Acidophilus Milk Products: 
A Review of Potential Benefits to Consumers

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ABSTRACT

During the past 80 yr considerable attention has been sporadically directed on benefits derived from consumption of milk products containing *Lactobacillus acidophilus*. Most earlier work dealt with the use of fermented acidophilus milk to treat intestinal infections. More recent studies have focused on other aspects of health or nutritional benefits that might be derived from this organism. These studies have shown that consumption of milk products containing *L. acidophilus* has the potential for: 1) preventing or controlling intestinal infections, 2) improving lactose digestion in persons classified as lactose maldigestors, 3) helping control serum cholesterol levels, and 4) exerting anticarcinogenic activity. Based on newer knowledge of *L. acidophilus*, a single strain of the organism probably will not produce all these benefits at optimal levels. Careful selection of specific strains of *L. acidophilus* combined with proper production and handling procedures will be necessary to ensure that desired benefits are provided to consumers.

INTRODUCTION

Interest in the role of lactobacilli in human health goes back at least as far as 1908 when Eli Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong life (34). His theory was that the lactobacilli would displace the microorganisms normally occurring in the intestinal tract that produced "toxins", resulting in reduction of life span.

Many reports since then have yielded variable results with regard to the benefits of consuming milk fermented with the lactobacilli. Some of these variations were most likely due to the use of inadequate microbiological techniques or poorly designed experiments. Many reports, for instance, did not include adequate control groups in the studies. Interest in the possible benefits to be derived from the lactic acid bacteria in health and nutrition has been renewed in the last 15 to 20 yr. Although the use of lactobacilli such as *Lactobacillus acidophilus* or bifidobacteria such as *Bifidobacterium bifidum* should not be considered a "cure-all", a number of possibilities for benefits do seem reasonable.

Among the possible benefits to health or nutrition from consuming milk containing lactobacilli or bifidobacteria include 1) control of intestinal infections, 2) anticarcinogenic activity, 3) improved lactose utilization, and 4) aid in controlling serum cholesterol levels. These represent the four main areas that have been addressed with regard to possible benefits derived from these dietary cultures. Many factors govern whether or not a dietary culture can benefit health and nutrition. Consideration of the cultures involved shows tremendous variations among strains. This combined with variations among persons makes for very complicated systems from which to evaluate potential benefits derived from the cultures. This paper will address each of the four areas of possible benefits from dietary cultures. Most attention will focus on *L. acidophilus*, since most scientific papers describing research using *B. bifidum* as a dietary culture are in foreign journals and are not readily available.

Control of Intestinal Pathogens

The possible role of certain cultured dairy
products in controlling intestinal pathogens has received much attention since the early 1900's. Both L. acidophilus and B. bifidum have been shown to be inhibitory toward many of the commonly known foodborne pathogens (10, 15, 23, 25, 31, 33, 44, 45, 47, 48, 51, 53, 56) in both in vivo and in vitro studies. There have been studies showing the preventive or therapeutic control of intestinal infections through administering milk cultured with one or both of these organisms (5, 21, 31, 33, 35, 44, 45, 55, 57, 58, 59). However, some studies have indicated that administration of milk cultured with the lactobacilli or bifidobacteria had no effect on the intestinal infections (4, 41, 43). Unfortunately, some of the studies were not well-documented, and it is rather difficult to evaluate whether or not they were done properly. For example, only a few of the studies had much basis for selecting the cultures used other than, in some cases, to identify them as L. acidophilus or B. bifidum. Cultures were not necessarily selected for their ability to inhibit intestinal pathogens.

Several studies can be cited that have shown a positive effect from utilizing milk containing the lactobacilli or bifidobacteria. Although it is not the intent of this paper to review all of the literature relative to this, a few examples will be reviewed.

Winkelstein (59) evaluated a tablet form of L. acidophilus for treating a number of different intestinal disorders. In this series of trials, the tablets were administered at the rate of three/d for 6 to 8 wk initially. Examples of the disorders being treated with the acidophilus tablets included functional diarrhea, mucous colitis, ulcerative colitis, diverticulitis, and antibiotic colitis. Several patients suffering from each of these forms of illness were included and treatment was successful in all cases except those involving ulcerative colitis.

Gordon et al. (21) conducted a study involving 66 patients receiving oral antibiotics. The patients were divided into two groups. One group was fed a dried preparation of an antibiotic-resistant strain of L. acidophilus and the other group served as the control. Fecal specimens were examined for numbers of staphylococci before, during, and after the antibiotic treatment. All of the subjects received oral antibiotics (tetracycline) for 5 d. The group receiving the L. acidophilus preparation continued receiving it for 4 d beyond the antibiotic treatment period. The number of patients having greater than $5 \times 10^3$ staphylococci/g in the feces was about equal for both groups initially. At the end of the experiment the group that had been receiving the dried preparation of L. acidophilus had fewer individuals exhibiting this level of staphylococci than in the control group. Their conclusion was that L. acidophilus inhibited or controlled the growth of the staphylococci in the intestines.

