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Prospects, Technological Aspects and Limitations of Probiotics – A Worldwide Review

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Review Article

ABSTRACT

The term "probiotics" (meaning "for life" in Greek) refers products containing live microorganisms, which increase the population of friendly intestinal bacteria upon ingestion. Several new dairy product formulations containing probiotic cultures are being developed with such microbes which when consumed offer potential health benefits viz. increased resistance to infectious diseases - particularly of the intestine, decreased duration of diarrhea, reduction in blood pressure, reduction in serum cholesterol concentration and allergy, stimulation of phagocytosis by peripheral blood leucocytes, modulation of cytokine gene expression, adjuvant effects, regression of tumors, reduction in carcinogen products, increased tolerance to lactose in lactose intolerant population etc. In addition, all potential benefits could not be achieved from just one type or strain of organism. Examples of probiotic microorganisms used in foods include *Lactococcus lactis, Lactobacillus* sp., *Streptococcus, Enterococcus, Bifidobacterium* sp., *Pediococcus, Propionibacteria* sp. This review paper highlights the benefits, technological aspects, world scenario and limitations of probiotic foods.

Keywords: Probiotics, Lactobacillus, Bifidobacterium, bile tolerance, heat tolerance;

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1. INTRODUCTION

Probiotics are defined as single or mixed cultures of live micro-organisms that when applied to animals or humans in adequate amounts confer a health benefit on the host by improving the properties of the indigenous micro flora (Tabbers and Benninga, 2007). According to Boirivant and Strober (2007) probiotics are a heterogeneous group of non-pathologic bacteria that are functionally defined by their ability to allay inflammation when introduced into the inflamed intestine. A number of health benefits such as antimutagenic effects, anticarcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol, and immune system stimulation have been claimed for probiotic foods. Because of the potential health benefits, probiotic organisms are increasingly being incorporated into a number of dairy foods. In addition, fermented functional foods with health benefits like Evolus® and Calpis®, have been introduced in the market, which are based on bioactive peptides released by probiotic organisms (Shah, 2007).

Foods containing probiotic microorganisms come within the category of functional foods, i.e., foods, which have a positive effect on health. Furthermore, increased commercial interest in exploiting the proposed health attributes of probiotics has contributed significantly to the rapid growth and expansion of this sector of the market (Stanton et al., 2001). Food manufacturers are enthusiastic about developing such products because the added ingredients give increased value to food. The global market for probiotic foods in the coming years is growing rapidly. Research is also being carried out to incorporate the probiotic microorganisms in non-dairy food products such as ice creams, chocolates, juices etc. The nondairy probiotic foods are very popular in European countries and a number companies are manufacturing probiotic juices, chocolates and other non-dairy probiotic foods (Stanton et al., 2001).

There are several technological factors related to probiotic microorganisms like oxygen tolerance, acid and bile tolerance, heat tolerance and type of food carrier, which are affecting the manufacturing of probiotic food products. A lots of researches are going on to overcome these impediments. This review focuses on the benefits, technological aspects, world scenario and limitations of probiotic foods.

2. PROBIOTIC FOODS AND THEIR POTENTIAL USES

Probiotics are described as ‘live micro-organisms which when administered in adequate numbers confer a health benefit on the host’ (FAO/WHO 2001, 2002). Probiotic foods are those foods which carry live mono or mixed culture of microorganisms which when consumed by humans, beneficially affects the host by improving the properties of intestinal microflora by selectively stimulating the growth and/or activity of one or a limited number of naturally present or introduced bacterial species in the colon, leading to improved host health (Anonymous, 2002). There are many examples, which show the health improvement associated with the consumption of probiotics including reduction in the incidence of childhood atopic, decrease in rotavirus shedding in infants and reductions in antibiotic-associated diarrhoea. Fermented foods are recognized as most healthy diets and carriers for microbes that take up residence in the intestine when consumed (Ross et al., 2005).

The main targets of probiotic intervention have been (Crittenden et al., 2005; Anonymous, 2010):

i. Increasing natural resistance to infectious disease in the gastrointestinal tract and a first line of defense against disease;
ii. Prevention of dangerous fungal overgrowth and some allergic reactions;
iii. Reducing putrefactive/toxic microbial metabolism in the gut;
iv. Promoting optimized digestive processes, allowing maximum nutritional benefit from food;
v. Improved resistance to toxic bowel problems and diarrhea;
vi. Stimulation of the immune system;
vii. Production of needed nutrients, like vitamin K, one form of which the body itself cannot make;
viii. Improving lactose intolerance conditions;
ix. Reduction of cholesterol levels;
x. Act as antioxidants;
xi. Controlling diseases where components of the intestinal microbiota have been implicated in aetiology.

To date, clinical studies where consumption of probiotics have shown health improvement include reduction in the incidence of childhood atopic eczema (Kalliomäki et al., 2001; Van Niel et al., 2002; Marteau et al., 2002), decrease in rotavirus shedding in infants (Saavedra et al. 1994) and reductions in antibiotic-associated diarrhoea (Plummer et al. 2004).

