2

Probiotics – What They Are, Their Benefits and Challenges

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1. Introduction

This chapter reviews literature on probiotics. Probiotics are defined and different microbial cultures used as probiotics will be considered. It further discusses delivery vehicles for probiotic cultures, with their advantages and disadvantages. Since the presence of viable probiotic cultures in products is vital to their functionality, different methods used for their detection in products will be examined. The beneficial health effects of probiotics, the methods that are currently used in an attempt to overcome some of the challenges faced will also be discussed. Different strategies for protection of probiotic cultures and challenges for the probiotic industry are highlighted. In addition to these, alternative strategies increasing numbers of beneficial microorganisms through administration of prebiotics and synbiotics are briefly mentioned.

2. What are probiotics?

The definition of probiotics has been modified with increasing knowledge in the field of how they function. The term is derived from the Greek language meaning 'for life'. In the past there have been many attempts to define the term 'probiotic', one of the first being described by Lilly & Stillwell in 1965. They defined probiotics as "substances secreted by one microorganism, which stimulates the growth of another". The focus of this definition was to distinguish them from and make it clear that they are the opposite of antibiotics. Subsequently, in 1974, Parker defined them as "organisms and substances which contribute to intestinal microbial balance" (Schrezenmier & de Vrese, 2001). In 1989, Fuller tried to improve on Parker's definition by proposing the following definition: "live microbial feed supplement, which beneficially affect the host (animal or human) by improving its intestinal microbial balance" (Salminen et al., 1999; Vilsojevic & Shah, 2008). Then, Havenaar & Huis In't Veld (1992) defined probiotics acceptably as 'a viable mono- or mixed culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora'. Schrezenmeir & de Vrese (2001) defined the term probiotic as "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 effects on host health". Among these descriptions and definitions, there were many others, until the Food and Agriculture Organization of the United Nations-World Health Organization (FAO-WHO) officially

defined probiotics as: "live microorganisms that when administered in adequate amounts confer a significant health benefit on the host" (FAO, 2001). This definition was later endorsed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) and is currently the most accepted definition of probiotics by scientists worldwide (Reid, 2006).

Probiotic food cultures have become popular due to appreciation of their contribution to good health (Desmond et al., 2002). In probiotic therapy, these beneficial microorganisms are ingested and thereby introduced to the intestinal microflora intentionally. This results in high numbers of beneficial bacteria to participate in competition for nutrients with and starving off harmful bacteria (Mombelli & Gismondo, 2000). The probiotics take part in a number of positive health promoting activities in human physiology (Chen & Yao, 2002).

The beneficial effects of the ingested probiotic bacteria are provided by those organisms that adhere to the intestinal epithelium (Salminen et al., 1998). The presence and adherence of probiotics to the mucous membrane of the intestines build up a strong natural biological barrier for many pathogenic bacteria (Chen & Yao, 2002). Adhesion is therefore regarded as the first step to colonization. Adhesion to the epithelium can be specific, involving adhesion of bacteria and receptor molecules on the epithelial cells, or non-specific, based on physicochemical factors.

2.1 Desirable properties for a probiotic strain

A microbial strain has to fulfil a number of specific properties or criteria for it to be regarded as a probiotic. These criteria are classified into safety, performance and technological aspects (Gibson & Fuller, 2000). The criteria are further dependent on specific purpose of the strain and on the location for the expression of the specific property. With regards to safety, the probiotic strain must be of human origin, isolated from the gastrointestinal tract (GIT) of healthy individuals. They should possess GRAS (generally regarded as safe) status, be non-pathogenic, and without previous association with diseases such as infective endocarditis or gastrointestinal disorders. Probiotic strains must not deconjugate bile salts and they should carry no antibiotic resistance genes that can be transferred to pathogens (Collins et al., 1998; Saarela et al., 2000). The strain must not induce an immune reaction in the host, i.e. the host must be immuno-tolerant to the probiotic (Havenaar & Huis int'Veld, 1992). The strain itself, its fermentation products or its cell components after its death, should be non-pathogenic, non-toxic, non-allergic, non-mutagenic or non-carcinogenic even in immunocompromised individuals (Collins et al., 1998; Havenaar & Huis int'Veld, 1992). It must have antimutagenic and anticarcinogenic properties and not promote inflammation in individuals (Collins et al., 1998). A probiotic strain should possess a desirable antibiogram profile. It must also be genetically stable with no plasmid transfer mechanism (Havenaar & Huis int'Veld, 1992; Ziemer & Gibson, 1998).

With respect to their performance, potential probiotic strains should be acid-tolerant and therefore survive human gastric juice and bile. They must be able to survive in sufficient numbers and adhere to the intestinal mucosal surface in order to endure the GIT. They should have antagonistic activity against pathogens such as *Salmonella* species, *Clostridium difficile* and *Listeria monocytogenes* that adhere to mucosal surfaces. Lastly, probiotic strains should also stimulate an immune response, thereby positively influencing the host (Biavati et al., 2000; Kolida et al., 2006; Mattila-Sandholm et al., 2002; Saarela et al., 2000;). The

probiotic should survive the environmental conditions in their target site of action and proliferate in this location (Havenaar & Huis int'Veld, 1992). That is, they should be able to adhere to and colonise the epithelial cell lining to establish themselves in the colon (Guarner & Schaafsma, 1998; Parracho et al., 2007). The ability to adhere to the epithelium secures the strain from being easily flushed out by peristaltic movements (Gupta & Garg, 2009). Technologically, a good probiotic strain should be easily, inexpensively reproducible (Charteris et al., 1998; Havenaar & Huis int'Veld, 1992). It must be able to withstand stress during processing and storage, with process and product application robustness (Charteris et al., 1998). The organism should be able to survive, in particular, the harsh environmental conditions of the stomach and small intestine (e.g. gastric and bile acids, digestive enzymes) (Dunne et al., 2001; Parracho et al., 2007).

In addition, technological aspects must be taken into account before selecting a probiotic strain. Strains should be capable of being prepared on a large scale and should be able to multiply rapidly, with good viability and stability in the product during storage. The strains must not produce off flavours or textures once incorporated into foods. They should be metabolically active within the GIT and biologically active against their identified target. Probiotic strains must be resistant to phages and have good sensory properties (Collins et al., 1998; Kolida et al., 2006; Lacroix & Yildirim, 2007; Mattila-Sandholm et al., 2002; Saarela et al., 2000;). Therefore probiotic containing foods and products need to be of good quality and must have high enough numbers of viable probiotic cells to ensure that consumers get the optimal benefits from the product (Alakomi et al., 2005). Probiotic strains have to be good vehicles for specific target delivery of peptides and recombinant proteins within the human GIT (Dunne et al., 2001; Parracho et al., 2007).

