Role of dietary lactobacilli in gastrointestinal microecology

Khem M. Shahani, Ph.D. and Amadu D. Ayebo, Ph.D.

At birth the human intestine is full but contains no microbes. However, it soon becomes populated with microbes of various genera, depending on the type of food ingested. The so-called indigenous biota is derived first from the mother and then from the rest of the external world. This point was first supported by the pioneer observation of Tissier (3) that feces of breast-fed infants contained enormous numbers of gram-positive bacilli, subsequently referred to as lactobacilli. Several studies (4) have confirmed that lactobacilli constitute one of the dominant groups of intestinal and fecal organisms (Table 1). Metchnikoff (5) postulated that intestinal bacteria produce putrefactive organisms could be minimized or prevented by establishing the proper lactobacillus flora in the gut. He believed that the harmful effects of putrefactive organisms could be minimized or prevented by establishing the proper lactobacillus flora in the gut. This consideration has contributed significantly to the popular interest in yogurt and other cultured milk products with the thought that such products may help a variety of gastrointestinal disorders.

Despite the inherent stability of the intestinal microflora, factors such as health, nutrition, oral administration of antibiotics, and possibly other factors not currently recognized do alter the intestinal flora. Accordingly, current research in intestinal microecology is geared toward better understanding of the interrelationships between intestinal flora and physiology, diet, nutrition and health of the host.

Effect of gut flora in nutrition

Experience with germ-free animals clearly demonstrates that intestinal flora is not essential for life (6–8). Animals raised in clean surroundings have a relatively simple gut flora and actually utilize their nutrients more efficiently (9). However, diets deficient in certain basic nutrients result in pronounced symptoms on germ-free animals compared to conventional animals (10). Feeding germ-free rats folate-deficient diet resulted in the development of folate deficiency symptoms, while the conventional rats showed no deficiency symptoms. Deficiency symptoms could apparently be prevented by the association of germ-free animals with any of a variety of enteric bacteria. Lactobacilli along with other enterobacteria are able to synthesize a complex variety of folate coenzymes that is very similar to dietary folate (11).

Shimada et al. (12) in a review noted that anaerobic microbes such as Bacteroides, Bifidobacterium, lactobacilli, and clostridia appear to be responsible for the deconjugation of bile acids in the intestinal tract. Gilliland and Speck (13) noted that lactobacilli isolated from intestinal contents possess the ability to grow on media containing bile, a property which partly accounts for their survival in the intestine.

The beneficial role of lactobacilli to the breast-fed infants has been related to the lower incidence of colic and other digestive disturbances in such infants. Robinson and Thompson (14) observed that infants partially nursed, even for 2 or 3 days postnatally, showed significantly greater weight gains during the 1st month than infants completely bottle-fed. Breast-fed infants developed a stable microflora consisting of about 99% Lactobacillus bifidus within 3 or 4 days. The authors also noted that bottle-fed infants on formulas supplemented with Lactobacillus

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acidophilus showed greater weight gains than control subjects. Mata et al. (15) clearly demonstrated that the colonization by bifidobacteria in breast-fed infants resulted in a lower incidence of shigellosis and other enteric diseases.

The significance of lactobacilli in gastroenterology is measured by the distribution of the organism in the intestinal tract and its metabolic contribution to the general health of the host. The ability of the organism to grow rapidly and tolerate the acid environment explains its predominance in the stomach.

**General characteristics of lactobacilli**

Lactobacilli vary in morphology from long slender rods to short coccobacilli, which frequently form chains (16). Metabolism is fermentative; some species are aerotolerant and may utilize O₂ via flavoprotein oxidase, while others are strictly anaerobic. Glucose fermentation produces at least 50% of the end-product carbon as lactic acid. Other products include acetate, formate, succinate, CO₂, and ethanol. By and large, growth is optimum at pH 5.5 to 5.8. Lactobacilli have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids, and carbohydrates. Growth on solid media is often improved by anaerobiosis with a 5 to 10% CO₂ atmosphere. The genus is subdivided into three groups based on fermentation patterns: 1) homofermentative—produce more than 85% lactic acid from glucose, 2) heterofermentative—produce only 50% lactic acid and considerable amounts of acetic acid, ethanol, and CO₂, 3) less well known heterofermentative species—these produce α-L-lactic acid, CO₂, and acetic acid.