In the vaginas of women different microorganisms including lactic acid bacteria can be found, which protect the woman from a vaginal infection by forming an acid milieu. After genital infection, the numbers of lactobacilli decrease, while the numbers of pathogenic organisms such as *Candida, Gardnerella* and/or *Trichomonas* increase. According to Sieber and Dietz (1999) several studies show that direct application of *Lactobacillus acidophilus* or yoghurt or ingestion of dairy products fermented by *Lactobacillus acidophilus* in the vagina might have a therapeutic benefit. Selected strains of probiotics like some strains of lactobacilli e.g. *Lactobacillus caseii* GG and *Lactobacillus reuteri* and *Saccharomyces boulardii* resulted in a statistically significant but clinically moderate benefit in reducing the duration of diarrhoea caused by acute infectious gastroenteritis (Vandenplas et al., 2007).

According to Tahri et al. (1996) the removal of cholesterol from the growth medium by Bifidobacteria strains like *Bifidobacterium longum*, *B. infantis*, *B. breve*, *B. animalis* and *B. thermophyllum* is due to both bacterial assimilation and precipitation of cholesterol. Lin and Chen (2000) studied the cholesterol-reducing abilities of six strains of *L. acidophilus* and reported that *in vivo* hypocholesterolaemic ability is likely due to the assimilation of cholesterol by *L. acidophilus* cells or/and attachment of cholesterol to the surface of *L. acidophilus* cells.

2.1 PROBIOTIC MICROORGANISMS

The traditional biotechnology of preservation of food through lactic acid fermentation has been known for thousands of years. Microorganisms had been tested in the 19th century to prevent and cure diseases and it is probably from the work of the Russian Nobel Laureate Elie Metchnikoff in 1908 that the first scientific assessments of probiotics were made. He first hypothesized that a high concentration of lactobacilli in intestinal flora were important for health and longevity in humans (Anonymous, 2002). Starter cultures, used for making thick sour cream in Copenhagen and Kiel in 1890 for the first time marked the beginning of modern industrial microbiology and food technology (Anonymous, 1999). The most commonly used probiotic microorganisms in foods for human consumption are *Lactobacillus* and *Bifidobacterium* species, which have given significant health benefits associated with ingestion of these micro-organisms (Stanton et al. 2003a,b). These microorganisms have a number of common characters such as generally regarded as safe (GRAS) status, acid and bile tolerance, and ability to adhere to intestinal cells (Dunne et al., 2001).

Studies of probiotic products by the genera *Lactobacillus* and *Bifidobacterium* in the European market can be found in (Anonymous, 1999):

1. The "*L. acidophilus* group" and its species *L. acidophilus and L. johnsonii*;
2. The *"L. casei* group" according to its current taxonomy and the heterofermentative species *L. reuteri*;
3. *Bifidobacterium sp.: B. animalis, (B. bifidum), B. longum, B. lactis, B. infantis* and *B. breve*. The form of this genus most frequently isolated from probiotic yogurt products in Germany was *B. animalis.*

2.2 PROBIOTIC STRAINS

2.2.1 US FDA recommended probiotic strains (Sanders, 2008)

United States Food and Drug Administration (US FDA) has recommended some of the probiotic microorganisms, which are known to be considered as safe. These probiotic microorganisms are generally used in probiotic foods. Some commercial probiotic strains and their sources have been presented in Table 1 (Sanders, 2008).

2.2.2 Australian Commercial Probiotic Strains (Crittenden et al., 2005)

A number of novel probiotic strains developed in Australia have now been commercialized, e.g., DSM Food Specialties (Sydney) produces *L. acidophilus* LAFTI® L10, *L. paracasei* LAFTI® L26 and *B. lactis* LAFTI® B94 for use in fermented dairy products. VRI BioMedical (Perth) produces *L. fermentum* VRI-002 in a capsule form under the brands Progastrim® and ProBio PCC®.

2.2.3 Commercial Probiotic Strains in New Zealand (Crittenden et al., 2005)

The strain *B. lactis* HN019 branded as DR10TM, and the strain *L. rhamnosus* HN001 as DR20TM have been successfully commercialized in products such as milk powder and cheese (DR20TM sold in Australia under the brand name “Inner Balance”) and in dietary supplements.

2.2.4 Probiotic Strains in China (Yuan, 2002; Crittenden et al., 2005)

Currently, there are about 100 types of probiotic organisms produced for the Chinese market, mostly sold as functional food ingredients. Clinical trials have been conducted for about 10% of the probiotic products, and these are approved by the Chinese regulatory authority to be marketed as pharmaceutical products (Table 2).

2.3 AMOUNT OF PROBIOTICS

The minimum effective microorganism count in the product is of critical importance with regard to the use of probiotics in food products and their intended health benefits.

