Probiotics: The Historic Milieu

The term probiotic is derived from Greek and means ‘for life’ as opposed to antibiotics which means ‘against life’. The history of probiotics began with the consumption of fermented foods. Consumption of fermented foods was first observed in ancient Greeks and Romans (Gismondo et al. 1999, Guarner et al. 2005). In 1907, Ellie Metchnikoff, a Nobel Prize winner, first proposed the beneficial effects of probiotic microorganisms on human health. Metchnikoff hypothesized that Bulgarians were healthy and lived long because of the consumption of fermented milk products which consists of rod shaped bacteria (*Lactobacillus* spp.). These bacteria affect the gut microflora positively and decrease the microbial toxic activity (Gismondo et al. 1999, Chuayana et al. 2003). The first documentation about health
promoting effects of fermented milk dates back to a Persian version of the Old Testament (Genesis 18:8) that states that ‘Abraham owed his longevity to the consumption of sour milk’. Kollath in 1953 and Vergio in 1954 were probably the first to introduce the term ‘Probiotic’ (Holzapfel and Schillinger 2002). The term ‘probiotic’ was first used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms. Since then the word ‘probiotic’ has been used in different contexts according to its mechanism and the affects on human health. The term, probiotic, as is used today was first used by Parker in 1974. Parker defined ‘probiotic’ as substances and organisms which contribute to intestinal microbial balance. In 1989, the term was modified further by Fuller. Thus, probiotic is a live microbial supplement which affects the host’s health positively by improving its intestinal microbial balance. This definition was broadened by Havenaar and Huis in’t Veld in 1992 to include mono or mixed culture of live microorganisms which benefits animals or man by improving the properties of the indigenous microflora (Çakır 2003, Guarner et al. 2005, Sanders 2003). Some authors have interpreted probiotics as microbrial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. Bacterial cell-wall components, heat-killed whole cells or metabolites can have a specific probiotic effect, for example, improvement of lactose digestion or treatment of acute or chronic diarrhoea (Ouwehand and Salminen 1998, Romond et al. 1998, Salminen et al. 1999, Simakachorn et al. 2000, Xiao et al. 2002). Through the years, lots of research has been done on probiotics and therefore, many definitions have been suggested. They are listed below in Table 1.

Probiotics offer challenges for industrial applications. The probiotic concept is open to lots of different applications in a large variety of fields relevant for human and animal health. Probiotic products consist of different enzymes, vitamins, capsules or tablets and some fermented foods contain microorganisms which have beneficial effects on the health of the host. They can contain one or several species of probiotic bacteria. Most probiotic products destined for human consumption are in the form of fermented milk or given in powder or tablet forms. These capsules and tablets are not used for medical applications but as health supporting products. The oral consumption of probiotic microorganisms produces a protective effect on the gut flora. Lots of studies suggest that probiotics have beneficial effects on microbial disorders of the gut, but it is really difficult to show the clinical effects of such products. The probiotic preparations used for traveller’s diarrhea, antibiotic associated diarrhea and acute diarrhea show that they have a positive therapeutic effect (Gismondo et al. 1999, Çakır 2003, Ouwehand et al. 1999).
Probiotics, Prebiotics and Synbiotics: An Introduction

Commercial Strains of Probiotics and their Sources

The probiotic bacteria generally belong to the genera Lactobacillus and Bifidobacterium. However, other bacteria and some yeast also have probiotic properties. Common probiotics include: 1) Lactobacilli such as Lactobacillus acidophilus, L. johnsonii, L. casei, L. delbrueckii ssp. bulgaricus, L. reuteri, L. brevis, L. ceblobiosus, L. curvatus, L. fermentum, L. plantarum 2) Gram-positive cocci such as Lactococcus lactis ssp. cremoris, Streptococcus salivarius ssp. thermophilus, Enterococcus faecium, S. diacetyllactis, S. intermedius and 3) Bifidobacteria such as Bifidobacterium bifidum, B. adolescentis, B. animalis, B. infantis, B. longum, B. thermophilum (Collins et al. 1998, Gibson 1999, Mercenier et al. 2002). Other microbial species, besides lactic acid bacteria (LAB), like Bacillus subtilis, Propionibacterium spp. and yeasts (Saccharomyces boulardii) have also been accepted and used as probiotics (Chukeatirote 2002, Jan et al. 2002). The mechanism of the action of probiotics (e.g., bifidobacteria and lactobacilli) relies on their metabolic end products; mainly organic acids which may lower the human gut pH and consequently inhibit the growth of pathogenic microbes. Other factors are occupation of normal colonisation sites by probiotics, competition for available nutrients and production of antimicrobial substances. The second generation of probiotics are genetically modified microorganisms that provide the host with some necessary components, e.g., production of immunomodulators.