2.2 Common probiotic microorganisms

A number of microorganisms are currently used as probiotics. However, the most commonly used are bacteria belonging to the genera *Lactobacillus,* the first and largest group of microorganisms to be regarded as probiotics (Mombelli & Gismondo, 2000; Wolfson, 1999) and *Bifidobacterium*. These bacteria are indigenous to the human GIT (Bielecka et al., 2002; Tannock, 2001). They are known to have no harmful effects, which is in contrast to other gut bacteria (Kimoto-Nira et al., 2007). Species of Lactobacilli include *L. acidophilus*, *L. rhamnosus* , *L. casei*, *L. delbrueckii* ssp. *bulgaricus*, *L. johnsonii* , *L. reuteri*, *L. brevis*, *L. cellobiosus*, *L. curvatus*, *L. fermentum*, *L. gasseri* and *L. plantarum* (Krasaekoopt et al., 2003; Meurman & Stamatova, 2007). The most recognized bifidobacteria species used are *Bifidobacterium breve*, *B. animalis* subsp *lactis* formerly *B. lactis* (Masco et al., 2004) and *B. longum* biotypes *infantis* and *longum* (Masco et al., 2005).

Probiotics now include other lactic acid bacteria (LAB) from genera such as *Streptococcus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Propionibacterium*, and *Pediococcus* (Krasaekoopt et al., 2003; O'Sullivan et al., 1992; Power et al., 2008; Vandenplas et al., 2007; Vinderola & Reinheimer, 2003). Some countries are, however, concerned about the possible transfer of antibiotic resistance genes by some members of the *Enterococcus* (Lund & Edlund, 2001). Other non-related microbes used include bacteria such as non-pathogenic *E. coli* Nissle 1917 and *Clostridium butyricum* (Harish & Varghese, 2006), yeasts (*Saccharomyces cereviciae*, *Saccharomyces boulardii*), filamentous fungi (*Aspergillus oryzae*), and some spore forming bacilli (Fuller, 2003; Mombelli & Gismondo, 2000; Wolfson, 1999).

2.3 Probiotic products

Probiotics can be consumed either as food components or as non-food preparations (Stanton et al., 1998). Foods containing probiotics are referred to by others as functional foods. This refers to foods with nutrient or non-nutrient components that affect targeted function(s) in the body resulting in a positive health effect (Bellisle et al., 1998). Thus, functional foods have a physiological or psychological effect beyond basic nutritional value (Clydesdale, 1997). Several probiotic LAB strains are available to consumers in both traditional fermented foods and in supplemented form (Kourkoutas et al., 2005). The majority of probiotics are incorporated into dairy products such as milk powders, yoghurt, soft-, semi-hard and hard cheeses and ice cream (Desmond et al., 2005; Dinakar & Mistry, 1994; Stanton et al., 2001; Stanton et al., 2005). These products offer a suitable environment for probiotic viability and growth (Özer et al., 2009; Ross et al., 2002). There is an increase in use of other foods as vehicles for probiotics. This is partly due to allergenicity of some consumers to milk products. Non-dairy products such as malt-based beverages and fruit juices (Champagne & Raymond, 2008; Rozada-Sanchez et al., 2007; Sheehan et al., 2007), meat sausages (Ruiz-Moyano et al., 2008), capsules, and freeze-dried preparations (Berni-Carnani et al., 2007) are among these alternatives. Growing vegetarian alternatives have also led to soy-based probiotic foods (Farmworth et al., 2007). Recently, Aragon-Alegro et al. (2007) added probiotic chocolate mousse to the list of alternatives.

2.4 Beneficial effects of probiotics

The benefits attributed to probiotics can either be nutritional or therapeutic (Prasad et al., 1998). Benefits associated are, however, strain specific (Saarela et al., 2000).

2.4.1 Nutritional benefits

Microbial action in the gut, specifically by beneficial cultures, has been shown to enhance the bioavailability, quantity and digestibility of certain nutrients (Parvez et al., 2006). Ingestion of probiotics is associated with improved production of riboflavin, niacin, thiamine, vitamin B_6, vitamin B_{12} and folic acid (Gorbach, 1997; Hargrove & Alford, 1978). Probiotics play a role in increasing bioavailability of calcium, iron, manganese, copper, phosphorous (Alm, 1982; McDonough et al., 1983) and increase the digestibility of protein and fat in yoghurt (Fernandes et al., 1987). Enzymatic hydrolysis of protein and fat leads to an increase in free amino acids and short chain fatty acids (SCFAs). Organic acids such as acetate and lactate produced during fermentation by LAB lower the pH of intestinal contents thereby creating undesirable conditions for harmful bacteria (Mack et al., 1999; Parvez et al., 2006).

2.4.2 Therapeutic benefits

Patients prefer medicine with little or no side effects for treatment of their ailments. Probiotics provide such an alternative, being living, non-pathogenic organisms, which are extremely safe as indicated by their GRAS status. Probiotic bacteria are claimed to alleviate and prevent conditions such as lactose intolerance, allergies, diarrheal diseases, lowering of serum cholesterol, reduction of the risk associated with mutagenicity and carcinogenicity and inhibition of pathogens, as well as stimulation of the immune system (Collins & Gibson,

1999; Shah, 2007). Positive effects of probiotics are not confined to the gut only, but can extend to other parts of the body. For instance, probiotics are known to have anti-inflammatory benefit when administered parenterally (Shiel et al., 2004).

Lactose malabsorption (also referred to as lactose intolerance or lactose indigestion) is the inability to hydrolyze lactose (Adams & Moss, 2000; Salminen et al., 1998a). It is caused by a deficiency of the enzyme β-D-galactosidase (lactase) (Buller & Grand, 1990). The undigested lactose passes to the colon where it is attacked by resident lactose fermenters (Adams & Moss, 2000). Colonic lactose fermentation results in high levels of glucose in blood and hydrogen gas in breath (Buller & Grand, 1990; Mombelli & Gismondo, 2000; Scrimshaw & Murray, 1988; Shah, 1993; Vesa et al., 2000). Probiotics strains and the traditional yoghurt cultures, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* produce β-D-galactosidase thereby improving tolerance to lactose (Adams & Moss, 2000; Fooks et al., 1999, Shah, 2000c)

Constipation, a disorder of motor activity of the large bowel characterized by bowel movements that are less frequent than normal (Salminen et al., 1998b), pain during defecation, abnormal swelling and incomplete emptying of colon contents (Salminen et al., 1998a), can also be relieved by probiotic use. *Lactobacillus reuteri, Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* are probiotic strains shown to improve the condition (Ouwenhand et al., 2002).

