Bile Salt Deconjugation and Cholesterol Removal from Media by *Lactobacillus casei* [1]

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ABSTRACT

Lactobacillus casei N19 and E5 and *Lactobacillus acidophilus* L1 and ATCC 43121 were compared for their ability to deconjugate bile salts and remove cholesterol from MRS broth during growth at pH 6.0 and during growth without pH control. Samples grown without pH control dropped to pH 4.2 to 4.5 during 20 h of incubation, depending on the culture. The plate counts indicated that populations in all cultures were near their maximum numbers after 16 h of growth. The amount of cholesterol removed from the broth was similar for both strains of *L. acidophilus* grown with and without pH control. However, the strains of *L. casei* differed significantly in the amount of cholesterol removed during growth with or without pH control. Both cultures of *L. casei* that were grown at pH 6.0 removed very little cholesterol from the broth, but cells grown without pH control removed up to 60 μg of cholesterol/ml. All cultures of both species deconjugated 60 to 90% of the bile salts. *Lactobacillus acidophilus* L1 was the only culture to demonstrate differences between the two pH treatments in the amount of bile salts deconjugated; however, there was no difference in the amounts of cholesterol removed. These results indicate that most of the cholesterol removal from broth by *L. acidophilus* was due to assimilation, perhaps by the incorporation of cholesterol into the cellular membrane. *Lactobacillus casei* most likely removes cholesterol from broth by means of the destabilization of cholesterol micelles and the coprecipitation of the cholesterol with the deconjugated bile salts at pH less than 6.0.
(**Key words**: *Lactobacillus acidophilus*, *Lactobacillus casei*, bile salt deconjugation, cholesterol)

INTRODUCTION

High serum cholesterol concentrations are associated with the development of coronary heart disease, the leading cause of death in the US (11, 14, 15, 19). During growth, *Lactobacillus acidophilus* can remove cholesterol from laboratory media that have been supplemented with cholesterol micelles and bile salts (2, 4, 9). *Lactobacillus casei* also can remove cholesterol from laboratory media during growth (17). Thus, both species may have the potential to reduce serum cholesterol concentrations in humans.

The mechanism by which the organisms remove the cholesterol from the laboratory media is not completely clear. Klaver and Van der Meer (12) reported that the removal of cholesterol from laboratory media by *L. acidophilus* was due to the disruption of the cholesterol micelles caused by bile salt deconjugation and precipitation of cholesterol with the free bile salts as the pH of the media dropped from acid production during growth. Both *L. acidophilus* and *L. casei* deconjugate bile salts during growth by producing the enzyme bile salt hydrolase (3, 5, 13). The solubility of cholic acid, a deconjugated bile acid, decreases as the pH of the media decreases (6, 7, 8). Cholic acid is especially insoluble at pH less than 5 because of its pK of 5 to 6. As the pH drops because of acid production during bacterial growth, the cholic acid precipitates from the broth and may cause the cholesterol to precipitate also if the cholesterol micelles are disrupted.

A study by Noh et al. (18) revealed that *L. acidophilus* incorporates some of the cholesterol removed from laboratory media into the cellular membrane during growth. Furthermore, during growth at pH 6.0, a pH that is sufficiently high to keep the deconjugated bile acids in solution, those researchers (18) still observed removal of cholesterol from a growth medium that had been supplemented with cholesterol micelles and conjugated bile salts.

Both the deconjugation of bile salts and the incorporation of cholesterol into the cellular membrane have the potential to lower serum cholesterol concentrations in humans. If incorporated into or attached

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to cells of lactobacilli during growth in the small intestine, cholesterol is likely to be unavailable for absorption into the blood. The release of free bile salts through the deconjugation of conjugated bile salts in the small intestine results in the excretion of more bile salts in the feces (25). The primary means by which cholesterol is removed from the body is by excretion in the form of deconjugated bile salts (27). Most conjugated bile salts are recirculated through the enterohepatic circulation. The bile salts that are excreted must be replaced by new bile acids, which are formed from cholesterol in the body. Thus, the more bile salts that are excreted, the more cholesterol is utilized from the pool within the body. Furthermore, free bile salts do not support the absorption of cholesterol and other lipids from the small intestine as well as do conjugated bile salts (8, 20).