Table 1. Some proposed definitions of probiotic.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Definition</th>
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<tr>
<td>1</td>
<td>A live microbial supplement which affects host’s health positively by improving its intestinal microbial balance</td>
<td>Fuller 1989</td>
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<tr>
<td>2</td>
<td>Living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition</td>
<td>Shaafasma 1996</td>
</tr>
<tr>
<td>3</td>
<td>A live microbial food ingredient that is beneficial to health</td>
<td>Salminen et al. 1998</td>
</tr>
<tr>
<td>4</td>
<td>A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract</td>
<td>Naidu et al. 1999</td>
</tr>
<tr>
<td>5</td>
<td>A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host</td>
<td>Schrezenmeir and de Vrese 2001</td>
</tr>
<tr>
<td>6</td>
<td>Live microorganisms which when administered in adequate amounts confer a health benefit on the host is accepted by FAO/WHO (report in October 2001)</td>
<td>Klaenhammer 2000, Sanders 2003, Guarner et al. 2005</td>
</tr>
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</table>
(e.g., interleukines) or Helicobacter pylori and rotavirus antigens (Mercenier et al. 2004). Probiotic preparations used as food supplements can consist of one single strain (e.g., Yakult, Japan—L. casei Sirota) or are mixed cultures of two (e.g., Bacilac, Belgium—L. acidophilus plus L. rhamnosus) or even more (e.g., food supplement VSL-3, Italy contains 8 LAB species) strains. Table 2 summaries the probiotic bacterial species and the strains primarily used in the food industry.

Table 2. Some commercial probiotic strains.

<table>
<thead>
<tr>
<th>Strain Source</th>
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<tbody>
<tr>
<td>L. acidophilus NCFM</td>
<td>Rhodia, Inc. (Madison, Wisconsin, USA)</td>
</tr>
<tr>
<td>Lactobacillus brevis KB290</td>
<td>Kagome Co., Ltd. (Tochigi, Japan)</td>
</tr>
<tr>
<td>L. acidophilus DDS-1</td>
<td>Nebraska Cultures, Inc. (Lincoln, NE)</td>
</tr>
<tr>
<td>L. acidophilus SBT-2062</td>
<td>Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)</td>
</tr>
<tr>
<td>B. longum SBT-2928</td>
<td></td>
</tr>
<tr>
<td>L. acidophilus R0011</td>
<td>Institut Rosell (Montreal, Canada)</td>
</tr>
<tr>
<td>L. rhamnosus R0052</td>
<td></td>
</tr>
<tr>
<td>L. paracasei CRL 431</td>
<td>Chr. Hansen (Horsholm, Denmark)</td>
</tr>
<tr>
<td>B. lactis Bb-12</td>
<td></td>
</tr>
<tr>
<td>L. casei Shirot &amp; breve strain Yakult</td>
<td>Yakult (Tokyo, Japan)</td>
</tr>
<tr>
<td>L. casei DN014001 (Immunitas)</td>
<td>Danone Le Plessis-Robinson (Paris, France)</td>
</tr>
<tr>
<td>L. fermentum RC-14</td>
<td>Urex Biotech Inc. (London, Ontario, Canada)</td>
</tr>
<tr>
<td>L. rhamnosus GR-1</td>
<td></td>
</tr>
<tr>
<td>Streptococcus thermophilus MN-ZLW-002</td>
<td>Inner Mongolia Mengniu Dairy Industry Co. Ltd., (Hohhot, China)</td>
</tr>
<tr>
<td>L. johnsonii L1 (same as Lj1)</td>
<td>Nestlé (Lausanne, Switzerland)</td>
</tr>
<tr>
<td>L. plantarum 299v</td>
<td>Probi AB (Lund, Sweden)</td>
</tr>
<tr>
<td>L. Rhamnosus 271</td>
<td></td>
</tr>
<tr>
<td>L. reuteri SD2112 (same as MM2)</td>
<td>BioGaia (Raleigh, North California, USA)</td>
</tr>
<tr>
<td>L. rhamnosus GG</td>
<td>Valio Dairy (Helsinki, Finland)</td>
</tr>
<tr>
<td>L. rhamnosus LB21</td>
<td>Essum AB (Umeå, Sweden)</td>
</tr>
<tr>
<td>Lactococcus lactis L1A</td>
<td></td>
</tr>
<tr>
<td>L. salivarius UCC118</td>
<td>University College (Cork, Ireland)</td>
</tr>
<tr>
<td>B. longum BB536</td>
<td>Morinaga Milk Industry Co., Ltd. (Zamak City, Japan)</td>
</tr>
<tr>
<td>B. lactis HN019 (DR10)</td>
<td>New Zealand Dairy Board</td>
</tr>
<tr>
<td>L. acidophilus LB</td>
<td>Lacteol Laboratory, (Houdan, France)</td>
</tr>
<tr>
<td>L. paracasei F19</td>
<td>Arla Dairy (Stockholm, Sweden)</td>
</tr>
<tr>
<td>L. crispatus CTV05</td>
<td>Gynelogix, Boulder, Colo.</td>
</tr>
<tr>
<td>L. casei DN 114</td>
<td>Danone, Paris, France</td>
</tr>
<tr>
<td>S. boulardii</td>
<td>Biocodex Inc. (Seattle, Washington, USA)</td>
</tr>
<tr>
<td>L. delbrueckii subsp. bulgaricus 2038</td>
<td>Meiji Milk Products (Tokyo, Japan)</td>
</tr>
</tbody>
</table>
Probiotics Attributes/Characteristics

Most probiotics are related to the Lactobacillus and Bifidobacterium genera (Bezkorovainy et al. 1997, Salminen and von Wright 1998, Sanders 2003, Guarner et al. 2005, Nagpal et al. 2007, 2012a, Kumar et al. 2009a). However, to be considered as probiotics, the different strains should be normal inhabitants of a healthy intestinal tract, survive the upper digestive tract and be capable of surviving and growing in the intestine (acid and bile resistant), safe for human consumption, produce antimicrobial substances like bacteriocins and have the ability of adherence to human intestinal cell lines and colonization (Guarner and Schaafsma 1998, Morelli 2000, Guarner et al. 2005).

