ASSIMILATION OF CHOLESTEROL BY STRAINS OF *LACTOBACILLUS ACIDOPHILUS* OF HUMAN INTESTINAL ORIGIN

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Bachelor of Science

Oregon State University

Corvallis, Oregon

1991

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1993

OKLAHOMA STATE UNIVERSITY

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PREFACE

New isolates of *Lactobacillus acidophilus* were obtained from humans which were more able to assimilate cholesterol and deconjugate bile salts than the previously isolated pig strain *L. acidophilus* ATCC 43121.

I would like to sincerely thank Dr. S.E. Gilliland for his guidance and assistance as my major advisor while I attended Oklahoma State University. I also thank Dr. P.L. Claypool for his assistance while serving on my graduate committee.

Special words of appreciation go to Dr. Thomas Rehberger, the final member of my committee, for his numerous words of encouragement, advice, and friendship.

I would also like to thank each of my sixteen volunteers for their contributions and every undergraduate assistant who aided me in the completion of my research. Thank you also to the Oklahoma Center for the Advancement of Science and Technology for the funding of this research.

Finally, I would like to thank Elizabeth Yoon and my mother, Marsha Buck, who offered the love, patience, and encouragement that made this degree possible.

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CHAPTER I

INTRODUCTION

Coronary heart disease (CHD) accounts for more deaths annually than any other disease. Increased levels of LDL cholesterol and total plasma cholesterol have been shown to correlate highly with the incidence of CHD. Individuals with hypercholesterolemia (elevated blood cholesterol levels) should therefore reduce serum cholesterol levels in order to decrease the risk of CHD. Serum cholesterol levels may be reduced through diet therapy or through the administration of drugs such as cholestyramine.

Lactobacillus acidophilus has been found to assimilate cholesterol under conditions found in the intestinal tract. When *L. acidophilus* is consumed in dairy products as a dietary adjunct, the assimilation of cholesterol by the microorganism may lead to increased cholesterol excretion from the body. Increased cholesterol excretion would potentially cause decreased serum cholesterol levels. The ability of *L. acidophilus* to assimilate cholesterol has been shown to vary by strain. Therefore, the strain used as a dietary adjunct to decrease serum cholesterol levels must be selected for its ability to assimilate cholesterol. The selected strain also must be bile tolerant in order to survive and grow in the presence of intestinal bile. Because *L. acidophilus* has been shown to exhibit host specificity, the strain chosen to reduce human serum cholesterol levels must also have originated from human intestinal sources.

Bile salt deconjugation may be yet another factor which may assist *L. acidophilus* in decreasing serum cholesterol levels. The enzyme bile salt hydrolase, which is produced by some bacteria including *L. acidophilus*, deconjugates taurine and/or glycine from primary bile acids such as taurocholic or glycocholic acid. The free acids produced by deconjugation are excreted more rapidly than conjugated bile acids. As the deconjugated bile salts are excreted from the body, their decreased concentration in the bile leads to the synthesis of new bile acids from cholesterol. This may decrease the total cholesterol concentration in the body.

The purpose of this study was to obtain new human intestinal isolates of *L. acidophilus* that were better able to assimilate cholesterol than currently available commercially used strains of *L. acidophilus* of human intestinal origin.

CHAPTER II

REVIEW OF LITERATURE

Role of Cholesterol in the Body

Cholesterol, a steroid normally found in the body, is intricately involved as a precursor of important compounds such as steroid hormones, bile salts, and Vitamin D (35, 50). Cholesterol also acts as a structural component of cellular membranes (50). It is acquired by the body in one of two ways: endogenous synthesis from acetate or absorption of dietary cholesterol (14). Ninety-seven percent of all cholesterol synthesis takes place in two organs, the liver and the gastrointestinal tract (14). In the liver, an increase in dietary cholesterol regulates endogenous cholesterol production because dietary cholesterol induces a feedback mechanism involving 3-hydroxy-3-methylglutaryl CoA reductase, the enzyme which controls the rate-limiting step in cholesterol synthesis (14, 50, 68). Cholesterol excretion from the body primarily occurs by excretion of cholesterol itself or of some bile acids synthesized from it.

Cholesterol and other lipids are transported in the body by lipoproteins. These lipoproteins have been placed into four categories according to increasing density: very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (68). The primary lipoproteins involved are LDL and HDL. LDL

molecules are the major carriers of cholesterol in the blood for uptake while HDL molecules are involved in cholesterol excretion (68). Thus in reducing serum cholesterol levels a high level of HDL cholesterol may be considered beneficial while a high level of LDL cholesterol is considered undesirable.

Relationship Between Cholesterol and Coronary Heart Disease

Atherosclerosis results from a plaque-like thickening of the wall of a major artery due to the accumulation of esterified cholesterol (27). This thickening is caused by lipid deposits in the interstitial spaces surrounding the smooth muscle cells and within the smooth muscle cells themselves (27). Atherosclerosis causes coronary heart disease, which accounts for more deaths annually than any other disease, including all forms of cancer combined (43).

Increased levels of LDL cholesterol and total plasma cholesterol have been shown to correlate highly with the incidence of coronary heart disease (37, 43, 44, 63). Levels of HDL cholesterol, on the other hand, are inversely related to the incidence of coronary heart disease (44). A 1% increase in HDL is said to correlate with a 5% decrease in the risk of coronary heart disease (42). Therefore, the most beneficial reduction of serum cholesterol levels must involve the reduction of levels of LDL cholesterol. The body has complex homeostatic regulatory mechanisms to control the levels of cholesterol in the body including alterations in the efficiency of intestinal absorption and rates of cholesterol biosynthesis, LDL-receptor activity, secretion of cholesterol into bile, and hepatic conversion of cholesterol into bile acids (37). In order to affect these internal regulatory mechanisms, the most common therapeutic methods for decreasing the risk of coronary heart disease involve lowering blood

cholesterol levels through diet therapy or through the administration of drugs such as cholestyramine (43, 44, 50). Dietary changes appear to be the most favorable method for control of cholesterol levels due to the fact that available hypocholesterolemic drugs tend to produce undesirable biochemical or systemic side effects (43).

Role of Intestinal Flora in Controlling Serum Cholesterol Levels

Germ-free animals raised in sterile atmospheres serve as models to study the potential importance of normal microbial flora. Using such models, experiments have been performed which demonstrate the effect microorganisms have in controlling serum cholesterol levels.

Experiments by Eyssen (16) showed that germ-free chicks, mice, and rats accumulated twice as much cholesterol in the blood and liver as did conventional animals housed in the same conditions. He also stated that germ-free animals eliminated less cholesterol through their feces than did conventional animals. He interpreted this to mean that intestinal organisms must interfere with the efficiency of cholesterol absorption. Germ-free animals also eliminated 30 to 40 % less bile acids than did conventional animals due to the fact that conventional animals can deconjugate the conjugated bile acids to free bile acids which are excreted more rapidly. In 1987 Chikai et al. (6) analyzed bile acids in feces from germ-free rats before and after inoculation of the animals with organisms including *Bacteroides vulgatus*, *Bifidobacterium longum*, *Escherichia coli*, or *Clostridium ramosum*. These organisms were chosen for this study because they were predominant strains in the human intestines from which they were isolated. Fecal excretion of bile acids increased dramatically after inoculation with all organisms except for *E. coli*,

which is unable to deconjugate bile acids. Deconjugation of bile acids in the intestines also could result in lower levels of serum cholesterol in conventional animals than in germ-free animals since the excretion of bile acids increases the rate of catabolism of cholesterol to new bile acids. This conversion of cholesterol to bile acids accounts for approximately 70% of the cholesterol disposed of daily (37).

Mott (53) indicated that microbial conversion of cholesterol to coprostanol and of primary bile acids to secondary bile acids was responsible for a concomitant decrease in serum cholesterol levels. In this experiment germ-free piglets were monocontaminated with *Lactobacillus acidophilus*. Following the monocontamination, some of the piglets were allowed to develop a normal flora. The latter group exhibited a dramatic decrease in serum cholesterol compared to the piglets containing only *L. acidophilus* and a four-fold increase in the excretion of neutral steroids. This experiment also showed that *L. acidophilus* was not the microorganism solely responsible for lowering serum cholesterol levels. Norin et al. (55) reiterated this conclusion based on an experiment involving the inoculation of germfree mice with *L. acidophilus* and *Bifidobacterium bifidum*. These organisms were chosen because they are normally present in the intestinal tract in high numbers. However, the strains of *L. acidophilus* in neither study were selected on the basis of ability to take up cholesterol or deconjugate bile acids. Norin et al. (55) concluded that the benefit of normal flora may be due to interaction of these two organisms with other microorganisms and not only to their direct influence on metabolism of the host.