Table 1. The US FDA recommended probiotic strains and their sources

Strains	**Sources**
L. acidophilus NCFM®	Danisco (Madison WI)
B. infantis 35264	Procter & Gamble (Mason OH)
L. fermentum VRI003 (PCC)	Probiomics, Eveleigh, Australia
L. rhamnosus R0011, *L. acidophilus* R0052	Institute Rosell (Montreal, Canada)
L. acidophilus LA-1, *L. paracasei* CRL 431, *B. lactis* Bb-12	Chr. Hansen (Milwaukee WI)
L. casei Shirota, *B. breve* strain Yakult	Yakult (Tokyo, Japan)
L. casei DN114001 (“L. casei Defensis ™”, *B. animalis* DN173 010 (“*Bifidis regularis*™”)	Danone (Paris, France), Dannon (Tarrytown NY)
L. reuteri RC-14™, *L. rhamnosus* GR-1™	Chr. Hansens (Milwaukee WI), Urex Biotech (London, Ontario, Canada)
L. johnsonii Lj-1 (same as NCC533 and formerly *L. acidophilus* La-1)	Nestlé (Lausanne, Switzerland)
L. plantarum 299V, *L. rhamnosus* 271	Probi AB (Lund, Sweden)
L. reuteri SD2112	Biogaia (Stockholm, Sweden)
L. rhamnosus GG (“LGG”)	Valio Dairy (Helsinki, Finland)
L. rhamnosus LB21, *Lactococcus lactis* L1A	Essum AB (Umeå, Sweden)
L. salivarius UCC118	University College (Cork, Ireland)
B. longum BB536	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
B. lactis HN019 (DR10), *L. rhamnosus* HN001 (DR20)	Danisco (Madison WI), Fonterra (Wellington, New Zealand)
L. acidophilus LB	Lacteol Laboratory (Houdan, France)
L. paracasei F19	Medipharm (Des Moines, Iowa)

Source: Sanders (2008)

Although the minimum recommended level of viable probiotics present in foods for any health benefits to be achieved can show a discrepancy, in general the food industry has adopted the recommended level of 10^6 CFU (Colony Forming Units) ml^{-1} at the time of consumption (Kurmann and Rasic, 1991; Kailasapthy and Chin, 2000; Boylston et al., 2004). This standard appears to have been introduced to achieve bacterial populations that are technologically attainable and cost effective (Boylston et al., 2004). US FDA has also recommended that the minimum probiotic count in a probiotic food should be at least 10^6 CFU ml^{-1}. Depending on the amount ingested and taking into account the best-before date, a regular - in most cases daily intake of 10^8 to 10^9 probiotic microorganisms is necessary to achieve probiotic action in the human organism. Table 3 specifies the recommended dose of *Lactobacilli* (Anonymous, 2011a). It is recommended that the probiotic culture must be present in the product at minimum numbers of 10^7 CFU ml^{-1} and even higher numbers have been recommended (Ishibashi and Shimamura 1993; Lee and Salminen 1995).

Table 2: Probiotics marketed as pharmaceutical products in China

Sl. No.	Probiotic bacteria	Company
1.	*Bifidobacterium adolescentis*	Lichu Drug House
2.	*Bifidobacterium longum; Lactobacillus acidophilus*	Shanghai Sinyi Drug Pte. Ltd.
3.	*Enterococcus faecalis*	Shansi Haishi Drug Pte. Ltd.
4.	*Bifidobacterium longum; L. delbrueckii* subsp*. lactis; Streptococcus thermophilus*	Mongolian Shuanchi Drug Co. Ltd.
5.	*Bacillus cereus*	Dalian Medical University Tayue Drug Co. Chendu Bioproduct Institute Anyang Yuanshou Biodrug Pte. Ltd.
6.	*Bacillus licheniformis*	Shenyang First Drug House
7.	*Bifidobacterium bifidum; L. acidophilus; Enterococcus faecalis; Bacillus cereus*	Jilin Weite Group
8.	*Clostridium tyrobutyricum*	Chongqing Taipin Drug Co. Ltd.
9.	*Bifidobacterium infantis; Clostridium tyrobutyricum*	Shantong Kesing Biodrug Pte Ltd.
10.	*Bacillus subtilis; Enterococcus faecalis*	Beijing Hanbei Drug Co.
11.	*L. debrueckii subsp. delbrueckii*	Dalian Medical University
12.	*L. acidophilus; Enterococcus*	Jianshu Taizhou Drug House
13.	*Bacillus subtilis*	Harbin
14.	*L. plantarum*	Shanghai Jiaotong University
15.	*Bifidobacterium; Lactobacillus; Enterococcus faecalis*	Shantong Jinan Sanzhu Co.
16.	*L. acidophilus*	Harbin Taige Co.

Source: Yuan (2002); Crittenden et al. (2005)

Table 3: Recommended dose of *Lactobacilli*

Strains	Effective dose in CFU day^{-1}
L. casei shirota	6.5×10^9
L. rhamnosus GG	109×10^{10}
L. plantarum 299 v	5×10^8
L. acidophilus NCFB 1748	3×10^{11}
L. reuteri	1×10^8-10^{11}
L. rhamnosus DSM 6594	16×10^9

Source: Anonymous (2011a)

3. WORLD SCENARIO OF PROBIOTICS FOODS

Consumer trends with respect to food choice are changing due to the increasing awareness of the link between diet and health (Mark-Herbert, 2004). The health benefits of *L. acidophilus* and *Bifidobacterium* spp. have resulted in their increased incorporation in dairy foods, particularly in yoghurts, leading to creation of a new generation of health foods. The growing health awareness among consumers has helped to spark a boom in the sale of probiotic yoghurts, especially in Europe, Japan and Australia (Sanders, 1998, Stanton et al., 2001). Consequently the global functional food market is thriving with recent estimates indicating up to a $50 billion annual share (Stanton et al., 2005). The world probiotic market is estimated at $15 billion. The world probiotic market was estimated to 10% of the lactic bacteria drink market. Today, this market is growing at a pace of 5 to 30% depending on the country and product type (Anonymous, 2007c).