Lactic acid bacteria have an established safety record as compared to the probiotics and are rarely involved in disease. The most commonly used probiotics are Lactobacillus spp., Bifidobacterium spp. and Lactococcus spp. and these have been accorded the GRAS (Generally Recognized As Safe) status (Salminen and von Wright 1998).

However, not all the probiotics available on the market have been shown to meet the requirements determined by FAO and WHO experts. These requirements hold particular importance because there is no information available on the risks related to the long-term use of probiotics. Experts have identified 4 kinds of potential adverse effects, i.e., systemic infections, harmful metabolic activities, excessive immune system stimulation in susceptible individuals and transfer of genetic material (De Groote et al. 2005, Herbrecht and Nivoix 2005, Salminen 2004b).

Indeed, as was very recently pointed out in a review (Paone 2012), additional characteristics that probiotics must possess are a demonstrated genetic stability and the capability to not develop antibiotic resistance. Such characteristics are rarely checked for in the currently available formulations. Lactobacillus F19 (species paracasei subsp. paracasei F19) has been developed in accordance with the abovementioned requirements, and in particular is genetically stable (Morelli et al. 2002) and capable of not developing antibiotic resistance (Sullivan et al. 2003, 2004). A worthwhile eco-friendly strategy of probiotic production has been originally developed by Kagome laboratories in Japan. Here culture collections of lactic acid bacteria from Japanese traditional fermented vegetables have been accumulated along the years while preserving the natural season sampling at proper microclimate conditions. Among almost a thousand isolated and tested strains, Lactobacillus brevis KB290 has been extensively studied in humans with proven safety and efficacy (Nobuta et al. 2009).
Mechanism of Actions of Probiotics

Probiotic microorganisms are considered to support the host health. However, the support mechanisms have not been explained (Holzapfel et al. 1998). There are studies on how probiotics work. These studies are trying to explain how probiotics could protect the host from intestinal disorders. The study mechanisms are listed below briefly (Rolfe 2000, Çakır 2003, Salminen et al. 1999, Castagliuola et al. 1999, Nagpal et al. 2007, 2010, 2011, 2012a,b, Kumar et al. 2009a,b, 2010, 2011, 2012).

- Production of inhibitory substances: Production of some organic acids, hydrogen peroxide and bacteriocins which are inhibitory to both gram-positive and gram-negative bacteria.
- Blocking of adhesion sites: Probiotics and pathogenic bacteria are in a competition. Probiotics inhibit the pathogens by adhering to the intestinal epithelial surfaces and blocking the adhesion sites.
- Competition for nutrients: Despite of the lack of studies in vivo, probiotics have been shown to inhibit the pathogens by consuming the nutrients which pathogens need.
- Stimulating of immunity: Stimulating of specific and nonspecific immunity may be one possible mechanism of probiotics to protect the host from intestinal disease. This mechanism is not well documented, but it is thought that specific cell wall components or cell layers may act as adjuvants and increase humoral immune response.
- Degradation of toxin receptor: Because of the degradation of toxin receptor on the intestinal mucosa, it was shown that S. boulardii protects the host against C. difficile intestinal disease.

Some other offered mechanisms are suppression of toxin production, reduction of gut pH, attenuation of virulence (Fooks et al. 1999).

Gastrointestinal Microflora Balance and Probiotics

More than 400 bacterial species exist in the human intestinal tract. It is an enormously complex ecosystem that includes both facultative anaerobic and anaerobic microorganisms (Naidu et al. 1999). The numbers of genera is nearly steady, because each of them has its own growth niches (Fooks et al. 1999). The composition of the gut microflora is constant but can be affected by factors such as age, diet, environment, stress and medication. To have a healthy intestine, the balance of the bacteria must be maintained but this is difficult due to the changing life style of the population. Lots of factors may change the balance away from potentially beneficial or health promoting bacteria to potentially harmful or pathogenic microorganisms. This makes the host more susceptible to illnesses. In this case, the prevalence of the
beneficial bacteria must be supported. The use of probiotics helps to protect the host from various intestinal diseases and disorders by increasing the number of beneficial bacteria and making the balance steady again (Fooks et al. 1999, Nagpal et al. 2007, 2010, 2012a,b, Kumar et al. 2009a,b, 2010, 2011, 2012). Probiotics are suggested as food to provide for the balance of intestinal flora (Holzapfel et al. 1998). Probiotics have been used for long times in food ingredients for human and animals without any side effects.