Incidences of antibiotic associated diarrhoea caused by *Clostridium difficile* (Fuller, 2003; Tuohy et al., 2003; Vasiljevic & Shah, 2008) and rotavirus diarrhoea (Salminen et al., 1998a) can also reduced by administration of probiotics. Strains associated with reduction of diarrhoea include *Bifidobacterium* spp, *B. animalis* Bb12 (Fuller, 2003, Guandalini et al., 2000), *L. rhamnosus* GG, *L. acidophilus, L. bulgaricus* (Fuller, 2003; Goldin, 1998; Gorbach, 2000; Sazawal et al., 2006) and *Saccharomyces boulardii* (Kotowska et al., 2005, Sazawal et al., 2006). The effect of probiotics against diarrhoea is the most researched and substantiated claim, with documented clinical applications (BergogneBérézin, 2000; Cremonini et al., 2002; Marteau et al., 2001, McNaught & MacFie, 2001; Reid et al., 2003; Sullivan & Nord 2005).

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are other intestinal disorders that can be treated with varying degrees of success using probiotics. IBD is a collection of disorders including ulcerative colitis, Crohn's disease and pouchitis, characterized by chronic or recurrent inflammation, ulceration and abnormal narrowing of the GIT resulting in abdominal pain, diarrhoea and gastrointestinal bleeding (Hanauer, 2006; Marteau et al., 2001). IBS is typically characterized by abdominal pain, excessive flatus, variable bowel habit and bloating (Madden & Hunter, 2002). Several studies have been conducted to investigate the efficacy of probiotics in treatment of IBD (Guandalini, 2002; Ma et al., 2004; Zhang et al., 2005). The tested strains against IBD include among others VSL#3 probiotic (Gionchetti et al., 2000), *Bifidobacterium longum (*Furrie et al., 2005) and *Lactobacillus rhamnosus* GG *(*Gupta et al., 2000). Combination of *Lactobacillus acidophilus and Bifidobacterium infantis* (Hoyos, 1999) and of *Bifidobacterium bifidus*, *Bifidobacterium infantis* and *Streptococcus thermophilus* were shown to reduce incidences of ulcerative colitis (Bin-Nun et al., 2005). Several studies reported the success of bifidobacteria for the alleviation of IBS (O'Mahony et al., 2005; Brenner et al., 2009; Jankovic et al., 2010). Alfredo (2004)

demonstrated the efficacy of *Lactobacillus plantarum* LP01 and *Bifidobaccterium breve* BR0 as short-term therapy for IBS. Although some of the results obtained were very encouraging, there is need for larger, randomized, double-blinded, placebo-controlled clinical trials to substantiate these claims.

Hereditary allergic conditions of increasing importance in developing countries such as eczema, asthma, atopic dermatitis and rhinitis can be treated with probiotics (Holgate, 1999; Kalliomaki et al., 2003; Salminen et al., 1998a). Tested probiotics with antiallergenic properties include *Bifidobacterium lactis* Bb-12 (Isolauri et al., 2000) and *Lactobacillus* GG (Isolauri et al., 2000; Kalliomaki et al., 2001; Kalliomaki et al., 2003; Lee et al., 2008; Mirkin, 2002; Vanderhoof & Young, 2003). However, contradictory studies report on the poor efficiency of probiotics in allergy alleviation (Helin et al., 2002; Vliagoftis et al., 2008) and highlight the need for more convincing and conclusive research in allergy treatment.

Probiotics have the ability to lower levels of cholesterol in serum, contributing to the prevention of cardiovascular disease (Fooks et al., 1999; Proviva, 2002). This ability has been shown for *Lactobacillus johnsonii* and *L. reuterii* using animal models (Mombelli & Gismondo, 2000). They also reduce the risk of cancer (Sanders, 1999) due to their activity against certain tumors (Chen & Yao, 2002). Several studies indicated that probiotics in a diet reduces the risk of cancer (Sanders, 1999). Anticarcinogenic effects of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were shown using clinical trials in humans (Fooks, 1999).

2.5 Mechanism of action of probiotics

Probiotic bacteria beneficially affect the individual by improving the properties of the indigenous microflora and its microintestinal balance (Betoret et al., 2003; Frost & Sullivan, 2000; Matilla-Sandholm et al., 2002; Saarela et al., 2000). They compete with disease causing bacteria for villi attachment sites and nutrients (Chen & Yao, 2002). Probiotic bacterial cultures encourage growth of beneficial microorganisms and crowd out potentially harmful bacteria thereby reinforcing the body's natural defence mechanisms (Saarela et al., 2000). They provide specific health benefits by modifying gut microflora, strengthening gut mucosal barrier, e.g. adherence of probiotics to the intestinal mucosa thereby preventing pathogen adherence, pathogen inactivation, modification of dietary proteins by intestinal microflora, modification of bacterial enzyme activity, and influence on gut mucosal permeability, and regulation of the immune system (Betoret et al., 2003; Krasaekoopt et al., 2003; Salminen et al., 1998).

The probiotic effect is accredited to their production of metabolic by-products such as acid, hydrogen peroxide, bacteriocins, e.g. lactocidin, and acidophilin that manifest antibiotic properties and inhibit the growth of a wide spectrum of pathogens and/or potential pathogens such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Salmonella*, *Serratia* and *Bacteroides* (Chen & Yao, 2002; Krasaekoopt et al., 2003). Lactic acid bacteria inhibit growth of pathogenic microorganisms by producing short chain fatty acids such as acetic, propionic, butyric as well as lactic and formic acids which reduces intestinal pH. Lactic acid produced by bifidobacteria in substantial amounts has antimicrobial activity against yeasts, moulds and bacteria (Adams & Moss, 2000; Percival, 1997). These species are also active in reducing the faecal activity of enzymes implicated in the production of genotoxic metabolites such as β-glucoronidase and glycolic acid hydroxylase (Collins & Hall, 1984; Mombelli & Gismondo,

2000). Probiotic organisms produce enzymes that help in digestion of proteins, fats and lactose (Frost & Sullivan, 2000). They also produce β-galactosidase, an enzyme that aid lactose intolerant individuals with breaking down or digestion of lactose (Krasaekoopt et al., 2003).