In Europe, the largest segment of this market comprises foods prepared with probiotics, prebiotics or synbiotics. The probiotic market, especially dairy products such as yogurts and fermented milks, has experienced rapid growth as functional foods in Europe (Stanton et al., 2001). Consumer acceptance varies greatly across Europe, with the most developed market in Northern European and Scandinavian countries - having a long traditional consumption of fermented dairy products (Anonymous, 2003). In 1997 these products accounted for 65% of the European functional foods market, valued at US$889 million, followed by spreads, valued at US$320 million and accounting for 23% of the market (Hilliam, 1998; Stanton et al., 2001).

Presently over 70 products all over the world including sour cream, buttermilk, yoghurt, powdered milk and frozen desserts contain bifidobacteria and lactobacilli (Shah, 2001). The probiotic yogurt market has experienced immense growth, as other big players like Nestle and Unilever have entered the fight. The spoonable probiotic yoghurt market alone was estimated by Euromonitor International to be worth US$1.6 billion at retail in 2005 (Anonymous, 2006a). According to the Financial Times of UK, the yoghurt, to be named Essensis, will have detoxifying properties that will reflect on skin health. The area of overlap between dietary supplement and cosmetics companies has been growing with a category, now estimated by Kline and Co to be worth around US$1 billion on a global basis. Kline calls these products 'nutricosmetics' (Anonymous, 2006a).

Properly formulated probiotic-containing foods having the potential to promote health benefits offer consumers a low risk and low cost dietary component. Several such products are available commercially, although markets in Japan and Europe are more developed than in the USA (Sanders and Veld, 1999). However, probiotics are gaining increasing significance in North America. Combined, the market size of both Canada and the United States is the fourth largest in the world and this region has tremendous potential with attractive growth rates for both probiotic foods and probiotic supplements. Probiotics applied to cosmeceutical products could make the North American market surpass Europe or even Asia in probiotic consumption (Anonymous, 2007a). Probiotics are available in the United States in foods, dietary supplements and medical foods. More than 100 companies in the United States market probiotic products in supplement form. A few of these are listed in Table 4 (Sanders, 2008).

Table 4: Probiotic products with targeted health benefits available in the US

Indication	Strains	Products
Infant diarrhea	*L. rhamnosus* GG	Culturelle (capsule) Danimals (drikable yogurt)
	L. casei DN-114001 (aka "Immunitas™")	DanActive (fermented milk)
Inflammatory bowel conditions	8-strain combination of 3 *Bifidobacterium* strains, 4 *Lactobacillus* strains and *S. thermophilus*	VSL#3 (powder)
Antibiotic associated diarrhea; *C. difficile*	*S. boulardii*	Florastor (powder)
	L. rhamnosus GG	Culturelle (capsule) Danimals (drikable yogurt)
	L. casei DN114001	DanActive (fermented milk)
Gut transit time	*B. animalis* DN-173 010 (aka "Bifidus regularis™")	Activia (yogurt)
Keeping healthy	*L. reuteri* ATCC 55730	Stonyfield yogurt
	L. casei DN-114001	DanActive (fermented milk)
	L. casei Shirota	Yakult
Allergy (atopic dermatitis in infants)	*L. rhamnosus* GG	Culturelle (capsule) Danimals (drikable yogurt)
Lactose intolerance	*L. bulgaricus* and/or *S. thermophilus* (most strains)	All yogurts with live, active cultures
Colic in infants	*L. reuteri* ATCC 55730	Reuteri drops
Immune support	*B. lactis* HN019 (aka HOWARU™ or DR10)	Naked Juice Probiotic Juice Smoothie Strain sold as an ingredient for dairy and supplement products - contact Danisco
	B. lactis Bb-12	Good Start Natural Cultures (infant formula) Nestle; Strain also sold as an ingredient for dairy and supplement products - contact Chr. Hansens (800-558-0802)
	L. casei DN114001	DanActive (fermented milk)
	L. rhamnosus GG	Culturelle (capsule) Danimals (drikable yogurt)
	L. reuteri ATCC 55730	Stonyfield yogurt
Vaginal applications	*L. rhamnosus* GR-1, *L. reuteri* RC-14	Fem-Dophilus (capsules)
Irritable bowel syndrome symptoms	*B. infantis* 35264 (aka "Bifantis™")	Align (capsules)

Source: Sanders (2008)

A Frost and Sullivan study estimates that the probiotic ingredient market in the US will reach $450 million by 2010. According to a data monitor estimation, functional foods and health immunity market in the US will reach $750 million by 2008, and overall probiotics health product market is set to reach $5 billion in the near future from the $3.9 billion in 2006 (Anonymous, 2007b). Market research by Euromonitor reveals that in 2003, global sales of dairy products reached €211.5 billion, with total value growth of 13.4% from 1998 to 2003 supported by good growth in the dominant Western European region. According to Euromonitor, probiotic 'little bottles' have grown by 52% in 2004, reaching a retail sales value of ₤28 million, the highest growth in the core European markets. This compares to a decline in plain and natural yoghurts of almost 2% (Anonymous, 2004a). But spoonable probiotic yoghurts are also growing fast, and account for more than €280 million in the French market, with growth of 10.4%, for both drinking and spoonable versions together. The probiotic trend is being particularly exploited in Sweden, with launching of organic and bio yoghurts in 2003 and 2004 by a number of companies, such as Proviva and Primaliv by Skånemejerier and Verum by Norrmejerier (Anonymous, 2004a).