Effect of Cultured or Culture Containing Dairy Products on Serum Cholesterol Levels

In 1974 Mann and Spoerry (47) reported a factor in a fermented milk product which was hypocholesterolemic. In this experiment they fed Maasai men at least 4L a day of milk fermented with wild strains of *Lactobacillus*. After 21 days of feeding the men showed an average decrease in levels of serum cholesterol of 14.8 mg/100ml. The amount of reduction was greatest in the men who drank the most fermented milk and gained the most weight. This experiment led to much research to identify the hypocholesterolemic milk factor, determine which milk products contain it, and to determine whether microorganisms play any part in the production or enhancement of this factor.

Since the milk product in Mann and Spoerry's study was similar to cultured yogurt, research was done to compare yogurt to other milk products for ability to decrease serum cholesterol levels. Mann (46) fed American subjects 4L a day of fresh, homogenized, Vitamin D enriched cow's milk fermented with commercially used yogurt cultures. After 12 days of feeding, serum cholesterol levels decreased 37%. This effect was decreased when less yogurt was consumed. He also found that 2L a day of skim-milk yogurt also caused a drastic decrease in serum cholesterol levels. In all cases, serum cholesterol gradually returned to initial levels after the feeding trials were completed. Hepner et al. (31) fed healthy volunteers less than 1L nonpasteurized yogurt, pasteurized yogurt, or lowfat (2%) milk. Serum cholesterol was significantly reduced by 5 to 10% after one week in the two groups fed yogurt, while the group fed the lowfat milk showed a smaller decrease. Thakur and Jha (71) fed rabbits yogurt, calcium, and milk. Each of these supplements reduced serum

cholesterol levels, with yogurt and calcium having similar effects. Yogurt caused a greater hypocholesterolemic effect than did milk.

Thompson et al. (72) fed volunteers having normal or low blood lipid levels 1L of 2% milk, whole milk, skim milk, yogurt, buttermilk, or sweet acidophilus milk daily for 3 weeks. Although they did not find great evidence of a hypocholesterolemic milk factor when the volunteers were fed the levels in this experiment, the greatest hypocholesterolemic effect was found in the volunteers fed skim milk. These same results were obtained by Rossouw et al. (62) who fed adolescent schoolboys 2L of skim milk, yoghurt, or full cream milk daily for 3 weeks. However, since the subjects in both experiments all had normal or low blood lipid levels initially, perhaps little or no decreases in serum cholesterol should have been expected.

There have been many hypotheses offered and tested to determine precisely what factor(s) in milk may cause a hypocholesterolemic effect. The suggested milk factors include: calcium (32, 71), casein (31), hydroxymethyl glutarate (31, 46, 54, 61), orotic acid (1, 3, 4, 61), uracil (1), whey (56), or a combination of these factors (3, 4, 61, 71). Rao and Reddy (59) reported that this effect is not the result of any changes in lipids in milk due to microbial fermentation. Hepner et al. (31) indicated direct alteration of the intestinal flora by fermented products was not responsible. The three most studied positive hypotheses are calcium, hydroxymethyl glutarate (HMG), and orotic acid. Each of these has been at least partially disproven (1, 32, 33, 36, 71) which suggests that the hypocholesterolemic effect may be due to a combination of factors.

Certain microorganisms have also been shown to have hypocholesterolemic effects when consumed in dairy products. Rao et al. (58) used rats to study the effect of milk and milk fermented with *Streptococcus thermophilus* on plasma cholesterol levels. Consumption of the fermented milk

by the rats resulted in a significant decrease in plasma cholesterol levels. The liver cholesterol levels were also lower in the rats receiving the fermented milk than in the rats fed skim milk. They concluded that fermentation may be at least partially responsible for the hypocholesterolemic effect of milk.

The consumption of *L. acidophilus* may also result in decreased serum cholesterol levels. Tortuero et al. (73) fed *L. acidophilus* to normal and cecectomized hens. Serum cholesterol levels of the cecectomized hens were higher than those of normal birds. The feeding of *L. acidophilus* to the hens resulted in a significant decrease in serum cholesterol but not in egg yolk cholesterol. Rats fed milk fermented with *L. acidophilus* for four weeks had significantly lower serum cholesterol levels than rats fed water or skim milk alone (28). Gilliland et al. (22) fed pigs on a high cholesterol diet milk containing cells of one of two strains of *L. acidophilus*. The pigs fed one of the strains had significantly decreased serum cholesterol levels compared to those receiving the other strain or control pigs. Danielson et al. (11) showed that mature boars fed a high cholesterol diet and yogurt fermented with selected strains of *L. acidophilus* for 56 days had reduced total serum cholesterol and LDL cholesterol levels. Harrison and Peat (30) fed newborn infants humanized milk or humanized milk inoculated with *L. acidophilus*. The infants given the milk supplemented with *L. acidophilus* had significantly decreased serum cholesterol levels compared to the babies fed the original milk formula. These experiments all support the conclusion that selected strains of *L. acidophilus* may have the ability to decrease serum cholesterol levels due to their ability to assimilate cholesterol. Selected strains of other organisms also have been shown to have the ability to assimilate cholesterol (51, 60).

Use of Lactobacilli as Dietary Adjuncts

Occurrence in the Intestinal Tract

Lactobacilli are part of the normal intestinal flora of many animal species. They also are present in other areas of the anatomy such as the human vagina (34). Lactobacilli have been isolated from intestinal contents of pigs (24, 49), rodents (52), cattle (21, 24), poultry (12, 18, 24), humans (24), and many other animal species. They are the predominant organism in the intestinal tract of the rat (52) and the small intestine of the chicken (12). In contrast, amphibians and reptiles contain no lactobacilli (18).

Potential Benefits

Lactobacilli potentially can provide several beneficial effects in addition to hypocholesterolemic actions. These include production of the enzyme ß-galactosidase to aid lactose malabsorbers (15, 20), activation of the immune system (15), tumor and cancer inhibition (15, 20, 65), bacteriocin production (20, 38, 65), and control of the population of harmful bacteria in the intestinal tract (8, 15, 20, 21, 30, 34, 38, 52, 64, 65).

Host Specificity

In order to select the strain of *Lactobacillus* with the greatest potential for use as a dietary adjunct, researchers should select a strain which originated from the same host animal or a highly related host animal (8, 15, 34, 38, 57, 66, 75).

Fuller (18) tested the ability of numerous strains of lactobacilli isolated from various animals to adhere to the crop epithelium of the chicken. Only lactobacilli isolated from fowl were able to adhere, and not all fowl isolates had this ability. Strains of the same organisms from laboratory culture collections also failed to adhere. Suegara (69) tested the ability of rat and chicken strains of lactobacilli to adhere to stomach or crop epithelial cells from each animal. Only rat isolates were able to adhere to rat stomach epithelial cells and only chicken isolates were able to adhere to chicken crop epithelial cells. Once again, strains showed different degrees of adherence to host cells.

Barrow et al. (2) obtained strains of normally occurring microorganisms from several animal and dairy food sources and tested their ability to adhere to pig squamous epithelial cells in vitro. Only isolates from domestic pigs and the closely related wild boar were able to adhere, with the exception of two chicken strains. They found that of their isolates, *L. fermentum* attached in the largest numbers. Gilliland et al. (21) fed isolates of *L. acidophilus* from humans and calves to young calves. The strain of calf origin was more effective as a dietary adjunct in the calves than was the strain of human origin. This conclusion was based on the ability of the strain to establish in the calf small intestinal tract and to control the number of fecal coliforms present. Tannock et al. (70) tested the ability of strains of lactobacilli to colonize the squamous epithelia of the gastrointestinal tract in gnotobiotic animals. In vivo studies showed that only lactobacilli from rodents could colonize rodent epithelium and only the fowl strain tested could colonize crop epithelium. Lin and Savage (41) tested the ability of lactobacilli from several animal species to colonize the lumens and form layers on the keratinized nonsecreting epithelium in the stomachs of monoassociated ex-germfree mice. With the exception of one calf isolate, only

strains previously isolated from rodents were able to form thick continuous layers on the gastric epithelial surface.