The objective of this study was to determine whether *L. acidophilus* and *L. casei* removed cholesterol from laboratory media in a similar manner by 1) determining whether *L. casei* incorporates cholesterol into the cellular membrane, 2) determining whether the amount of cholesterol removed from the laboratory media by both species differed during growth when it was maintained at pH 6.0 than when pH was not controlled, 3) determining the amount of sodium taurocholate deconjugated by the cultures during growth with and without pH control, and 4) determining whether *L. casei* preferentially deconjugates sodium glycocholate or sodium taurocholate during growth with or without pH control.

MATERIALS AND METHODS

Source and Maintenance of Cultures

All cultures used in this study were from the stock culture collection of the Dairy Microbiology Laboratory at Oklahoma State University (Stillwater). *Lactobacillus acidophilus* ATCC 43121 was originally isolated from a pig. Both *L. acidophilus* L1 and *L. casei* strains A3, A17, E5, E10, L15, L19, M5, M12, N7, and N19 were isolated from human intestine by Buck and Gilliland (2). The cultures were maintained by subculturing 1% inocula into MRS broth (Difco Laboratories, Detroit, MI) and incubating them for 18 h at 37°C. The cultures were stored at 7°C between transfers. Stock cultures were stored in MRS agar stabs. The cultures were subcultured at least three times immediately before experimental use.

Bacterial Growth Media

The MRS broth (Difco Laboratories) was used for subculturing and was prepared according to instructions of the manufacturer. The MRS agar was prepared by adding 1.5% agar to MRS broth prior to sterilization. The MRS-THIO broth was prepared by supplementing MRS broth with 0.2% sodium thioglycolate (Sigma Chemical Co., St. Louis, MO). This broth was further supplemented with 6 m*M* sodium taurocholate (Sigma Chemical Co.) or with 2.8 m*M* sodium glycocholate (Sigma Chemical Co.) and 1.2 m*M* sodium taurocholate when needed. All media were sterilized by autoclaving for 15 min at 121°C. All media containing sodium thioglycholate were prepared the day of use.

Initial Screening of Cultures for Cholesterol Removal and Bile Salt Deconjugation

The cultures of *L. casei* were isolated from human intestine in a previous study (2). The cultures represented some of the lactobacilli other than *L. acidophilus* described in that study. Determinations of identity characteristics and the relative abilities to remove cholesterol from the medium and to deconjugate sodium taurocholate were done as described earlier (2). The medium contained 90 to 100 μg of cholesterol/ml.

Incorporation of Cholesterol into the Cellular Membrane of *L. casei*

Strains E5 and N19 of *L. casei* were examined for their ability to incorporate cholesterol into their cellular membrane. The isolation and evaluation of the cholesterol content of the membrane was done as described by Noh et al. (18).

Growth of Cultures with and Without pH Control

On the day of the experiment, 800 ml of MRS-THIO broth containing 6 m*M* sodium taurocholate was prepared. After the broth was cooled, it was supplemented with 80 ml (10%) of cholesterol-phosphatidylcholine micelles that were prepared according to the method of Razin et al. (21). The micelles were prepared using egg yolk-lecithin (Type III-e; Sigma Chemical Co.). The concentration of cholesterol in the broth was 90 to 100 μg/ml. The medium was mixed thoroughly, and 10 ml were removed, placed into a sterile tube, and held at 5°C (uninoculated control or time 0). The broth was then inoculated with 8 ml of a freshly prepared MRS broth culture of the appropriate strain to be evaluated. (*Lactobacillus acidophilus* L1 and ATCC 43121 and

L. casei E5 and N19 were all examined.) The broth was then aseptically transferred in equal portions into each of two sterile fermentor jars (each approximately 1-L capacity). One of the fermentor jars was equipped with a combination pH electrode and ports for the addition of neutralizer, continuous sparging with nitrogen gas, and sample removal. The automatic pH controller was adjusted to maintain the pH at 6.0 during growth by adding a neutralizer containing 5% sodium carbonate in 5% ammonium hydroxide (11). Nitrogen gas was purged through the broth at a rate of 10 ml/min to help maintain a low oxidation reduction potential. The culture in the other fermentor jar was incubated statically without pH control at 37°C. The temperature for both was maintained at 37°C in a water bath.