Production of short chain fatty acids in the colon during fermentation by colonic microflora is the main process that prevents colorectal cancer (Holzapfel & Schillinger, 2002). Probiotic strains also reduce levels of some colonic enzymes such as glucoronidase, β-glucoronidase nitroreductase, azoreductase (Adams & Moss, 2000; Chen & Yao, 2002; Fooks et al., 1999; Gorbach, 2000) and glycoholic acid hydrolase. These enzymes convert procarcinogens to carcinogens such as nitrosamine or secondary bile acids (Chen & Yao, 2002). Low levels of these enzymes therefore decrease chances of cancer development in the colon (Gorbach, 2000; Kasper, 1998).

2.6 Methods for quantification of probiotic cultures

The methods used for detection of viable probiotic cells include conventional plate counts (culture dependent) and molecular techniques (culture-independent). The culture dependent method has been criticized for underestimation of counts due to bacteria forming chains and/or clumping and unsuitability (inappropriateness) of media for growing of viable but non-culturable cells (Auty et al., 2001; Lahtinen et al., 2006; Veal et al., 2000). Isolation media used may be insufficiently selective, affecting the reproducibility of results (Roy, 2001). These limitations of plate counting techniques prompted the use of molecular techniques and other alternative methods (Vitali et al., 2003). New methods include molecular based techniques such as quantitative real-time polymerase chain reaction (PCR), fluorescent *in situ* hybridization (FISH) (Boulos et al., 1999; Veal et al., 2000), confocal scanning laser microscopy (CSLM) (Auty et al., 2001; Gardiner et al., 2000; Palencia et al., 2008), flow cytometry (Alakomi et al., 2005) and microplate scale fluorochrome staining assay (Filoche et al., 2007; Mättö et al., 2006).

Flow cytometry is a rapid and sensitive technique that measures physiological characteristics such as membrane integrity, enzyme activity, respiration, membrane potential and intracellular pH (Bunthof et al., 2001) of each cell individually (Bunthoff & Abee, 2002). Microplate scale fluorochrome staining assay is appropriate for assessing viability of fresh, freeze-dried and stressed cells. It can detect changes in the condition of probiotic cells earlier than can be done with conventional cultivation methods (Filoche et al., 2007; Mättö et al., 2006).

The fluorescence based molecular techniques are used in conjunction with viability staining techniques. A number of commercial techniques are available. LIVE/DEAD® *Bac*Light™ and BD Cell viability assay kit (BD Biosciences, Oxford, UK) are some examples. LIVE/DEAD® *Bac*Light consists of two nucleic acid stains SYTO® 9 and propidium iodide (PI). Green-fluorescent SYTO9 (excitation and emission maxima, 480 and 500 nm, respectively) penetrates both viable and nonviable cells. Red-fluorescent PI (excitation and emission maxima, 490 and 635 nm, respectively) penetrates cells with damaged cell membranes (Auty et al., 2001). The BD Cell viability assay kit (BD Biosciences, Oxford, UK) contains the stains, thiazole orange and propidium iodide (Doherty et al., 2009). Cells stained using these kits can also be assessed using microscopes, which will also distinguish between 'live' (e.g. green-stained) from 'dead' (e.g. red-stained) cells (Berney et al., 2007).

All the above mentioned methods have their own disadvantages. For example, the viability kits and real time PCR are based on bacterial DNA which is not only present in live cells but can also be retained by dead cells in significant amounts. Both PCR and FISH are not independent as they require determination of a standard curve which is determined most of the times using standard plate counts. PCR requires expensive reagents which cannot be afforded by everyone in the industry. Detection limits for PCR and FISH are relatively high, being about 10^4 cells/ml and 10^6 cells/ ml, respectively. FISH is based on detection of 16s rRNA whose presence is not a direct proof of metabolic activity but rather an indication of potential viability (Biggerstaff, 2006). Real-time PCR and FISH have a limitation whereby counts of bacteria decreased but PCR and FISH results remained higher over the experimental period. This is due to detection of high levels of rRNA and DNA in dead cells. The intensity of rRNA in dead cells may still be strong enough for visually counting (detection) though it is expected to decrease upon cell death. Thus, the RNA content of the cell detected by fluorescent probes cannot be regarded as reliable indicator of cellular viability (Vives-Rego et al., 2000). Also, real time PCR detects both viable and non-viable bacteria, thus does not provide information on the condition of the cells and results in an overestimation of metabolically active cells (Kramer et al., 2009; Masco et al., 2007).

The appropriateness of PCR for quantification of viable cells can be improved by staining the samples with DNA binding dyes prior to DNA extraction and amplification. Treatment with DNA-binding dyes and subsequent PCR analysis uses membrane integrity as the criterion in determining viability of cells. Live cells are able to exclude DNA-binding dyes such as ethidium monoazide (EMA) and propidium monoazide (PMA), while dead cells or those whose membrane integrity has been compromised are able to pick-up these stains (Kramer et al., 2009). These dyes form covalent bonds with DNA upon exposure to visible bright light and thus inhibit subsequent PCR amplification. Only DNA from live cells with intact membranes is selectively amplified (Nocker et al., 2009).

Despite some of its drawbacks, the plate count method is traditionally used to assess cell viability in probiotic preparations (Alakomi et al., 2005). Though plate counting is arduous and time consuming, no method has yet been found that completely replaces it. Therefore it is still being routinely used in assessing viability of probiotic cultures in various foods, often in conjunction with culture-independent methods (Lopez-Rubio et al., 2009; Masco et al., 2005; Temmerman et al., 2003b).

2.7 Probiotic challenges

Commercially, viable probiotic strains are incorporated into fermented food products or are supplied as freeze-dried supplements or pharmaceutical preparations (Holzapfel & Schillinger, 2002). The basic requirement for probiotics is that products should contain sufficient numbers of microorganisms up to the expiry date (Fasoli et al., 2003). Thus, probiotics must contain specific strains and maintain certain numbers of live cells for them to produce health benefits in the host (Mattila-Sandholm et al., 2002). Different countries have decided on the minimum number of viable cells required in the probiotic product for it to be beneficial. In Australia, a minimum viable count of 10^6 organisms per gram should be available in fermented milk products at the end of the shelf life (Wahlqvist, 2002). However, according to Krasaekoopt et al. (2003), there are no specifications as to how many probiotics should be available in Australian fermented products. The same minimum count (10^6

organisms per gram) was approved by countries of MERCOSUR which includes Argentina, Paraguay, Brazil and Uruguay (Krasaekoopt et al., 2003). In products containing multiple probiotic organisms, at least a million of each of them per gram should be present to produce required beneficial effects (Wahlqvist, 2002). In Japan, a minimum of 10^7 viable cells per millilitre of fresh dairy product is required. The South African legislation states that functional foods containing probiotic bacteria must deliver 1 x 10^8 bacterial cells per day. A daily intake of 10^9 to 10^{10} cfu viable cells is considered the minimum dose shown to have positive effects on host health (Fasoli et al., 2003). This could be achieved by consuming 100 g of a product containing between 10^6 and 10^7 viable cells g^{-1} daily (Boylston et al., 2004).