In studying the possible role of L. acidophilus in inhibiting intestinal pathogens, Watkins and Miller (57) tested both the prophylactic and therapeutic aspects of L. acidophilus in gnotobiotic chicks. Two-day-old chicks were divided into two groups, a therapeutic group and a prophylactic group. In the therapeutic group, chicks were dosed with the desired pathogen initially, then divided into three subgroups. One of the subgroups was not treated further, but the other two both received L. acidophilus at 2-d intervals for up to five additional treatments. The pathogens evaluated were Salmonella typhimurium and Staphylococcus aureus. In the prophylactic group the 2-d-old chicks were treated initially with cells of L. acidophilus then divided into five subgroups. One of the groups did not receive additional treatments. Two of the remaining groups were challenged with the S. typhimurium 2 d later and two were challenged with Staph. aureus. One of the subgroups challenged with each pathogen was then retreated with L. acidophilus five times at 2-d intervals following the treatment with the pathogens. In their results, the percentage of mortality was lowest for the group in which L. acidophilus was fed first as a "prophylactic treatment". This was true for both L. acidophilus and Staph. aureus, indicating that the prophylactic treatment was much more effective than the therapeutic approach at the level of L. acidophilus (1 ml of broth culture/bird) being used in these experiments. Results were similar against enteropathogenic Escherichia coli in a related study, again using gnotobiotic chicks (58).

Fuller (8) compared two strains of L. acidophilus in controlling E. coli using gnotobiotic chicks. Chickens in two experiments were divided into two groups. One group was inoculated with E. coli and a second group was inoculated with both E. coli and the desired test
strain of L. acidophilus. The birds were sacrificed after 7 d and the intestinal contents analyzed for the number of E. coli. Both strains of L. acidophilus exerted inhibitory action toward the E. coli in that lower counts were obtained in both the upper and lower small intestines in the birds that received the lactobacilli in the diet. Because the two experiments were conducted at different times, it is not possible to determine whether or not one strain of L. acidophilus was more effective than the other.

In a study to evaluate the influence of feeding a dried preparation of L. acidophilus on the numbers of coliforms in commercial laying hens, Miles et al. (35) was able to show a benefit. Hens were divided into four groups, a control group plus three groups receiving increased amounts of the dried preparation of L. acidophilus in their daily ration. Ten birds from each group were killed on d 28 and 245 of the experiment. The large and small intestinal contents were combined and analyzed for the numbers of coliforms. The numbers of coliforms in all groups increased from d 28 to 245. However, there were fewer coliforms in the hens receiving the dried preparation of L. acidophilus.

Studies using humans to evaluate the effectiveness of dietary preparations of L. acidophilus or B. bifidum are difficult to conduct. This is especially true of those involving a challenge with intestinal pathogens. Many reports in the earlier literature regarding such treatments were rather poorly designed and did not include appropriate controls. Furthermore, most of those studies used the therapeutic approach to treat existing illnesses without knowing for sure that all patients included were infected with the same pathogen. Thus, even if the patients were divided into two groups, including a control group, there is no assurance that the control group had been infected with the same pathogenic organism.

The exact mechanism whereby dietary cultures of L. acidophilus may inhibit intestinal pathogens is not completely clear. However, a number of laboratory studies have shown that L. acidophilus exerts antagonistic actions toward these pathogenic microorganisms. Gilliland and Speck (15), for instance, showed that L. acidophilus exerted inhibitory action toward Staph. aureus, S. typhimurium, and enteropathogenic E. coli when grown with each pathogen in associative culture. In their experiments the pathogenic organism was inoculated into a milk-based medium and divided into two portions. One portion was additionally inoculated with L. acidophilus. The associative and control cultures were incubated 6 h at 37°C after which they were examined for the numbers of the pathogens using selective media. In all cases there were fewer pathogens in the presence of L. acidophilus than in the control samples. This was true for each of the three pathogenic organisms included. This study also revealed that the antagonistic action was not due just to acid produced by the lactobacilli since inhibition was also obtained when the associative culture was maintained at pH 6.5. This same study revealed that the intensity of the antagonistic action produced by L. acidophilus varied among strains of the organism tested. In an experiment involving five different strains of L. acidophilus the intensity of the inhibition of S. aureus in associative cultures varied from 55 to 85% and for Salmonella typhimurium from 49 to 67%. Based on our knowledge of other lactic acid bacteria it is not surprising that there would be variation among strains of L. acidophilus with regard to the intensity of any antagonistic action they produce.