Samples were aseptically removed and placed into sterile tubes for evaluation 16, 18, 20, and 22 h of growth. Samples were stored in a mixture of ice and water until they were examined (not more than 30 min). Samples were examined for pH, plate counts on MRS agar, bile salt deconjugation, and cholesterol content.

Deconjugation of Sodium Glycocholate and Sodium Taurocholate

Experiments were conducted in a manner similar to that described in the previous section. *Lactobacillus casei* E5 and N19 were compared. The growth medium differed from that in the experiments described in the previous section in that no cholesterol was added and 2.8 m*M*/ml of sodium glycocholate and 1.2 m*M*/ml sodium taurocholate were added to the broth instead of 6 mm of sodium taurocholate. These concentrations were chosen because they resemble the molar ratio of the two salts in human bile (3, 23). Samples were aseptically removed, placed into sterile tubes, and held in ice water for not more than 30 min before evaluation at 16, 18, 20, and 22 h of incubation for concentrations of bile salts.

Measurement of Cholesterol Removal

The initial sample (0 h) removed from the fermentor served as an uninoculated control for the measurement of cholesterol assimilation. The amount of cholesterol remaining in MRS broth was determined at 16, 18, 20, and 22 h after inoculation for cultures grown with and without pH control. The cells were removed from broth samples by centrifugation at 12,000 × *g* at 4°C for 10 min. The supernatant was recovered, and the amount of cholesterol remaining in it was determined. The *o*-phthalaldehyde method described by Rudel and Morris (22) was used to determine the amount of cholesterol in the sample. The amount of cholesterol removed from the broth was determined by subtracting the amount in each broth sample (micrograms per milliliter) from the amount present in the uninoculated control.

Bile Salt Deconjugation

The amounts of bile salt (sodium taurocholate or sodium glycocholate) deconjugated by the lactobacilli were determined using HPLC analysis as described by Corzo (3). Methanol-acetate buffer, pH 5.0, was used as the mobile phase. Two milliliters of sample were suspended in 8 ml of 0.9% NaCl in 0.1 *M* NaOH and 6 ml of mobile phase. The bile salts were recovered by passage of the solution through a Sep-Pac cartridge (Waters Associates), and the bile salts were eluted from the cartridge with 3 ml of the mobile phase. Dexamethasone (0.2 mg/ml; Sigma Chemical Co.) was added to the eluate as an internal standard to permit quantitation of the bile salts. The amount of each bile salt deconjugated was based on the disappearance of each conjugated bile salt from the growth medium.

Plate Counts of Lactobacilli in Samples

The total numbers of lactobacilli were determined using the pour plate method (26) on MRS agar. The samples were diluted in 0.1% peptone (Sigma Chemical Co.) dilution blanks (99 ml) containing 0.01% silicone antifoamer (Sigma Chemical Co.). Plates were incubated in a 37°C incubator for 48 h. Colonies were counted with the aid of a Québec Colony Counter (American Optical Co., Buffalo, NY), and the colony-forming units per milliliter were determined. Colony-forming units per milliliter were converted to $\log_{10}$ colony-forming units per milliliter for statistical examination.

Statistical Analyses

The experimental design of this experiment was repeated measures over time in a split plot. The main units were in a completely randomized design. The main unit treatments were cultures, and the subunit treatments were pH 6 and no pH control. The repeated measures were sampling times. The PROC MIXED command of the SAS system was used to separate means (24).