Probiotics, naturally found in the mouth, lower intestine and vagina of healthy individuals, help defend the body against invading pathogenic bacteria. Due to the dominance of common antibiotic treatment, many people lack healthy intestinal flora. The composition of the intestinal flora is relatively stable in healthy human beings between harmful and beneficial or natural bacteria. Among the beneficial bacteria are *Lactobacillus* spp. and *Bifidobacterium* spp. which play a useful role in the production of vitamins, organic acids and antimicrobial factors that inhibit pathogens. Any imbalance in the gut microflora leads to dominance of harmful bacteria in the intestinal flora, which affects the production of essential nutrients and increases the level of damaging substances, including carcinogens, putrefactive products and toxins (Mitsuoka 1996, Salminen and Gueimonde 2004). Therefore, to maintain a well-balanced microflora in the gastrointestinal tract, it has been suggested that live bacteria be introduce to stimulate growth of beneficial bacterial population groups which prevent harmful effects and promote beneficial actions of the intestinal microflora (Salminen et al. 1996, Shah 2000). Consuming probiotics with dairy foods buffers the stomach acid and increases the likelihood that the bacteria will survive in the intestine. Dairy products containing probiotics also provide a number of essential nutrients including calcium and protein (Stanton et al. 2001, Nagpal et al. 2011, 2012a).

**Site of Action of Probiotics: The Small Intestine**

The intestinal epithelium is a highly organized, single-cell layer covering the interface between tissues and the intestinal lumen. This monolayer is mainly constituted of enterocytes, which are the cells responsible for taking up nutrients, Paneth cells, which secrete the mucus bathing the epithelium, and intra-epithelial lymphocytes, which are part of the mucosa-associated lymphoid tissue (MALT). Yet, all epithelial cells arise from common non-differentiated precursors present in the epithelium (Brandtzaeg 1995). This monolayer is constantly being renewed as epithelial cells undergo a lifecycle, which starts deep within the crypts, from where they arise, continue with their differentiation and migration towards the tip of the villi and end with apoptosis and exfoliation (Stadnyk 1994, Turner 2003, Dommett et al. 2005). This cycle takes about 3 to 5 days in humans and allows
epithelial self-renewal. Because of this turnover, the gut surface is covered by dead and exfoliating cells, which provide together with the mucus and the nutrients passing through the lumen an excellent growth substrate for microorganisms (Stadnyk 2002, Tlaskalova-Hogenova et al. 2005).

Colonization of Probiotics in the Gut

It is generally agreed that to permanently establish a bacterial strain in the host intestine, the microorganism must be able to attach to the intestinal mucosal cells (O’Sullivan et al. 1992). Moreover, many pathogens cannot exert their deleterious effects on the gut unless they become so attached (Hoepelman and Tuomanen 1992) and the beneficial action of probiotics has been studied for their purported ability to interfere with the adherence of pathogens to intestinal mucosal cells (Fuller 1991). The normal colonization of the sterile newborn intestine is a complex process. Initial colonization is achieved with maternal vaginal and fecal bacterial flora. The first colonizers have a high reductive potential and include species such as enterobacter, streptococcus, and staphylococcus. These metabolize oxygen, thus encouraging the growth of anaerobic bacteria including lactobacilli and bifidobacteria.

Effects of Probiotics on Health

There are lots of studies on the health benefits of fermented foods and probiotics. However, in most of these studies, researchers did not use sufficient test subjects or the used microorganisms were not identified definitely (Mohania et al. 2008). So, while a number of reported effects have been only partially established, some can be regarded as well established and clinically well documented for specific strains. These health-related effects can be considered as indicated below (Schrezenmeir and De Vrese 2001, Dunne et al. 2001, Dugas et al. 1999, Nagpal et al. 2007, 2010, 2011, 2012a,b, Kumar et al. 2009a,b, 2010, 2011, 2012).

- Managing lactose intolerance
- Improving immune system
- Prevention of colon cancer
- Reduction of cholesterol and triacylglycerol plasma concentrations
- Lowering blood pressure
- Reducing inflammation
- Reduction of allergic symptoms
- Beneficial effects on mineral metabolism, particularly bone density and stability
• Reduction of *Helicobacter pylori* infection
• Suppression of pathogenic microorganisms (antimicrobial effect)
• Prevention of osteoporosis
• Prevention of urogenital infections

**Lactose intolerance**

Most humans, commonly non-Caucasians, become lactose intolerant after weaning. These lactose intolerant people cannot metabolize lactose due to the lack of an essential enzyme, $\beta$-galactosidase. If lactose passes through the small intestine, it is converted to gas and acid in the large intestine by the colonic microflora. Also, the presence of breath hydrogen is a signal for lactose maldigestion. The studies provide that the addition of certain starter cultures to milk products allows lactose intolerant people to consume these products without the usual rise of breath hydrogen or associated symptoms (Fooks et al. 1999, Scheinbach 1998, Quewand and Salminen 1998, Lin et al. 1991). The beneficial effects of probiotics on lactose intolerance are due to the lower lactose concentration in the fermented foods due to the high lactase activity of bacterial preparations used in the production. Given that lactose is converted to, lactic acid by $\beta$-galactosidase enzyme, which is contained in yogurt, this becomes more suitable than milk for individuals with lactose intolerance. Furthermore, the LAB which is used to produce yogurt, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are not resistant to gastric acidity. Hence, products with probiotic bacteria are more efficient for lactose intolerant humans.