Several antibiotic-like substances such as acidolin (23), acidophilin (51), and lactocidin (56) are produced by various strains of L. acidophilus. More recently Mehta et al. (33) reported a broad spectrum inhibitor producer by L. acidophilus, which was active against a number of both gram-positive and gram-negative organisms. However, it was not active against all organisms tested. This inhibitory material was characterized as being a protein having a molecular weight of 5.4 kdal. The organisms against which it was active included a number of the common foodborne and intestinal pathogens. Silva et al. (53) reported another broad spectrum inhibitory compound produced by a culture of lactobacilli isolated from human intestines. This inhibitory system also was active against both gram-negative and gram-positive organisms. It was characterized as being resistant to proteolytic enzymes, heat resistant, and of low molecular weight. The investigators indicated that it resembled a short-chain fatty acid although it was not lactic or acetic acid. In addition to these types of inhibitory compounds, L. acidophilus also produces bacterio-
cins (1, 2, 9). Although bacteriocins by definition are active only against closely related species of bacteria and thus may not be of much benefit in controlling intestinal pathogens, they may be very important in enabling selected strains of *L. acidophilus* to establish in the intestinal tract in the presence of other lactobacilli.

Thus, *L. acidophilus* apparently is capable of producing more than one inhibitory system, and the antagonistic action they exert toward intestinal pathogens likely results from the involvement of more than one particular inhibitor. Recognizing that variations occur among strains of *L. acidophilus* it is not likely that any given strain produces all of the types of inhibitory compounds that have been attributed to this particular species of bacteria.

Information on specific experiments evaluating the ability of bifidobacteria to inhibit intestinal pathogens is not as readily available as for *L. acidophilus*. However, the organisms has received much attention in the literature as a beneficial dietary adjunct. Most papers involving this organism have been published in foreign journals with little being reported in scientific journals in this country with regard to specific experiments. Rasic and Kurman (44) have reviewed the beneficial aspects of this group of bacteria in their book, *Bifidobacteria and Their Role*.

The bifidobacteria produce acetic, formic, and lactic acids from the fermentation of sugars. The volatile fatty acids, particularly acetic acid, is much more inhibitory toward gram-negative bacteria than is lactic acid. Thus, the bifidobacteria may possess an advantage over *L. acidophilus* in some cases in inhibiting the growth of gram-negative intestinal pathogens. In infants, the presence of acetic acid in the intestines is more important than the pH in controlling gram-negative microorganisms. Bullen and Tearle (3) reported that feces from breast-fed infants contained higher levels of acetic acid and acetate than did the feces from bottle fed infants. Breast-fed infants also contained a much higher level of bifidobacteria than did bottle-fed infants. However, the number of gram-negative bacteria in the bottle-fed infants' intestines were considerably higher than those in the breast-fed infants. This suggests a benefit from the presence of the bifidobacteria in the intestines, since they produce large amounts of acetic acid in their fermentation of sugars.

Rasic and Kurman (44) cited a number of reports showing that *B. bifidum* can be used both as a prophylactic and therapeutic treatment for various intestinal disorders. Many of the experiments and results resemble those reported for *L. acidophilus*.

**Anticarcinogenic Actions**

Several lactic acid bacteria have been shown to produce anticarcinogenic or antimutagenic activity (6, 26, 39, 46, 50). Some of these activities are apparently due to compounds or substances produced by the organism during growth. It also is possible that the overall action may be due to antagonistic action of the lactic acid bacteria, particularly during growth in the intestines, toward those organisms that might convert procarcinogens into carcinogens.

Shahani et al. (50), using rats as an animal model, studied the influence of *L. acidophilus* on Ehrlich ascites tumor cells. After tumor cells were implanted, rats were divided into two groups (six rats each) including a control and a group that was fed milk fermented with *L. acidophilus*. The control group received regular rat chow and the acidophilus group received the regular rat chow plus milk fermented with *L. acidophilus*. After 7 d the animals were killed and the number of tumor cells determined. The experiment was repeated in a series of three trials. In all three, lower numbers of tumor cells were observed in rats that received the milk fermented with *L. acidophilus* than in the control group. The conclusion was that *L. acidophilus* produced something during growth that was antagonistic toward the proliferation of these tumor cells.

Takano et al. (54) studied the influence of a "sour" milk product on proliferation of colon tumors in rats. The soured milk was produced by fermenting milk with a mixture of *Lactobacillus helveticus* ss *jugurti* and *Candida utilis*. Rats were divided into four groups after being injected intraperitoneally with 1,2- dimethylhydrazine (DMH), which will induce tumor formation. One group of the animals served as a control, a second group received the sour milk, a third group received milk that had been acidified with DL-lactic acid, and the fourth group received an aqueous suspension of the starter
culture cells that had been grown in broth. These treatments were in addition to the normal rat chow. The injection of DMH was repeated weekly for 16 wk. Ten weeks later the rats were examined for numbers of colon tumors. The results indicated that the rats receiving the sour milk had significantly fewer tumors than did the control group. Neither the acidified milk nor the starter culture cells alone had any significant effect.