Although the worldwide market for probiotics is growing fast, however, in India, the market has just started to conceive with leading companies like Amul, Nestle and Mother Dairy making the first move (Anonymous, 2011b). In India, these companies have come up with their probiotic products, which are very popular. Some of these are presented in Table 5. Yakult-Danone India, a 50:50 joint venture of Japanese global probiotics leader, Yakult, with the French food major, Group Danone, has entered the Indian probiotics market with launching of their product ‘Yakult’, a probiotic curd in December 2008.

Table 5: Probiotics products marketed in India

Sl. No.	Probiotic Products	Company
1.	Probiotic curd	Heritage Foods (India) Ltd.
2.	‘b-Activ’ probiotic curd (*L. acidophilus* and *B. lactis* strain *BB12*)	Mother Dairy
3.	‘Nesvita’ probiotic yoghurt	Nestle
4.	Probiotic ice creams, ‘Amul Prolife’ ‘Prolite’ and ‘Amul Sugarfree’	Amul (Brand of Gujarat Cooperative Milk Marketing Federation Ltd.)
5.	Yakult, Probiotic curd with *L. casei* strain *Shirota*	Yakult Danone India (YDI) Private Limited
6.	Probiotic drugs	Ranbaxy (Binifit)
7.	Probiotic drugs	Dr. Reddy's Laboratories
8.	Probiotic drugs	Zydus Cadila
9.	Probiotic drugs	Unichem
10.	Probiotic drugs	JB Chem
11.	Probiotic drugs	GlaxoSmithKline
12.	Fructo-Oligo Saccharides, Probiotic drugs	Glenmark Alkem Labs

Source: Anonymous (2007b; 2011b)

Yakult is the only probiotic drink that contains more than 6.5 billion beneficial bacteria (*Lactobacillus casei* strain *Shirota*, named after its founder Dr Minoru Shirota). Yakult-Danone India have already invested Rs 136 crore in India and going to invest another Rs

100 crore in 2-3 years to develop the market nationally (Anonymous, 2011b). At least 39 probiotic drug brands, mainly in the area of gastroenterology, from 30 major Indian companies have already created a probiotics drug market in India worth Rs 80 crore, with an year-on-year growth of 41.1% in 2006 (Anonymous, 2007b). Major players in the probiotics drug market in India include companies like Ranbaxy (Binifit), Dr. Reddy's Laboratories, which has four probiotic brands, Zydus Cadila, Unichem, JB Chem, and Glaxo SmithKline (Anonymous, 2007b).

Many Indian research institutes are working on the probiotic technologies (Probiotic curd - Punjab Agricultural University, Ludhiana, Punjab; Probiotic ice cream - National Dairy Research Institute (NDRI), Karnal, Haryana; Cereal based probiotic foods - Haryana Agricultural University, Haryana, etc.) (Anonymous, 2011c). National Research Development Corporation, a Govt. of India enterprise under Ministry of Science and Technology, has also taken active role in commercialization of the probiotic technologies developed in various Indian research institutes and laboratories for popularization of this new concept.

In the Japanese market new products resulting from totally new concepts have been launched in 2003. For example, yogurts that fight tooth decay or beverage, such as Kirin Noale, using KW lactic acid bacteria against allergy. Morinaga sells yogurts with BB356 and lactoferrin. Calpis mixes lactic acid bacteria and lactotripeptides in Ameal yogurt drink to reduce blood pressure. With Interbalance L-92, using *Lactobacillus acidophilus* developed by the company, Calpis also fight seasonal allergies but additionally claims to relieve fever (Anonymous, 2007c). In Japan, the functional foods market is over $16 billion and growing at the rate of 12% a year for the last 10 years (Anonymous, 2006b). Probiotics, with a market of $3.23 billion is a large part of Japanese functional foods. The Japanese spent $126 per person per year on functional foods and it is higher than other countries like US ($67.9), Europe ($51.2) and Asia ($3.20). Six percent of Japanese food expenditure is spent on functional foods, which is also higher than any other country. In 1991 functional foods were given legal status in Japan, where they are described as FOSHU means “Foods for Specific Health Use” (Sanders, 1998). A FOSHU is defined as a food expected to have certain health benefits and that has been licensed to bear a label to that effect (Shinohara, 1995). Nutraceuticals ingredients like peptides and proteins, n-3 and n-6 fats and oils, sugar alcohols, oligosaccharides, and lactic acid bacteria come under FOSHU food category (Sanders, 1998). Since 1990, over 5,500 new functional foods have been introduced in Japan. On any given day there are 1,500 to 2,000 functional foods on the market and 400 of them qualify for FOSHU status (Anonymous, 2004b).