**Intestinal implantation of lactobacilli**

Early attempts to implant lactobacilli in the intestine to help alleviate various gastrointestinal disorders were made using yogurt and cultures of Lactobacillus bulgaricus. Cohendy (17, 18) observed that feeding milk soured by L. bulgaricus to subjects with intestinal putrefaction when on normal diet resulted in decreased putrefaction, their stools became normal, and showed relatively high L. bulgaricus counts in the stools. The organism appeared in the stools 8 days after initiation of treatment and persisted for at least 12 days. However, Rahe (19) questioned the ability of L. bulgaricus to establish in the intestine and noted that even though the organism showed limited period of survival in the small intestine, this was of little value to the host. It, therefore, became apparent that care should be taken in choosing lactic bacteria for dietary supplementation. Morishita et al. (20) observed that a L. acidophilus isolated from a human infant failed to establish when administered to chickens, while a strain of L. acidophilus isolated from chicken could be established in the digestive tract of germ-free chickens. The authors therefore concluded that the strains of L. acidophilus investigated have specific affinity for each animal species.

Bacteria used for dietary supplementation should possess certain basic qualities: 1) they should be normal inhabitants of the host intestine or be capable of adapting to the host intestinal environment; 2) they must survive passage into the intestine and be capable of establishing in the intestine, particularly the small intestine where physiological activities associated with digestion and absorption of nutrients would be expected to occur; 3) they must perform functions that are advantageous to the host; and 4) the addition of the culture should not be detrimental to the quality of the food, and neither should the food nor its treatment harm the culture.

L. bifidus (Bifidobacterium bifidum), L. acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus salivaroes, Lactobacillus brevis, Lactobacillus leichmannii, Lactobacillus plantarum, and Lactobacillus cellobiosus, normal inhabitants of human intestinal tract, possess most of the qualities described and most are usually considered for dietary supplementation (21). The species most often used as dietary supplements are L. acidophilus, L. casei, and L. bifidus (22, 23).

**TABLE 1**

Viable fecal flora in men and mice

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Clostridium perfringens</th>
<th>Strepto-cocci</th>
<th>Bacteroides</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>10₀⁻¹⁰⁶</td>
<td>0⁻¹⁰¹</td>
<td>10⁰⁻¹⁰⁷</td>
<td>10⁻¹⁰⁻¹⁰⁵</td>
<td>10⁻⁰⁻¹⁰⁶</td>
</tr>
<tr>
<td>Men</td>
<td>10⁻¹⁰⁻¹⁰⁶</td>
<td>0⁻¹⁰¹</td>
<td>10⁻¹⁰⁻¹⁰⁵</td>
<td>10⁻¹⁰⁻¹⁰⁷</td>
<td>10⁻¹⁰⁻¹⁰⁶</td>
</tr>
</tbody>
</table>

* Adapted from Smith and Crabb (4).
Nutritional and therapeutic aspects of lactobacilli

Nutritionally, lactobacilli have been associated with 1) the synthesis of B vitamins, 2) the elaboration of enzymes resulting in the partial hydrolysis of milk proteins, fat, and lactose, thus increasing the digestibility of culture containing products, 3) anticholesteremic effect, and 4) alleviation of lactose intolerance. Several of these factors have been examined extensively in our laboratory (24).

Synthesis of B-vitamins

As early as 1960 Shahani et al. (25) observed marked increases in concentrations of the B-complex vitamins (niacin, pantothenic acid, pyridoxine, biotin, folic acid) in 30 varieties of cheeses and processed cheeses, indicating that the lactic cultures synthesized B vitamins during ripening. Reif (26) also reported increased niacin, B6, B12, and folic acid synthesis by lactic acid organisms used in cheese manufacture (Table 2). Reddy et al. (27) observed that cultured products generally contain slightly higher levels of B-vitamins compared to directly acidified products.

