

BIFIDOBACTERIA AS POTENTIAL PROBIOTIC IN YOGURT

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ABSTRACT

Nowadays, probiotics are considered in the development of “functional food” products due to their benefits to human health. Food products containing bifidobacteria have largely been of dairy origin including yogurts. Yogurt is a fermented milk product that has been prepared traditionally by allowing milk to sour at 40–45°C. Modern yogurt production is a well-controlled process that utilises ingredients of milk, milk powder, sugar, fruit, flavours, colouring, emulsifiers, stabilisers, and specific pure cultures of lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) to conduct the fermentation process. A number of health benefits have been attributed to bifidobacteria, which are colonization resistance, anticarcinogenic activity, reduction of the risk of colon cancer, stimulation of immune functions, improving digestibility of lactose, improving vitamin and antibiotic activity, and cholesterol-lowering ability

Keywords: *bifidobacteria, probiotic, yogurt, health benefits*

1. INTRODUCTION

Nowadays, food products containing bifidobacteria have largely been of dairy origin including yogurts. As much as 70% of milk products on the market in some European countries, such as Sweden, contain bifidobacteria.

Recently, in the food industry, probiotics are considered in the development of “functional food” products due to their benefits to human health. Many lactobacilli are used in functional foods. Dairy products appear to be the best vehicles for delivery of them to humans because of the LAB properties of most probiotics (Hattingh & Viljoen, 2004).

Foods fortified with health-promoting probiotic bacteria are mainly produced using fresh milk or milk derivatives such as yogurt, cheese, ice-cream, desserts, etc (Lavermicocca, 2006). It is estimated that there are 80 probiotic-containing products in the world. Some of the commercial dairy products with probiotics are listed in Table 1.

2. BIFIDOBACTERIA

2.1. Physiology

Bifidobacterium can grow between 20°C to 46°C and dies at 60°C. Optimum pH for growth as 6.5 -7 and no growth at pH < 5.1 or > 8.0. These organisms do not grow in the synthetic medium. Bifidobacteria are able to survive under different oxygen conditions

and ferment wide range of substrates. Bifidobacteria are anaerobic organisms, but some species can tolerate oxygen (Arunachalam, 1999). The enzymes like superoxide dismutase and catalase, help the organism to defence against the toxic effects of superoxide an hydrogen peroxide. Some different bifidobacterial species and their origin can be seen in Table 2.

Bifidobacteria do not have aldolase and glucose-6-phosphate dehydrogenase; they thus ferment hexose via a phosphoketolase pathway that is known as the ‘bifid shunt’, where the final products are acetic and lactic acids in a molar ratio of 3:2. The key enzyme in the bifid shunt is fructose-6-phosphate phosphoketolase (F6PPK), which converts fructose 6-phosphate into acetyl 1-phosphate and eritrose 4-phosphate.

2.2. Fermentation of Sugars

All Bifidobacteria species can ferment lactose and grow well in milk. *B. adolescentis*, *B. breve*, *B. infantis* and *B. longum* can utilize many carbohydrates while *B. bifidum* can utilize fructose, galactose and lactose. Bifidobacteria ferment glucose via the fructose 6 - phosphate shunt, which has been reported and the key enzyme involved in the hexose metabolism, fructose-6-phosphate phosphoketolase (F6PPK) is present in the cellular extracts and the path way is called

as ‘bifid shunt’(Figure 1) (Vries & Stouthamer, 1967).

3. YOGURT AS PROBIOTIC CARRIER FOOD

Yoghurt is prepared by fermentation of milk using two types of bacteria, namely *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Tamime and Marshall, 1997). Fermentation contribute to the hydrolysis of milk proteins, pH drops, increase of viscosity, and bacterial metabolites are produced that give the taste and the health promoting properties of yogurt.

Several health benefits have been reported for traditional yogurt (Rachid et al., 2002), and this healthy image is enhanced by supplementation with probiotic bacteria. Fermented foods that have potential probiotic properties are produced worldwide from a variety of food substrates. Probiotics have been used for the treatment of various types of diarrhoea, urogenital infections and gastrointestinal diseases such as Crohn's disease (Bousvaros et al., 2005).

