

Assimilation of Cholesterol by *Lactobacillus acidophilus*†

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Considerable variation was found among strains of *Lactobacillus acidophilus* isolated from the fecal flora of pigs with regard to the ability to grow well in the presence of bile and to assimilate cholesterol from a laboratory growth medium. The uptake of cholesterol occurred only when the culture(s) was growing in the presence of bile under anaerobic conditions. Consumption of *L. acidophilus* RP32, which was selected for its ability to grow well in the presence of bile and to assimilate cholesterol from the laboratory medium, significantly inhibited increases in serum cholesterol levels of pigs ($P < 0.05$) fed a high-cholesterol diet. Consumption of *L. acidophilus* P47, which was selected for its ability to grow in the presence of bile and lack of ability to remove cholesterol from the growth medium, failed to have a similar effect. This indicates that certain strains of *L. acidophilus* act directly on cholesterol in the gastrointestinal tract, and thus may be beneficial in reducing serum cholesterol levels.

According to the latest U.S. census (26), heart disease is the number one cause of death in America. A recent report from the Lipid Research Clinics Program (10) included results from a 7-year study indicating that reduction of total plasma cholesterol can lower the incidence of coronary heart disease in that relatively small portion of the population suffering from primary hypercholesterolemia. Because of the associated risk of heart disease, there has been a thrust toward finding ways to lower plasma cholesterol levels in such people.

Several studies have indicated that consumption of certain cultured dairy products resulted in reduction of serum cholesterol. Mann and Spoerry (12) found that serum cholesterol levels in men from a tribe of African Maasai warriors decreased after consumption of large amounts of milk fermented with a wild *Lactobacillus* strain. Harrison and Peat (8) found that serum cholesterol levels in bottle-fed babies decreased as the numbers of *Lactobacillus acidophilus* in their stools increased. Consumption of yogurt also has been shown to decrease serum cholesterol levels in humans (9, 11) and rabbits (23). Rao et al. (16) reported a significant decrease in plasma cholesterol in rats fed milk fermented by *Streptococcus thermophilus*. Grunewald (7) observed a significant decrease in serum cholesterol in rats fed milk fermented with *L. acidophilus*. Mott et al. (14) reported decreased serum cholesterol in germ-free pigs after monocontamination with *L. acidophilus* and subsequent development of a "normal" flora. Tortuero et al. (25) found that feeding *L. acidophilus* to laying hens resulted in a significant decrease in serum cholesterol.

Conclusions from some studies showing beneficial influences of consuming cultured milk products on serum cholesterol have indicated that the starter culture bacteria produce metabolites during their growth in milk which inhibit cholesterol synthesis in the body (11, 16, 18, 23). None have suggested that the decrease in cholesterol levels was related to a direct action of the bacteria in the cultured milk products on cholesterol in the intestinal tract. Little or no information has been reported on the direct action of lactobacilli on cholesterol or other steroid compounds. Gil-

land and Speck (4) did show that certain intestinal lactobacilli, including *L. acidophilus*, would deconjugate bile acids under anaerobic conditions.

The objectives of this study were to determine whether *L. acidophilus* would assimilate cholesterol and to confirm whether consumption of selected strains would significantly prevent an increase of serum cholesterol in pigs fed a high-cholesterol diet.

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MATERIALS AND METHODS

Source and maintenance of cultures. Cultures of *L. acidophilus* NCFM (of human origin) and P47 (of pig origin) were obtained from the Food Science Department at North Carolina State University. Their identity was confirmed as previously described (4), except that mannose was not included in the sugars tested as fermentation substrates.

Cultures were maintained by subculturing weekly with 1% inocula in 10-ml portions of sterile lactobacilli MRS broth (Difco Laboratories, Detroit, Mich.) and incubating for 18 h at 37°C. Cultures were subcultured twice in a like manner immediately before using. They were stored at 4°C between subcultures.

