripts differ ($P < 0.05$).

[1]Cultures were incubated at 37°C for 18 h in MRS broth supplemented with 0.2% sodium thioglycolate, 0.004 *M* sodium taurocholate, the indicated amounts of Tween 80, and the indicated cholesterol source.

[2]Cholesterol-phospholipid micelles prepared using phosphatidylcholine, dioleoyl (final cholesterol concentration in broth = 101 μg/ml); water-soluble cholesterol = polyoxyethanyl-cholesteryl sebacate (final cholesterol concentration in broth = 134 μg/ml).

[3]Absorbance at 620 nm of 1:10 dilution of each sample; all values are the means (SE = 0.014) of three trials. 20 df.

[4]All values are the means (SE = 11.77) of three trials. 20 df.

harvested from a culture that had been maintained at pH 6.0 during growth, a pH at which free bile acids would remain in solution. In the present study, however, we obtained uptake of cholesterol by *L. acidophilus* ATCC 43121 during growth at pH 6.0, which indicates that the cholesterol did not merely coprecipitate with free bile acids as has been indicated by Klaver and Van der Meer (11). Those researchers, however, relied on flushing the head space of their fermentor with nitrogen to maintain anaerobic conditions. We used sodium thioglycollate in the medium coupled with sparging nitrogen gas through the medium to maintain anaerobic conditions. Those researchers also used a different source and lower level of cholesterol. These differences could account for the differences observed between studies. Additionally, in the present study, some cholesterol was recovered in the cell membrane of *L. acidophilus* grown in a medium containing cholesterol. Furthermore, in a previous study (28), we showed that there is no relationship between the ability of *L. acidophilus* to deconjugate bile acids and the ability to assimilate cholesterol.

Incorporation of cholesterol into the membranes of mycoplasmas also has been reported (13, 18). Cholesterol in the cell membranes of mycoplasmas protects the cells from lysis by increasing the tensile strength of the membranes (16). Cells of *L. acidophilus* ATCC 43121 that were grown in the presence of oxgall and cholesterol micelles showed greater resistance to lysis by sonication than did cells grown in control broth. These results suggest that cholesterol may have altered the cellular membrane or wall of the lactobacilli so that they were more resistant to sonic disruption. In addition, in a preliminary experiment (data not shown), cells of *L. acidophilus* that were grown in the presence of oxgall and cholesterol micelles did not all stain Gram-positive, but those grown without cholesterol micelles did. This result further suggests that changes occur in the cells of lactobacilli as a result of growth in the presence of cholesterol and bile salts.

The specific ATPase activity of whole cells of these organisms would be expected to be lower than the activity observed in this study. However, the cells of *L. acidophilus* were grown in a medium containing the bile salt, sodium taurocholate. We have shown in another study (14) that growth of *L. acidophilus* in media containing bile salts increases the cellular permeability. Thus, the higher than expected ATPase activity of whole cells likely is due to increased permeability of the cells because of growth in the presence of sodium taurocholate.

Oleic acid is an important growth factor for lactobacilli (29). Smittle et al. (24) reported that growth of *L. bulgaricus* in media containing Tween 80 resulted in cells that survived freezing much better than did cells grown in its absence. Oleic acid in the Tween 80 was identified as a component responsible for the improved survival of the cells during frozen storage. Growth in its presence modified the fatty acid composition of the cells (25), which was significantly related to the increased survival of the cells during freezing. Cholesterol uptake by *L. acidophilus* ATCC 43121 in the present study was affected by the presence or absence of Tween 80. Without Tween 80, little, if any, cholesterol uptake was observed, probably because growth was greatly reduced in its absence. Of the concentrations tested, the presence of 0.05% Tween 80 supported the highest level of cholesterol uptake; the amount that was assimilated decreased as the concentration of Tween 80 increased beyond 0.05%. The differences in cholesterol assimilation in the media containing 0.05 to 0.2% Tween 80 were not due to differences in amounts of growth. These findings suggest that *L. acidophilus* has an optimum level of Tween 80 (or oleic acid) required to maximize cholesterol assimilation. Smittle et al. (24) reported that the optimum level of Tween 80 in the growth medium for growing cells that survive freez-

ing varied among strains of *L. bulgaricus*. This influence of Tween 80 was related to alterations in the cellular membrane of the organism (25). A similar relationship among strains of *L. acidophilus* may exist with respect to the influence of oleic acid on cholesterol uptake.

The observed amounts of cholesterol in the membrane fraction of *L. acidophilus* were greater than the concentrations reported for other bacterial species including *B. megaterium* and *M. lysodeikticus* (17, 26). Cells that were grown at pH 6.0 assimilated cholesterol as well as those grown without pH control. However, the membranes of cultures grown without pH control contained more cholesterol per milligram of protein than did those from the pH 6.0 culture. This difference in ability to assimilate cholesterol into the membranes might be due to differences in the membranes resulting from two growth conditions. More work is needed to identify the composition of the membranes from cells grown under both conditions.