There have been several hypotheses offered to explain the factors that mediate host specificity. These hypotheses include specificity due to plasmids (49), surface protein adhesive molecules (10, 66, 69) and acidic carbohydrate polysaccharides (19, 38).

Characteristics of *Lactobacillus acidophilus* for Use as Dietary Adjuncts to Produce Hypocholesterolemic Effects

Cholesterol Assimilation

Cholesterol assimilation is the ability of certain microorganisms to take up cholesterol from laboratory media containing bile and a cholesterol source. To test the effectiveness of cholesterol assimilation in vivo, Gilliland et al. (22) obtained swine isolates of *L. acidophilus* which were bile tolerant and had the ability to assimilate cholesterol. The ability to assimilate cholesterol varied greatly among strains of *L. acidophilus*. The isolate that was best able to assimilate cholesterol and that was also bile tolerant was labelled RP32 and later given the ATCC designation 43121. This isolate and an isolate unable to assimilate cholesterol were fed in nonfermented acidophilus milk to pigs on a high cholesterol diet. The pigs fed the strain able to assimilate cholesterol had significantly decreased serum cholesterol levels compared to control pigs and pigs receiving the strain of *L. acidophilus* that did not assimilate cholesterol. It has been postulated that human strains selected for this ability may also decrease human serum cholesterol levels when used as dietary adjuncts.

Since the ability of *L. acidophilus* to assimilate cholesterol varies among strains (22, 26), those chosen for use as dietary adjuncts to reduce serum cholesterol levels must be selected carefully (29, 42). Lin et al. (42) tested the ability of the commercially available preparation Lactinex™ to decrease serum cholesterol levels in humans. Lactinex™ is a pharmaceutical preparation containing *L. acidophilus* and *Lactobacillus bulgaricus*. This preparation did not affect serum cholesterol levels, perhaps due to the fact that the strain of *L. acidophilus* used in the product was not chosen for ability to assimilate cholesterol. The *Lactobacillus* strains were also shown to be bile sensitive. Similar results were seen when Grunewald (29) fed mice fermented and nonfermented acidophilus milk and saw no decrease in serum cholesterol levels. Again, the culture of *L. acidophilus* was not tested for the ability to assimilate cholesterol before it was used as a dietary adjunct.

Klaver and van der Meer (39) recently postulated that the "apparent" assimilation of cholesterol by *L. acidophilus* resulted solely from bile salt-deconjugating activity. They stated that in a laboratory medium, cholesterol was not assimilated by the lactobacilli but was instead precipitated with bile salts that were deconjugated by the culture. However, recent research by Walker and Gilliland (74) found that there is no significant statistical correlation between the ability of cultures of *L. acidophilus* to assimilate cholesterol and to deconjugate bile salts. Some cultures of *L. acidophilus* exist that are able to deconjugate bile salts but that assimilate little if any cholesterol. Cholesterol assimilation, bile tolerance, and bile salt deconjugation, however, should be maximized when selecting a culture for use as a dietary adjunct.

Bile Tolerance

Bile tolerance is based on the ability of an organism to survive and grow in the presence of bile. In order to survive and grow in the intestine, organisms used as dietary adjuncts must be able to grow in the presence of bile since bile is normally present in the environment of the intestine. Numerous experiments have shown that the amount of bile tolerance varies among strains of *L. acidophilus* (25, 26, 49, 57, 74). The researchers suggest that bile tolerant strains should be more able to adapt to conditions in the intestine and therefore can be more beneficial to the host.

The importance of bile tolerance has been shown by Gilliland et al. (25) who fed newborn dairy calves two strains of *L. acidophilus*, one strain that was highly bile tolerant and another which was only slightly bile tolerant. The calves fed the bile tolerant strain showed a greater increase in number of facultative lactobacilli in the upper small intestine than calves fed the strain exhibiting lower resistance to bile. This showed that bile tolerance may increase intestinal colonization. Overdahl and Zottola (57) examined the possible relationship between bile tolerance and the presence of a Ruthenium Red staining layer which indicates the outer polysaccharide. They found no apparent relationship between the staining layer and bile tolerance.

Gilliland et al. (22) tested the influence of bile concentration on cholesterol uptake in *L. acidophilus*. They showed that bile was necessary for cholesterol assimilation and that increased levels of bile (from 0 to .5%) tended to increase the amount of cholesterol assimilated. This suggested that more bile tolerant isolates might therefore have a greater ability to assimilate cholesterol. Danielson et al. (11) isolated *L. acidophilus* from pigs and tested the isolates for bile tolerance and cholesterol assimilation. Two of three strains

assimilated more cholesterol at .2% oxgall than at .4% oxgall while the third strain showed the opposite. They preferentially selected one of the strains for use in yogurt based on its bile tolerance and cholesterol assimilation ability. Gilliland and Walker (26) compared twelve commercially available cultures of *L. acidophilus* of human intestinal origin. They agreed that the strain of *L. acidophilus* most appropriate for use as a dietary adjunct for humans should be a bile tolerant human strain and have a high capacity to assimilate cholesterol. None of the human strains used in this study, however, compared favorably to the pig strain, ATCC 43121 which significantly influenced serum cholesterol in earlier pig feeding trials (22). They further found no significant relationship between bile tolerance and cholesterol assimilation ability. Walker and Gilliland (74) also found no similar relationship between bile tolerance and bile salt deconjugation.

Bile Salt Deconjugation

Cholesterol is the precursor for bile acids. The main bile acids in humans, cholic and chenodeoxycholic acids (40% each) and deoxycholic acid (10%) (65), are conjugated with taurine and glycine in the liver before they are secreted in bile (50). Some bacteria present in the intestine can deconjugate these bile salts. In conventional animals, 100% of the excreted bile acids are deconjugated (13, 67). Germfree animals, on the other hand, excrete only conjugated bile acids (6, 13). The total amount of bile acids excreted by germ-free animals is also only half the amount of conventional animals (6).

Many microorganisms have been shown to deconjugate taurine and/or glycine conjugated bile salts (6, 9, 12, 13, 17, 23, 40, 45, 48, 67). Anaerobic organisms have a greater ability to deconjugate bile salts than aerobic

organisms (67). Also, coliforms are rarely able to deconjugate (9). Many lactobacilli including *L. acidophilus* have been shown to deconjugate taurine and/or glycine conjugated bile salts (12, 23, 65). Lundeen and Savage (45) stated that gastric lactobacilli are responsible for approximately 86% of total deconjugation occurring in the ileum and 74% in the cecum of mice. Because the ability to deconjugate bile salts varies within bacterial strains and species (9, 23, 48), not all bacteria of the same species may be able to deconjugate the same substrates. Also, in vitro tests of deconjugation ability do not always correlate with comparable in vivo results (13).

The enzyme responsible for the deconjugation of bile salts is bile salt hydrolase (12, 45, 48). It is produced constitutively by lactobacilli and other bacteria (23, 48) in the presence and absence of conjugated bile acids and released into the surrounding media (48). However, bile salt hydrolase is stimulated threefold by the presence of conjugated bile acids. Neither unconjugated bile acids or taurine stimulated the enzyme (45). Experiments by Kobashi et al. (40) showed that there are enzymes specific for only taurine-conjugates in addition to enzymes which hydrolyze both glycine and taurine-conjugates. Lundeen and Savage (45) showed that bile salt hydrolase is composed of two forms of the enzyme. This could explain the results of Kobashi et al. and the variability of bile salt deconjugation in similar bacterial strains. The bile salt hydrolase gene has been characterized by Christiaens et al. (7). Southern blot experiments showed that this gene is well conserved among the different *Lactobacillus* species tested.

Cholesterol levels in blood can be partially controlled by the microbial metabolism of bile acids through deconjugation (5). Free bile acids are excreted more rapidly than conjugated bile acids in part because free bile acids adhere to dietary fibers (6). As bile salts are removed from the body, the

decrease in bile concentration leads to the synthesis of new bile acids from cholesterol. The conversion of cholesterol to bile acids accounts for approximately 70% of the cholesterol disposed of daily (37). Total cholesterol concentration in the body is therefore reduced when bile acid excretion is increased (15, 72). This is an important mechanism for the removal of excess cholesterol in dogs and rats and may also occur in humans (50). In this way, the ability of organisms used as dietary adjuncts to deconjugate bile acids may help control atherosclerosis and reduce serum cholesterol levels. Walker and Gilliland (74) showed that bile salt deconjugation and assimilation of cholesterol by *L. acidophilus* are not significantly correlated. Thus deconjugation may reduce serum cholesterol in ways unrelated to cholesterol assimilation instead of augmenting or aiding cholesterol assimilation by intestinal lactobacilli.