Measurement of cholesterol uptake. Preliminary tests indicated that *L. acidophilus* would, during anaerobic growth, remove cholesterol from laboratory media when bile was contained in the media. A freshly prepared MRS broth culture of *L. acidophilus* was inoculated (1%) into 10 ml of sterile MRS broth containing 1% pleuropneumonia-like organism (PPLO) serum fraction as the cholesterol source and the desired concentration of oxgall (Difco). The tube(s) was incubated anaerobically for 24 h at 37°C in a GasPak hydrogen-carbon dioxide anaerobic system (BBL Microbiology Systems, Cockeysville, Md.). Cells were removed from the broth by centrifugation for 10 min at $12,000 \times g$ and 1°C. The cell pellet was resuspended in a volume of distilled water equal to that of the original broth culture. The o-phthalaldehyde method for measuring cholesterol described by Rudel and Morris (19) was used to determine the amount of cholesterol in the resuspended cells and spent broth. Uninoculated sterile broth was also analyzed in some experiments. Since some modifications with respect to sample

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volume and reagent volumes were used, the procedure is described here for convenience. The sample (0.5 ml) was placed into a clean test tube (duplicates for each sample). Three milliliters of 95% ethanol was added to each tube, followed by 2 ml of 50% potassium hydroxide. The contents of all tubes were mixed thoroughly after addition of each component. Tubes were heated for 10 min in a 60°C water bath, and after cooling, 5 ml of hexane was dispensed into each tube. After mixing thoroughly with a Vortex-Genie vibrator (model K-550-0) on setting 5 for 20 s, 3 ml of distilled water was added, and the mixing was repeated. Tubes were allowed to stand for 15 min at room temperature to permit phase separation. Then 2.5 ml of the hexane layer was transferred into a clean test tube. The hexane was evaporated from each tube at 60°C under the flow of nitrogen gas. *o*-Phthalaldehyde reagent (4 ml) was added to each tube. The reagent contained 0.5 mg of *o*-phthalaldehyde (Sigma Chemical Co., St. Louis, Mo.) per ml of glacial acetic acid. The tubes were allowed to stand at room temperature for 10 min, and then 2 ml of concentrated sulfuric acid was pipetted slowly down the inside of each tube. The contents of each tube were immediately mixed thoroughly on the Vortex mixer as described previously. After standing at room temperature for an additional 10 min, the A_{550} was read against a reagent blank. The A_{550} was compared with a standard curve to determine the concentration of cholesterol. Results were expressed as micrograms of cholesterol per milliliter. The same procedure was used for the standard curve, except the following amounts of cholesterol (99% standard for chromatography; Sigma) were assayed in place of the samples: 0, 10, 20, 30, 40, and 50 μ g. The A_{550} values were plotted against micrograms of cholesterol.

Influence of bile concentration on cholesterol uptake. *L. acidophilus* NCFM was used to evaluate the influence of bile concentration on the uptake of cholesterol. Lactobacilli MRS broth containing 1% PPLO serum fraction and 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5% oxgall was prepared and dispensed in 10-ml volumes and sterilized by heating at 121°C for 15 min. *L. acidophilus* NCFM was inoculated (1%) into duplicate tubes of each concentration of oxgall. All tubes were incubated anaerobically in a GasPak hydrogen-carbon dioxide anaerobic system at 37°C for 24 h. Cells and spent broth were separated and analyzed for cholesterol as described above.

Isolation of *L. acidophilus* from pigs. Rectal swabs were obtained from 4- to 6-month-old pigs in the Oklahoma State University swine herd and from a commercial producer by using sterile dacron swabs (Fisher Scientific Co., Pittsburgh, Pa.). The fabric portion of the swab was broken off into a sterile plastic Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) and placed in ice water for transport to the laboratory. The swab was aseptically transferred to a tube containing 10 ml of sterile 0.1% peptone water. After the tube was shaken vigorously, appropriate dilutions were prepared by using sterile 0.1% peptone water and plated by using the pour plate method on lactobacillus selection agar. The lactobacillus selection agar was prepared from individual ingredients according to the directions of the manufacturer (BBL). Plates were placed in plastic bags and flushed with carbon dioxide gas for 30 s. The bags were then sealed and incubated at 37°C for 48 h (6). Cultures were isolated from individual colonies and identified by procedures previously described (4).