Low viability of probiotic cultures in yoghurt has been reported (Kailasapathy & Rybka, 1997; Lourens-Hattingh & Viljoen, 2001; Shah, 2000).

Retention of viability of the probiotic bacteria presents a major marketing and technological challenge for application of probiotic cultures in functional foods (Desmond et al., 2002; Mattila-Sandholm et al., 2002). Many active cultures die during manufacturing, storage or transport of the finished product (Siuta-Cruce & Goulet, 2001) and also during the passage to the intestine (Sakai et al., 1987; Siuta-Cruce & Goulet, 2001; Park et al., 2002). Thus, the majority die even before the consumer receives any of the health benefits (Siuta-Cruce & Goulet, 2001). A serious problem of shelf instability had been encountered with dried cultures. Refrigerated products also have short lives due to negative effects of low temperature and formation of crystals on bacterial cells. The numbers of viable bacteria continually decrease with time during refrigerated storage (Porubcan et al., 1975). Market surveys have revealed much lower counts in the products even before the expiry date (Talwalkar et al., 2001). Shelf life for probiotics is thus unpredictable; hence, the industry has had difficulty backing up label claims (Siuta-Cruce & Goulet, 2001). Excesses of 50 to 200 % cells have been incorporated into products in an attempt to make-up for cells that die during storage. For example, in tablets containing dry cells, where the tablets are labelled as containing a certain minimum count of active cells per tablet, to be safe, the manufacturer must incorporate an excess of cells at the time the tablets are manufactured, thereby assuring that the labelling will remain accurate while the product is in stock by the retailers. This practice increases the cost and makes the use instructions inaccurate (Porubcan et al., 1975).

Probiotics, after surviving food processing, are upon consumption then exposed to conditions prevailing in the stomach and small intestine before they reach their site which is the colon (Siuta-Cruce & Goulet, 2001; Hansen et al., 2002; Lian et al., 2002). The microbes may die during their transit through the upper intestinal tract to the colon and therefore they may not be able to colonize the colon (Talwakar et al., 2001). They must therefore survive gastric acidity and bile salts which they encounter during their passage through the GIT (Hansen et al., 2002; Lian et al., 2002; Sakai et al., 1987; Siuta-Cruce & Goulet, 2001;). Their survival in the GI T depends on the strain and species-specific resistance to low pH (pH values ranging from 1.3 to 3.0) in gastric juice and to bile salts found in the small intestine (Hansen et al., 2002; Lian et al., 2002).

Probiotic bacteria can only perform when they find adequate environmental conditions and when they are protected against stresses (e.g extreme temperatures, high pressure, shear forces) they encounter during their production at the industry level or in the GIT (gastric

acids and bile salts) (Siuta-Cruce & Goulet, 2001). Factors affecting viability during storage such as temperature, moisture, light and air should also be taken into consideration (Percival, 1997; Mattila-Sandholm et al., 2002). Oxygen toxicity is another major problem in the survival of probiotic bacteria in dairy foods. High levels of oxygen in the product are detrimental to the viability of these anaerobic bacteria (Talwakar et al., 2001).

Manufacturers of probiotics are facing the challenge that they should produce probiotic cultures that can survive for long periods, and are resistant to acidity in the upper intestinal tract so that they can reach the colon in high numbers to colonize the epithelium. Probiotic cultures should therefore be produced in a way that will protect these sensitive bacteria from unfavourable interactions with detrimental factors (Siuta-Cruce & Goulet, 2001).

In view of the health benefits associated with probiotics, it is not surprising that there is increasing interest in their viability. Probiotics do not have a long shelf life in their active form. Refrigeration is required in most cases to maintain shelf life as high temperatures can destroy probiotic cultures (Saxelin et al., 1999). However, most probiotics still have a short shelf-life even under low temperature storage (Lee & Salminen, 1995). There is low recovery of viable bacteria in products claiming to contain probiotic bacteria (Hamilton-Miller et al., 1999; Temmerman et al., 2003a).

The preservation of these probiotic microorganisms presents a challenge because they are affected by exposure to temperature, oxygen and light (Bell, 2001; Chen et al., 2006). Survival of most bifidobacteria in most dairy products is poor due to low pH and/or exposure to oxygen (Gomes & Malcata, 1999). Naturally many LAB may excrete exo-polysaccharides to protect themselves from harsh conditions but this is usually not enough to give them full protection (Shah, 2002).

2.8 Methods for improving probiotic viability

In view of the health benefits associated with probiotics, it is not surprising that there is an increasing interest in their viability. The common practice is storage at refrigerated temperatures to prolong their shelf life (Saxelin et al., 1999). Nevertheless, most of them still have a short shelf-life (Lee & Salminen, 1995). There is low recovery of viable bacteria in products claiming to contain probiotic bacteria (Hamilton-Miller et al., 1999; Temmerman et al., 2003a).

Viability of probiotics is reduced as a result of their exposure to high temperature, oxygen, low pH and light (Bell, 2001; Chen et al., 2006; Gomes & Malcata, 1999). Naturally many LAB may excrete exo-polysaccharides (EPS) to protect themselves from harsh conditions. However, protection afforded by these EPS is not sufficient (Shah, 2002). As a result, researchers are continuously searching for ways to improve survival of probiotic cultures during processing, storage and GIT transit. Different approaches are used in an attempt to preserve viability of probiotic cultures. These include among others, pre-exposure of probiotic cultures to sub-lethal stresses (Desmond et al., 2002) and incorporation of micro-nutrients such as peptides and amino acids (Shah, 2000). The disadvantage of pre-exposure to sublethal stresses is that it may result in significant decreases in cellular activity, cell yield and process volumetric productivity (Doleyres & Lacroix, 2005). Other alternative methods for improving probiotic viability are genetic modification (Sheehan et al., 2006; Sheehan et al., 2007; Sleator & Hill, 2007), immobilization (Doleyres et al., 2004), two-step fermentations,

use of oxygen-impermeable containers and microencapsulation (Özer et al., 2009). Of these techniques, microencapsulation is relatively new and has been investigated by various researchers.