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CHAPTER 3

COMPARISONS OF FRESHLY ISOLATED STRAINS OF *LACTOBACILLUS ACIDOPHILUS* OF HUMAN INTESTINAL ORIGIN FOR ABILITY TO ASSIMILATE CHOLESTEROL DURING GROWTH

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ABSTRACT

Fecal isolates of *Lactobacillus acidophilus* were obtained from human volunteers and tested for bile tolerance, the ability to deconjugate bile salts, and the ability to assimilate (take up) cholesterol during growth. One hundred and twenty-three out of the 304 isolates of lactobacilli obtained were identified as *L. acidophilus*. In most cases, isolates of *L. acidophilus* from the same volunteer varied significantly ($P < .05$) in the amount of cholesterol assimilated, bile salt deconjugated, and bile tolerance. The two cultures from each of 9 volunteers that assimilated the most cholesterol were compared to select the most active ones. *L. acidophilus* ATCC 43121 (an isolate from the intestines of a pig which significantly benefitted serum cholesterol in pigs) was included in this comparison. Significant ($P < .05$) variation in ability to assimilate cholesterol was observed among these isolates from different volunteers. Eight of seventeen isolates assimilated numerically but not significantly ($P > .05$) more cholesterol than *L. acidophilus* ATCC 43121 while 4 isolates assimilated significantly ($P < .05$) less. Bile tolerance and bile salt deconjugation also varied significantly ($P < .05$) between the selected isolates. Six of the selected isolates were quantitatively but not significantly ($P > .05$) better able to deconjugate bile salts than *L. acidophilus* ATCC 43121 while none were significantly more bile tolerant. Of the selected isolates, B7, D3, L1, O16, and O17 were determined to have the most potential for use as dietary adjuncts to lower human serum cholesterol levels.

INTRODUCTION

Increased levels of LDL cholesterol and total plasma cholesterol correlate highly with the incidence of coronary heart disease, a major cause of death and disability in the United States (18, 22, 26). Dietary adjustment is one way to decrease LDL and total plasma cholesterol thereby reducing the risk of coronary heart disease. Consumption of dairy products supplemented with cultures of *Lactobacillus acidophilus* can aid in the control of serum cholesterol levels in animals and humans (3, 8, 13, 15, 29). Reports in the literature have shown that strains of *L. acidophilus* exhibit host specificity (6, 20, 27, 28). Therefore, to be most effective for use as a dietary adjunct for humans the *L. acidophilus* should likely originate from human intestinal sources. Factors which allow *L. acidophilus* to decrease serum cholesterol levels include ability to assimilate cholesterol, bile tolerance, and bile salt deconjugation ability.

Selected strains of *L. acidophilus* have been shown to assimilate (take up) cholesterol during anaerobic growth in laboratory media containing bile and a cholesterol source (8). The assimilation of cholesterol by *L. acidophilus* may increase the amount of cholesterol excreted while reducing the amount of dietary cholesterol absorbed into the body. Since the ability of *L. acidophilus* to assimilate cholesterol during growth varies by strain (8, 12), the *L. acidophilus* chosen for use as a dietary adjunct to potentially reduce serum cholesterol levels must be selected carefully (14, 21). Pigs fed a high cholesterol diet and a strain of *L. acidophilus* able to actively assimilate cholesterol had significantly lower serum cholesterol levels than did control pigs fed the same diet without

lactobacilli or pigs fed a strain of *L. acidophilus* which did not assimilate cholesterol in laboratory media (8).

In order to survive and grow in the intestinal tract *L. acidophilus* must be able to tolerate physiological concentrations of bile. Gilliland et al. (11) fed newborn dairy calves isolates of *L. acidophilus* that differed in tolerance to bile. The calves supplemented with the more bile tolerant strain had increased numbers of lactobacilli in their intestinal tracts compared to the calves fed the less bile tolerant strain.

Some species of bacteria present in the intestinal tract including *L. acidophilus* are able to deconjugate taurine and/or glycine from primary bile acids such as taurocholic or glycocholic acid (9). Deconjugation of bile acids by *L. acidophilus* may help decrease human serum cholesterol levels due to the fact that free bile acids are excreted more rapidly than conjugated bile acids (2). As bile salts are excreted from the body, the decreased bile concentration leads to the synthesis of new bile acids from cholesterol. This therefore can reduce the total cholesterol concentration in the body (4). Also, cholesterol absorption into the blood from the intestines is not supported as well by deconjugated bile acids as it is by conjugated bile acids (5, 16).

Gilliland and Walker (12) compared the assimilation of cholesterol by 12 commercially available cultures of *L. acidophilus* said to be of human origin. The isolates were all much less active than the strain of pig origin (*L. acidophilus* ATCC 43121) shown in an earlier study to be effective in helping control serum cholesterol in pigs (8). They concluded that the cultures currently commercially available probably were not sufficiently active at cholesterol assimilation for human dietary use to aid in the control of serum cholesterol levels.

The purpose of this study was to obtain new human intestinal isolates of *L. acidophilus* that were better able to assimilate cholesterol than currently available commercially used strains of *L. acidophilus* of human intestinal origin. New isolates were also tested for bile tolerance and the ability to deconjugate bile salts.

MATERIALS AND METHODS

Source of Cultures

Human fecal samples were obtained from volunteers on sterile dacron swabs (Fisher Scientific Co., Pittsburgh, Pa.). The dacron ends of the swabs were placed into tubes containing 10 ml of sterile MRS broth (Difco Laboratories, Detroit, Mi.) by the volunteer. The broth tubes containing the fecal samples were held in a refrigerator in ice water at 0 to 5 °C for a maximum of 48 hours before microbiological analysis was done. The tubes containing the samples were vortexed (Fisher Scientific Co., Model 232) for approximately 15 seconds then appropriate dilutions were made using sterile 9 ml 1% peptone dilution blanks. One tenth ml of each dilution was plated using the spread plate method onto plates of LBS (*Lactobacillus* selection; BBL, Cockeysville, Md.) agar. LBS agar was prepared the day prior to plating from individual ingredients according to the formulation and procedure as described by the manufacturer (BBL). The plates were placed in plastic bags and flushed for approximately 30 seconds with carbon dioxide gas. Following incubation for 48 hours at 37 °C, twenty isolated colonies were picked with a flame sterilized needle into tubes of sterile MRS broth (Difco) and incubated at 37 °C until growth was indicated by turbidity.

The *L. acidophilus* ATCC 43121 culture used in these experiments was from our laboratory stock culture collection. It was originally isolated from intestinal contents of a pig.

Identification of Isolates

An MRS broth culture of each isolate was streaked onto a plate of sterile MRS agar (lactobacilli MRS broth plus 1.5% agar) using a flame sterilized metal loop. The plates were incubated anaerobically in a GasPak system (BBL) at 37 °C for 18 to 20 hours. Purity of the culture was ascertained from morphology of colonies appearing on the plate. If more than one colony type appeared, one of each was picked into sterile MRS broth. Following growth at 37 °C they were restreaked onto MRS agar for identification. Characteristics used to identify the isolates included: Gram-stain reaction, catalase reaction, ability to grow at 15 and 45 °C, and biochemical reactions determined using the BBL Minitek system as described by Gilliland and Speck (10). The Minitek system was used to test the ability of the isolates to produce ammonia from arginine, to hydrolyze esculin, and to ferment the following carbohydrates: amygdalin, arabinose, cellobiose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. The identity of each isolate was then determined by comparison of the test results to the characteristics of each species of lactobacilli as given in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (1).

Maintenance of Cultures

All isolates were maintained by subculturing into lactobacilli MRS broth using a 1% inocula and 19 to 20 hours of incubation at 37 °C. The isolates were stored at 1 to 2 °C between transfers. Each isolate was subcultured at least two times in MRS broth prior to experimental use. Each isolate also was maintained by monthly subculture in MRS agar stabs until tests were completed.