Comparing cultures for bile tolerance. Strains of *L. acidophilus* were compared for the ability to grow in the presence of bile by inoculating them individually (1%) into 5-ml portions

of sterile MRS broth with and without 0.3% oxgall. They were incubated in a 37°C waterbath. Growth was monitored by measuring the A_{600} at hourly intervals for 8 h. Growth curves were constructed by plotting the increases in absorbance against incubation time. The number of hours required for the absorbance to increase by 0.3 U in each broth was used to compare the cultures.

Screening cultures for cholesterol uptake. The test medium for screening cultures for cholesterol uptake was sterile MRS broth containing 0.3% oxgall and 1% PPLO serum. Strains were inoculated (1%) into 10-ml volumes of the broth and incubated anaerobically in a GasPak system for 24 h at 37°C. Cells and spent broth were analyzed for cholesterol as described above.

Preparation of frozen concentrated cultures. Cultures of selected strains of *L. acidophilus* were grown statically (1% inoculum) in 2.4 liters of sterile MRS broth for 18 h at 37°C. Cells were harvested by centrifugation for 20 min at 4,000 $\times$ *g* and 0°C. Cell pellets were resuspended in two times their weight of cold sterile 10% nonfat milk solids (NFMS), dispensed in 2-g portions into sterile cryogenic vials, frozen, and stored in liquid nitrogen until used.

Plate counts for the concentrated cultures were determined 24 h after freezing. Frozen concentrated cultures were thawed for use by placing the frozen vials in 500 ml of water at 27°C for 5 min. The appropriate dilutions were prepared by using sterile 0.1% peptone water. Dilutions were plated by using MRS agar (MRS broth plus 1.5% agar) via the pour plate method. Plates were incubated for 48 h at 37°C. Colonies were counted with the aid of a Quebec colony counter.

Feeding trial. Eighteen five-week-old Yorkshire gilts from seven litters were randomly assigned to individual metal pens and subsequently to three treatment groups to provide six pigs per treatment. The location of each pig was random with respect to the treatments.

All pigs were fed a corn base diet without added crystalline cholesterol (Table 1) twice daily (0.28 kg each feeding) for a 1-week adjustment period. The experimental period began at the second week. All pigs were then fed the corn diet supplemented with crystalline cholesterol (Table 1) to supply ca. 1,500 mg of cholesterol per day for each pig at the start of the trial. The amount of cholesterol thus increased as the amount of feed was increased during the trial. Pigs were

TABLE 1. Composition of corn base diet used in the pig feeding trial

Component	Amt ^a (kg)
Ground shelled corn	153.5
Butter	27.2
Dried sweet whey	72.6
Soybean meal	98.0
Salt	0.9
Dicalcium phosphate	5.4
Calcium carbonate	3.3
Vitamin and trace mineral premix ^b	1.5

^a After mixing with a Marion mixer (Rapids Machinery Co., Marion, Iowa), 78.7 kg was removed for adjustment period feeding, and 450 g of cholesterol (purity at least equivalent to USP; Sigma) was added and mixed into the remaining 283.7 kg. Including that from the butter, the diet contained 1,775 mg of cholesterol per kg.

