Some Beneficial Effects of Probiotic Bacteria

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A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus*, bifidobacteria, and *Lactobacillus casei*. Because of the potential health benefits, these organisms are increasingly incorporated into dairy foods. A number of health benefits have been claimed including antimicrobial, antimutagenic and anticarcinogenic properties, reduction in serum cholesterol, improvement in lactose tolerance in lactose intolerant people and adherence to intestinal cells. This review will cover some health benefits of probiotic bacteria.

Key words: *Lactobacillus acidophilus*; bifidobacteria; beneficial effects; mutagens; adherence; lactose intolerance

INTRODUCTION

The health benefits derived by the consumption of acidophilus and bifidus products (called AB products) are well documented and more than 90 probiotic products are available worldwide. Probiotic food can be defined as “food containing live microorganisms believed to actively enhance health by improving the balance of microflora in the gut” (6, 7).

Yogurt containing *Lactobacillus acidophilus* and bifidobacteria is referred to as ‘AB’ yogurt. The trend is to incorporate *L. casei* in addition to *L. acidophilus* and bifidobacteria and such products are known as ‘ABC’ yogurt (47). Traditionally, yogurt is manufactured using *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* as starter cultures. These yogurt organisms have been claimed to offer some health benefits; however, they are not natural inhabitants of intestine and cannot survive under acidic conditions and bile concentrations usually encountered in the gastrointestinal tract. Therefore, for yogurt to be considered as a probiotic product, *L. acidophilus* and bifidobacteria (and *L. casei* or both) are incorporated as dietary adjuncts. Fermented milk with only *L. acidophilus* and bifidobacteria could be manufactured; however, the longer incubation period and product quality are the two main factors that are sacrificed when fermenting milk with only ‘AB’ or ‘ABC’ bacteria. Thus, the normal practice is to make product with both yogurt and probiotic bacteria.

A number of health benefits have been claimed for *L. acidophilus* and bifidobacteria. Because of the potential health benefits, these organisms are increasingly incorporated into dairy products. It seems reasonable to assume that the beneficial effects of probiotic bacteria can be expected only when viable cells are ingested. This paper will focus on some beneficial effects of probiotic bacteria.

BENEFICIAL EFFECTS OF PROBIOTIC PRODUCTS

Metchnikoff (31) in his fascinating treatise “The Prolongation of Life” propounded the theory that the longevity of Bulgarians was in part due to ingesting large quantities of fermented milks containing lactobacilli. This observation has led to burgeoning activity on the elucidation of the role of lactic acid cultures and cultured milk products in alleviation of human and animal disorders. The benefits offered by these *L. acidophilus* and bifidobacteria include improvement in intestinal disorders and lactose tolerance, antimicrobial properties, reduction in serum cholesterol, antimutagenic and anti-carcinogenic activities and adherence to intestinal cells.

*Lactobacilli* and bifidobacteria establish an intimate relationship with humans from the time of birth and this relationship continues throughout life. The digestive tract is colonised soon after birth by a variety of microorganisms. *Lactobacilli* commonly encountered in the intestinal tract include *L. acidophilus*, *L. leichmanii*, *L. plantarum*, *L. casei* and *L. fermentum*. The organisms often used as beneficial for dietary adjuncts are *L. acidophilus*, *Bifidobacterium* spp., and *L. casei* (8, 33).

There is sufficient experimental evidence to support that oral administration of lactobacilli (preferably *L. acidophilus*) and bifidobacteria is able to restore the normal balance of microbial population in the intestine...
Table 1. Therapeutic properties of acidophilus and bifidus milk products.