Comparison of Isolates for Assimilation of Cholesterol

Each freshly prepared culture of *L. acidophilus* was inoculated (1%) into 10 ml MRS broth containing .2% sodium thioglycollate (Sigma Chemical Co., St. Louis, Mo.), .3% Oxgall (Difco), and 1 ml freshly prepared cholesterol-phosphatidylcholine micelles (24). The oxgall used in all of these experiments was from the same lot. The tubes were incubated at 37 °C for 12 or 24 hours. After incubation, cells were removed by centrifugation for 10 minutes at 12, 000 x *g* and 1 °C. The o-phthalaldehyde method for measuring cholesterol described by Rudel and Morris (25) was used to determine the amount of cholesterol in the spent broth and uninoculated sterile broth.

Comparison of Isolates for Bile Tolerance and Bile Salt Deconjugation

The bile tolerance of each isolate was measured by the procedure described by Gilliland and Walker (12) except that 2% inocula were used. Results were expressed as hours required for the optical density (O.D.) at 620 nm to increase by .3 units. The methods described by Walker and Gilliland (30)

were used to compare the ability of the cultures to deconjugate sodium taurocholate. Results were expressed as uMol/ml bile salt deconjugated during a 15 hour incubation period at 37 °C.

Comparison of the Best Isolates from Each Volunteer

All isolates from a single volunteer were tested for ability to assimilate cholesterol, bile tolerance, and bile salt deconjugation before a fecal sample was obtained from the next volunteer. Each assay was replicated three times for each isolate. After the isolates from each volunteer were compared, the two isolates from each volunteer which were able to assimilate the most cholesterol were compared along with *L. acidophilus* ATCC 43121. These evaluations included ability to assimilate cholesterol, bile tolerance, and bile salt deconjugation.

Statistical Analyses

This study was arranged in a completely randomized design with subsampling. A volunteer served as a treatment while the isolates obtained from each volunteer were considered experimental units. The three replications of each experimental test were equivalent to subsampling since they were run using the same isolate.

Data from the replications of the experiments were analyzed using the analysis of variance (ANOVA) procedure from SAS (17). The least significant difference (LSD) procedure was used to determine if statistically significant differences occurred among means. Results of the assays of each volunteer were analyzed as well as the results of the final comparison of the top two

isolates from each volunteer and *L. acidophilus* ATCC 43121. Statistical analyses also were done to determine if there were statistical differences between the top two isolates from the same person.

RESULTS

Identification of Isolates

Gram-positive, catalase-negative, rod-shaped bacteria isolated on LBS agar were assumed to be lactobacilli. Three hundred and four isolates of lactobacilli were obtained from sixteen volunteers (Table 1). One hundred and twenty-three of these isolates were identified as being *L. acidophilus*. The identification characteristics of each isolate of *L. acidophilus* are listed in Appendix A. No isolates obtained from seven (A, E, F, I, M, N, and P) of the sixteen volunteers were identified as *L. acidophilus*. All 20 isolates from volunteer H were identified as *L. acidophilus*. The majority (> 80%) of isolates from volunteers B, D, G, H, J, and O were identified as *L. acidophilus*. Thus the majority of lactobacilli from 6 of the 16 volunteers were *L. acidophilus*.

Comparison of Isolates for Assimilation of Cholesterol

The amounts of cholesterol assimilated varied among isolates of *L. acidophilus* from individual volunteers. As an example, the results for the isolates of *L. acidophilus* from volunteer K are presented in Table 2. They are listed in order of decreasing amounts of cholesterol assimilated during 24 hour incubations at 37 °C. The amounts of cholesterol assimilated ranged from 83.3 ug/ml to 20.5 ug/ml. Isolate K4 assimilated significantly ($P < .05$) more than did

TABLE 1

INCIDENCE OF *LACTOBACILLUS ACIDOPHILUS* AMONG LACTOBACILLI FROM FECAL MATERIAL OBTAINED FROM VOLUNTEERS

Volunteer	Number of Isolates From LBS Agar[1]	
	Total	*Lactobacillus acidophilus*
A	16	0
B	20	16
C	19	6
D	22	17
E	20	0
F	20	0
G	20	16
H	20	20
I	2	0
J	20	16
K	25	12
L	20	1
M	20	0
N	20	0
O	20	19
P	20	0
Total	304	123

[1] Fecal samples were diluted and plated onto LBS agar. These plates were incubated for 48 hr at 37 °C in a carbon dioxide enriched atmosphere.

isolates K16, K2, K19, K15, K11, and K7 but not significantly ($P > .05$) more than the other 5 isolates. Isolates K4 and K10 were selected for comparison with the best isolates from other volunteers and *L. acidophilus* ATCC 43121.

The isolates from volunteers H and O also showed significant ($P < .05$) differences in amounts of cholesterol assimilated (Appendix B). Isolates H11, H13, O16, and O17 were selected for further comparison. The isolates from volunteers B, C, D, G, and J, however, all assimilated similar ($P > .05$) amounts of cholesterol (Appendix B). For these volunteers, the two isolates from each which exhibited the numerically highest amounts of cholesterol assimilated were selected for further comparisons. They were isolates B7, B11, C14, C18, D3, D5, G5, G20, J12, and J18. Statistical analysis (Table 25, Appendix B) showed that the two selected isolates from each individual were not significantly ($P > .05$) different in ability to assimilate cholesterol. Because only one isolate of *L. acidophilus* was obtained from volunteer L, it too was selected for further comparisons.

Comparison of *L. acidophilus* ATCC 43121 and the two isolates selected from each of 9 volunteers showed significant ($P < .05$) variation in ability to assimilate cholesterol (Table 3). The isolates are listed in order of decreasing amounts of cholesterol assimilated during a 12 hour incubation at 37 °C. Isolate O16 assimilated the most cholesterol at 50.9 ug/ml while only 28 ug/ml of cholesterol was assimilated by isolate D5. Isolate O16 assimilated significantly ($P < .05$) more cholesterol than did G5, C18, K10, and D5. Eight of the isolates (O16, C14, L1, B11, D3, H11, H13, and G20) were quantitatively better at cholesterol assimilation than ATCC 43121 although not statistically better ($P > .05$).

TABLE 2

COMPARISON OF ASSIMILATION OF CHOLESTEROL BY ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER K

CULTURE	CHOLESTEROL ASSIMILATED [1,2]
K4	83.3 [a]
K10	70.6 [a,b]
K12	69.0 [a,b]
K9	68.6 [a,b]
K14	64.2 [a,b]
K20	62.1 [a,b]
K16	55.6 [b]
K2	53.9 [b]
K19	51.7 [b]
K15	50.1 [b]
K11	22.0 [c]
K7	20.5 [c]

1 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 ^{o}C.

2 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

TABLE 3

COMPARISON OF ASSIMILATION OF CHOLESTEROL BY ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM EACH OF NINE HUMAN VOLUNTEERS

CULTURE	CHOLESTEROL ASSIMILATED [1,2]
O16	50.9 [a]
C14	47.1 [a,b]
L1	46.3 [a,b]
B11	46.3 [a,b]
D3	45.4 [a,b,c]
H11	45.1 [a,b,c]
H13	44.9 [a,b,c]
G20	43.3 [a,b,c]
ATCC 43121[3]	42.7 [a,b,c]
J12	41.7 [a,b,c]
J18	38.9 [a,b,c]
B7	36.3 [a,b,c]
K4	35.5 [a,b,c]
O17	33.2 [a,b,c]
G5	31.3 [b,c]
C18	29.9 [b,c]
K10	28.5 [c]
D5	28.0 [c]

1 ug/ml cholesterol taken up by the culture during a 12 hour incubation at 37 °C.

2 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

3 Originally isolated from pig intestinal contents. 1985. Appl. and Environ. Microbiol. 49:377.

Comparison of Isolates for Bile Tolerance

The isolates of *L. acidophilus* obtained from individual volunteers exhibited varying degrees of bile tolerance. As an example, the results for isolates from volunteer K which varied significantly ($P < .05$) are presented in Table 4. They are listed in order of decreasing bile tolerance. Bile tolerance ranged from 2.0 hours for isolate K20, the most bile tolerant, to 2.8 hours for the least bile tolerant isolate K19. Isolate K20 was significantly ($P < .05$) more bile tolerant than isolates K16, K12, and K19 but not significantly ($P > .05$) more tolerant than the other 8 isolates. The isolates from all other individual volunteers except for volunteer O also exhibited significant ($P < .05$) differences in bile tolerance (Appendix B).