Other evidence supporting the fact that the lactobacilli can have an influence on cancer formation in the intestinal tract has been provided by Goldin and Gorbach (18, 19). They studied the influence of feeding cells of \textit{L. acidophilus} on the activity of three enzymes encountered in fecal material. The enzymes were β-glucuronidase, azoreductase, and nitroreductase. Each of these enzymes can convert procarcinogens into carcinogens in the intestinal tract. Thus, a treatment that would reduce the level of activity in these enzymes in the intestinal tract would be expected to reduce the chances of carcinogens being formed. In their studies, rats were injected with DMH weekly by subcutaneous injection. The rats were placed on a meat-based diet and divided into two groups. The control group, in addition to the meat-based diet, received daily \(0.4\) ml of milk each and the acidophilus group received \(0.4\) ml of milk each containing \(10^{10}\) cells of \textit{L. acidophilus}. The level of the three enzymes was monitored in the feces of the animals during a 7-wk period. The results indicated significant reductions in the activity of all three enzymes in the rats receiving the milk supplemented with cells of \textit{L. acidophilus}.

Goldin and Gorbach (20) also showed the consumption of milk containing cells of \textit{L. acidophilus} also can cause significant reductions in the activity of the three enzymes mentioned in humans. Twenty-one test subjects were included in the study. After enzyme levels in the fecal materials were monitored for several days, subjects were fed milk on a regular basis to establish the base line for the control period. This was followed by another period without milk and finally subjects were fed milk containing cells of \textit{L. acidophilus}. Consuming the milk alone (i.e., control period) caused no influence on the level of the enzymes. However, when milk was consumed that contained cells of \textit{L. acidophilus}, activity for these enzymes declined significantly.

Macrophages are reported to play a role in suppressing the growth of tumor cells. Perdigon et al. (42) showed that consumption of milk containing cells of \textit{L. casei} activated macrophages in mice. In one experiment involving nine groups of mice the influence of feeding milk containing cells of either \textit{L. casei} or \textit{L. bulgaricus} was evaluated. In addition to the control group, there were four groups of mice for each \textit{L. casei} and \textit{L. bulgaricus}. Mice were fed increasing numbers of cells of the lactobacilli. Following an 8-d feeding period, mice were killed and macrophage enzyme activity determined. Increase in enzyme activity from the macrophages suggests activation of the macrophages. (In this particular experiment they measured lactic dehydrogenase (LDH) activity). Consumption of the milk containing cells of \textit{L. casei} resulted in increased levels of LDH activity, while consumption of the milk containing cells of \textit{L. bulgaricus} had little or no effect on the level of this enzyme. This suggests another mechanism whereby dietary culture of lactobacilli may influence proliferation of tumor cells in the body.

**Improved Lactose Utilization in Lactose Maldigestors**

The inability of a person to digest lactose has been referred to by a number of terms including “lactose malabsorption”, “lactose intolerance”, and more recently “lactose maldigestion”. “Lactose maldigestion” probably is the most accurate term used to describe this condition in humans since it refers to the inability of the person to digest lactose adequately. This inability to digest lactose adequately is due to the absence of sufficient amounts of the enzyme β-galactosidase in the small intestines.

The fact that microorganisms in the gastrointestinal tract can influence the ability to digest lactose was reported by Siddons and Coates (52), who were comparing the influence of dietary lactose on lactase activity in the intestines of germ-free and conventional chicks. The chicks were fed a diet that included either starch or lactose as the only carbohydrate. For the germ-free chicks there was very little lactase activity in either group, but for the conventional chicks there was lactase activity in both groups. However, lactase activity was much
larger in the birds receiving lactose as a dietary carbohydrate. Thus, the intestinal flora is responsible for enabling the chicks to digest lactose. In fact, germ-free chicks simply do not tolerate lactose, and many consuming it do not survive.