It is thought that the major factor that improves the digestibility by the hydrolyses of lactose is the bacterial enzyme $\beta$-galactosidase. Another factor is the slower gastric emptying of semi-solid milk products such as yogurt. So the $\beta$-galactosidase activity of probiotic strains and other lactic acid bacteria used in dairy products is really important. $\beta$-galactosidase activity within probiotics varies in a huge range. It has to be considered both the enzyme activity of probiotic strain and the activity left in the final product for their use in lactose intolerant subjects (Salminen et al. 2004).

**Immuno-modulation**

The effect of probiotics on the immune system are promising. However, the mechanism is not well understood. Human studies have shown that probiotic bacteria can have positive effects on the immune system of their hosts (Mombelli and Gismondo 2000). Several researchers have studied the effects of probiotics on immune system stimulation. Some *in vitro* and *in vivo* searches have been carried out in mice and with humans. The data indicates
that oral bacteriotherapy and living bacteria feeding in fermented milks supported the immune system against some pathogens (Scheinbach 1998, Dugas et al. 1999). Probiotics affect the immune system in different ways such as: producing cytokines, stimulating macrophages and increasing secretory IgA concentrations (Scheinbach 1998, Dugas et al. 1999). Some of these effects are related to adhesion while some of them are not (Quwehand et al. 1999). Link-Amster et al. (1994) examined whether eating fermented milk containing \textit{Lactobacillus acidophilus} La1 and bifidobacteria could modulate the immune response in humans. They gave volunteers the test fermented milk over a period of three weeks during which attenuated \textit{Salmonella typhi} Ty21a was administered to mimic an enteropathogenic infection. After three weeks, the specific serum IgA titre rise to \textit{S. typhi} Ty21a in the test group was >4-fold and significantly higher (p=0.04) than in the control group which did not eat fermented foods but received \textit{S. typhi} Ty21a. These results showed that LAB which can survive in the gastrointestinal tract can act as adjuvants to the humoral immune response (Lime-Amster et al. 1994, Quwehand et al. 1999).

Perdigon et al. (1988) fed the mice with lactobacilli or yogurt and it stimulated macrophages and increased secretory IgA concentrations (Scheinbach 1998). Also in a human trial Halpern et al. (1996) fed humans or human subjects with 450 g of yogurt per day for 4 months and at the end a significant increase was observed in the production of \(\gamma\)-interferon (Fooks et al. 1999). Mattila-Sandholm and Kauppila (1998) showed that \textit{Lactobacillus rhamnosus} GG and \textit{Bifidobacterium lactis} Bb-12 derived extracts suppress lymphocyte proliferation \textit{in vitro}. A very fine study recently published, showed that \textit{Streptococcus thermophilus} MN-ZLW-002 fermented milk may stimulate non-specific cell-mediated immunity at plasma and epigenomic pulmonary levels which are involved in the protection of the mammals from respiratory infections, thus paving the way to a promising clinical application (Kang et al. 2012). Finally, a recent editorial from Rastmanesh et al. (2012) has envisaged the potential use of probiotics in enhancing the efficacy of HIV vaccination.

\textbf{Diarrhea}

Diarrhea has many causes and many types so it is difficult to evaluate the effects of probiotics on diarrhea. But there is a lot of research and evidence that probiotics have beneficial effects on some types of diarrhea. Diarrhea is a major cause/reason of childrens deaths worldwide and rotavirus is its common cause (Scheinbach 1998). In the treatment of rotavirus diarrhea, \textit{Lactobacillus GG} is reportedly really effective. The best documented probiotic effect is a shortened duration of rotavirus diarrhea using \textit{Lactobacillus GG}. This has been proved in several studies around the world by some
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Probiotics, like Guandalini (2000) and Pant et al. (1996). Also, Lactobacillus acidophilus LB1, Bifidobacterium lactis and Lactobacillus reuterii are reported to have beneficial effects on shortening the duration of diarrhea (Salminen et al. 2004). One type of diarrhea is travellers’ diarrhea (TD) which affects healthy travellers not only in developing countries but also in Europe. Probiotics have beneficial effects in preventing some forms of TD. Oksanen et al. (1990) evaluated the efficacy of Lactobacillus GG in preventing diarrhea in 820 people travelling from Finland to Turkey. In a double-blind study by Black et al. (1989) lyophylised bacteria (L. acidophilus, B. bifidum, L. bulgaricus, S. thermophilus) were given to 56 Danish tourists on a 2-week trip to Egypt. The occurrence of diarrhea in the group receiving the lactic acid bacteria was 43% while it was 71% in the placebo group (Gismondo et al. 1999).

Antibiotic therapy causes outbreaks of diarrhea. The normal microflora is suppressed during the microbial therapy and may result in a predominance of pathogenic strains. The changes in microflora may also encourage resistant strains like Clostridium difficile which is the reason for antibiotic associated diarrhea (ADD). Several clinical trials (McFarland 1998, McFarland and Elmer 2005) have used Saccharomyces boulardii, Lactobacillus spp. and Bifidobacterium spp. in ADD. Probiotics which are able to restore and replace the normal flora should be used. Also, they should be used in high risk patients such as old, hospitalized or immunocompromised. Studies have shown that Clostridium difficile decreases in the presence of Saccharomyces boulardii (Gismondo et al. 1999).