Comparisons of *L. acidophilus* ATCC 43121 and the two isolates selected on the basis of amounts of cholesterol assimilated from each of the 9 volunteers also showed significant ($P < .05$) variation in bile tolerance (Table 5). *L. acidophilus* ATCC 43121 was the most bile tolerant, requiring only 2.0 hours for the O.D. to increase by .3 units while J18 and J12 did not reach an O.D. of .3 in seven hours of incubation at 37 °C. *L. acidophilus* ATCC 43121 was significantly ($P < .05$) more bile tolerant than isolates C14, G20, G5, H13, H11, J18, and J12 but not significantly ($P > .05$) more tolerant than the other 10 isolates. Isolate O17 was the most bile tolerant of all of the intestinal isolates from the volunteers. This isolate was significantly ($P < .05$) more bile tolerant than isolates G5, H13, H11, J18, and J12 but not significantly different than the other eleven human intestinal isolates. In all but one instance (D5 and D3), the two isolates from the same person had extremely similar bile tolerance values.

TABLE 4

COMPARISON OF THE BILE TOLERANCE OF ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER K

CULTURE	BILE TOLERANCE[1,2]
K20	2.0 [d]
K9	2.0 [d]
K15	2.0 [d]
K14	2.0 [d]
K2	2.0 [c,d]
K10	2.0 [b,c,d]
K11	2.2 [a,b,c,d]
K7	2.2 [a,b,c,d]
K4	2.6 [a,b,c,d]
K16	2.6 [a,b,c]
K12	2.7 [a,b]
K19	2.8 [a]

[1] Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

[2] Values are the means of three replications. Means with no commom superscript letters differ significantly ($P<.05$).

TABLE 5

COMPARISON OF THE BILE TOLERANCE OF ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM EACH OF NINE HUMAN VOLUNTEERS

CULTURE	BILE TOLERANCE[1,2]
ATCC 43121[3]	2.0 [a]
O17	2.3 [a,b]
O16	2.5 [a,b]
D5	2.7 [a,b,c]
B11	2.7 [a,b,c,d]
B7	2.8 [a,b,c,d]
L1	3.0 [a,b,c,d]
D3	3.2 [a,b,c,d]
K4	3.3 [a,b,c,d]
K10	3.3 [a,b,c,d]
C18	3.3 [a,b,c,d]
C14	3.4 [b,c,d]
G20	3.6 [b,c,d]
G5	4.0 [c,d]
H13	4.2 [d]
H11	5.7 [e]
J18	7.0 [e]
J12	7.0 [e]

1 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall. For those isolates requiring > 7.0 hours to reach an O.D. of .3, a value of 7 hours was used for comparisons.

2 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

3 Originally isolated from pig intestinal contents. 1985. Appl. and Environ. Microbiol. 49:377.

Comparison of Isolates for Bile Salt Deconjugation Ability

The amount of bile salt deconjugation varied among isolates of *L. acidophilus* from individual volunteers. As an example, the results for isolates of volunteer K are presented in Table 6. These isolates are listed in order of decreasing amounts of bile salt deconjugated. The values ranged from 1.2 uMol/ml for isolate K15 to .4 uMol/ml for isolate K7. Isolate K15 deconjugated significantly ($P < .05$) more than isolates K12 and K7 but not significantly ($P > .05$) more than the other isolates. Significant ($P < .05$) differences in bile salt deconjugation values also were seen in the isolates of all volunteers except for volunteers G and H (Appendix B).

Comparison of *L. acidophilus* ATCC 43121 and the two isolates selected from each of 9 volunteers showed significant ($P < .05$) variation in ability to deconjugate bile salts (Table 7). Isolate D3 deconjugated the most taurocholate (1.4 uMol/ml) while isolate C18 deconjugated the lowest amount (.3 uMol/ml). Isolate D3 was significantly ($P < .05$) better at bile salt deconjugation than isolates H11, B11, K4, K10, D5, and C18 but not significantly ($P > .05$) better than the other isolates. Six of the intestinal isolates from humans (D3, G20, O16, B7, H13, and J18) were quantitatively better at bile salt deconjugation than *L. acidophilus* ATCC 43121 although not statistically ($P > .05$) better.

DISCUSSION AND CONCLUSIONS

Adults vary in the number and species of lactobacilli present in their intestinal tracts. Individual isolates of lactobacilli also vary in bile tolerance (8, 11, 12, 30), the ability to assimilate cholesterol (8, 12, 30), and the ability to

TABLE 6

COMPARISON OF BILE SALT DECONJUGATION BY ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER K

CULTURE	BILE SALT DECONJUGATED[1,2]
K15	1.2 [a]
K4	1.2 [a]
K19	1.2 [a]
K14	1.1 [a]
K20	1.1 [a]
K2	1.1 [a]
K10	1.1 [a,b]
K9	1.1 [a,b]
K11	.8 [a,b,c]
K16	.7 [a,b,c]
K12	.6 [b,c]
K7	.4 [c]

[1] uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

[2] Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

TABLE 7

COMPARISON OF BILE SALT DECONJUGATION BY ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM EACH OF NINE HUMAN VOLUNTEERS

CULTURE	BILE SALT DECONJUGATED[1,2]
D3	1.4 [a]
G20	1.4 [a]
O16	1.3 [a,b]
B7	1.3 [a,b]
H13	1.3 [a,b]
J18	1.3 [a,b]
ATCC 43121[3]	1.3 [a,b]
O17	1.3 [a,b]
L1	1.2 [a,b]
J12	1.1 [a,b,c]
G5	1.0 [a,b,c]
C14	1.0 [a,b,c]
H11	.9 [b,c]
B11	.7 [c,d]
K4	.4 [d,e]
K10	.3 [d,e]
D5	.3 [e]
C18	.3 [e]

1 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

2 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

3 Originally isolated from pig intestinal contents. 1985. Appl. and Environ. Microbiol. 49:377.

deconjugate bile salts (9, 30). Results of this study showed that isolates from most individual volunteers also varied significantly ($P < .05$) with respect to these three characteristics.

Bile tolerance, bile salt deconjugation, and cholesterol assimilation are factors which may affect the ability to some strains of *L. acidophilus* to potentially help control human serum cholesterol levels when used as dietary adjuncts. Gilliland and Walker (12) evaluated 12 commercially available cultures of *L. acidophilus* said to be of human intestinal origin. When they found that none of the commercially available cultures compared favorably to *L. acidophilus* ATCC 43121, which had been shown to beneficially influence serum cholesterol levels in pigs, they recommended that additional research focus on the isolation of more active strains of *L. acidophilus* of human intestinal origin. In this study eight isolates (O16, C14, L1, B11, D3, H11, H13, and G20) were obtained that were quantitatively but not significantly ($P > .05$) more able to assimilate cholesterol than *L. acidophilus* ATCC 43121.

Isolates from each individual were tested for the ability to assimilate cholesterol in 24 hours of incubation at 37 °C. This incubation time was selected to allow for maximal growth and assimilation by each isolate. The 24 hour incubation also allowed for the slower growth of the less bile tolerant isolates. When the 17 selected isolates and *L. acidophilus* ATCC 43121 were compared for ability to assimilate cholesterol, the cholesterol assimilation tubes were incubated for 12 hours. Because these isolates were already selected based on their ability to assimilate cholesterol, the decreased incubation selected for the isolates which had the greatest ability to assimilate cholesterol in a shorter period of time.

Thirteen of the selected 17 isolates did not differ significantly ($P > .05$) from *L. acidophilus* ATCC 43121 in ability to assimilate cholesterol. Of these 13

isolates, six (C14, G20, H11, H13, J12, and J18) were significantly ($P < .05$) less bile tolerant than *L. acidophilus* ATCC 43121. Since bile tolerance is considered important in order to permit the lactobacilli to survive and grow well in the intestines (14), these six cultures would be less likely candidates for use as dietary adjuncts. Of the remaining seven isolates, isolates B11 and K4 had significantly ($P < .05$) lower bile salt deconjugation values than the others. Thus, if bile salt deconjugation is important in helping control serum cholesterol levels, these isolates also would not be optimal for use as dietary adjuncts to aid in controlling serum cholesterol levels. The remaining isolates, B7, D3, L1, O16, and O17 were not significantly ($P > .05$) different than *L. acidophilus* ATCC 43121 at any of the three characteristics tested. Thus, these five isolates should be considered better candidates for use as dietary adjuncts to aid in controlling serum cholesterol than the 12 commercially available cultures of *L. acidophilus* which were compared to *L. acidophilus* ATCC 43121 earlier (12).