Microencapsulation is a process by which solids, liquids or gases are packaged into miniature, sealed microcapsules that can release their contents at controlled rates under influences of specified conditions (Anal et al., 2006; Anal & Singh, 2007). It stabilizes the probiotics, increases their survival during processing and storage, controls the oxidative reaction, ensures sustained or controlled release at a specific target site (both temporal and time-controlled release) and improves shelf life (Anal & Singh, 2007; Dembczynski & Jankowski, 2002). The encapsulated probiotics are released from the microparticles as a result of many factors such as changes in pH and/or temperature (Gibbs et al., 1999; Vasishtha, 2003). These changes may cause microcapsule walls to swell and rupture or dissolve (Franjione & Vasishtha, 1995; Brannon-Peppas, 1997).

Microparticles reduce loss of probiotic cell viability by blocking reactive components such as atmospheric moisture, oxygen (Kim et al., 1988; Krasaekoopt et al., 2003; Reid, 2002; Siuta-Cruise & Goulet, 2001; Vasishtha, 2003), high temperature, pressure, bacteriophage attack and cryoeffects (Krasaekoopt et al., 2003). Studies have indicated that probiotic cultures enclosed within solid fat microcapsules retain both their activity and vitality (Krasaekoopt et al., 2003).

Methods of microencapsulation used in pharmaceutical and food industries are classified as either physical or chemical. Physical methods include pan coating, air-suspension coating, centrifugal extrusion, vibrational nozzle and spray drying (Anal & Singh, 2007), spray coating, annular jet, spinning disk, spray cooling, spray drying and spray chilling (Versic, 1988), extrusion coating, fluidized bed coating, liposome entrapment, coarcervation, inclusion complexation, centrifugal extrusion and rotational suspension separation (Vasishtha, 2003). Chemical methods include interfacial polymerization, *in-situ* polymerization, matrix polymerization(Vidhyalakshmi et al., 2009) and extrusion. Extrusion and emulsion techniques are the mostly commonly used methods (Krasaekoopt et al., 2003).

There is a diversity of materials used for encapsulation of probiotics. These include among others, alginate (Chandramouli et al., 2004; Dembczynski & Jankowski, 2002; Hansen et al., 2002; Krasaekoopt et al., 2003; Sultana et al., 2000), κ-carrageenan, locust bean gum, cellulose acetate phthalate, chitosan, gelatin (Krasaekoopt et al., 2003), cellulose (Chan & Zhang, 2002), pectin, whey protein (Guerin et al., 2003) and rennet (Heidibach et al., 2009). These materials are used either as supporting materials or gelling agents by different investigators.

Although generally there are positive effects of microencapsulation, this method is not without disadvantages. Some types of the resulting microparticles may shrink and lose mechanical strength due to their sensitivity to acids (Sun & Griffiths, 2000), may present problems for large scale production, others require use of potassium ions that should not be taken in large amounts in diet, (Sun & Griffiths, 2000), some of the polysaccharides used are prohibited in specific foods in other countries (Picot & Lacroix, 2004). Additionally and possibly the key disadvantage, is that the mentioned microencapsulation methods use water and other organic solvents whose use is less favoured due to their high costs and concerns about their negative environmental impacts (Sihvonen et al., 1999).

Progress towards elimination of use of organic solvents has been made with development of microencapsulation technique using supercritical technology (Moolman et al., 2006). This microencapsulation technique is based on the formation of an interpolymer complex between poly (vinyl pyrrolidone) (PVP) and poly (vinyl acetate-co-crotonic acid) (pVA-CA) in supercritical carbon dioxide ($scCO_2$). A supercritical substance is neither a gas nor a liquid but possesses properties of both, making it unique (Moshashaee et al., 2000). Since supercritical fluids have a wide spectrum of solvent characteristics, they can be used as solvents in different techniques (Frederiksen et al., 1997). Microparticles produced using this method have suitable morphological characteristics, encapsulation efficiency and affords encapsulated probiotic cultures protection in simulated gastrointestinal fluids (Mamvura et al., 2011; Thantsha et al., 2009).

2.9 Concerns about probiotics

Although there are numerous advantages and health benefits associated with probiotics or probiotic food products, there are risks associated with probiotic therapy. These risks are mainly concerned with respect to safety in vulnerable target groups such as immunocompromised individuals (pregnant women, babies and the elderly) or critically ill or hospitalized patients (Boyle et al., 2006; Jankovic et al., 2010).

Probiotic cultures are resistant to some antibiotics. There is concern about the possible transfer of antimicrobial resistance from probiotic strains to pathogenic bacteria in the gut. For example, many *Lactobacillus* strains are naturally resistant to vancomycin, which poses a potential threat of transfer of this resistance to other pathogenic bacteria such as *Staphylococcus aureus*. However, these vancomycin-resistant genes in lactobacilli are chromozomal and not readily transferred to other species.

Another important area of concern is the risk of sepsis. There have been several reports of cases of *Lactobacillus* sepsis and other bacterial sepsis due to the intake of probiotic supplements (Boyle et al., 2006). One case included a 67 year-old man who was taking probiotic capsules daily for mitral regurgitation and developed *Lactobacillus rhamnosus* endocarditis after a dental procedure (Borriello et al., 2003; Mackay et al., 1999). In another case, a 4-month old infant with antibiotic-associated diarrhoea, who was given *Lactobacillus rhamnosus* after cardiac surgery, developed *Lactobacillus* endocarditis 3 weeks after *Lactobacillus rhamnosus* treatment (Boyle et al., 2006; Kunz et al., 2004). However, there have been no reports to date on the occurrence of *Bifidobacterium* sepsis. All cases of bacterial sepsis from the use of probiotics (*Lactobacillus* spp.) have occurred in immunocompromised individuals or patients who have a chronic disease or debilitation. No cases have been reported in healthy individuals (Boyle et al., 2006). There have also been several cases of fungemia associated with *Saccharomyces boulardii*. However, investigation of these cases revealed that the infection was due to contamination of inserted catheters. It is therefore now recommended that *Saccharomyces boulardii* probiotics be prepared in powdered form under stringent hygienic conditions to prevent contamination (Borriello et al., 2003; Salminen et al., 1998). There is a small risk of adverse metabolic effects from manipulation of the microbiota with the use of probiotics, although probiotic studies to date have not shown significant adverse effects on growth or nutrition (Boyle et al., 2006). A review of safety assessments of probiotics was recently published (Sanders et al., 2010).