Improved digestibility of food constituents

Although a cultured product such as yogurt is similar in caloric value to the milk from which it was made, yogurt has been reported to be more easily digestible, with improved biological value (28, 29). The improved nutritional quality has been attributed to the predigestion of milk components by enzymes elaborated by the lactic acid bacteria (24). The lipolytic and proteolytic activities of various lactic cultures were investigated by Chandan et al. (30), and they observed that lactic cultures possessed higher lipolytic and proteolytic activities.

Table 2

<table>
<thead>
<tr>
<th>Product</th>
<th>Folic acid</th>
<th>Biotin</th>
<th>Niacin</th>
<th>Pantothenic acid</th>
<th>B6</th>
<th>B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>0.13-0.73</td>
<td>2.9-4.9</td>
<td>71-96</td>
<td>330-460</td>
<td>17-40</td>
<td>0.27-0.57</td>
</tr>
<tr>
<td>Yogurt</td>
<td>3.9</td>
<td>4.0-5.1</td>
<td>130-141</td>
<td>280-381</td>
<td>0.35-0.52</td>
<td></td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>2.3-5.0</td>
<td>3.2</td>
<td>70-257</td>
<td>463</td>
<td>24-56</td>
<td>0.8-2.1</td>
</tr>
<tr>
<td>Sour cream cultured</td>
<td>10.8</td>
<td>2.6</td>
<td>11-67</td>
<td>320-360</td>
<td>16</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Cheddar cheese</td>
<td>4.21</td>
<td>0.65-2.5</td>
<td>13-212</td>
<td>111-711</td>
<td>49-147</td>
<td></td>
</tr>
</tbody>
</table>

* Adapted from Shahani and Chandan (24). * μg/100 g or μg/ml.

Lactose intolerance

Deficiency of the enzyme lactase or β-galactosidase in humans results in the inefficient utilization of milk sugar lactose, a condition known as lactose intolerance. The ability of lactobacilli to produce the lactase enzyme during the fermentation process has led to the advocacy of their use in cultured dairy foods. In experiments to determine lactase activity of cultured and acidified dairy foods, Kilara and Shahani (31) observed that cultured yogurt possessed considerable lactase activity due to the inherent production of the endoenzyme lactase by the culture.

Anticholesteremic effect of yogurt and lactobacilli

There exists considerable evidence that dairy foods possess antihypercholesterolemic factors. Mann and Spoerry (32) reported that despite consumption of large quantities of saturated fat and cholesterol through fermented milk and meat, the Massai tribesmen of Africa had fairly low serum cholesterol levels. Mann and his associates subsequently postulated that the effective agent in yogurt and milk is hydroxymethylglutarate which inhibits hydroxymethylglutaryl CoA reductase, the regulatory enzyme in cholesterol synthesis (33, 34). Hepner et al. (35) in a recent study to determine the effect of dietary supplementation of yogurt on serum cholesterol and triglycerides of human volunteers observed significant reduction in serum cholesterol levels after 1 week.

Therapeutic properties of lactobacilli

Kopeloff (36) and Retter et al. (37) reported on studies describing the use of L. acidophilus in the therapy of various intestinal illnesses. However, the initial interest in the
use of these organisms to alleviate certain gastrointestinal maladies soon diminished due to lack of consistent results. It is evident now that the initial enthusiasm far exceeded the basic scientific understanding that was necessary for successful preparation and administration of the organism. Attention was not paid to cell viability and culture identity, which consequently resulted in lack of consistency. Recently, interest in intestinal microflora and its effects on health has been rekindled by improved knowledge of the normal intestinal microflora of humans of all ages (38, 39) and the factors affecting viability and establishment of lactobacilli in the intestine.