Colon and other cancer treatment

Probiotic bacteria play an important role in retarding colon carcinogenesis by possibly influencing metabolic, immunologic, and protective functions in the colon. The use of probiotics in prevention and cancer treatment has been undergoing a recent evaluation in a number of studies. Although we should not expect miraculous outcomes in cancer treatment following probiotics administration, their immunomodulatory properties have been tested and need to be brought to the publics’ attention. It is important to note that the desired effects are strain and dose specific and therefore more clinical studies are needed to screen each strain and corresponding disorder. In animals, LAB ingestion was shown to prevent carcinogen induced preneoplastic lesions and tumors. In the study by McIntosh et al. (1999), Lactobacillus acidophilus (Delvo Pro LA-1), Lactobacillus rhamnosus (GG), Bifidobacterium animalis (CSCC1941), and Streptococcus thermophilus (DD145) strains were examined for their influence on 1, 2-dimethylhydrazine (DMH) induced intestinal tumors in 100 male Sprague-Dawley rats when added as freeze-dried bacteria. This study concluded that the strain of L. acidophilus supplied as freeze-dried bacteria in the diet was protective because it significantly

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inhibited tumors within the rat colon. There is a substantial amount of study done by Perdignon et al. dealing with anti-inflammatory properties of probiotic bacteria. In the study by Galdeano and Perdigon (2006), the probiotic bacterium *Lactobacillus casei* was screened for its influence on the expression of receptors involved in the innate immune response in colorectal cancer BALB/c model mice. Further, a complex nature of kefir was studied in BALB/c mice. Kefir is fermented milk produced by the action of lactic acid bacteria, yeasts and acetic acid bacteria, trapped in a complex matrix of polysaccharides and proteins. In addition, it is an excellent source of proteins and calcium. A study concluded that since LAB contained in kefir along with yeasts and acetic acid bacteria have an *in vivo* role as oral biotherapeutic substances capable of stimulating immune cells of the innate immune system they are able to promote cell-mediated immune responses against tumors and also against intracellular pathogenic infections. In another kefir related study by Vinderola et al. (2000), the immunomodulating capacity of kefir on the intestinal mucosal immune response in mice of viable or heat-inactivated bacteria at different doses was determined. However, in humans, there is no evidence available on whether probiotics can prevent the initiation of colon cancer. Epidemiologic studies are contradictory; some studies could not find an association between the consumption of fermented-milk products and the risk of colon cancer whereas other studies showed a lower incidence of colon cancer in persons consuming fermented-milk products or yogurt.

There is *in vitro* and *in vivo* evidence not only from animal studies but also from human studies that probiotics have beneficial effects on suppression of cancer. Oral administration of lactic acid bacteria has been shown to reduce DNA damage caused by chemical carcinogens, in gastric and colonic mucosa in rats (Marotta et al. 2003). Moreover, improved gastrointestinal motility due to probiotic consumption (Metugriachuck et al. 2006) may be a further protecting factor against endoluminal carcinogens. The consumption of lactobacilli by healthy volunteers has been demonstrated to reduce the mutagenicity of urine and feaces associated with the ingestion of carcinogens in cooked meat. When it comes to epidemiological studies, they show an association between fermented dairy products and colorectal cancer. The consumption of a large quantity of dairy products especially fermented foods like yogurt and fermented milk containing *Lactobacillus* or *Bifidobacterium* may be related to a lower occurrence of colon cancer (Rafter 2003, Hirayama and Rafter 2000). A number of studies have shown that predisposing factors (increase in enzyme activity that activate carcinogens, increase procarcinogenic chemicals within the colon or in population of certain bacterial genera and species) are altered positively by consumption of certain probiotics (Brady et al. 2000, Kumar et al. 2010, 2011, 2012).
Cholesterol reduction

Lots of researchers have proposed that probiotics have cholesterol reduction effects. However, the mechanism of this effect has not been explained definitely. There are two hypotheses trying to explain the mechanism. One of them is that bacteria may bind or incorporate cholesterol directly into the cell membrane. The other one is, bile salt hydrolysis enzymes deconjugate the bile salts which are more likely to be exerted resulting in increased cholesterol breakdown (Çakır 2003, Scheinbach 1998, Prakash and Jones 2005, Nagpal et al. 2010). A study on the reduction of cholesterol showed that Lactobacillus reuteri CRL 1098 decreased total cholesterol by 38% when it was given to mice for 7 days in the rate of $10^4$ cells/day. This dose of Lactobacillus reuteri caused a 40% reduction in triglycerides and a 20% increase in the ratio of high density lipoprotein to low density lipoprotein without bacterial translocation of the native microflora into the spleen and liver (Kaur et al. 2002).