^b The vitamin trace mineral premix supplied 1,760 mg of riboflavin; 8,800 mg of pantothenic acid; 8,800 mg of niacin; 8.8 mg of vitamin B₁₂; 176,000 mg of choline chloride; 1,760,000 IU of vitamin A; 176,000 IU of vitamin D₃; 4,400 IU of vitamin E; 44 mg of menadione dimethyl-primidonol bisulfite; 39.6 mg of selenium; 299.2 mg of iodine; 19.8 g of iron; 11 g of manganese; 2.2 g of copper; and 39.6 g of zinc per kg of premix.

fed twice daily. On days 1 through 7 of the feeding trial, all pigs were fed 0.41 kg of feed at each feeding. For days 8 through 10 the amount of feed for each was increased to 0.69 kg per feeding. If any feed was left after a 2-h period, it was removed from the pens. Water was available at all times.

In addition to the corn base diet, group 1 (control group) was given 50 ml of sterile 10% NFMS, group 2 was given 50 ml of 10% NFMS containing 5×10^{10} cells of *L. acidophilus* P47, and group 3 was given 50 ml of 10% NFMS containing 5×10^{10} cells of *L. acidophilus* RP32 once daily (at the evening feeding). The desired numbers of *L. acidophilus* cells were provided by adding the appropriate amount of frozen concentrated cultures after thawing. The milk with and without lactobacilli was fed to the pigs in individual bowls just before feeding the corn base diet each evening.

Blood samples were taken from each pig after a 12-h fast by anterior vena cava puncture by using sterile Vacutainers (Becton, Dickinson & Co., Rutherford, N.J.) with sterile 20-gauge needles during the experimental period on days 0, 5, and 10 to be analyzed for total serum cholesterol. The tubes containing the blood samples were placed in an ice water bath immediately after collection and were held at 0°C overnight. The samples were centrifuged for 10 min at $3,000 \times g$ and 1 to 2°C. Each sample of serum was pipetted into a clean test tube and mixed thoroughly. Portions were dispensed into two cryogenic vials and frozen at -20°C. All serum samples were kept frozen for the duration of the feeding trial before analysis for total serum cholesterol.

Serum samples were thawed for analysis by placing the vials in 500 ml of tap water at 27°C for 5 min. Samples were analyzed for total serum cholesterol by using the Sigma enzymatic method for determination of total cholesterol in serum or plasma. The modified procedure for spectrophotometers requiring greater than 1 ml of reaction volume was followed as per the instructions of the manufacturer.

Statistical analysis. Serum cholesterol data were analyzed by using the general linear models procedure from the Statistical Analysis System package (1). The least-significant-difference mean separation technique (22) was used to determine the statistical significance among means of serum cholesterol levels for the three treatment groups on days 0, 5, and 10.

RESULTS

Culture identification. The identity of the cultures of lactobacilli used in this study were confirmed as *L. acidophilus* as described in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (2). All strains were gram-positive, catalase-negative, nonsporeforming rods and grew at 45°C but not at 15°C. None produced ammonia from arginine, whereas all hydrolyzed esculin. All strains fermented cellobiose, galactose, glucose, lactose, maltose, and sucrose. None fermented mannitol, melezitose, rhamnose, sorbitol, and xylose. Fermentation of melibiose and raffinose was variable, as expected. The identity characteristics of all strains fit those of *L. acidophilus* more closely than any other species (2). Reactions differing from those reported for *L. acidophilus* included the following. Strains C1-5, C2-5, GP2B, and RP43 did not ferment amygdalin. Strain C1-5 did not ferment salicin. Strain GP2B fermented arabinose. All strains except NCFM and GP4A did not ferment trehalose.

Influence of oxgall on cholesterol uptake. No cholesterol was removed from the broth medium containing PPLO serum and oxgall during aerobic growth of *L. acidophilus*. However, when grown anaerobically, the amount in the broth decreased, whereas the amount in the cells increased.