Even though the bacteria used to make yogurt normally do not include _L. acidophilus_ or _B. bifidum_, it is important to consider the benefits of this product in relation to lactose malabsorption in humans. In our laboratories we conducted a study to determine whether or not the presence of viable starter culture cells in yogurt would be beneficial for lactose malabsorption in humans (12). The yogurt was manufactured using milk supplemented with nonfat milk solids and a typical yogurt starter culture containing both _L. bulgaricus_ and _Streptococcus thermophilus_. Following the fermentation, the yogurt was cooled, mixed thoroughly, and divided into two portions, one of which was heated at 65°C for 3 min to ensure destruction of the starter culture bacteria and the other portion was left unheated. The breath hydrogen test (BHT) was used to evaluate the ability of the products to influence lactose utilization in human test subjects classified as lactose malabsorbers. The BHT provides a measure of lactose malabsorption and is based on the level of hydrogen excreted in the breath following ingestion of lactose. Each subject was tested using the heated and unheated yogurt as the test dose on 2 separate d. Comparison of the overall mean values obtained for breath hydrogen concentration revealed significantly lower breath hydrogen for the subjects tested after they consumed unheated than heated yogurt. These results were confirmed in a separate study by Kolars et al. (28).

A product such as yogurt is able to provide a benefit for lactose malabsorbers primarily because the starter culture bacteria contain the enzyme β-galactosidase. This enzyme, being intracellular, is able to survive passage through the stomach to reach the intestines. The yogurt starter bacteria, however, are not bile resistant and thus are not expected to survive and grow in the intestinal tract. However, the bile increases the permeability of the bacterial cells, which enables them to hydrolyze lactose more rapidly than would nongrowing cells.

Although the evidence strongly supports the fact that cultured yogurt is beneficial for persons unable to digest lactose adequately, it is an "acid tasting" product, which many people may not wish to include in their diet as a prominent source of milk products. In our research prior to investigating the benefits of yogurt on lactose malabsorption, we studied the benefits of a nonfermented acidophilus milk product (non acid tasting) on lactose malabsorption (27). The first series of experiments was to determine the influence of consuming the nonfermented acidophilus milk over time on the ability of lactose malabsorbers to digest lactose. Briefly, it involved the test subjects coming into the laboratory on d 0 and being subjected to the BHT using control milk (milk without cells of _L. acidophilus_) as a test dose. The individuals randomly assigned to the first group were then instructed to consume control milk on the daily basis on d 1 through 6 and on d 7 they returned to the laboratory for a second BHT using the control milk again as a test dose. Subjects in the second group were assigned milk containing cells of _L. acidophilus_ to be consumed daily; on d 7 they were brought back to the laboratory and the BHT was repeated using the milk containing cells of _L. acidophilus_ as a test dose. There were six test subjects in each group. The BHT results for the group consuming the control milk on d 0 and 7 were not significantly different, indicating that consuming milk daily did not affect their ability to digest lactose. For the group consuming the milk containing cells of _L. acidophilus_ (2.5 × 10⁸/ml), the mean level of hydrogen excreted on d 7, when they were retested using the milk containing cells of _L. acidophilus_, was significantly lower than obtained on d 0, indicating that the bacteria did improve the utilization of lactose. Results were similar using milk containing 2.5 × 10⁶ _L. acidophilus/ml_.

In another experiment involving a different group of test subjects classified as lactose malabsorbers, the individuals were tested over 21 d for breath hydrogen at 7-d intervals using different types of milk (27). On d 0 and 7, individuals were tested using control milk as a test dose for the BHT and on d 14 and 21, they were tested using milk containing 2.5 × 10⁶ cells of _L. acidophilus/ml_ as the test dose. They were instructed not to consume any milk products between the tests. There was no difference between the group averages on d 0 and 7 for which the control milk was used as the test.
acidophilus milk products

dose. Neither was there any significant difference between d 14 and 21 for which milk containing cells of \( L. \) acidophilus was used as a test dose. (These data confirm the value of the BHT for such experiments in that the same results were obtained on 2 different d using the same test dose for the same groups of individuals.) However, comparison of the overall averages for each type of milk revealed that the mean breath hydrogen value produced from the group consuming the milk containing cells of \( L. \) acidophilus was significantly lower than that for the group consuming the control milk as a test dose. This shows that the nonfermented acidophilus milk can have a benefit without having to be first consumed over a period of time.

The nonfermented acidophilus milk can improve lactose digestion by a mechanism similar to that observed for yogurt. The cells of \( L. \) acidophilus, if grown and prepared properly before being added to the milk, would contain \( \beta \)-galactosidase. In the intestines the presence of bile increases the ability of the cells to hydrolyze lactose in a manner similar to that observed with the yogurt cultures. Additionally, since \( L. \) acidophilus is bile resistant, the culture can grow in the intestinal tract and produce more of the enzyme.