Functional Foods and Probiotics

Although the primary purpose of food is to provide enough nutrients to fulfill body requirements, various functions of the body are modulated by diet. In order to compensate for deficiency of certain nutrients in the diet due to changes in nutritional habits of developed industrial countries, the concept of functional food has been developed. A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effect, in a way which is relevant to either an improved state of health and well being and/or reduction of disease risk” (ILSI Europe 1999). Functional food is intended for a population generally in normal health and must demonstrate beneficial effects in amounts that are usually consumed in the diet. Functional food is a natural food, to which a component has been added/removed or a food in which the bioavailability of the components has been modified by technological or biotechnological means (Korhonen 2002). Functional food can be classified into different groups according to their effect: fat replacers, probiotics, probiotics and dietary fibres, antioxidants, vitamins, polyphenols, plant sterols, polyunsaturated fatty acids and minerals. The most promising targets for functional food are the functions and particularly control of nutrient bioavailability (Roberfroid 2000). The gastrointestinal (GI) functions include balanced colonic microflora, control of transit time and mucosal motility, modulation of epithelial cell proliferation, balance of redox and antioxidant systems, metabolism of macronutrients, especially amino acids, carbohydrates and fatty acids. The term functional food originates from the 1980s (Sanders 1999). In 1991, a legal status to
functional foods was granted in Japan, indicating foods for special health use. The first functional food probiotic fermented milk drink Yakult has been available in Japan since 1935 (Karimi and Peña 2003). Currently various probiotic supplemented functional foods, i.e., dairy products are available in the market, worldwide, but very few of them have been studied for their claimed health beneficial effects; therefore we think systemic studies should be conducted before claiming the health benefits of known functional foods. National Dairy Research Institute (India) developed a probiotic dahi (Indian yogurt) supplemented with two health beneficial probiotic bacterial strains named *Lactobacillus casei* and *Lactobacillus acidophilus* (Yadav et al. 2005) and has been systematically studied for health beneficial effects, i.e., anti-diabetic (Yadav et al. 2007, 2008) and immunomodulatory (Jain et al. 2009a,b) effects, before taking it to the market. Such studies support the health beneficial effects of foods available for consumers and provide more consumer confidence in market.

**Safety of Probiotics**

The best evidence for the general safety of lactic acid bacteria and bifidobacteria is their long tradition of use without any harmful effects on human health. With the exception of one strain belonging to the *L. rhamnosus* species, lactobacilli and bifidobacteria used for food production are “generally recognized as safe” (GRAS) by the Food and Drug Administration in USA. Moreover, certain strains of probiotic bacteria have been proven to be free of risk factors like: transferable antibiotic resistances, cancer-promoting and/or putrefactive enzymes and metabolites, hemolysis, activation of thrombocyte-aggregation or mucus degradation in the mucus layer of the gastrointestinal tract. Despite the absence of a pathogenic potential, lactic acid bacteria were found in < 0.1% (enterococci 1%) of clinical samples from severe infections (endocarditis, meningitis, or bacteremia (Gasser 1994). Most probably these bacteria originated from the indigenous microflora, whereby in many cases the translocation was facilitated by underlying disease, lesions or inflammations in the oral cavity and in the gastrointestinal tract, or by an impaired immune system. However, there is no evidence for a higher risk due to the ingestion of probiotic products in comparison with conventional products. This conclusion is supported by a study from Finland, where the consumption of *L. rhamnosus* GG has increased considerably during the last two decades without an increase in the incidence of infections by lactobacilli (Rautio 1999). Moreover, studies in immuno-compromised persons (HIV-positive subjects, patients with leukemia) did not show undesired effects, but rather positive effects, e.g., lower incidence of Candida during chemotherapy. Health risks due to overdosage or long term ingestion have also not been observed.
Prebiotics: Foods for Probiotics

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating growth, activity, or both, of one or a limited number of bacterial species already resident in the colon (Gibson and Roberfroid 1995, Nagpal et al. 2007, Nagpal and Kaur 2011). To exhibit such effects, a prebiotic must neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract, and must be selectively fermented by one or a limited number of potentially beneficial bacteria residing in the colon (Collins and Gibson 1999). The number of probiotics in the human gut tend to decrease with age (Mitsuoka 1996). Two major strategies have been proposed to maintain a high level of probiotics to sustain beneficial health effects; 1) continuous ingestion of probiotics containing foods or 2) supplementation of food with prebiotics (Gomes and Malcata 1999). These prebiotics are fermented by one or a limited number of potentially beneficial bacteria form the resident colonic microflora. A prebiotic is expected to improve the composition of the colonic microbiota and through this serve as beneficial to the host health (Gibson 1999).

Since the 1980s, awareness of the healthier food and drink market has increased all over the world, and these are named as Functional Foods (Roberfroid 2002). The popularity of these foods reflects nutritional guidelines recommending an increase in the dietary fibre intake. The uses of insoluble fibre ingredients (Gibson 2004), such as bran, have been used in products such as breakfast cereals, bread and pasta, but the acceptability of these materials is limited in different systems, which decreases their incorporation into foods. Soluble fibre ingredients such as oligosaccharides are currently of more interest in formulation of healthy foods because they are more acceptable. Moreover, some of them can be used as thickening in food system to add viscosity or form gel (Dreher 1999). The main reason of prebiotics supplementation to human diet is to beneficially enhance the gut microflora (Kolida et al. 2002), which is Bifidobacterium spp., the most dominant and important flora in breast-fed and healthy infants. The beneficial effects of the presence of bifidobacteria in the gastrointestinal tract are dependent on their viability and metabolic activity. Their growth is dependent on the presence of complex carbohydrates known as oligosaccharides. Some oligosaccharides, because of their chemical structure, are resistant to digestive enzymes and therefore pass into the large intestine. Therefore, prebiotics are used as bifidogenic factors in diet applications, especially because of their ability not to degrade in the stomach and small intestine (Crociari et al. 1994). Inulin and oligofructose are recognized as safe ingredient supplements to food without limitation but the European Commission confirmed that oligofructose (FOS) and inulin could be used in foods targeted towards infants older than six months of age.
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...age at a concentration of 0.8 g/day (Rao 2002). Kaplan and Hutkins (2000) have shown the ability of a selection of twenty-eight lactic acid bacteria and bifidobacteria to ferment inulin and oligofructose on MRS agar.