Although Japan currently accounts for about one-half of this market, the fastest rate of growth is expected to be in the Europe and United States (Stanton et al., 2001). A study by Leatherhead Food RA, revealed that the probiotic yogurt market in the United Kingdom, France, Germany, Spain, Belgium, Netherlands, Denmark, Finland totaled more than 250 million kg in 1997 (Hilliam, 1998; Stanton et al., 2001), with the largest market by France having sales of about 90 million kg, valued at US$219 million. The German market for probiotic yogurts is growing rapidly and during 1996-1997, it increased by 150%, whereas the UK market rose by 26% during the same period. On an average, probiotic yogurts accounted for 10% of all yogurts sold in the above mentioned nine countries, with Denmark having the highest proportion (20%) followed by Germany and the United Kingdom (both at 13%), France (11%), Netherlands and Belgium (both at 6%) and then Finland and Sweden (both at 5%) (Hilliam, 1998; Stanton et al., 2001). Although the market for dairy probiotic food products is not very significant in Finland and Sweden but these two countries are the leaders in non-dairy probiotic food products such as probiotic juices and chocolates (Stanton et al., 2001).

4. TECHNOLOGICAL ASPECTS OF THE PROBIOTIC FOODS

From a technological perspective, it would be advantageous if probiotics are capable of growing in milk-based media and survive during product manufacture and shelf life (Stanton et al., 2003a). In addition, from a food processing point of view, probiotic strains should be suitable for large-scale industrial production with the ability to survive under both food-processing conditions and storage (Stanton et al., 2003a) and the presence of the probiotic culture in the food product should not adversely affect product quality or sensory properties (Ross et al., 2005). Probiotic strains must have demonstrable benefits to host health and have GRAS (generally recognized as safe) status. The technological properties associated with the incorporation of probiotic strains into food products have been presented in Table 6 (Ross et al., 2005).

Table 6: Technological properties of probiotics

Physiological traits	References
Oxygen tolerance	Kulisaar et al. (2002)
Acid tolerance	Shah (2000)
Bile tolerance	Charteris et al. (1998)
Heat tolerance	Desmond et al. (2001)
Ability to grow in milk	Klaver et al. (1993)
Ability to metabolize prebiotics	Ziemer and Gibson (1998)

Source: Ross et al. (2005)

The use of oxygen-impermeable containers, microencapsulation (Desmond et al., 2002), incorporation of nutrients, and selection of stress-resistant strains (Shah et al., 2000) are applied to solve these problems. Modern researches in genomics and proteomics have made it possible to identify of genes involved in *Lactobacillus* stress responses, such as the molecular chaperone *groESL* and *dnaK* genes for heat stress (Prasad et al., 1999; Schmidt et al., 1999; Walker et al., 1999), methionine sulfoxide reductase genes for oxygen stress (Walter et al., 2003), and F_1F_0-ATPase genes for acid stress (Kullen et al., 1999).

4.1 OXYGEN TOLERANCE

The viability of *L. acidophilus* and *Bifidobacteria* in probiotic yoghurts reported in the market surveys differ with some studies reporting low counts of these bacteria (Iwana et al., 1993; Rybka and Fleet, 1997; Shah et al., 1995, 2000) while others mentioning satisfactory viability (Lourens et al., 2000; Shin et al., 2000). Several factors has been reported to be responsible for the survival of *L. acidophilus* and *Bifidobacterium* spp. in yoghurts, which include acid and hydrogen peroxide produced by yoghurt bacteria, type of strain, culture conditions, concentrations of lactic and acetic acids, whey proteins and interaction of the probiotic species with the yoghurt starters (Kailasapathy and Supraidi, 1996; Godward et al., 2000; Shah, 2000; Vinderola et al., 2000). Among these, the oxygen content in the product and oxygen permeation through the package is considered most significant in reducing the viability of *L. acidophilus* and bifidobacteria in fermented milk products (Klaver et al., 1993; Dave and Shah, 1997c). The sensitivity of probiotic lactobacilli and bifidobacteria to oxygen is considered an important factor affecting their extended survival in yoghurts. Many studies employing only qualitative techniques have been conducted so far to measure the oxygen tolerance of probiotic bacteria (Shimamura et al., 1992; Meile et al., 1997; Shin and Park, 1997; Talwalkar et al., 2001). Talwalkar et al. (2001) successfully modified the Relative Bacterial Growth Ratio (RBGR) methodology, a quantitative technique to enumerate the oxygen tolerance of several probiotic bacteria for the first time. The utilization of this methodology by yoghurt manufacturers or commercial culture companies can assist in differentiating the oxygen sensitive strains from oxygen tolerant strains and thereby facilitate the maintenance of high numbers of probiotic bacteria in yoghurts throughout its manufacture and storage period (Talwalkar et al., 2001). *L. acidophilus* is considered as microaerophilic and bifidobacteria are considered more susceptible to oxygen than *L. acidophilus* due to their anaerobic nature, however, the oxygen susceptibility of bifidobacteria are strain dependent (Talwalkar and Kailasapathy, 2004). Meile et al. (1997) also isolated a moderately oxygen tolerant species of *Bifidobacterium*, *B. lactis* sp. *nov* from fermented milk and thereby reiterated the strain dependent phenomenon of oxygen sensitivity.

In a study Dave and Shah (1997b) found that bifidobacteria survived well over a 35 days period in yoghurt, regardless of the oxygen content and redox potential of the yoghurt. Even the dissolved oxygen of the yoghurt was seen to rise steadily over the shelf, counts of bifidobacteria remained above the recommended 10^6 CFU g^{-1} throughout the shelf life of the yoghurt, while *L. acidophilus* counts were found to decrease below 10^3 CFU g^{-1} by the third week of storage (Miller et al., 2002).