Production of antimicrobial agents

The species of lactobacilli most often suggested as beneficial dietary supplements have all been reported to exert antagonistic actions toward such organisms as enteropathogenic Escherichia coli, Salmonella typhimurium, Clostridium perfringens, and Staphylococcus aureus (40, 41). L. casei has been shown to inhibit Salmonella species, Vibrio species, Escherichia coli and Staphylococcus aureus (42), and L. bifidus exerts similar actions against E. coli, Shigella species and S. typhimurium in vitro (43). Even though the mechanism and the exact nature of the inhibitory principle in each case is not completely understood, numerous workers have reported isolating natural antibiotics from lactic acid bacteria (40, 41). The production of natural antibiotics by lactic cultures has been evaluated in our laboratory. Shahani et al. (41) isolated acidophilin from Lactobacillus acidophilus and bulgarican from Lactobacillus bulgaricus. These natural antibiotics were shown to be inhibitory to a number of pathogenic and nonpathogenic bacteria. Besides antibiotics, several species of lactobacilli produce hydrogen peroxide (44, 45), fermentation end products (lactic acid, acetic acid) and other factors not presently elucidated which are reported to be inhibitory to other microorganisms. L. acidophilus and Lactobacillus lactis have been shown to produce enough hydrogen peroxide to inhibit S. aureus (46) even at low temperatures (5 C). Similarly, Lactobacillus plantarum, L. bulgaricus or lactic streptococci produce enough hydrogen peroxide in refrigerated milk to retard growth of psychotropic bacteria (47).

Anticarcinogenic effects

Earlier observation by Bogdanov et al. (48) linking L. bulgaricus with antitumor activity has led to studies utilizing cultured dairy products for possible regression of certain tumors. Reddy et al. (49) and Farmer et al. (50) investigated the inhibitory effect of yogurt on the proliferation of Ehrlich ascites tumor cells in male Swiss mice. They observed that feeding yogurt resulted in 28 to 35% reduction in the number of tumor cells when compared to another group fed milk. Bailey and Shahani (51) reported that feeding milk and colostrum fermented with L. acidophilus DDS; resulted in 16 to 41% reduction in tumor proliferation. To elucidate the nature of these tumor inhibitory factors of yogurt, Ayebo (52) reported that yogurt dialyze rather than retentate fraction possessed the antitumor principle(s) (Table 3). Upon further purification, using ion exchanged chromatography (Fig. 1) the anionic fraction of yogurt dialyze was observed to cause significant inhibition of ascites tumor proliferation in vivo (Table 4).

There exists a basic relationship between intestinal bacteria, their metabolic products, and the nutrition and health of the host. Goldin and Gorbach (53) observed that supplementation of the normal diet of rats with L. acidophilus lowered the activity of fecal nitroreductase, azo-reductase, β-glucuronidase. The significance of these enzymes should be considered in the light of their involvement in chemical carcinogenesis (54).

Development of lactobacilli for dietary use

Acidophilus milk (milk fermented using L. acidophilus) is by nature somewhat sour with

TABLE 3

<table>
<thead>
<tr>
<th>Series</th>
<th>Control (millions/mouse)</th>
<th>Experimental (millions/mouse)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.3</td>
<td>13.6</td>
<td>32.9</td>
</tr>
<tr>
<td>2</td>
<td>16.9</td>
<td>11.5</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Each series is a mean of three replicates. Significant (P ≤ 0.05).
the limited consumer appeal and limited culture viability (24, 55) due to processing and storage. Attempts to improve viability by addition of L. acidophilus to other cultures did not produce any promising results, since the organism failed to grow optimally when in association with other lactic cultures. To overcome the undesirable flavor and improve viability of the organism in acidophilus milk, recent advances in the development of frozen concentrates and freeze-drying have facilitated the addition of viable acidophilus cells.
to unfermented low fat milk (56, 57). The product does not ferment as long as the milk is held under refrigeration (4 to 10°C) during manufacture, distribution, and storage. When manufactured with the appropriate strain of *L. acidophilus*, the consumer enjoys the flavor of milk, while also ingesting the viable culture. Several investigators have used different concentrations of *L. acidophilus* in the preparation of nonfermented culture containing milk. Myers (58) reported that adding about 34 × 10⁷ cells/ml of *L. acidophilus* to pasteurized milk resulted in a product which retained the acceptable flavor of milk for 7 days when stored below 10°C, and upon feeding, the viable culture was implanted in the human intestine. Ayebo et al. (59) used a similar acceptable product containing 4 billion *L. acidophilus* DDS₁ cells/liter in a human dietary supplementation trial with very satisfactory results. The ingestion of 10⁶ to 10⁸ viable *L. acidophilus* cells daily has been suggested as the most satisfactory level since excessive numbers may induce mild gastrointestinal disturbances (23).