The amounts of cholesterol recovered in the membrane fraction of *L. acidophilus* do not account for the total amounts assimilated by the culture. (Based on the amount in the whole cells, we estimated 10 to 15% of the assimilated cholesterol was recovered in the membrane fraction.) Some cholesterol likely would have been lost in the isolation procedure; however, some probably was more loosely associated with the cells and not incorporated into the membrane.

REFERENCES

1 Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248.

2 Chen, H., E. Born, S. N. Mathur, F. C. Johlin, Jr., and F. J. Field. 1992. Sphingomyelinase content of intestinal cell membranes regulates cholesterol absorption: evidence for pancreatic and intestinal cell sphingomyelinase activity. Biochem. J. 286: 771.

3 Daneilson, A. D., E. R. Peo, K. M. Shahani, A. J. Lewis, P. J. Whalen, and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966.

4 Efrati, H., S. Rottem, and S. Razin. 1981. Lipid and protein membrane components associated with cholesterol uptake by mycoplasmas. Biochim. Biophys. Acta 641:386.

5 Field, F. J., N.T.P. Kam, and S. N. Mathur. 1990. Regulation of cholesterol metabolism in the intestine. Gastroenterology 99: 539.

6 Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

7 Gilliland, S. E., C. R. Nelson, and C. Maxwell. 1984. Assimilation of cholesterol of *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.

8 Gilliland, S. E., and C. N. Rich. 1990. Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. J. Dairy Sci. 73:1187.

9 Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078.

10 Harrison, V. C., and G. Peat. 1975. Serum cholesterol and bowel flora in the newborn. Am. J. Clin. Nutr. 28:1351.

11 Klaver, F.A.M., and R. Van der Meer. 1993. The assumed assimilation of cholesterol by lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120.

12 Mann, G. V., and A. Spoerry. 1974. Studies of a surfactant and cholesteremia in the Maasai. Am. J. Clin. Nutr. 27:464.

13 Melchior, D. L., and S. Rottem. 1981. The organization of cholesterol esters in *Mycoplasma capricolum* membranes. Eur. J. Biochem. 117:147.

14 Noh, D. O., and S. E. Gilliland. 1993. Influence of bile on cellular integrity and β-galactosidase activity of *Lactobacillus acidophilus*. J. Dairy Sci. 76:1253.

15 Pusch, D. J., F. F. Busta, W. A. Moats, and A. E. Schulze. 1976. Direct microscopic count. Page 132 *in* Compendium of Methods for the Microbiological Examination of Foods. M. L. Speck, ed. Am. Publ. Health Assoc., Washington, DC.

16 Razin, S. 1967. The cell membrane of mycoplasma. Ann. New York Acad. Sci. 143:115.

17 Razin, S. 1975. Cholesterol incorporation into bacterial membranes. J. Bacteriol. 124:570.

18 Razin, S., S. Kutner, H. Efrati, and S. Rottem. 1980. Phospholipid and cholesterol uptake by mycoplasma cells and membranes. Biochim. Biophys. Acta 598:628.

19 Razin, S., M. Wormser, and N. L. Gershfeld. 1974. Cholesterol in mycoplasma membrane. II. Components of *Acholeplasma laidlawii* cell membranes responsible for cholesterol binding. Biochim. Biophys. Acta 352:385.

20 Rottem, S., and S. Razin. 1966. Adenosine triphosphatase activity of mycoplasma membranes. J. Bacteriol. 92:714.

21 Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *o*-phthalaldehyde. J. Lipid Res. 14:364.

22 Safonova, I. G., D. D. Sviridov, J.-L. Nano, P. Rampal, and V. S. Repin. 1994. Regulation of cholesterol uptake in the rat intestinal cell line. Biochim. Biophys. Acta 1210:181.

23 SAS® Procedures Guide for Personal Computers, Version 6. 1985. SAS Inst., Inc., Cary, NC.

24 Smittle, R. B., S. E. Gilliland, and M. L. Speck. 1972. Death of *Lactobacillus bulgaricus* resulting from liquid nitrogen freezing. Appl. Microbiol. 24:551.

25 Smittle, R. B., S. E. Gilliland, M. L. Speck, and W. M. Walter, Jr.. 1974. Relationship of cellular fatty acid composition to survival of *Lactobacillus bulgaricus* in liquid nitrogen. Appl. Microbiol. 27:738.

26 Tani, H., M. Sato, H. Tsuchiya, and I. Namikawa. 1993. Cholesterol incorporation into *Bacillus megaterium* without compositional modification of membrane lipids in response to changes of the membrane functions. Current Microbiol. 26:253.

27 Thorne, K.J.I., and D. C. Barker. 1972. The occurrence of bactoprenol in the mesosome and plasma membranes of *Lactobacillus casei* and *Lactobacillus plantarum*. J. Gen. Microbiol. 70:87.

28 Walker, D. K., and S. E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956.

29 Williams, W. L., H. P. Broquist, and E. E. Snell. 1947. Oleic acid and related compounds as growth factors for lactic acid bacteria. J. Biol. Chem. 170:619.

30 Zacconi, C., V. Bottazzi, A. Rebecchi, E. Bosi, P. G. Sarra, and L. Tagliaferri. 1992. Serum cholesterol levels in axenic mice colonized with *Enterococcus faecium* and *Lactobacillus acidophilus*. Microbiologica 15:413.