TABLE 1. Comparison of the amounts of cholesterol removed from suspension in growth medium by isolates of *Lactobacillus casei* originating from human volunteers.

Culture	Cholesterol removed[1]
	(μg/ml)
E5	73.3[a]
E10	61.4[ab]
M5	56.6[abc]
L15	48.8[abcd]
N19	45.4[abcd]
A17	34.5[bcd]
N7	32.2[bcd]
L19	30.9[bcd]
A3	28.6[cd]
M12	16.9[d]

[a,b,c,d]Values are the means of three replications. Means with no common superscript letters differ ($P < 0.05$).

[1]Cholesterol removed by the culture during a 24-h incubation at 37°C.

RESULTS

Initial Screening of Cultures for Cholesterol Removal and Bile Salt Deconjugation

The identities of the *L. casei* cultures in this study were confirmed. The identity characteristics of all 10 were confirmed to be *L. casei*, based on the identity characteristics for the genus *Lactobacillus* (1). There were differences ($P < 0.05$) among the 10 strains of *L. casei* in the amounts of cholesterol removed from suspension in the growth medium during 24 h of incubation (Table 1). Differences ($P < 0.05$) in the deconjugation of sodium taurocholate also were observed among cultures (Table 2). *Lactobacillus casei* E10, E5, and L15, which deconjugated greater amounts of bile salt than the other 7 strains, also were among the most active in removing cholesterol from suspension in the growth medium. Strains E5 and N19 were among the most active in removing cholesterol from the growth medium but exhibited a difference ($P < 0.05$) in deconjugation of sodium taurocholate were selected for further comparisons. Two strains of *L. acidophilus*, L1 and 43121, which had been shown to be very active in the removal of cholesterol from growth media in previous studies (2, 10), were selected for comparison with *L. casei* E5 and N19.

Removal of Cholesterol from the Broth

Statistical analysis indicated interactions ($P < 0.05$) of culture and time, of culture and treatment, and of culture, time, and treatment. Because of these interactions, we examined the results at each sampling time at each level of the other factors in the interaction. The pattern indicated that each culture behaved differently in the removal of cholesterol from the broth at each sampling time and pH treatment.

Because the maximum amounts of cholesterol had been removed after 20 h of growth for all cultures, the results are summarized for that time in Table 3. *Lactobacillus casei* E5 and N19 had removed 56.6 and 36.3 μg of cholesterol/ml, respectively, after 20 h of growth without pH control (static culture). These amounts were not different ($P > 0.05$). Both cultures of *L. casei* grown at pH 6.0 removed very little cholesterol from the broth. Strain E5 removed 8.0 μg/ml, and N19 removed 10.4 μg/ml. Neither of these amounts differed ($P > 0.05$) from the amount that was present in the broth initially. Thus, little or none was removed during growth at pH 6.0.

Both strains of *L. acidophilus* removed significant ($P < 0.05$) amounts of cholesterol from the broth when grown at pH 6.0 or without pH control. *Lactobacillus acidophilus* L1 grown at pH 6.0 removed 46.9 μg/ml; without pH control, 44.7 μg/ml were removed. The amount removed from the broth by strain 43121 grown at pH 6.0 was not different ($P > 0.05$) from the amounts removed by strain L1. However, strain 43121 grown without pH control removed 60.6 μg of cholesterol/ml, which was more ($P < 0.05$) than when grown at pH 6.0.

Deconjugation of Sodium Taurocholate

There was an interaction ($P < 0.05$) between cultures and time with respect to deconjugation of sodium taurocholate, which indicates that the cultures

TABLE 2. Comparison of the deconjugation of sodium taurocholate by isolates of *Lactobacillus casei* from human volunteers.

Culture	Bile salt deconjugated[1]
	(μM/ml)
E10	1.6[a]
E5	1.5[a]
L15	1.4[a]
M5	0.8[b]
N7	0.3[c]
A17	0.2[c]
M12	0.2[c]
A3	0.2[c]
N19	0.1[c]
L19	0.1[c]

[a,b,c]Values are the means of three replications. Means with no common superscript letters differ ($P < 0.05$).