The nutritional and therapeutic qualities of unfermented milk containing *L. acidophilus* organisms have been examined in our laboratory. Sinha (60) observed that feeding nonfermented acidophilus milk to mice infected with Ehrlich ascites tumor cells significantly reduced the growth of the tumor. The degree of tumor inhibition was similar to that reported when yogurt was fed.

Ayebo et al. (59) reported that supplementation of the normal diet of human subjects with unfermented acidophilus milk resulted in reduced fecal β-glucosidase and β-glucuronidase activities. In a study using rats, Sinha (60) also observed reduced activities of these enzymes when unfermented acidophilus milk was added to rat diet (Figs. 2 and 3). The addition of viable cells of lactic acid bacteria, especially *L. acidophilus* in nonfermented milk, besides providing a product with the “sweet flavor” of milk also has the improved nutritional and therapeutic qualities of fermented milk products.

**Significance of lactobacilli in relation to other intestinal microorganisms**

Based upon Metchnikoff's (5) hypothesis of replacing "undesirable" types of biota with lactobacilli, early attempts were made to establish *L. acidophilus* as the dominant species in the intestine. Now interest has shifted...
slightly to the use of lactobacilli to maintain its predominance, without necessarily interfering with the other flora in the interest of general health. In studies using laboratory animals, an intimate association between anaerobic streptococci, lactobacilli, fusiform bacteria, and yeasts in certain areas of gastrointestinal epithelium has been demonstrated (61–63), thus, emphasizing the importance of a balanced microbial population in healthy animals. Gilliland et al. (64) observed that ingestion of low fat milk containing \textit{L. acidophilus} by human subjects resulted in a slight increase in the total number of facultative lactobacilli in their feces and a shift in the types of lactobacilli. These authors did not observe any decrease in coliform counts. Decrease in intestinal \textit{E. coli} population is significant because these organisms have been reported to synthesize ethionine (65) and nitrosamine from nitrates and nitrites (66), compounds that are known to be carcinogenic (67, 68). Sinha (60), feeding unfermented milk containing \textit{L. acidophilus} DDS₁ to rats, observed increased acidophilus counts, with a concomitant decrease in coliform counts in the intestine (Figs. 4 and 5). Muralidhara et al. (1) made similar observations when they fed lactobacilli to piglets, while Tanami (42) also observed decreasing intestinal \textit{E. coli} counts when \textit{L. acidophilus} was fed to specific pathogen free chickens (Fig. 6). In a study supplementing the regular diet of human subjects with low fat milk containing viable culture of \textit{L. acidophilus}, we observed that the total lactobacilli counts increased during the period of supplementation and remained at that level for at least 4 weeks after culture supplementation was discontinued. The total coliform count, during acidophilus supplementation, decreased; however, upon discontinuation of acidophilus supplementation, the coliform counts increased again to prestudy levels (59).

There is a growing consensus on the beneficial attributes of lactobacilli in human and animal nutrition. However, for consistent and positive results, investigators should be encouraged to first understand the microbiology of the cultures they use. Data now being evolved on culture identity, viability when ingested, possible implantation criteria, and

![Figure 4](https://example.com/figure4.png)

**FIG. 4.** Effect of feeding nonfermented acidophilus milk (experimental) and low fat milk containing 1% fat (control) on the lactobacilli count in the feces of Sprague-Dawley rats.
FIG. 5. Effect of feeding nonfermented acidophilus milk (experimental) and low fat milk containing 1% fat (control) on the coliform count in the feces of Sprague-Dawley rats.

FIG. 6. Change of fecal flora of specific pathogen free chickens which survived after simultaneous administration of *L. acidophilus* and *E. coli*. (From Reference 42.)
other qualities of the organisms will help in the choice of the best suitable cultures.

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LACTOBACILLI IN GASTROINTESTINAL MICROECOLOGY

47. **SHAHANI AND POUPARD.** Inhibitory effect of yogurt components.