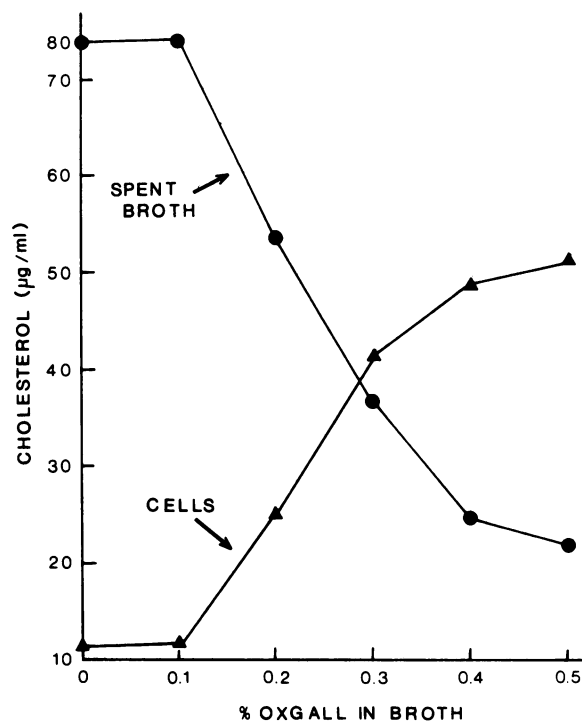


FIG. 1. Influence of oxgall on the uptake of cholesterol by *L. acidophilus* NCFM during anaerobic growth in MRS broth. (Each value represents an average from four trials.)

The amount of bile in the growth medium influenced the assimilation of cholesterol by *L. acidophilus* (Fig. 1). The amounts of cholesterol in the spent broth and in the resuspended cells after a 24-h growth period are plotted against the oxgall concentration (each value is the average from four trials). As the amount of oxgall was increased in the growth medium, the amount of cholesterol detected in the spent broth decreased. This was associated with increases in the amounts of cholesterol in the resuspended cells. Cholesterol was not removed from the broth during the growth of the lactobacilli, nor did appreciable amounts appear in the cells until more than 0.1% oxgall was present in the growth medium. The amount of uptake appeared to level off at oxgall concentrations greater than 0.4%.

Comparisons of cultures for bile tolerance. Results from the comparison of cultures for bile tolerance are summarized in Table 2. The strains exhibited considerable variation with regard to growth in the control broth. Strains RP32, C1-5, GP1B, and GP4A grew more rapidly than did the other strains. Strains also varied considerably with regard to growth in the broth containing 0.3% oxgall (MRSO). Strains C1-5, GP1B, GP2B, GP4A, RP32, and RP43 appeared to be most resistant to bile, since the added times required for the A_{600} to increase to 0.3 in the MRSO broth compared with the MRS broth were comparatively less than those for the other strains. The slower growth in MRSO than in MRS broth was not entirely due just to the increased lag times. For the most part, those strains that grew more slowly in the control broth were less bile tolerant.

Screening cultures for cholesterol uptake. Results from the screening of cultures for cholesterol uptake are shown in Table 3. Cholesterol concentrations in the cell suspensions ranged from 8.2 µg/ml for strain P47 to 29.8 µg/ml for strain RP43. Based on the concentrations of cholesterol in the

TABLE 2. Comparison of bile tolerance of cultures of *L. acidophilus* isolated from fecal samples from pigs

Culture	No. of h taken for A_{600} to increase by 0.3 U ^a	
	Control	0.3% Oxgall
C1-5	3.90	4.00
C2-5	6.35	>8.00 ^b
GP1B	3.90	4.20
GP2B	4.55	4.70
GP4A	3.95	3.95
P47	4.90	6.25
RP32	2.70	2.90
RP43	4.20	4.20
S-1	5.45	>8.00 ^c

^a Each value represents an average from two or three trials.^b A_{600} at 8 h was 0.214.^c A_{600} at 8 h was 0.282.

control and spent broths, minimal amounts of cholesterol were removed from the broth by cultures of strains C1-5, C2-5, GP2B, and P47. All other strains caused reductions in the amount of cholesterol in the broth portion during the 24-h growth period. It is somewhat difficult to compare the first three strains with the last six in the table since two lots of PPLO serum with differing concentrations of cholesterol were used. However, some strains were much more active than others with regard to the uptake of cholesterol.