4.2 ACID AND BILE TOLERANCE

Bacterial sensitivity to acidic conditions implies that only strains which can remain viable for an acceptable shelf life to ensure actual benefits to the consumer (Sheehan et al., 2007). Initial assessment of strains for use as probiotic cultures using assays such as acid and bile tolerance, can provide useful information for predicting their performance during gastric transit. Selection of strains based on tolerance to acid stresses as may also be useful indictors of technological performance in fermented foods. Fasting pH in the stomach may be as low as 1.5 (Waterman and Small 1998), and therefore one of the most important characteristics of probiotics following ingestion is ability of acid tolerance (Shah, 2000). There is great variation between strains and species in the ability of potentially probiotic strains to survive in acidic conditions. According to Hood and Zottola (1988) no cells of a *Lactobacillus acidophilus* culture were recovered following 45 min exposure to pH 2.0, while the number of cells was not significantly reduced after 2 h exposure at pH 4.0. Similar trends have been shown for survival of *Lactobacillus rhamnosus* GG in human gastric juice at pH values ranging from 1.0 to 7.0 (Goldin et al., 1992). According to Dunne et al. (1999), reduced tolerance of *Bifidobacterium* cultures to human gastric juice proved that these are less acid tolerant than *Lactobacillus cultures*.

The probiotic bacteria for commercial probiotic applications like *Lactobacillus* and *Bifidobacterium* are traditionally added to fermented milk and other dairy products; however, these can also be added to beverages such as fruit juices, which have an established market sector. Calcium and vitamin fortified juices as functional drinks are consumed regularly and it is essential if the full benefits of probiotics are to be gained. The fruit juices have low pH, typically between pH 2.5 and 3.7 (Ross et al., 2005). After isolating from the harsh environment of the gastrointestinal tract (GIT), Lactobacilli are mainly acid tolerant (McLauchlan et al. 1998) and this theory has become useful in selection of human faecal *Bifidobacterium* resulting into yielding of strains, which are both acid and bile tolerant (Chung et al. 1999). Utilizing the similar approach, Chou and Weimer (1999) isolated acid and bile resistant *Lactobacillus acidophilus* variants. Acid-adapted *Bifidobacterium breve* exhibits superior survival characteristics compared with the presence of other environmental stresses such as bile, hydrogen peroxide and cold storage as reported by Park et al. (1995). Acid-resistant strains screened in this way may prove useful for probiotic applications and may result to better survival both in host environmental conditions and in food systems (Ross et al., 2005).

4.3 HEAT TOLERANCE

Control of the resistance of probiotic bacteria to temperature stress has potential practical benefits in industrial fermentation processes, which requires bacteria with enhanced thermo tolerance (Ross et al., 2005). Heat inducible thermo tolerance allows bacteria, after a non-lethal heat shock to tolerate a second heat stress higher in intensity and it has been found that the heat adaptation increases the thermo tolerance of Lactobacilli (Boutibonnes et al., 1992). Teixeira et al. (1994) and Gouesbet et al. (2002) reported that heat adaptation increased the thermo tolerance of lactobacilli.

According to Desmond et al. (2004) the chaperone proteins are considered essential components of the heat shock response and they guide proteins along the proper pathways for folding. Many are termed as heat shock proteins, because they are formed in great amounts when cells are exposed to heat, which makes misfolding more common. Previous researches revealed that heat adaptation of live microorganisms prior to heat stress has positive effect to improve the thermo tolerance of lactococci and lactobacilli by up to 300-fold compared with untreated parent strains (Boutibonnes et al., 1992; Desmond et al., 2001).

Ding and Shah (2007) tested eight strains of probiotic bacteria, including Lactobacillus rhamnosus, Bifidobacterium longum, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, B. lactis type Bl-O4, and B. lactis type Bi-07, for their acid, bile, and heat tolerance. To enhance survival of the bacteria in acid and bile as well as a brief exposure to heat, microencapsulation in alginate matrix was used and free probiotic organisms were used as a control. HCl in MRS broth over a 2-h incubation period was used for testing acid tolerance of probiotic organisms. Two types of bile salts, oxgall and taurocholic acid were used over an 8-h incubation period for testing bile tolerance. Heat tolerance was tested by exposing the probiotic organisms to 65°C for 1 h. Results revealed that microencapsulated probiotic bacteria survived better than free probiotic bacteria in MRS containing HCl. Viability was reduced by 6.51-log CFU ml-1 when free probiotic bacteria were exposed to oxgall, whereas in microencapsulated strains only 3.36-log CFU ml-1 was lost. At 30 min of heat treatment, microencapsulated probiotic bacteria survived with an average loss of only 4.17-log CFU ml-1 as compared to 6.74-log CFU ml-1 loss with free probiotic bacteria. However, after 1 h of heating both free and microencapsulated probiotic strains showed comparable losses in viability. It could be mentioned that microencapsulation improved the survival of probiotic bacteria when exposed to acidic conditions, bile salts, and mild heat treatment. Overexpression of stress-induced proteins, the GroES/EL chaperone complex can be exploited to prepare and improve the performance of probiotic lactic acid bacteria for industrial processes (Desmond et al., 2004).