[1]Bile salt deconjugated after incubation for 15 h at 37°C.

TABLE 3. Cholesterol removal, bile salt deconjugation, pH, and plate counts on MRS agar of *Lactobacillus casei* and *Lactobacillus acidophilus* grown for 20 h with or without pH control at 37°C.

Culture	Growth conditions	Cholesterol removed[1]	Deconjugation[2]	Plate counts[3]	pH
E5	pH 6.0	8.0^{c}	4.2^{b}	9.77^{a}	6.0
	Static	56.6^{ab}	4.0^{b}	8.56^{ab}	4.2
N19	pH 6.0	10.4^{c}	3.6^{b}	9.21^{a}	6.0
	Static	36.3^{b}	3.4^{b}	8.62^{a}	4.2
L1	pH 6.0	46.9^{ab}	2.1^{c}	8.84^{a}	6.0
	Static	44.7^{ab}	4.1^{b}	7.99^{b}	4.6
43121	pH 6.0	41.6^{b}	4.8^{a}	9.35^{a}	6.0
	Static	60.6^{a}	4.8^{a}	8.36^{b}	4.3

^{a,b,c}Values represent the means of three replications. Means with no common superscript letters within each column differ ($P < 0.05$). Because of the nesting design of this experiment, standard errors have wider ranges when two different cultures are compared than when single cultures are used.

[1]Cholesterol reported as micrograms of cholesterol removed per milliliter of broth.

[2]Deconjugation of sodium taurocholate; initial concentration of 6 m*M*; reported as millimoles deconjugated.

[3]Reported as $\log_{10}$ colony-forming units per milliliter.

had not deconjugated comparable amounts of sodium taurocholate at the various sampling times. However, the cultures behaved similarly after either pH treatment. We analyzed the results from each sampling time at each level of the other factors in the interaction. The data for 20 h are summarized in Table 3.

Of all cultures, *L. acidophilus* 43121 deconjugated the most sodium taurocholate (4.8 m*M*) after 20 h of growth at pH 6.0 and without pH control. *Lactobacillus acidophilus* L1 grown at pH 6.0 deconjugated the least amount (2.1 m*M*). When grown without pH control, *L. acidophilus* deconjugated 4.1 m*M*, which was more ($P < 0.05$) than the amount deconjugated (2.1 m*M*) by the culture grown at pH 6.0, but was not different ($P > 0.05$) from the amount deconjugated by either strain of *L. casei*.

Lactobacillus casei E5 and N19 deconjugated similar amounts of sodium taurocholate under both growth conditions. Strain E5 deconjugated 4.2 and 4.0 m*M* grown at pH 6.0 and without pH control, respectively; strain N19 deconjugated 3.6 m*M* when grown at pH 6.0 and 3.4 when grown without pH control.

Plate Counts of Lactobacilli

There were no interactions ($P > 0.05$) among cultures, times, or treatments for the plate count data. Generally, cultures grown at pH 6.0 had higher counts than those grown without pH control. There were no differences ($P > 0.05$) between the two growth conditions for *L. casei* E5 and N19 and no differences ($P > 0.05$) in the counts reached by the two stains.

There were differences ($P < 0.05$) between strains of *L. acidophilus* grown with or without pH control. Strain 43121, grown at pH 6.0, reached a population of 9.35 $\log_{10}$ cfu/ml after 20 h of growth; cells grown without pH control were about one log cycle lower at 8.36 $\log_{10}$ cfu/ml. Strain L1 had a similar growth pattern. At pH 6.0, the culture reached a population of 8.84 $\log_{10}$ cfu/ml; without pH control, the population was lower ($P < 0.05$) at 7.99 $\log_{10}$ cfu/ml.

pH of the Broth

The pH of all samples began at 6.5. Cells of all cultures grown with pH control were allowed to produce enough acid to reduce the pH to 6.0, which was then maintained throughout the remainder of the sampling period. Static cultures (no pH control) of *L. casei* E5 and N19 and *L. acidophilus* L1 and 43121 produced enough acid to lower the pH to 4.2, 4.2, 4.3, and 4.6, respectively, after 20 h of growth.