<table>
<thead>
<tr>
<th>No.</th>
<th>Therapeutic properties</th>
<th>Possible causes and mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colonisation of gut and inhibition of spoilage types organisms</td>
<td>Survive gastric acid, resist lysozyme, tolerate high bile concentration, adhere to intestinal surface and production of inhibitory compounds i.e. acids, H₂O₂ and bacteriocins.</td>
</tr>
<tr>
<td>2</td>
<td>Improved digestibility and enhanced growth</td>
<td>Partial breakdown of protein, fat, carbohydrates and improved bioavailability of nutrients.</td>
</tr>
<tr>
<td>3</td>
<td>Lactose tolerance</td>
<td>Reduced lactose in the product and further availability of bacterial lactase enzymes for lactose hydrolysis.</td>
</tr>
<tr>
<td>4</td>
<td>Hypocholesterolaemic effect</td>
<td>Production of inhibitors of cholesterol synthesis, deconjugation of biles, assimilation of cholesterol.</td>
</tr>
<tr>
<td>5</td>
<td>Anticarcinogenic effect</td>
<td>Inhibition of carcinogens and enzymes involved in converting procarcinogens to carcinogens, inhibition of growth of putrefying organisms and stimulation of host immune system.</td>
</tr>
<tr>
<td>6</td>
<td>Stimulation of the host immunological system</td>
<td>Enhancement of macrophage formation, stimulation of suppressor cells and production of interferon.</td>
</tr>
<tr>
<td>7</td>
<td>Control of vaginal infections</td>
<td>Inhibition of fungi and bacteria responsible for the infection.</td>
</tr>
<tr>
<td>8</td>
<td>Increased vitamin contents</td>
<td>Synthesis of group B vitamins.</td>
</tr>
<tr>
<td>9</td>
<td>Prevention of constipation</td>
<td>Improvement in bowel movement and stabilisation of ecological balance in the intestinal tract.</td>
</tr>
<tr>
<td>10</td>
<td>Recovery of damaged liver, effectiveness against aspects of nausea, liver diseases, acne, etc.</td>
<td>Reduction in concentration of metabolites and enterotoxins produced by putrefying organisms.</td>
</tr>
<tr>
<td>11</td>
<td>Antihypersensitivity</td>
<td>Yet to be established.</td>
</tr>
<tr>
<td>12</td>
<td>Prolongation of life</td>
<td>Reduced intestinal putrefaction and auto-intoxication.</td>
</tr>
</tbody>
</table>

(Sources: Refs. 5, 17, 18, 21, 24, 31, 32, 36, 38, 52, 54).

(12, 42). In addition to their established role in gastrointestinal therapy, L. acidophilus and bifidobacteria are also claimed to offer several other nutritional and therapeutic benefits. Several authors have reviewed and reported the therapeutic roles and importance of these bacteria in detail (18, 21, 24, 32, 36, 38, 52, 53). Some of these benefits are summarised in Table 1 and discussed below.

Antimicrobial and Antimutagenic Properties of Probiotic Bacteria

With the emergence of antibiotic-resistant bacteria and natural ways of suppressing pathogens, the concept of ‘probiotics’ has attracted much attention. The belief in the beneficial effect of probiotic approach is based on the knowledge that the intestinal microflora provides protection against various diseases.

Antimicrobial properties. Microorganisms that are considered probiotic must have several important characteristics including their ability to produce antimicrobial substances such as organic acids (e.g., lactic and acetic acids), hydrogen peroxide and bacteriocins to suppress the multiplication of pathogenic and putrefying bacteria (26). Because of these virtues, these probiotic bacteria show strong antimicrobial properties against Gram +ve bacteria such as Staphylococcus aureus, Clostridium perfringens than against Gram –ve bacteria such as Salmonella typhimurium and Escherichia coli. Hydrogen peroxide in the presence of organic acids such as lactic acids is more inhibitory to bacteria (23).

These probiotic bacteria produce several organic acids. Acetic, lactic and pyruvic acids are the main acids. Other acids produced in small quantities include citric acid, hippuric acid, orotic acid and uric acid (23). Lactic and acetic acids are the major acids produced and these acids account for >90% of the acids produced. Several researchers believe that lactic acid is the only antimicrobial agent of any importance. Lowering of pH due to lactic acid or acetic acid produced by these bac-
BENEFICIAL EFFECTS OF BACTERIA

Two types of lactic acid, L(+) and D(−) are produced during fermentation by lactic acid bacteria. Some species of bacteria such as L. delbrueckii ssp. bulgaricus and Lactococcus lactis produce only D(−) lactic acid, whereas some lactic streptococci and L. casei produce L(+) lactic acid. Lactobacillus helveticus and L. acidophilus produce a racemic mixture of L(+) and D(−) lactic acid. D(−) lactic acid is not metabolised to pyruvic acid in the body due to lack of D2-hydroxy acid dehydrogenase and this results in acidosis in neonatal infants. L(+) isomer is completely harmless.