Recently Klaver and van der Meer (19) postulated that the "apparent" assimilation of cholesterol by *L. acidophilus* resulted solely from the bile-salt deconjugating ability of the culture. They stated that in a laboratory medium, cholesterol was not assimilated by the lactobacilli but was instead precipitated along with free bile salts liberated from bile in the medium through deconjugation by the culture. However, Walker and Gilliland (30) have reported that no significant relationship occurred between cholesterol assimilation and bile salt deconjugation. In the present study some isolates (examples: B11 and H11) deconjugated significantly less bile acids than others (examples: G20 and D3), however they did not assimilate significantly less cholesterol.

In summary, isolates of *L. acidophilus* from human intestinal material were obtained which were better able to assimilate cholesterol and more active at bile salt deconjugation than commercially used cultures of *L. acidophilus*

based on comparisons with the pig isolate *L. acidophilus* ATCC 43121. Based on the results, isolates B7, D3, L1, O16, and O17 have the most potential for use as dietary adjuncts to aid in the control of human serum cholesterol levels because these isolates were not significantly ($P > .05$) different than *L. acidophilus* ATCC 43121 at any of the three characteristics tested.

Variations in stability during frozen and refrigerated storage occur among strains of *L. acidophilus* (7, 23). Thus additional evaluations of isolates B7, D3, L1, O16, and O17 should be done to ascertain whether or not they would survive in commercial production and handling applications. This must be done before selecting the best one(s) for use as dietary adjuncts to provide potential for helping control serum cholesterol levels in humans.

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APPENDIX A

IDENTIFICATION OF ISOLATES

TABLE 8

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER B

Test[1]	La[2]	B3	B4	B5	B6	B7	B8	B9	B11	B12	B13	B14	B15	B16	B17	B18	B20
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+	+/-	+/-	+	+	+
Arabinose	-	+	-	-	+/-	-	-	+	-	+/-	-	+/-	-	-	-	+/-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-			-	-	-	-	-	-		-	+/-	-	-	-	-	-
Melibiose	+/-	+/-	+/-	-	-	-	-	+/-	-	+/-	+	+	-	+/-	+	+/-	-
Raffinose	+/-	-	-	-	-	-	-	+/-	-	-	-	+	-	+	+	-	-
Rhamnose	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology*.

TABLE 9

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER C

Test[1]	La[2]	C7	C11	C14	C17	C18	C19
Amygdalin	+	+	+/-	-	+	-	+
Arabinose	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+
Esculin	+	+	+			+	+
Galactose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+/-
Maltose	+	+	+	+	+	+	+
Mannitol	-	-	+	+	+	-	+
Mannose	+	+	+	+	+	+	+
Melezitose	-	+	+	+	-	-	-
Melibiose	+/-	-	-	-	-	-	-
Raffinose	+/-	-	-	-	-	-	+/-
Rhamnose	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+
Sorbitol	-	-	+/-			-	-
Sucrose	+	+	+	+	+	+	+
Trehalose	+	+	+	-	+	-	+
Xylose	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology.*

TABLE 10

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER D

Test[1]	La[2]	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D13	D14	D15	D16	D17	D19	D20
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+		+		+			+	+	+							+	
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
Melibiose	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-		-		+/-			-	-	-							-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology*.

TABLE 11

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER G

Test[1]	La[2]	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G12	G13	G14	G16	G19	G20
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	+	-	-	-	-	-	+/-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	+/-	+	-	+/-	+	+	-	+	+	+	+	-	+	-	+	+
Melibiose	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	+/-	-	-	-	-	-	+/-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology.*

TABLE 12

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER H

Test[1]	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Amygdalin	+	+	+	+	+/-	+	+/-	+	+	+/-	+	+	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	+/-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	+/-	-	-	-	+/-	-	-	-	-	-	-	+/-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

TABLE 13

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER J

Test[1]	La[2]	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14	J15	J16	J17	J18	J19	J20
Amygdalin	+	+/-	+	+	+	+	+/-	+	+	+	+	+	+/-	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	+/-	+/-	+/-	+/-	+/-	-	+/-	-	-	+/-	+/-	+/-	+/-	-	+/-	-
Melibiose	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology*.

TABLE 14

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEERS K AND L

Test[1]	La[2]	K2	K4	K7	K9	K10	K11	K12	K14	K15	K16	K19	K20	L1
Amygdalin	+	-	+	+	+	+/-	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+/-	+	+	+	+	+	+	+	+	+	+/-	-	+	+
Raffinose	+/-	+	+	+	+	+	+	+	+	+	+	-	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology.*

TABLE 15

IDENTITY CHARACTERISTICS OF ISOLATES OF
LACTOBACILLUS ACIDOPHILUS
FROM VOLUNTEER O

Test[1]	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O15	O16	O17	O18	O19	O20
Amygdalin	+	+/-	+	+	+	+	+	-	+	+	+	+	+	+	+/-	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	+/-	+	+	+/-	+/-	+/-	-	+/-	+/-	+	+	+/-	+/-	+	+	+/-	+/-	+/-	+/-
Melibiose	-	+/-	+/-	-	-	-	-	-	-	-	+/-	+/-	-	-	+/-	+/-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	-	-	+/-	+/-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

TABLE 16

IDENTITY CHARACTERISTICS OF THE FINAL ISOLATES
OF *LACTOBACILLUS ACIDOPHILUS*

Test[1]	La[2]	B7	B11	C14	C18	D3	D5	G5	G20	H11	H13	J12	J18	K4	K10	L1	O16	O17
Amygdalin	+	-	-	-	-	+/-	+/-	-	+/-	-	-	-	-	+	+/-	+	+/-	+/-
Arabinose	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+/-	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	-	+/-	+/-	+/-	+/-	+/-	+/-	+	+	+/-	+/-	+/-	+/-	+	+	+	+/-	+/-
Melibiose	+/-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-
Raffinose	+/-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-			-	-			-	-		-					-	-	
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	-	-	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] All isolates were Gram positive, catalase negative rods which grew at 45 °C but not 15 °C.

[2] La = *Lactobacillus acidophilus*. Reactions as listed in the 8th Edition of *Bergey's Manual of Determinative Bacteriology*.

APPENDIX B

DATA FROM ISOLATES FROM EACH VOLUNTEER

TABLE 17

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER B

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
B11	23.8 [a]	4.7 [c,d]	1.1 [a,b]
B7	23.4 [a]	4.5 [d]	1.2 [a]
B20	23.4 [a]	6.1 [b,c,d]	1.1 [a,b]
B9	22.3 [a]	5.5 [b,c,d]	1.2 [a,b]
B16	21.9 [a]	5.0 [b,c,d]	1.1 [a,b]
B6	21.8 [a]	5.0 [b,c,d]	1.1 [a,b]
B4	20.5 [a]	5.7 [b,c,d]	1.1 [a,b]
B5	18.9 [a]	7.2 [a,b,c,d]	1.1 [a,b]
B12	17.7 [a]	6.7 [a,b,c,d]	1.2 [a,b]
B3	16.9 [a]	5.9 [b,c,d,e]	1.1 [a,b]
B8	14.5 [a]	7.8 [a,b,c]	1.3 [a]
B13	10.0 [a]	8.0 [a,b]	1.1 [a,b]
B18	9.1 [a]	6.7 [a,b,c,d]	1.0 [b]
B17	8.5 [a]	6.8 [a,b,c,d]	1.1 [a,b]
B15	7.5 [a]	9.7 [a]	1.1 [a,b]
B14	4.6 [a]	8.1 [a,b]	1.1 [a,b]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 18

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER C

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
C14	41.5 [a]	6.0 [b]	.3 [a]
C18	38.6 [a]	6.0 [b]	.3 [a,b]
C7	37.9 [a]	5.9 [b]	.2 [b]
C17	36.6 [a]	5.0 [b]	.2 [b]
C19	32.5 [a]	8.3 [a]	.3 [a]
C11	16.4 [a]	6.0 [b]	.3 [a]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 19