Deconjugation of Sodium Glycocholate and Sodium Taurocholate

When cultures were grown in broth containing both 2.8 m*M* sodium glycocholate and 1.2 m*M* sodium taurocholate, *L. casei* E5 and N19 varied in the amount of each bile salt deconjugated (Table 4). Strain E5 was more active on both bile salts than was strain N19. Generally, both strains preferred sodium glycocholate over sodium taurocholate. Strain E5 had deconjugated most of the 2.8 m*M*/ml of sodium glycocholate by the end of 18 h under either growth

TABLE 4. Amount of sodium glycocholate and sodium taurocholate deconjugated by *Lactobacillus casei* during growth with and without pH control at 37°C in MRS-THIO broth supplemented with 1.2 m*M*/ml of sodium taurocholate and 2.8 m*M*/ml of sodium glycocholate.

Sample time	Sodium taurocholate[1]				Sodium glycocholate[1]			
	L. casei E5		*L. casei* N19		*L. casei* E5		*L. casei* N19	
	pH 6[2]	No pH	pH 6	No pH	pH 6	No pH	pH 6	No pH
16	0.3^{cd}	0.4^{c}	0.1^{e}	0.1^{e}	1.8^{bc}	2.1^{abc}	1.0^{de}	0.5^{e}
18	0.6^{bc}	0.9^{b}	0.1^{e}	0.1^{e}	2.1^{abc}	2.8^{a}	1.3^{cd}	1.2^{de}
20	0.8^{b}	0.9^{b}	0.2^{de}	0.2^{de}	2.8^{a}	2.8^{a}	1.7^{bc}	1.1^{de}
22	0.7^{bc}	0.8^{b}	0.5^{bc}	0.3^{cd}	2.8^{a}	2.8^{a}	2.4^{ab}	1.0^{de}

[a,b,c,d,e]Means followed by the same superscript letter are not different ($P > 0.05$). Because of the nesting design of this experiment, standard errors have wider ranges when two different cultures are compared than when a single culture is used.

[1]All values represent the mean from three trials, reported as millimoles deconjugated per milliliter.

[2]pH 6 = pH maintained at 6.0; no pH = cultures grown without pH control.

condition. By the end of 22 h of incubation, the *L. casei* E5 grown under either condition had deconjugated all of the 2.8 m*M*/ml (100%) of the sodium glycocholate in the broth but only 58 and 67% of the sodium taurocholate when grown at pH 6 or without pH control, respectively. *Lactobacillus casei* N19 deconjugated less ($P < 0.05$) sodium taurocholate than did strain E5 at 16, 18, and 20 h of growth at pH 6.0 and at all sampling times when grown without pH control. Strain N19 also had deconjugated less ($P < 0.05$) sodium glycocholate after 16 and 20 h of growth at pH 6.0 and at all sampling times for cells grown without pH control.

Examination of Cellular Membrane of *L. casei* for Cholesterol

Examination of the cellular membranes that were isolated from the two strains of *L. casei* grown in the broth medium containing cholesterol and sodium taurocholate at pH 6.0 and without pH control revealed no cholesterol. Thus, there is no evidence that cholesterol was incorporated into the cellular membrane of *L. casei* under the conditions of growth utilized in this study.

DISCUSSION

Several reports (2, 4, 9) indicate that some lactobacilli can remove cholesterol from suspension in laboratory media during growth. Some scientists (12) theorize that the cholesterol is removed by lactobacilli because of bile salt deconjugation. They theorize that, when bile salts are deconjugated and the pH of the media drops because of the natural acid production by the culture, the cholesterol micelles destabilize and cholesterol precipitates with the free bile acids (12). Bile acids are less soluble and are more likely to precipitate at pH below 6.0 (6, 8).