Good nutrition and health promote the optimum ‘balance’ in microbial population in the digestive tract (Rybka & Kailasapathy, 1995). The microorganisms primarily associated with this balance are lactobacilli and bifidobacteria. Factors that

negatively influence the interaction between intestinal microorganisms, such as stress and diet, lead to detrimental effects in health. Increasing evidence indicates that consumption of ‘probiotic’ microorganisms can help maintain such a favourable microbial profile and results in several therapeutic benefits (Hattingh & Viljoen, 2001).

In recent years probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts. One of the most popular dairy products for the delivery of viable *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cells is bio-yogurt. Adequate numbers of viable cells, namely the ‘therapeutic minimum’ need to be consumed regularly for transfer of the ‘probiotic’ effect to consumers. Consumption should be more than 100 g per day of bio-yogurt containing more than 10^6 cfu mL⁻¹ (Rybka & Kailasapathy, 1995). Survival of these bacteria during shelf life and until consumption is therefore an important consideration.

Since the renewed interest in probiotics, different types of products are proposed as carrier foods for probiotic microorganisms by which consumers can take in large amounts of probiotic cells for the therapeutic effect. Yogurt has long been recognized as a product

with many desirable effects for consumers, and it is also important that most consumers consider yogurt to be 'healthy'. In recent years, there has been a significant increase in the popularity of yogurt (Hamann & Marth, 1983) as a food product, accentuating the relevance of incorporating *L. acidophilus* and *B. bifidum* into yogurt to add extra nutritional-physiological value. The conventional yogurt starter bacteria, *L. bulgaricus* and *Streptococcus thermophilus*, lack the ability to survive passage through the intestinal tract and consequently do not play a role in the human gut (Gilliland, 1979).

3.1. Bio-yogurt

Recently, live strains of *L. acidophilus* and species of *Bifidobacterium* (known as AB-cultures) are used in formulation of yogurt products in addition to the conventional yogurt organisms, *S. thermophilus* and *L. bulgaricus*. Therefore, bio-yogurt is yogurt that contains live probiotic microorganisms, the presence of which may give rise to claimed beneficial health effects.

For the production of AB-yogurt, similar processing procedures to traditional yogurt are applied with the exception of the incorporation of live probiotic starter cultures. Heat treated, homogenised milk with an increased protein content (3.6–3.8%) is inoculated with the conventional

starter culture at 45 °C or 37 °C and incubated for 3.5 and 9 h, respectively. The probiotic culture can be added prior to fermentation simultaneously with the conventional yogurt cultures or after fermentation to the cooled (4 °C) product before packaging.

3.2. Regulatory requirements for starter cultures in a bio-yogurt

Bio-yogurt, containing *L. acidophilus* and *B. bifidum* (AB-yogurt), is a potential probiotic. The number of probiotic bacteria required to produce a beneficial effect has not been established. Kurmann and Rasic (1991) suggested to achieve optimal potential therapeutic effects, the number of probiotic organisms in a probiotic product should meet a suggested minimum of $>10^6$ cfu mL⁻¹. These numbers required, however, may vary from species to species, and even among strains within a species. One should aim to consume 10^8 live probiotic cells per day. Regular consumption of 400–500 g/week of AB-yogurt, containing 10^6 viable cells per ml would provide these numbers (Tamime et al., 1995). Ishibashi and Shimamura (1993) reported that the Fermented Milks and Lactic acid Bacteria Beverages Association of Japan has developed a standard which requires a minimum of 10^7 viable bifidobacteria cells/mL to be present in fresh dairy products. The criteria developed by the

National Yogurt Association (NYA) of the United States specifies 10^8 cfu g^{-1} of lactic acid bacteria at the time of manufacture, as a prerequisite to use the NYA 'Live and Active Culture' logo on the containers of products (Kailasapathy & Rybka, 1997). The Australian Food Standards Code regulations, requires that the lactic acid cultures used in the yogurt fermentation must be present in a viable form in the final product, the populations are not specified. At the same time, attainment of pH 4.5 or below is also legally required to prevent the growth of any pathogenic contaminants (Micanel, Haynes, & Playne, 1997). It has been claimed that only dairy products with viable microorganisms have beneficial health effects. However, in the case of lactose tolerance, treatment of acute gastro-enteritis and treatment of candidiasis, probiotics used showed the same beneficial effect in viable and non-viable form (Ouweland and Salminen, 1998).