Feeding trial. All pigs appeared to remain healthy, and there were no significant differences in weight gains among the treatment groups during the feeding trial. Consumption of the diet fed was not a problem, and intake did not differ among treatment groups. The pigs were fed a diet high in cholesterol to cause an increase in serum cholesterol. This ability to increase serum cholesterol by increasing dietary cholesterol in pigs has been demonstrated in other studies (3, 21). All groups exhibited increases in serum cholesterol during the feeding trial (Table 4). On day 0, the mean serum cholesterol concentration for all treatment groups was between 52 and 56 $\mu\text{g/dl}$; there were no significant differences among groups ($P > 0.05$). There were no significant interactions among treatments and days ($P > 0.05$). By day 5, the mean serum cholesterol values for the control group and the P47 group had increased significantly ($P < 0.05$) to 69.10 and 72.01 $\mu\text{g/dl}$, respectively. The group receiving *L. acidophilus*

ilus RP32 did not exhibit a significant ($P > 0.05$) increase from day 0 to day 5. The mean concentration for the RP32 group on day 5 was significantly lower ($P < 0.05$) than that for the P47 group. The mean value for the RP32 group on day 10 was also significantly lower ($P < 0.05$) than that for both the control and P47 groups. None of the groups exhibited significant increases from day 5 to day 10 ($P > 0.05$).

DISCUSSION

Information recently published by the Lipid Research Clinics (10) indicates that the higher the total serum cholesterol level is in humans, the greater the risk for development of coronary heart disease. Some studies have reported that ingestion of *L. acidophilus* has resulted in decreased serum cholesterol levels in humans (8) and animals (7, 15, 25). None of these studies has suggested that the *L. acidophilus* was acting directly on the cholesterol.

Certain strains of *L. acidophilus* have the ability to assimilate cholesterol. This was shown by the appearance of cholesterol in the cells during growth which was associated with decreases in the concentrations of cholesterol in the growth medium. This uptake of cholesterol occurred only when the culture was growing anaerobically in the presence of bile. The amount of bile required to enable the cultures to remove cholesterol from the growth medium was not in excess of the levels normally encountered in the intestines (20). Thus, the conditions required in the in vitro system for cholesterol uptake by *L. acidophilus* would also be expected to occur in the intestinal tract. This should enable the organism to assimilate at least part of the cholesterol ingested in the diet, thus making it unavailable for absorption into the blood. A similar action could be exerted on endogenous cholesterol in the intestines.

Cholesterol was apparently not being merely precipitated from the growth medium during incubation and thus being removed along with the cells by centrifugation, since it was not detected in the cells grown in broth without oxgall. Furthermore, not all strains of *L. acidophilus* were able to effect removal of cholesterol from the growth medium.

Gilliland and Speck (5) reported that certain strains of *L. acidophilus* have the ability to deconjugate bile salts. This only occurred in an anaerobic environment. No attempt was made to determine whether or not the steroid portion of the deconjugated bile salts was incorporated into the cells of the lactobacilli. Their findings and the uptake of cholesterol shown in the present study suggest that *L. acidophilus*, when grown under appropriate conditions, may be able to incorporate steroids into the cellular structure. Additional research is needed to further evaluate this and to determine

TABLE 3. Assimilation of cholesterol by *L. acidophilus* during anaerobic growth in MRS broth containing PPLO serum and oxgall

Culture	Cholesterol ($\mu\text{g/ml}$) ^a		
	Control broth	Spent broth	Cells
GP1B	41.9 ^b	24.2 (3.1)	23.2 (5.4)
GP4A	41.9	22.2 (6.0)	24.4 (8.4)
RP43	41.9	18.8 (2.1)	29.8 (4.4)
RP32	67.2	40.6 (0.3)	28.6 (2.1)
C1-5	67.2	62.6 (0.7)	19.0 (7.0)
C2-5	67.2	63.0 (3.2)	12.0 (0.3)
GP2B	67.2	60.0 (1.6)	10.0 (1.1)
P47	67.2	61.4 (1.6)	8.2 (2.4)
S-1	67.2	52.6 (6.1)	22.6 (4.2)