However, a study by Noh et al. (18) revealed that cholesterol was removed from laboratory media by *L. acidophilus* ATCC 43121 and L1 when the pH was maintained at 6.0. At this pH, very little precipitation of bile acids should have occurred. Also, some of the cholesterol was found to have been incorporated into the cellular membrane of the organism. In the present study, the isolation of the cellular membrane of two strains of *L. casei* and the examination of the membranes for cholesterol revealed that cholesterol was not incorporated into the cellular membrane of this species in measurable amounts.

In our study, the amount of cholesterol that was removed from the broth was variable, depending on the culture and the pH during growth. *Lactobacillus acidophilus* L1 was the only culture among those evaluated that did not differ in the amount of cholesterol removed when grown with or without pH control. Although significant differences existed in the amounts of cholesterol removed during growth with and without pH control, *L. acidophilus* 43121 was similar to *L. acidophilus* L1 in that it removed large amounts of cholesterol even when the broth was maintained at pH 6.0. Because free sodium cholate stays in solution at pH 6.0 (6, 8), these results further suggest that the cholesterol removed from the broth by *L. acidophilus* was not due just to bile salt deconjugation and coprecipitation of cholesterol with free sodium cholate. Thus, it is likely that much of the cholesterol removed from the broth by this species was not entirely due to the destabilization of cholesterol micelles.

The amount of bile salts deconjugated by *L. acidophilus* L1 was significantly different between

the two growth conditions, but the amount deconjugated by *L. acidophilus* 43121 was not. The difference in the amounts of bile salts deconjugated during growth with or without pH control and the lack of difference in the amount of cholesterol removed from the broth between the two treatments, and vice versa, further suggests that the cholesterol removal was not entirely related to bile salt deconjugation for *L. acidophilus* L1.

Both strains of *L. casei* exhibited differences in the amount of cholesterol that was removed from the broth during growth with or without pH control. Very little cholesterol was removed from the broth maintained at pH 6.0; the amounts removed were comparable with the amounts removed by *L. acidophilus* when the cultures were grown with no pH control. These results suggest that most of the cholesterol that had been removed from the broth by *L. casei* was perhaps from coprecipitation of the cholesterol with the deconjugated bile salts. Very little difference was found in the amount of sodium taurocholate deconjugated for either strain for the two pH treatments. However, the bile salts deconjugated by the cultures grown at pH 6.0 would have remained in solution. Therefore, the cholesterol likely stayed in suspension.

Further experiments indicated that both strains of *L. casei* deconjugated sodium glycocholate better than did sodium taurocholate. Sodium glycocholate predominates in the intestinal tract of adult humans (16, 23). Strains that prefer to deconjugate sodium glycocholate thus may have more potential to lower serum cholesterol concentrations if deconjugation of bile salts is important in controlling serum cholesterol.

Either mechanism, bile salt deconjugation or cholesterol assimilation, has potential importance in exerting control of serum cholesterol concentrations in humans. Cholesterol incorporated into or adhered to the bacterial cells likely would be less available for absorption from the intestine into the blood. Deconjugated bile salts are less well absorbed in the enterohepatic circulation and thus are more likely to be excreted in the feces. To maintain the necessary levels of conjugated bile salts for the enterohepatic circulation, excreted bile salts are replaced by synthesis of new ones in the body from cholesterol, thus providing the potential to reduce the pool of cholesterol in the body. Furthermore, free bile salts do not support the absorption of lipids, including cholesterol, from the intestines as well as do conjugated bile salts (8, 20). Results from this study suggest that strains of *L. acidophilus* should be selected not only on the basis of bile salt deconjugation but also for cholesterol assimilation. When strains of *L. casei* are being selected for use as a dietary adjunct, it is important that they have the highest possible level of bile salt deconjugating activity in order to maximize the potential to reduce serum cholesterol concentrations. Based on our observation, cholesterol assimilation of *L. casei* does not appear to be a major factor in controlling serum cholesterol because little or no cholesterol was removed from the growth medium maintained at pH 6, and it is not likely that the pH of the intestine would be much lower, if any, than this.

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