4. POTENTIAL HEALTH BENEFITS OF BIFIDOBACTERIA

A number of health benefits have been attributed to bifidobacteria, which are colonization resistance, anticarcinogenic activity, reduction of the risk of colon cancer, stimulation of immune functions, improving digestibility of lactose, improving vitamin and antibiotic activity and cholesterol-lowering ability

4.1. Colonization Resistance

One of the most common claims associated with dairy foods containing bifidobacteria, and other LAB, is the 'maintenance or re-establishment of healthy intestinal microflora. The normal colonic flora provides an important barrier function against pathogenesis, often termed 'colonization resistance.' Multiple mechanisms may be involved in the exclusion of undesirable organisms by bifidobacteria, including competition for receptor sites or nutrients and production of inhibitory factors or conditions, e.g., organic acids or antimicrobials, as well as physiological factors (lowering of pH or stimulating the immune system)(Mc Cartney, 2003).

The production of organic acids (acetic and lactic acid) by bifidobacteria inhibits the growth of pathogenic organisms (directly and indirectly) and stimulates intestinal peristalsis. Acetic acid is a stronger antagonist against Gram-negative bacteria than lactic acid. As such, the potential applications of bifidobacteria against microbial perturbation may surpass those of lactobacilli. Additionally, the organic acids produced by bifidobacteria have been shown to inhibit the growth of many nitrate-reducing bacteria (Mc Cartney, 2003).

4.2. Anticarcinogenic Activity

Increasing evidence, from in vitro experiments and animal studies, indicates the potential protective influence of probiotic bacteria (including bifidobacteria) against cancer. To date, three conceivable mechanisms have been identified: (1) inhibition of putrefactive organisms that produce carcinogens (such as N-nitroso compounds, phenolic products of tyrosine and tryptophan, and metabolites of biliary steroids); (2) binding and/or inactivation of carcinogens; and (3) inhibition of tumor cell formation. Bacterial enzymes (including β -glucuronidase, β -glucosidase, nitroreductase, and azoreductase) are responsible for converting some procarcinogens into carcinogens. As such, the levels/activities of these enzymes are considered a useful biomarker for cancer risk in humans, enabling noninvasive estimation of carcinogen levels (Mc Cartney, 2003).

4.3. Reduction of the Risk of Colon Cancer

Research in the past fifteen years has focused on the potential role of BIB in the prevention of cancer initiation. *B. bifidum* have been shown in human clinical studies to reduce the levels of some colonic enzymes (β -glucuronidase, nitroreductase, azoreductase, and glycolic acid hydrolase), which are implicated in the

conversion of procarcinogens to carcinogens such as nitrosamines or secondary bile salts (Ling et al, 1994). Most studies report a decrease in these enzymes during the study period when live BIB were consumed with a return to baseline levels during follow-up when no BIB were consumed. The mechanisms and long-term effects of these changes are not clear. Recent epidemiological studies (Kampman et al, 1994) have found that colon cancer risk was inversely related to the consumption of diets which included fermented milks. Other dietary factors have been considered in the prevention of colon cancer, including fibre and calcium fermented milks may be one factor of many that affect risk of colon cancer.

4.4. Stimulation of Immune Functions

Several probiotics, including some bifidobacterial strains, are claimed to enhance the immune system in a nonspecific manner, thereby stimulating immunity to a number of antigens. A number of studies have also shown the ability of certain LAB strains to alter cytokine production and/or increase secretory IgA levels. Preliminary data demonstrate the potential of probiotics in modulating certain immune responses and indicate their potential role in allergy, autoimmunity, and gastrointestinal disease (Mc Cartney, 2003).