3. Prebiotics

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of colonic bacteria and thereby improve host health (Femia et al., 2002; Gibson & Roberfroid, 1995; Roberfroid, 1998; Theuer et al., 1998; Tuohy et al., 2003; Young, 1998). Gibson et al. (2004) redefined them as 'a selectively fermented ingredient that allows specific changes; both in the composition and/or activity in the gastrointestinal microflora that confers benefits to host well-being and health'. The refined definition takes into account both the microbial changes and the nutritional and physiological benefits attributed to prebiotics. Just like probiotics, they modulate the composition of the natural ecosystem though probiotics does so through introduction of exogenous bacteria into the colon (Bouhnik et al., 2004). Prebiotics pass through the upper GIT unfermented and are only utilized in the colon and are therefore called 'colonic food' (Roberfroid, 2000). Non-digestibility can be demonstrated *in vitro* by subjecting the carbohydrates to pancreatic and small intestinal enzymes. It can be shown *in vivo* on human subjects with an ileostomy (i.e people who have had their large intestine removed and have a stoma at the end of the ileum) (van Loo et al., 1999). Compounds that are not digested and absorbed by the host but are preferentially fermented by *Bifidobacterium* species in the colon are called 'bifidogenic' factors (Shah, 2007). Prebiotics are not readily digested by pathogenic bacteria (Annika et al., 2002; Farmer, 2002; Femia et al., 2002). They favour or promote growth of potentially health-promoting bacteria such as lactobacilli and bifidobacteria, thereby allowing them to be predominant (Bouhnik et al., 2004; Flamm et al., 2001; Gibson et al., 1999; Roberfroid, 1998; Wang, 2009). This subsequently leads to predominant numbers of the stimulated endogenous bacteria in faeces as well (Femia et al., 2002; Losada & Olleros, 2002). Scientific studies in Japan indicated that consumption of prebiotics increases the populations of bifidobacteria and other beneficial microorganisms even in the absence of probiotics in diet. The selective stimulation of growth of bifidobacteria by prebiotics is characterized by a substantial decrease in numbers of potentially pathogenic bacteria (Losada & Olleros, 2002).

The following criteria are used for selection of a carbohydrate as a prebiotic: It must not be absorbed in the stomach or small intestine; It must be selectively fermented by the beneficial gut microflora; It should also stimulate the growth and/or activity of beneficial bacteria; Its fermentation should induce the beneficial luminal or systemic effects within the host; It must be resistant to gastric acidity and mammalian enzyme hydrolysis (Kolida, et al., 2002; Manning and Gibson, 2004).

Classes of non-digestible oligosaccharides (NDOs) commercially available are cyclodextrins, fructooligosaccharides (FOS), gentiooligosaccharides, glycosylsucrose and isomaltulose (also known as palatinose). Other classes include lactulose, lactosucrose and maltooligosaccharides (Sako et al., 1999). NDOs such as inulin, fructooligosaccharide, lactulose and dietary fibre are common prebiotics (Davis & Milner, 2009). There are conflicting views on the prebiotic classification of resistant starch. Some researchers classify it as a prebiotic (Douglas & Sanders, 2008) while others (Shah, 2004) differ, arguing that resistant starch is not digested by some beneficial bacteria, and therefore cannot be classified as a prebiotic. Inulin and FOS are the only NDOs that have been sufficiently studied to give adequate data to analyze their functional properties (Roberfroid, 2000). Galacto-oligosaccharides (GOS), gluco-oligosaccharides, lactulose, isomalto-oligosaccharides, raffinose, transgalacto-oligosaccharides,

xylo-oligosaccharides, soya bean oligosaccharides and oat β-glucans are considered as prebiotic candidates (Lomax & Calder, 2009). Other NGOs such as lactulose (Crittenden & Playne, 1996) and xylobiose (Vazquez et al., 2000) are also included in the prebiotic category. All the prebiotic candidates except maltooligosaccharides, glycosylsucrose and cyclodextrins are reported as being bifidogenic (Ziemer & Gibson, 1998).

Prebiotics are found naturally in a number of materials. Honey, fruits and vegetables such as artichoke, asparagus, banana, barley, chicory, garlic, leeks, oats, onion, rye, soybeans, tomatoes and wheat are sources of non-digestible oligosaccharides, especially inulin (Davis & Milner, 2009; Femia et al., 2002; Losada & Olleros, 2002; Manning & Gibson, 2004; Mussatto & Mancilha, 2007; Sangeetha et al., 2005). They are also present in burdock, Chinese chives, garlic, graminae (fodder grass), pine, even bacteria and yeasts (Bengmark et al., 2001; Ziemer & Gibson, 1998). Honey and bamboo shoots are natural sources of isomaltulose (Lina et al., 2002). Raffinose and stachyose can be found in soyabeans and other leguminous seeds and pulses (Voragen, 1998). Milk is a good source of glycoproteins and oligosaccharides (lacto-N-tetraose and lacto-N-neotetraose), including those believed to be prebiotic (Alander et al., 2001; Newburg, 2000; Petschow & Talbott, 1991). Human milk contains more galactooligosaccharides than cow's milk. Oligosaccharides can be found in levels as high as (12g/l), making them the third bulkiest constituent of human milk (Newburg et al., 2004). Taking foods containing prebiotic oligosaccharides is not enough for modulation of gut flora as they are present in only small concentrations in these foods. Instead, prebiotics are extracted from these foods and transferred into more commonly ingested foodstuffs like biscuits and other carbohydrate based materials (Taylor et al., 1999).

These natural compounds can also be produced commercially through enzyme hydrolysis and extraction processes (Losada & Olleros, 2002; Mussatto & Mancilha, 2007; Sako et al., 1999). The enzymes employed include β-D-fructofuranosidase or fructosyltransferase, which joins the fructose molecules by means of transfructosylation mechanisms (Losada & Olleros, 2002). These mechanisms are employed for production of oligosaccharides with the exception of raffinose, soybean oligosaccharides and lactulose (Mussatto & Mancilha, 2007; Sako et al., 1999). Raffinose and soybean oligosaccharides are extracted directly from plant materials using solvents such as water, aqueous methanol or ethanol (Johansen et al., 1996; Mussatto & Mancilha, 2007). Lactulose is produced by enzymatic action of β-galactosidases (E.C 3.2.1.23) on lactose. The glucose moiety is converted to a fructose residue by alkali isomerization and the process results in a lactulose disaccharide (Villamiel et al., 2002). Inulin can also be extracted from chicory (*Cichorium intybus*) and then partially hydrolyzed to short-chain fructans (oligofructose) using inulase (E.C 3.2.1.7) or to long chain fructans by applying an industrial separation technique (Roberfroid, 2000). Additionally, inulin type fructans can be manufactured through transfer of fructosyl residue to and between sucrose molecules using fungal fructosyl transferases (E.C 2.4.1.9) (Cummings & Roberfroid, 1997). FOSs are manufactured from sucrose using the trans-fructosylation activity of β-fructofuranosidase (E.C 3.2.1.26). Alternatively, FOS can are produced by controlled enzymatic hydrolysis of inulin (Crittenden & Playne, 1996; Frost & Sullivan, 2000). Lactose is also used in the industrial production of GOS through the transgalactosylation activity of β-galactosidases (Sako et al., 1999). The production of FOS and GOS requires high concentrations of the starting material for efficient transglycosylation (Park & Almeida, 1991). GOS are synthesized from lactose syrup using the enzyme β-galactosidase (Frost & Sullivan, 2000; Gibson, 2004). They are neither hydrolyzed nor absorbed in the human