4.4 ABILITY TO GROW IN FOOD CARRIERS

The food carriers such as dairy products may enhance microbial survival in gastric juice, most likely due to a buffering or protective effect (Ross et al., 2005). Researches revealed that for delivery of viable probiotic *Lactobacilli* (Stanton et al., 1998) and *Enterococci* (Gardiner et al., 1999) to the gastrointestinal tract, Cheddar cheese shows more protective effect as a food carrier as compared to the yoghurt. According to Ross et al. (2005) this effect may be due to the buffering capacity of the food product. Addition of milk or milk proteins to gastric juice or media simulating gastric juice significantly increased the pH and enhances survival of some *Lactobacillus* and *Bifidobacterium* species (Charteris et al., 1998). The *L. paracasei* NFBC 338 strain satisfies a number of characteristics as a probiotic strains, viz., being of human intestinal origin, exhibiting bile and acid tolerance (Desmond et al., 2004), and being technologically compatible with cheese manufacture (Gardiner et al., 1998) and spray drying (Gardiner et al., 2000).

5. LIMITATIONS

Probiotics are restricted to products that

1) contain live microorganisms (e.g., as freeze-dried cells or in a fresh or fermented product),
2) improve the health, growth and well-being of humans or animals, and
3) can affect all host mucosal surfaces, including the mouth and gastrointestinal tract (e.g., applied in food, pill, or capsule form), the upper respiratory tract (e.g., applied as an aerosol), or the urogenital tract (Havenaar and Veld, 1992).

Though probiotics are "generally regarded as safe (GRAS)", but side effects such as septicemia and fungaemia have rarely been reported in high-risk situations (Vandenplas et al., 2007).

Not all probiotic strains are effective, and considerable strain-to-strain variation in properties relevant to probiotic efficacy is observed within bacterial species (Crittenden et al., 2005). Just one type or strain of organism cannot provide all potential benefits. Several probiotic microorganisms like *Lactococcus lactis, Lactobacillus* sp., *Streptococcus, Enterococcus, Bifidobacterium* sp., *Pediococcus, Propionibacteria* sp. etc. are used in foods (Anonymous, 2007d). The choice of strain of microorganism is important to avoid removal of micronutrients from the food, to avoid production of adverse components such as vasoactive amines and to avoid opportunistic lactic acid bacterial pathogens. Because of the potential side effects and interactions with medications, dietary supplements should be taken only under the supervision of a healthcare provider. Mild gastrointestinal upset may occur in some individuals (not on antibiotic therapy) who take more than 1 to 2 billion *L. acidophilus* cells per day (Anonymous, 2007d).

It is interesting to note that under similar genus of a microorganism there may be wide range of species and within each of these species are separate strains of which there can be hundreds, which may have different effects on health. As for example the genus *Lactobacillus* contains up to 60 species including *L. acidophilus* and *L. casei* - the ones most commonly added to yoghurts and drinks (Wahlqvist, 2002). To have any effect in the colon, the bacteria in probiotic foods should survive food processing and storage in large numbers, then survive the passage through the acids and digestive enzymes in the stomach and small intestine in appreciable numbers, and still survive once they reach the colon. Limited evidences regarding the survival of bacteria to the colon are available (Wahlqvist, 2002).

To have the desired effect, scientists believe at least a million of each probiotic bacteria per gram of yoghurt or drink are needed e.g. if a yoghurt contains three different types of probiotic bacteria, it should contain at least a million of each of them per gram. The yoghurt Vaalia contains three different types of bacteria at these desirable levels; Yoplus has two different bacteria and LC1 and Yakult have one bacterium at these levels (Wahlqvist, 2002).

If a person is currently being treated with any of the Sulfasalazine, a medication used to treat ulcerative colitis, he/she should not use *Lactobacillus* or other probiotics without first talking to healthcare provider. A laboratory study suggests that *L. acidophilus* speeds up metabolism of sulfasalazine. The importance of this information is not known presently (Wahlqvist, 2002).

6. CONCLUSION

Today probiotics are gaining importance because of the numerous benefits, e.g. treating lactose intolerance, asymptomatic bacterial vaginosis, hypercholesterolemia, irritable bowel disease, cardiac diseases, atherosclerosis and arteriosclerosis. The ability of probiotics to prevent diseases and improve health at all ages is increasing the market potential at a high rate. However, the development of successful probiotic products depends on proof of a probiotic effect as well as on the foods where high numbers of viable organisms survive at the time of consumption as well as at the time it reach to the colon. Identification and characterization of genus and species of probiotic organisms by using internationally accepted methodologies, such as DNA-DNA hybridization, sequencing of DNA encoding 16S rRNA, Pulsed Field Gel Electrophoresis or Randomly Amplified Polymorphic DNA and thereby labeling the product will help the consumers to know exactly what strains are present in the products. Keeping in mind the losses in cell viability during gastric transit, to deliver the relevant dose of live bacteria to the gut, the probiotic food product should be regularly consumed in sufficient quantity.

Consequently, the technological issues related to the development of foods containing these bacteria in sufficient numbers throughout shelf-life need to be overcome, as well as means of stabilization following ingestion, i.e. during exposure to the adverse conditions of the human gastrointestinal tract. In spite of many health benefits of probiotics, the mechanisms by which the probiotic organisms exert their effects are still at nascent stage. Therefore, researches should focus on understanding the mechanisms of health-promoting effects of probiotic cultures for safe future of probiotics as functional food ingredients.

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