4.5. Lactose Intolerance

Lactose malabsorption affects large portions of the population (estimated by some to affect over half the world's population), with a higher prevalence in those of Oriental or African ancestry. Symptoms normally include abdominal discomfort, flatulence, and/or diarrhea. However, lactose-intolerant individuals can consume cultured milk products (containing bifidobacteria and/or lactobacilli) without any deleterious effects. Two mechanisms have been proposed for the improved digestibility of lactose in such products: (1) the β -galactosidase activity of the probiotic strains; and (2) stimulation of host mucosal β -galactosidase activity by the ingested strains (Mc Cartney, 2003).

4.6. Nutritional Value

Bifidobacteria are known to produce thiamine, riboflavin, vitamin B6, and vitamin K. There have also been reports of their ability to synthesize folic acid, niacin, and pyridoxine. These vitamin B complexes are slowly absorbed in the human body. However, the impact on human nutrition of such vitamin synthesis by bifidobacteria in the colon is unknown. Available information on the nutritional properties of fermented milks containing bifidobacteria indicates that they have lower residual lactose and higher levels of free amino acids and vitamins than non-fermented milks. Additionally, they preferentially contain

L(+)- lactic acid (produced by bifidobacteria in addition to acetic acid, whereas lactobacilli produce D/L(-)- lactic acid), which is more easily metabolized by humans. This is particularly important for infants less than 1 year old, in whom metabolic acidosis can be a problem. Consuming bifidobacterial food products may also improve the bioavailability of certain minerals, including calcium, zinc, and iron, by lowering the gastric pH (facilitating ionization of minerals, which is necessary for their uptake).

4.7. Improving Protein Metabolism

Bifidobacteria have phosphoprotein phosphatase activity which helps in increase absorption of human milk protein by breaking down the casein in human milk. This is thought to contribute to the satisfactory absorption of human milk (91). Nitrogen retention is good in infants with a bifidus microflora; bifidobacteria promotes the aminoacids metabolism. One of the roles which bifidobacteria fulfil in the intestinal tract of the infants is to suppress the multiplication of putrefactive bacteria thereby stopping losses of nutrients (Arunachalam, 1999).

4.8. Improving Vitamin Metabolism

Bifidobacteria are predominant in the intestinal microflora of healthy people irrespective of age and the vitamins

produced by them needs warrant attention. The values reported for vitamins produced by bifidobacteria are : vitamin B1: 7.5 µg and B2: 25 µg per g dry weight for intracellular bacterial vitamins, and B1: 25 – 250 µg, B2: 100 µg, B12: 0.06 µg, nicotinic acid 400 µg, and folic acid 25 µg per litre of medium for vitamins produced outside the bacterial cells. With bifidus microflora it would also be enable the beneficial utilization of the extracellular vitamin B, produced by the bifidobacteria (Arunachalam, 1999).

4.9. Antibiotic Activity

In vitro, bifidobacteria have been noted to have antibacterial activity against pathogenic *E. Coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus spp.* and *Candida albicans*. The antibacterial action shown by bifidobacteria is from the organic acids they produce. Bifidobacteria make 1 mol lactic acid, 1.5 mol acetic acid and small amount of formic acid from 1 mol of glucose. Rasic and Kurmann (1983) reported that the intensity of the antibiotic action varies with acids, for example the minimum pH at which *Salmonella spp.* can grow is 5.4 for acetic acid, 4.4 for lactic acid and 4.05 for citric/hydrochloric acid. Bifidin is stable to heating 100°C for 30 min; it gives a positive ninhydrin reaction, and its main components are phenylalanine and glutamic acid. It

shows antibacterial activity against *Micrococcus jlavus* and *Staphylococcus aureus*, by being active at pH 4.8 to 5.5 (Ramakrishna et al, 1985). Ferrari et al. (1980) have shown that the bifidobacterial cells breakdown the conjugated bile acids to free bile acids which intern inhibit the growth of pathogens.