The nonfermented acidophilus milk can have a definite advantage over cultured yogurt for improving lactose utilization in humans in that it is not an acid product and thus may be better accepted by many individuals. However, it is absolutely necessary that a culture of \( L. \) acidophilus be used that contains adequate levels of \( \beta \)-galactosidase and that the enzyme be stable during production and storage of the milk. Some reports have indicated that nonfermented milk containing cells of \( L. \) acidophilus is not beneficial for lactose maldigestors (37, 40, 49). However, in those studies, little information was given relative to the culture used or the manner in which it was handled during preparation and storage of the product prior to testing. Thus, it is possible that inadequate \( \beta \)-galactosidase activity was present in the culture when used. In fact, in one study (49) no \( \beta \)-galactosidase activity was detected in the acidophilus milk used in the feeding trial.

We have in a related study compared the levels of \( \beta \)-galactosidase activity in three different cultures of \( L. \) acidophilus and found them to possess different levels of the enzyme (13). Thus, it would be important to select the strain having the highest level of enzyme activity for use in a product to benefit individuals classified as lactose maldigestors. Because the milk is made by the addition of a frozen concentrated culture of the bacteria to refrigerated pasteurized milk, the organism must survive freezing and storage at frozen temperatures for extended periods prior to use. Furthermore, it must remain viable and maintain adequate enzyme activity during subsequent refrigerated storage of the nonfermented acidophilus milk. We have compared the stability of the enzyme activity in the three cultures of \( L. \) acidophilus during frozen storage at \(-196^\circ \)C (13). In all three cases, there was no significant decline in enzyme activity as a result of being frozen for 1 d. However, \( L. \) acidophilus NCFM did exhibit a significant decline in activity during 28 d of storage at \(-196^\circ \)C. Neither of the other two cultures exhibited any decline in enzyme activity during frozen storage. When the concentrated cultures were thawed on d 28 they were added to refrigerated milk to make nonfermented acidophilus milk, which was stored an additional 21 d at 5\(^\circ \)C. During this period, enzyme activity in the milk was monitored. \( Lactobacillus \) acidophilus strains 1 and RAM-1 exhibited no appreciable losses in enzyme activity during storage of the refrigerated milk; however, culture NCFM did decline significantly.

Control of Serum Cholesterol

For hypercholesterolemic individuals, significant reductions in plasma cholesterol levels are associated with significant reduction in risks of heart attacks (30). The intestinal microflora may influence serum cholesterol levels. Eyssen (6) reported that conventional animals excreted much higher levels of cholesterol in the feces than did germ free animals. Furthermore, germ-free animals on an elevated cholesterol diet accumulated approximately twice as much cholesterol in the blood as did conventional animals on a similar diet. This suggests that the intestinal microflora may interfere with cholesterol absorption from intestines. The types of microorganisms involved were not indicated.

Mann and Spoerry (32) conducted a study to evaluate the influence of surfactant compounds
such as Tween 20 on cholesterol absorption in man. Tween 20 was added to a fermented milk. Their theory was that the surfactant would increase the uptake of cholesterol from the milk in the intestinal tract. The trial involved 24 Maasai men who generally have a much lower incidence of coronary heart disease than observed in other populations in the world. The men were divided into two groups; one received a placebo (olive oil), the other group received the Tween 20. The compounds were added to milk that had been fermented with a "wild strain" of lactobacillus. The milk was consumed for 6 d (4 to 5 L per man/d). The results were a bit surprising. Because of the large intakes of milk, all men on the trial gained weight. However, serum cholesterol level in both groups decreased. The decline was greatest in those that gained the most weight. The conclusion from this study was that a factor was produced in the fermented milk that impaired cholesterol syntheses in the body, thus resulting in the reduced serum cholesterol levels. Neither the organism used to ferment the milk nor the factor responsible was identified.

Harrison and Peat (24) evaluated the effect of adding cells of L. acidophilus to infant formula on increases of serum cholesterol levels during the first 6 to 9 d of life. Three groups of infants (25 each) were included in the study. Treatments for the groups were as follows: 1) control, 2) 1.5 meq sodium bicarbonate per feeding added to the formula, and 3) formula supplemented with a culture of L. acidophilus. The serum cholesterol levels in the infants receiving the formula containing either L. acidophilus or the sodium bicarbonate decreased significantly during the experimental period. Decreases in the serum cholesterol level in both the groups were associated with increased numbers of lactobacilli in the stools obtained from the infants. The conclusion was that the bacteria acted on cholesterol in the intestines to reduce its absorption from the intestinal tract. This adds support to the theory that bacteria in the intestinal tract can and do influence serum cholesterol levels.

Using rats as an animal model, Grunewald (22) evaluated the influence of skimmed milk that had been fermented with L. acidophilus on serum cholesterol levels. One group received control milk and the other milk fermented with L. acidophilus. Both groups had free access to rat chow during the trials. After 4 wk, the rats that received the fermented milk had significantly lower serum cholesterol than did rats fed the control milk. She concluded that during fermentation of the milk some factor was produced by the L. acidophilus that resulted in lowering the serum cholesterol level after consuming the milk. However, no investigation was made to confirm this nor did she suggest what the compound might be.