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER D

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
D3	65.4 [a]	5.3 [a,b]	1.1 [a]
D5	64.7 [a]	3.7 [e]	.2 [c]
D2	62.1 [a]	3.7 [e]	.3 [c]
D7	61.1 [a]	4.1 [c,d,e]	.2 [c]
D14	60.5 [a]	3.8 [e]	.2 [c]
D9	58.5 [a]	5.3 [a,b]	.9 [a,b]
D6	58.1 [a]	4.7 [a,b,c]	.9 [a,b]
D10	58.1 [a]	3.8 [e]	.2 [c]
D19	57.6 [a]	3.8 [e]	.2 [c]
D16	57.2 [a]	5.2 [a,b]	.7 [b]
D1	56.9 [a]	5.5 [a,b]	.9 [a,b]
D4	55.7 [a]	5.6 [a]	.9 [a,b]
D15	55.1 [a]	4.9 [a,b,c]	.9 [a,b]
D8	55.0 [a]	4.9 [a,b,c]	.9 [a,b]
D17	52.9 [a]	4.9 [a,b]	.9 [a,b]
D20	52.2 [a]	3.9 [d,e]	.2 [c]
D13	50.9 [a]	4.7 [b,c,d]	.8 [a,b]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 20

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER G

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
G5	37.7 [a]	3.2 [a,b,c]	1.0 [a]
G20	35.3 [a]	3.8 [a,b,c]	1.1 [a]
G16	35.1 [a]	2.6 [c]	1.0 [a]
G6	32.3 [a]	2.6 [b,c]	1.0 [a]
G19	31.1 [a]	3.7 [a,b,c]	1.1 [a]
G14	29.4 [a]	3.4 [a,b,c]	.9 [a]
G3	29.1 [a]	3.4 [a,b,c]	1.0 [a]
G4	27.9 [a]	3.9 [a,b,c]	1.1 [a]
G10	26.3 [a]	3.7 [a,b,c]	1.1 [a]
G7	26.1 [a]	6.0 [a,b,c]	1.0 [a]
G12	24.3 [a]	4.5 [a,b,c]	1.1 [a]
G2	23.8 [a]	3.7 [a,b,c]	1.0 [a]
G9	20.2 [a]	5.3 [a,b,c]	1.1 [a]
G8	18.7 [a]	4.0 [a,b,c]	1.0 [a]
G1	15.6 [a]	7.0 [a]	1.0 [a]
G13	14.8 [a]	6.7 [a,b]	1.1 [a]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 21

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER H

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
H13	47.5 [a]	2.3 [b]	1.2 [a]
H11	45.1 [a,b]	2.5 [b]	1.1 [a]
H3	44.7 [a,b]	2.3 [b]	1.3 [a]
H4	44.0 [a,b]	2.4 [b]	1.3 [a]
H5	43.3 [a,b]	2.3 [b]	1.2 [a]
H9	43.2 [a,b]	2.3 [b]	1.4 [a]
H18	41.4 [a,b]	2.2 [b]	1.3 [a]
H17	41.3 [a,b]	2.3 [b]	1.3 [a]
H2	40.9 [a,b]	2.2 [b]	1.3 [a]
H20	40.7 [a,b]	2.3 [b]	1.1 [a]
H12	40.3 [a,b]	2.4 [b]	1.3 [a]
H10	39.5 [a,b]	2.4 [b]	1.2 [a]
H8	39.4 [a,b]	2.2 [b]	1.2 [a]
H6	39.1 [a,b]	2.4 [b]	1.3 [a]
H16	38.3 [a,b]	2.4 [b]	1.3 [a]
H1	37.5 [a,b]	2.2 [b]	1.0 [a]
H15	35.8 [a,b]	2.3 [b]	1.3 [a]
H7	35.6 [a,b]	2.6 [a,b]	1.1 [a]
H19	35.5 [a,b]	3.2 [a]	1.4 [a]
H14	34.7 [b]	2.3 [b]	1.2 [a]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P < .05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 22

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER J

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
J12	63.2 [a]	2.2 [d,e]	.9 [a,b]
J18	61.2 [a]	2.3 [b,c,d,e]	.5 [a,b]
J17	58.6 [a]	2.2 [d,e]	.5 [a,b]
J19	57.5 [a]	2.2 [c,d,e]	.6 [a,b]
J7	57.3 [a]	2.2 [c,d,e]	.6 [a,b]
J13	57.1 [a]	2.1 [e]	1.0 [a,b]
J20	55.6 [a]	3.4 [a,b]	.4 [a,b]
J9	55.2 [a]	3.3 [a,b,c,d]	.5 [a,b]
J14	55.1 [a]	2.4 [b,c,d,e]	.5 [a,b]
J5	55.0 [a]	2.1 [e]	1.0 [a]
J10	54.8 [a]	3.8 [a]	.4 [b]
J16	53.8 [a]	2.2 [d,e]	.7 [a,b]
J6	53.5 [a]	3.7 [a]	.5 [a,b]
J15	52.0 [a]	3.8 [a]	.4 [b]
J11	51.9 [a]	2.2 [c,d,e]	1.0 [a,b]
J8	50.7 [a]	3.3 [a,b,c]	.6 [a,b]

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P<.05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 23

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEERS K AND L

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
K4	83.3 [a]	2.6 [a,b,c,d]	1.2 [a]
K10	70.6 [a,b]	2.0 [b,c,d]	1.1 [a,b]
K12	69.0 [a,b]	2.7 [a,b]	.6 [b,c]
K9	68.6 [a,b]	2.0 [d]	1.1 [a,b]
K14	64.2 [a,b]	2.0 [d]	1.1 [a]
K20	62.1 [a,b]	2.0 [d]	1.1 [a]
K16	55.6 [b]	2.6 [a,b,c]	.7 [a,b,c]
K2	53.9 [b]	2.0 [c,d]	1.1 [a]
K19	51.7 [b]	2.8 [a]	1.2 [a]
K15	50.1 [b]	2.0 [d]	1.2 [a]
K11	22.0 [c]	2.2 [a,b,c,d]	.8 [a,b,c]
K7	20.5 [c]	2.2 [a,b,c,d]	.4 [c]
L1[5]	62.6	3.3	.9

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P < .05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

5 Volunteer L had only one isolate of *Lactobacillus acidophilus.*

TABLE 24

RESULTS OF CHOLESTEROL ASSIMILATION, BILE TOLERANCE, AND BILE SALT DECONJUGATION TESTS ON ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM VOLUNTEER O

ISOLATE	CHOLESTEROL ASSIMILATION[1,2]	BILE TOLERANCE[1,3]	BILE SALT DECONJUGATION[1,4]
O16	58.6 a	2.5 a	1.0 a,b
O17	56.9 a,b	2.7 a	.8 b,c
O18	55.6 a,b	2.5 a	1.1 a
O10	55.5 a,b	2.4 a	.9 a,b,c
O2	55.5 a,b	2.6 a	1.1 a,b
O7	55.1 a,b	2.9 a	1.0 a,b
O15	54.8 a,b	2.6 a	1.0 a,b
O6	54.5 a,b	2.4 a	.8 a,b,c
O3	54.5 a,b	2.8 a	.9 a,b,c
O14	54.3 a,b	2.6 a	1.0 a,b,c
O4	53.8 a,b	2.5 a	.9 a,b,c
O13	53.8 a,b	2.4 a	.9 a,b,c
O12	53.8 a,b	2.6 a	1.0 a,b,c
O9	53.7 a,b	2.6 a	1.0 a,b
O8	53.4 a,b	2.7 a	.9 a,b,c
O20	52.8 a,b	2.6 a	1.0 a,b
O11	52.5 a,b	2.5 a	1.0 a,b,c
O19	52.0 a,b	2.5 a	1.0 a,b
O5	49.9 b	2.5 a	.7 c

1 Values are the means of three replications. Means with no common superscript letters differ significantly ($P < .05$).

2 ug/ml cholesterol taken up by the culture during a 24 hour incubation at 37 °C.

3 Time in hours for the culture to increase the O.D. by .3 at 620 nm in MRS containing .3% oxgall.

4 uMol/ml bile salt deconjugated after incubation for 15 hours at 37 °C.

TABLE 25

ANALYSIS OF VARIANCE TABLE - COMPARISON OF THE TWO ISOLATES OF *LACTOBACILLUS ACIDOPHILUS* FROM EACH VOLUNTEER THAT WERE BEST ABLE TO ASSIMILATE CHOLESTEROL

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Individual	7	654.66	93.52	.41	.8710
Best (Individual)	8	1822.21	227.78	1.83	.11
Replications within Individuals	32	3973.23	124.16		
Corrected Total	47	6450.09			

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VITA

Lys M. Buck

Candidate for the Degree of

Master of Science

Thesis: ASSIMILATION OF CHOLESTEROL BY STRAINS OF *LACTOBACILLUS ACIDOPHILUS* OF HUMAN INTESTINAL ORIGIN

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