4.10. Cholesterol-lowering Ability

Subsequent in vitro work has demonstrated the ability of bifidobacteria to both assimilate cholesterol and coprecipitate it with deconjugated bile acids. Such observations have led to great interest in the cholesterol-lowering capacity of a diet containing fermented milks. However, much contradictory data exist regarding the effects of consuming foods containing bifidobacteria on serum cholesterol levels. Confounding the issue has been the use of different strains, dosages, and food vehicles in the various studies carried out so far. Additional criticisms of the current data have included lack of stabilization of baseline cholesterol levels, inadequate size and/or duration of studies, and difficulty in controlling the diet and physical activity of subjects. Influence of yoghurt in human serum cholesterol level can be seen in Table 3. Consumption of yogurt itself may not help in controlling cholesterol but some factor produced by the yogurt bacteria during

fermentation of the milk is responsible. So, there may be some ways to concentrate the active factor(s) into usable volume for practical use.

5. CONCLUSION

Results to date clearly indicate the potential benefits of consuming a diet incorporating foods containing bifidobacteria. Bifidobacteria is popular as one of the most important groups of intestinal organisms regarding human health. Dairy products appear to be the best vehicles for delivery of them to humans because of the LAB properties of most probiotics. Lactic acid bacteria including lactobacilli and bifidobacteria are the most common bacterial species considered as potential probiotics. Bio-yogurt is yogurt that contains live probiotic microorganisms, including species of *Bifidobacterium* that give the beneficial health effects. A number of health benefits have been attributed to bifidobacteria, which are colonization resistance, anticarcinogenic and antibiotic activities, reduction of the risk of colon cancer, stimulation of immune functions, enhancement of nutritional value and lowering cholesterol. Essential to the future of functional foods are adequate studies confirming the safety, efficacy, and viability of such products. Current developments within the scientific community, food

industry, and regulatory bodies are all pursuing this end.

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Table 1. Examples of the commercial yogurt products containing probiotic cultures (Champagne & Gardner, 2005)

Examples of commercial products	Microorganisms used mainly as starter	Microorganisms used mainly as a probiotic adjunct culture
Lunebest, Mil-Mil	<i>S. thermophilus</i> <i>Lb. bulgaricus</i>	<i>Lactobacillus</i> , <i>Bifidobacterium</i>
Olifus	<i>S. thermophilus</i> <i>Lc. Lactis</i>	<i>Lactobacillus</i> , <i>Bifidobacterium</i>
Biogarde, Aktifit	<i>S. thermophilus</i>	<i>Lactobacillus</i> , <i>Bifidobacterium</i>
Progurt	<i>Lc. Lactis</i>	<i>Lactobacillus</i> , <i>Bifidobacterium</i>
BA, Biobest, Yoplait Basket	<i>S. thermophilus</i> <i>Lb. bulgaricus</i>	<i>Bifidobacterium</i>
Biokys		<i>Lactobacillus</i> , <i>Pediococcus</i> , <i>Bifidobacterium</i>
Gaio, Praghurt	<i>S. thermophilus</i> <i>Lb. bulgaricus</i>	<i>Enterococcus</i>
Bioghurt	<i>S. thermophilus</i>	<i>Lactobacillus</i>
Bifighurt, Yoke	<i>S. thermophilus</i>	<i>Bifidobacterium</i>

Table 2. Bifidobacteria species and their origin (Ventura, 2007)

Strain	Origin
<i>B. breve</i> , <i>B. bifidum</i> , <i>B. pseudocatenulatum</i>	Infant feces
<i>B. adolescentis</i> , <i>B. catenulatum</i> , <i>B. longum</i> biotype <i>longum</i>	Intestine of adult
<i>B. gallicum</i>	Human feces
<i>B. animalis</i> subsp. <i>Lactis</i>	Yogurt
<i>B. longum</i> biotype <i>infantis</i>	Intestine of infant
<i>B. scardovii</i>	Human blood

Table 3. Influence of Yoghurt in Human Serum Cholesterol Level (Mann, 1977).

Product	Serum cholesterol (mg/l)		
	Before	After	Significant reduction
Whole Milk	196	177	No
Yogurt from Whole milk	193	175	Yes
Yogurt from Skim Milk	211	150	Yes