Bifidobacteria produce L(+) lactic acid. Thus the lactic acid produced by bifidobacteria is easily metabolised, while providing antimicrobial properties.

Antimutagenic properties. Antimutagenic activity of fermented milk has been demonstrated in vitro against a large spectrum of mutagens including 4-nitroquinoline-N'-oxide, 2-nitrofluorene, and benzopyrene. Antimutagenic effect of fermented milks has been detected against a range of mutagens and promutagens in various test systems based on microbial and mammalian cells (2). Epidemiological evidences have indicated a negative correlation between the incidences of certain cancers and consumption of fermented milk products (40).

Some strains of L. acidophilus and bifidobacteria have been reported to show antimutagenic and anticarcinogenic properties. The evidence of anticancer effect can be due to decrease in faecal enzymes involved in conversion of procarcinogens to carcinogens. These probiotic bacteria also lower levels of harmful enzymes such as β-glucosidase and β-glucuronidase responsible for catalysing the conversion of harmful amines (25).

The mechanism of antimutagenicity of probiotic bacteria have not been understood or identified so far and the mechanism of antimutagenicity remains speculative. It has been suggested that microbial binding of mutagens could be the possible mechanism of antimutagenicity (40).

Lankaputhra and Shah (23) studied the levels of acetic, butyric, lactic and pyruvic acids produced by the probiotic bacteria as determined by HPLC. All strains produced acetic, lactic and pyruvic acids. Butyric acid was produced by most of the strains of L. acidophilus and bifidobacteria. Lactic acid and acetic acid accounted for >90% of organic acid produced. Other acids produced in small quantities were citric, hippuric, orotic...
and uric acid.

Lankaputhra and Shah (23) studied the antimutagenic activity of these acids against eight mutagens and promutagens including N-methyl, N'-nitro, N-nitrosoguanidine (MNNG); 2-nitrofluorene (NF); 4-nitro-O-phenylenediamine (NPD); 4-nitroquinoline-N-oxide (NQO); Aflatoxin-B (AFTB); 2-amino-3-methyl-3H-imidazoquinoline (AMIQ); 2-amino-1-methyl-6-phenyl-imidazo(4,5-6)pyridine (AMPIP), and 2-amino-3-methyl-9H-pyrido(3,3-6)indole (AMPI). MNNG is one of the most potent direct chemical mutagen. NF, NPD and NQO are chemical pro-mutagens, which need metabolic activation for mutagenicity. AFTB is a diet related potent mutagen produced by a fungal strain of Aspergillus flavus. Aspergillus flavus is a major food contaminant species prevalent in most Asian countries. AMIQ, AMPIP and AMPI are heterocyclic amine mutagens. These are major mutagens formed in heat processed beef in Western diet. TA-100 mutant of S. typhimurium (His') strain is used as mutagenicity indicator organism. Mutagenicity test is carried out using the Ames Salmonella test. Antimutagenic activity of acetic, butyric, lactic, and pyruvic acids against the 8 mutagens or promutagens is shown in Fig. 1. Acetic acid showed higher antimutagenic activity than lactic or pyruvic acids against NQO, NF and NPD, whereas butyric acid showed the highest antimutagenic activity against all the 8 mutagens or promutagens studied. Lactic and pyruvic acids showed lower antimutagenic activities against all the mutagens studied except NQO. Thus it appears that lactic acid produced by lactic acid bacteria plays a minor role in antimutagenic activity. Butyric acid is mainly produced by probiotic bacteria and these bacteria are likely to provide antimutagenic properties through the production of butyric acid.