^a Each value represents the average from two to three trials; values in parentheses represent the standard deviation.^b Two batches of PPLO were used during the experiments: batch 1 had 4.19 mg of cholesterol per ml, and batch 2 had 6.72 mg of cholesterol per ml.TABLE 4. Influence of feeding *L. acidophilus* cells on serum cholesterol levels in pigs on a high-cholesterol diet

Group	Cholesterol (mg/dl) ^a		
	Day 0	Day 5	Day 10
Control	52.23 (1.88) ^{C1}	69.10 (3.91) ^{CD2}	74.44 (4.64) ^{C2}
<i>L. acidophilus</i> P47	55.58 (4.70) ^{C1}	72.01 (3.02) ^{C2}	73.48 (4.68) ^{C2}
<i>L. acidophilus</i> RP32	52.84 (3.00) ^{C1}	61.48 (3.30) ^{D1}	62.29 (4.91) ^{D1}

^a Each value represents the mean from six pigs; numbers in parentheses represent the standard deviation. Values in the same column followed by different superscript letters are significantly different ($P < .05$); values in the same row followed by different superscript numbers are significantly different ($P < .05$). There were no significant interactions among days and treatments ($P > .05$).

what, if any, effect it might have on the characteristics of cultures.

It is reasonable to assume that a strain must be able to grow well in the presence of bile to remove cholesterol from its environment, since bile must be present for this activity to occur. However, a highly bile-tolerant strain may not necessarily have the ability to assimilate cholesterol, since there were some notable exceptions. Strains C1-5 and GP2B were among the strains that were bile tolerant, and they were also among the strains that assimilated little or no cholesterol from the growth medium.

Both strains of *L. acidophilus* selected for use in the pig feeding trial exhibited resistance to bile; however, strain RP32 grew faster than did P47 in the presence of bile. Strain RP32 exhibited maximal ability and strain P47 exhibited minimal ability to assimilate cholesterol. These two strains were selected for the feeding trial to determine whether the difference in the ability to assimilate cholesterol would influence any effect that consuming cells of *L. acidophilus* might have on serum cholesterol levels in the pigs.

Pigs were selected as an animal model since their digestive system, distribution of coronary arteries, and atherosclerotic tendencies resemble those of humans (17). Since *L. acidophilus* exhibits host specificity in the intestinal tract (4, 13), it was deemed necessary to use strains of *L. acidophilus* of pig origin. There were significant differences between the two strains with regard to their influence on serum cholesterol levels. This further supports the idea that the ability of *L. acidophilus* to cause reductions in serum cholesterol is a result of the direct action of the culture on cholesterol.

The variance among strains in the ability to assimilate cholesterol coupled with results from the feeding trial points out the need to select strains of *L. acidophilus* for use as dietary adjuncts that possess the desired activity. For example, some studies have reported that ingestion of *L. acidophilus* did not affect serum cholesterol levels (16, 24). Perhaps this could be due to differences among strains with regard to cholesterol uptake activity.

Mott et al. (14) observed that there was a decrease in serum cholesterol of pigs fed *L. acidophilus* cells with an associated increase in the amount of cholesterol detected in fecal material. Their conclusion was that factors other than microbial metabolism of steroids in the gastrointestinal tract were responsible for the observed reduction in serum cholesterol. However, the lactobacilli could have been absorbing the cholesterol in the gastrointestinal tract, thus keeping it from being absorbed by the body.

Further research is needed to determine the mechanism of cholesterol uptake and to determine whether or not ingestion of cells of a selected strain of *L. acidophilus* could decrease serum cholesterol levels in adult humans with primary hypercholesterolemia.

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