Conclusions from most of the studies thus far mentioned have not considered the possible direct action of lactic acid bacteria on cholesterol on the intestinal tract. In our studies (14), we have shown that such action does occur: L. acidophilus, when grown under proper conditions, will remove cholesterol from laboratory media. The conditions required for uptake of cholesterol include an anaerobic environment and the presence of bile in the growth medium containing a source of cholesterol.

Because the assimilation of cholesterol by L. acidophilus required anaerobic conditions and the presence of bile, we theorized that the action should occur in the intestinal tract. To test this theory we selected young pigs as an animal model since their digestive system and blood circulatory system resemble those of humans. For this feeding trial, we used strains of L. acidophilus that had been isolated from the intestines of a pig since the organism tends to show host specificity.

To ensure selection of a culture that would most actively assimilate cholesterol, strains were screened for cholesterol uptake in MRS broth supplemented with 3% oxgall and 10% PPLO serum. The inoculated medium was incubated anaerobically at 37°C for 24 h. The resulting cells and spent broth were assayed for cholesterol content. Uninoculated broth was also assayed for cholesterol. The results indicated tremendous variation among strains with regard to the ability to assimilate cholesterol. Cholesterol concentrations in the cell suspensions ranged from 8.2 mg/ml for strain P47 to 29.8 mg/ml for strain RP43. Based on the concentrations of cholesterol in the control and spent broths, culture C1-5, C2-5, GP2B, and P47 assimilated minimal amounts of cholesterol in the broth.

Strain RP32, which assimilated a considerable amount of cholesterol, and strain P47, which assimilated little or none in laboratory
tests, were selected for the feeding trial. The objective of the feeding trial was to determine the effect of ingestion of cells of *L. acidophilus* on serum cholesterol levels of piglets fed a high cholesterol diet. Pigs housed in individual pens were randomly assigned to three treatment groups with six pigs per treatment. Following 1-wk dietary adjustment, all pigs were fed twice daily a diet supplemented with cholesterol (1000 mg/feeding). Additionally, group 1 (control) was given 50 ml milk/d. Group 2 was given 50 ml of milk containing $5 \times 10^{10}$ cell of *L. acidophilus* P47/d, and group 3 was given 50 ml of the milk containing $5 \times 10^{10}$ *L. acidophilus* RP32/d. Blood samples were taken by vena cava puncture during the experimental period on d 0, 5, and 10 and analyzed for total serum cholesterol using an enzymatic method.

Results from the analyses of the blood samples revealed that on d 0 the mean serum cholesterol levels for all treatment groups were between 52 and 56 mg/dl. On d 5, the mean serum cholesterol level for the group-fed cells of *L. acidophilus* RP32 was significantly lower than the mean values for either the pigs receiving strain P47 or the control pigs. On d 10 the mean value for the group receiving strain RP32 also was significantly lower than mean values for the other two groups. Thus, ingestion of selected strains of the organism can significantly reduce the extent to which serum cholesterol increases when swine are on a diet containing a high level of cholesterol. It may be possible to ingest cells of appropriately selected strains of *L. acidophilus* and have beneficial effect in helping control serum cholesterol levels in persons having abnormally high serum cholesterol levels.

Prior to testing the influence of *L. acidophilus* on serum cholesterol in humans it will be necessary to select a strain of human origin that most actively assimilates cholesterol. Preliminary screening of cultures of *L. acidophilus* originating from human intestinal tracts has been conducted in our laboratory (38). Ten strains were included in the initial screening. There was some variation among strains with regard to assimilation of cholesterol, although the variation was not as great as observed among isolates from the pig. None of the cultures was as active as strain RP32 isolated from a pig. Although all the strains included in this evaluation assimilated considerable amounts of cholesterol from the broth medium, we were not necessarily satisfied that we had the most desired strains for use in a human feeding trial. As a part of this study, we have also been interested in looking at other species of intestinal lactobacilli with regard to their ability to assimilate cholesterol. Four strains of *L. casei* exhibited considerable variation in the ability to assimilate cholesterol. Two of the strains, *L. casei* LN and *L. casei* 7, were isolated from human intestines. Strain 7 was significantly more active than the other strains in assimilating cholesterol. We also have shown in other experiments that *L. plantarum* possesses the capability for assimilating cholesterol.

Another activity exhibited by *L. acidophilus* that may influence cholesterol levels is its ability to deconjugate bile acid. We reported earlier (16) that various species of lactobacilli occurring in the intestinal tract deconjugate both taurocholic and glycocholic acids. Although the data from this study represented a limited number of strains from each species tested, it is interesting to note the considerable amount of variation among the organisms in their ability to deconjugate either or both of these bile acids. Such deconjugation activity becomes significant in relation to serum cholesterol levels when it is considered that deconjugated bile acids function more poorly in supporting absorption of lipids from the intestinal tract than do conjugated ones (6). This could result in reduced absorption of cholesterol from the intestines and thus influence serum cholesterol levels.