Butyric acid is claimed to prevent carcinogenic effects at molecular (DNA) level (49, 50, 54). Yanagi et al. (54) reported that addition of butyric acid to a diet containing 20% margarine prevented mammary tumour formation by 7,12-dimethylbenz(a)anthracene in rats. Thus, it appears that antimutagenic effects of probiotic bacteria may be due to both inhibition by bacterial cells and production of organic acids, especially butyric acid.

In general, live probiotic bacterial cells showed higher antimutagenicity against the mutagens studied. This suggests that live bacterial cells may metabolise or bind the mutagens. Inhibition of mutagens and promutagens by probiotic bacteria appeared to be permanent for live cells and temporary for killed cells. Killed cells released mutagens and promutagens when extracted with dimethyl-sulfoxide. The results emphasised the importance of consuming live probiotic bacteria and of maintaining their viability in the intestine in order to provide efficient inhibition of mutagens.

Anticarcinogenic Activity

Lactic acid bacteria and fermented products made from lactic acid bacteria have potential anticarcinogenic activity (17, 34). Anti-tumor effect of L. acidophilus was reported by Goldin and Gorbach (13, 14). Oral supplementation of diet containing viable cells of L. acidophilus decreased bacterial enzymes, β-glucuronidase, azoreductase, and nitroreductase. These enzymes catalyse conversion of procarcinogens to carcinogens. Potential anticarcinogenic effects of bifidobacteria may be possibly due to the result of one of the two mechanisms. The first mechanism is claimed to be due to direct removal of procarcinogens, and the second indirect removal of procarcinogens or activation of the body’s immune system.

Direct removal of procarcinogens by probiotic bacteria may involve reduction in the rate at which nitrosamines are produced. Probiotic bacteria may remove the sources of procarcinogens or the enzymes that lead to the formation of carcinogens. It has been shown that probiotic bacteria can greatly reduce the mutagenicity of nitrosamines. This may be due to the fact that certain species of probiotic bacteria, such as B. breve, have high absorbing properties for carcinogens, such as produced upon charring of meat products (17, 34). A reduction in excreted carcinogens and that in bacterial procarcinogenic enzymes has been observed in mice when fed with B. breve 4006 and fructooligosaccharides.

Mitsuoka (34) observed large numbers of liver tumours that developed in mice when the intestinal flora contained Escherichia coli, Enterococcus faecalis and Clostridium paraputrificum. However, in the presence of probiotic bacteria, the proliferation of tumours decreased considerably. Even in the presence of undesirable bacteria, there was decrease in the number of tumours. Tumour suppression via the body’s immune response system was studied by Sekine et al. (44). Injection of cell wall fractions into growing tumours caused regression of the tumours and activation of the immune response. Cell wall fractions of B. infantis are claimed to contain active antitumor constituents (31). The enhancement in body’s defences may be due to increased production of IgA antibody by probiotic bacteria.

Anticarcinogenic effect of cultured milk products using 71 strains of lactic acid bacteria, including L. aci-
of Lactobacillus and Bifidobacterium, on the mutagenicity of N-methyl-N'-nitro-N-nitrosoguanidine was investigated. Milk cultured with L. acidophilus LA 106 (LA2) showed the highest inhibition (16). Bifidobacterium longum and B. infantis are cited as being effective antitumor agents. Their mode of action may be by suppression of bacterial enzymes, which form them or by activation of the host immune system and/or reduction of intestinal pH. Activation of macrophages would be expected to have an antitumor effect. Macrophages play a significant role in the immune response to tumours and macrophages have been observed clustering around tumours.

Presence of B. longum in the gut of gnotobiotic C3H/He male mice has been found to reduce liver tumours (35). The effect is claimed to be due to stimulation of immune response of the host, or due to decreasing the activity of some faecal bacterial enzymes by bifidobacteria. However, further research is needed in this area and more evidence is required to verify these claims.