A range of dietary compounds have been suggested as prebiotics, and these have been selected for their health benefits on the host. Gibson et al. (1995) presented the popularity of Inulin, Fructo-oligosaccharides (FOS) and Galcto-oligosaccharides (GOS) as health benefit substrates. In human studies the addition to bread of 7g of FOS has been shown to beneficially affect the dominant bifidobacteria as compared to common bread. The authors clearly proved that the use of FOS exerted a profound effect upon bifidobacteria (Gibson 2004). The only prebiotics for which sufficient data has been generated to allow an evaluation of their possible classification as functional food ingredients are the inulin type fructans, which include native inulin, enzymatically hydrolyzed inulin or oligofructose, and synthetic fructooligosaccharides (Roberfroid et al. 1998a, 1998b, Nagpal et al. 2007, 2011). The two basic types of fermentations taking place in the gut are saccharolytic fermentation and proteolytic fermentation. The main end products of carbohydrate metabolism are the short chain fatty acids: acetate, propionate and butyrate. These may be further metabolised systematically or locally to generate energy for the host. The end products of the proteolytic fermentation include more or less toxic compounds as amines, ammonia and phenolic compounds. Fermentation in the gut can be modulated towards saccharolytic by prebiotic consumption. Much of the interest is aimed at non-digestible oligosaccharides and indeed more than 36000 plants worldwide contain FOS; some common sources of inulin are onion (2–6%), garlic (9–16%), leek (3–10%), banana (0.3–0.7%), asparagus (10–15%), Jerusalem artichokes (15–20%), chicory (13–20%), and even wheat (1–4%). Yet the levels are too low for a significant tract effect (Crow 2004). Consumption of more than 4 grams of FOS daily is needed to induce changes in LAB levels in the gut, though estimated daily consumption differs in the US and Europe (Roberfroid 2000, Gibson 2001).

Prebiotics are increasingly used in development of new food products, e.g., drinks, yoghurts, biscuits and table spreads (Gibson and Roberfroid 1995, Gibson 1999). Several prebiotics are available in Europe. The positive effects of prebiotic consumption are: improvement of bowel habit; reduction of diarrhoea and constipation; modulation of lipid metabolism by normalizing cholesterol values; reduction of osteoporosis by improved mineral absorption; reduction of allergy risk through immune system modulation; reduction of colon cancer risk (Roberfroid 2000, Conway 2001). Still, many of the above mentioned health claims require further research.
Synbiotics: Blending Probiotics and Prebiotics

Another possibility of gut microflora management is the use of synbiotics, where probiotics and prebiotics are used in combination. The combination of suitable probiotics and prebiotics enhances survival and activity of the organism, for example a FOS in conjunction with a *Bifidobacterium* strain or lactitol in conjunction with *Lactobacillus* (Gibson and Roberfroid 1995). The combination of prebiotic and probiotic has synergistic effects because in addition to promoting growth of existing strains of beneficial bacteria in the colon, synbiotics also act to improve the survival, implantation and growth of newly added probiotic strains (Nagpal et al. 2007, Nagpal and Kaur 2011). The synbiotic concept has been widely used by European dairy drink and yoghurt manufacturers such as Aktifit (Emmi, Switzerland), Proghurt (Ja Natürlich Naturprodukte, Austria), Vifit (Belgium, UK) and Fysiq (Netherlands) (Niness 1999). The combination of *Bifidobacterium* and oligofructose was reported to synergistically improve colon carcinogenesis in rats compared to when both were given individually (Gallaher and Khil 1999). Another study reported that a synbiotic containing *Pediococcus pentoseceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei*, and *L. plantarum* with four fermentable fibres namely β-glucan, inulin, pectin, and resistant starch, reduced the occurrence of post-operation infections from 48% to 13% in 66 liver transplant patients (Rayes et al. 2005). Most of the claims on benefits of different synbiotics are on general health (Gibson and Roberfroid 1995). There have yet to be any clinical trials on suitable combinations of synbiotics that specifically target reduction of serum cholesterol levels in animals and humans. Bifidobacteria and lactobacilli are the most frequent target organisms for prebiotics. Probiotics and prebiotics used in synergistic combination are termed synbiotics. Synbiotics are mixtures that improve the survival and implantation of live microbial dietary supplements in the tract, either by stimulating growth or by metabolically activating the health promoting bacteria (Kaur et al. 2002). Although there is growing interest in the development of new functional foods with synbiotics, combination of prebiotics and probiotics into a synbiotic has been studied to a limited extent and needs further investigations, because of the afore mentioned different substrate requirements for individual probiotic LAB species and strains. Only a few human studies have been carried out on the effectiveness of synbiotics (Morelli et al. 2003, Tsuchiya 2004, Lighthouse 2004, Lamiki 2010).
References


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