**Research Needs**

Development of adequate screening tests are needed to select strains of *L. acidophilus*, *B. bifidum*, or related organisms for use as dietary adjuncts. This would help ensure that the desired benefits would be realized when the product containing the dietary culture(s) is consumed.

If either the lactobacilli or the bifidobacteria are to be used effectively as dietary adjuncts, it is critical that proper strains of the organisms be selected for such use. Based on the possible benefits addressed in this paper, it is important that the strain(s) of the organism(s) to be utilized as a dietary adjunct possess the ability to achieve the desired purpose. Examples include
the control of enteric pathogens, assimilation of cholesterol, hydrolysis of lactose, production of anticarcinogenic activity, or others. Based on expected variation among strains of the lactic acid bacteria, it is not likely that any one strain of either organism would be best suited to achieve all of these purposes. However, the strain selected for a particular purpose should be selected on the basis of being able to produce that desired effect at a maximum level.

In addition to possessing the ability to produce the desired effect, it is important that the selected organism possess certain other desirable characteristics. One of these is the ability to grow and establish in the intestinal tract. Factors that must be considered here include the fact that the lactobacilli have been shown to be host specific (7, 11, 29, 36), and it is likely that the bifidobacteria also possess this characteristic. Tests are needed to characterize the cultures with respect to the host system in which they will best function.

If a strain of the selected organism is to be utilized for producing a desired function in the host, it is important that it possess the ability to compete with other lactobacilli that might occur naturally in the gut. One factor that may be important here is the production of bacteriocins by the lactobacilli. If the culture selected as a dietary adjunct possesses the ability to produce a desired function and also produces bacteriocins, it should have a greater chance of becoming established and growing in the intestinal tract.

Bile resistance is another factor important in enabling the lactobacilli or bifidobacteria to survive and grow in the intestinal tract. At present it is not known what level of bile resistance is needed; however, a previous study (17) has shown that a strain of *L. acidophilus* possessing a high level of bile resistance produced higher numbers of lactobacilli in the intestinal tract than did a strain having lower bile resistance. Investigations are needed concerning the degree of bile resistance needed by the dietary cultures.

In addition to possessing the desired characteristics, the organism selected for use as a dietary adjunct must maintain those characteristics during production and storage of the culture and, subsequently, the milk product. Storage stability studies should be included when considering the use of lactobacilli or bifidobacteria to produce a specific nutritional or health benefit.

Within the four areas of possible health or nutritional benefits from the consumption of lactobacilli or bifidobacteria addressed in this paper there are some specific areas of research that should be addressed. To control intestinal pathogens, it would be desirable to conduct challenge studies using selected enteric pathogens. These studies most likely would have to include the use of animal models and a number of different intestinal pathogens such as pathogenic species of *Salmonella*, *Staphylococcus*, *Clostridium*, *Campylobacter*, and *Yersinia*. Studies are also needed relative to the influence of the lactobacilli or bifidobacteria on viruses.

With regard to the production of anticarcinogenic activity, the mechanisms of action whereby the organisms promotes such activity should be elucidated and ways to enhance such activity should be investigated. This activity may possibly be related to the control of undesirable bacteria in the intestinal tract; thus these studies may be related to those designed to control intestinal pathogens.

If the *L. acidophilus* is to be used in a nonfermented milk product to benefit persons who cannot adequately digest lactose, it is important that investigations be made into the levels of β-galactosidase activity needed to provide optimum benefit. Similar investigations are needed with regard to bifidobacteria. It is also important that investigations be made into using the lactobacilli and bifidobacteria in products other than fluid milk, such as ice cream and dried milk products.

Research relating to the assimilation of cholesterol by *L. acidophilus* should better define the factors necessary for the assimilation process. Investigations are needed into the possible relationship between the ability of the organisms to deconjugate bile salts and the assimilation of cholesterol. A clearer understanding of the factors permitting the organism to assimilate cholesterol should make it possible to enhance such activity in the intestinal tract and, thus, make greater use of these organisms in aiding the control of serum cholesterol. Ultimately, of course, feeding trials using hypercholesterolemic individuals as test subjects are needed to determine if consuming strains of these organisms that assimilate cholesterol will be beneficial in lowering serum cholesterol levels.
As with many other bacteria, the lactobacilli and bifidobacteria possess plasmids. Research pertaining to the functions controlled by plasmids and the development of efficient plasmid transfer systems could lead to the development of improved cultures for use as dietary adjunct.

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