Reduction in Serum Cholesterol

Cholesterol lowering effects of fermented milks and their culture organisms has been the subject of a number of studies. Studies have shown that consuming certain cultured dairy products can help reduce serum cholesterol level. Feeding of fermented milks containing very large numbers of bifidobacteria (10^9 bacteria/g) to hypercholesterolemic human subjects has resulted in lowering cholesterol from 3.0 to 1.5 g/l (15). The role of bifidobacteria in reducing the serum cholesterol is not completely understood. Mann and Spoerry (27) suggested that consumption of fermented dairy products could help lower serum cholesterol. They observed a decrease in serum cholesterol levels in men fed large quantities of milk fermented with Lactobacillus. This may have been due to the production of hydroxymethylglutarate by LAB, which inhibit hydroxymethylglutaryl-CoA reductases required for the synthesis of cholesterol. Rao et al. (41) reported that metabolites from orotic acid formed during fermentation of fermented dairy products may help lower cholesterol level. According to Jaspers et al. (19), uric acid inhibits cholesterol synthesis and orotic acid and hydroxymethylglutamic acid reduce serum cholesterol.

However, the role of bifidobacteria in lowering cholesterol is still debated. No significant changes in cholesterol concentration were found in human subjects who were fed yogurt. Klaver and Meer (20) reported that removal of cholesterol from the culture medium by L. acidophilus RP32 and other species is not due to bacterial uptake of cholesterol, but results from bacterial bile salt deconjugating activity. The deconjugation of bile acid by the intestinal flora may influence the serum cholesterol level. The deconjugated bile acid does not absorb lipid as readily as conjugated counterpart, leading to a reduction in cholesterol level.

Lactobacillus acidophilus and bifidobacteria actively assimilated cholesterol and other organic acids. Reports by Gilliland et al. (11) show that L. acidophilus itself may take up cholesterol during the growth in the small intestine and make it unavailable for absorption into the blood stream. The effects of lactic acid bacteria on cholesterol levels are therefore inconsistent and range from a significant reduction to no reduction. The exact mechanism is unknown.

Improvement in Lactose Tolerance

Lactose intolerance is a condition in which lactose, the principal carbohydrate of milk, is not completely digested into its component monosaccharides, glucose and galactose (39, 43, 45, 46, 48). Since lactose is cleaved into its monosaccharides by the enzyme β-D-galactosidase (β-gal), lactose intolerance results from a deficiency of this enzyme. The traditional cultures used in making yogurt i.e., L. delbrueckii ssp. bulgaricus and S. thermophilus contain substantial quantities of β-gal, and it has been suggested that the consumption of yogurt may assist in alleviating the symptoms of lactose intolerance. Bifidobacteria are resistant to bile, which gives them an increased chance of colonising the gut, and delivering the enzyme to its site of action (17).

Many Oriental and African people are lactose intolerant. This is due to the deficiency of β-gal in the small intestine. People who are unable to digest lactose often complain of “gastric distress” after consuming fresh, unfermented products, such as milk. The gastric distress is due to the formation of hydrogen gas by the unfermented lactose as a result of microbial action (10, 17, 39, 45, 46, 48).

It is well accepted that lactose digestion from yogurt made with live L. delbrueckii ssp. bulgaricus and S. thermophilus is significantly improved in lactase-deficient individuals as compared with that of milk or heated yogurt (45, 46, 48). Mechanisms for improved lactose digestion are not well understood, but at least three factors have been suggested. One possible reason for an improvement in the lactose tolerance level could be due to the fermentation of lactose by yogurt bacteria, another reason could be that the enzyme autodigests the
lactose intracellularly before reaching the intestines (9, 17), and the third reason may be slower oral caecal transit time, allowing more time for residual β-gal in the intestine to hydrolyse lactose. Slower oral caecal transit time has been observed for yogurt as compared with milk (48).

Although, there are limited studies conducted on the efficacy of bifidus products in management of lactose malabsorption, effects of acidophilus milk in alleviation of lactose malabsorption is well studied and general review and research papers have been published (9, 28–30, 32, 46). Mixed results have been obtained with consumption of sweet acidophilus milk. However, the reports suggest that sweet acidophilus milk is not effective in management of lactose malabsorption (46).

Alm (1) studied the suitability of fermented milk products including acidophilus and bifidus milks for lactose intolerant individuals and found the fermented products to be suitable for lactose malabsorbers. Martini et al. (28) fed several dairy based meal, including milk fermented with *L. acidophilus* and *B. bifidum* to 12 lactase-deficient subjects and concluded that the products containing *L. acidophilus* or *Bifidobacterium* were less effective in alleviation of lactose-intolerance symptoms. The contribution of bifidobacteria to the alleviation of lactose intolerance is speculative. Only few publications are available on this topic.

### Adherence

Adherence is one of the most important criteria for selection of strains of probiotic bacteria. The desirable effects of these organisms will be produced only if they are able to adhere, multiply and colonise in the intestine. The ability of probiotic bacteria to adhere to the intestine will improve their chances in winning the competition against ‘unfriendly bacteria’ to occupy the intestinal ‘niches.’ Adherence to the intestinal cell wall is an important prerequisite for colonisation to the gastrointestinal tract (3, 4). However, thus far only a few *Lactobacillus* species such as *L. gasseri* ADH, *L. acidophilus* BG2FO4 and *L. casei* GG have been studied for their colonisation property. Among bifidobacteria, *B. breve*, *B. longum*, *B. bifidum* and *B. infantis* have been studied (3). Coconnier et al. (4) reported that an adhesion promoting factor was present in the spent broth supernatant of *L. acidophilus* BG2FO4, a human isolate. This factor was reported to promote adhesion of poorly adhering *L. casei* GG. *Lactobacillus plantarum* produced a protein substance, which promoted adherence to Ht-29 cells. Mukai and Arihara (37) reported the presence of lectin binding glycoproteins on the cell surface of *L. acidophilus*.

Lankaputhra and Shah (22) studied adherence properties of probiotic bacteria. Bifidobacteria showed better adherence to Ht-29 colonic cancer cell monolayers as compared with *L. acidophilus*. Among bifidobacteria, *B. infantis* 1912 and *B. longum* 1941 showed highest level of adherence. Among the strains of *L. acidophilus* 2400 and 2415 showed better adherence (Table 2). Thus, in general, bifidobacteria may be preferred as dietary adjuncts as compared with *L. acidophilus*.

<table>
<thead>
<tr>
<th>Strain/species of bacteria</th>
<th>No. of bacterial cells adhered to Ht-29 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium</em> spp.</td>
<td></td>
</tr>
<tr>
<td>1900 (<em>B. bifidum</em>)</td>
<td>185 ± 4</td>
</tr>
<tr>
<td>1901 (<em>B. bifidum</em>)</td>
<td>170 ± 8</td>
</tr>
<tr>
<td>1912 (<em>B. infantis</em>)</td>
<td>665 ± 15</td>
</tr>
<tr>
<td>1920 (<em>B. adolescentis</em>)</td>
<td>180 ± 12</td>
</tr>
<tr>
<td>1930 (<em>B. breve</em>)</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>1941 (<em>B. longum</em>)</td>
<td>546 ± 13</td>
</tr>
<tr>
<td>20097 (<em>B. longum</em>)</td>
<td>73 ± 14</td>
</tr>
<tr>
<td>20099 (<em>B. pseudolongum</em>)</td>
<td>8 ± 5</td>
</tr>
<tr>
<td>20210 (<em>B. thermophilum</em>)</td>
<td>195 ± 17</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td></td>
</tr>
<tr>
<td>2400</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>2401</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>2404</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>2405</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>2409</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>2415</td>
<td>380 ± 17</td>
</tr>
</tbody>
</table>

All strains of bacteria were used at a concentration of 1 × 10⁹/ml.

*Mean ± S.D. (n = 4).*

Source: Lankaputhra and Shah (23).
in adherence. As it was illustrated in the electron micrographs, adherence was mediated by a bridging structure (possibly a protein-polysaccharide structure) formed between bacterial and HT-29 cells (23).

CONCLUSIONS

Several health benefits have been claimed for probiotic bacteria, however, not all probiotic bacteria are effective in providing health benefits. Proper strain selection should be carried out in order to incorporate these strains for providing the claimed health benefits. There is some evidence that probiotic microorganisms could enhance gut flora and provide benefits to lactose intolerant people, however, further work is necessary to substantiate other health benefits.

REFERENCES