intestine and act as a substrate for bifidobacteria (Frost & Sullivan, 2000). Soy oligosaccharides are extracted directly from soybean whey. Their bifidogenecity has been confirmed in humans (Frost & Sullivan, 2000).

3.1 Beneficial effects of prebiotics

In the last decade there have been numerous investigations on the health-promoting effects of prebiotics. Bifidogenic oligosaccharides increase the level of nutrient supplementation and enhance nutrient solubility (Farmer, 2002). Some of these effects include better mineral absorption, alleviation of constipation and irritable bowel syndrome, protection against colon cancer, enhancement of the immune system, anticarcinogenic effects and lowering of cholesterol (Davis & Milner, 2009; Manning & Gibson, 2004; Tuohy et al., 2003; Venter, 2007). Supplementation of diet with oligofructose and inulin-type oligosaccharides significantly lowers serum triglycerols and phospholipids. This hypotriacylyglycerolaemic effect could be caused by a decrease in the concentration of plasma very low-density lipoproteins (VLDL) (Delzenne et al., 1993; Fordaliso et al., 1995). Prebiotics acidifies colonic contents by increasing the concentration of short-chain carboxylic acids. They also aid in colonic absorption of minerals, particularly Mg^{2+} and Ca^{2+}. Mineral absorption is promoted through establishment of an osmotic effect whereby entry of water into the colon increases dissolution of minerals (Roberfroid, 2000).

Ingestion of prebiotics is also associated with relief of constipation due to faecal bulking and possible effects on intestinal motility, aiming at daily defecation. They suppress diarrhoea when it is associated with intestinal infections. They also reduce the risk of osteoporosis when improved bioavailability of calcium due to use of inulin-type fructans is followed by significant increase in bone density and bone mineral content. Prebiotics reduce the risk of obesity and possibly type-2 diabetes (van Loo et al., 1999).

Most of the studies on prebiotic effects on bone development have been done on animal models, particularly rats (Scholz-Ahrens, 2007). *In vivo* studies carried out on humans showed that inulin and oligofructose increase the absorption of calcium but not iron, zinc or magnesium (Coudray et al., 1997). More research needs to be done to substantiate these claims, including that of bowel cancer prevention (Ziemer & Gibson, 1996). Studies that have been carried out on animal models so far have shown promise, though human studies are required (Reddy et al., 1997; Rowland et al., 1998). It is vital for studies conducted *in vitro* on prebiotic effects to be carried out *in vivo* in well-designed and reproducible experiments.

Prebiotics, unlike probiotics, are not living organisms, and therefore they do not have survival problems both in the products and the gut (Frost & Sullivan, 2000). Prebiotics have an advantage over probiotics in that they are not affected by factors such as oxygen and heat in industrial applications. This attribute led to increased interest in their health issues (Kolita et al., 2002). Some commercially available prebiotic supplements are water-soluble. Solubility in water allows their possible incorporation in any type of food and also renders them undetectable once dissolved (Douglas & Sanders, 2008).

However their excess levels can cause symptoms such as flatulence, bloating and diarrhoea. This may be caused by a change in osmotic potential or due to excessive fermentation.

Undesirable effects occur when very high doses are ingested. This is advantageous as it allows a relatively broad "therapeutic window", i.e. the dose above the minimal effective level (Holzapfel & Schillinger, 2002). However, FOSs are slightly laxative and produce flatulence when taken in high doses (Losada & Olleros, 2002).

4. Synbiotics

Synbiotics are preparations containing a mixture of a probiotic and a prebiotic, or a combination of probiotics and prebiotics (Davis & Milner, 2009; Holzapfel & Schillinger, 2002; Touhy et al., 2003). These preparations aim to improve the viability of the proven probiotic *in vivo* as well as stimulate the indigenous gut microflora. They provide both the beneficial microbial culture and a specific substrate that can be readily available for fermentation by this culture (Gallaher & Khil, 1999). The presence of the readily fermentable substrate could enhance the survival of the probiotic. The prebiotic ingredient could also offer protection of probiotic against gastric acidity and proteolysis, probably through steric hindrance and coating of the probiotic.

Synbiotic supplements available include combinations of bifidobacteria and FOS, *Lactobacillus* GG and inulin and a combination of bifidobacteria lactobacilli with either FOS or inulin. Fermented milks contain both live beneficial bacteria and fermentation products that may positively stimulate the intestinal microflora also fall within this category. Crittenden et al. (2006) encapsulated a *B. infantis*-FOS synbiotic within a film-forming protein-carbohydrate-oil emulsion to improve its survival and viability during non-refrigerated storage and GIT transit. Gallaher et al. (1996) observed a comparable synbiotic effect with oligofructose and bifidobacteria. Lactobacilli/lacitol, and bifidobacteria/GOS combinations have also been tried as synbiotics in addition to bifidobacteria/FOS (Mountzouris et al., 2002). A well-known benefit of synbiotics is that they increase the persistence of the probiotic in the GIT (Tuohy et al., 2003). The use of synbiotics has not been extensively explored, though studies indicated positive results with regard to maintenance of gut microflora (Shimizu et al., 2009). Their possible use for prevention of allergic diseases is just an example of the studies that still needs to be covered (Johannsen & Prescott, 2009).

5. Conclusion

Probiotics are beneficial microorganism with a host of benefits for the consumer though some of these benefits still have to be confirmed using clinical trials. There is however still a problem of maintenance of viability of these cultures both in storage and in the gastrointestinal tract. The search for methods that can protect and retain viability of probiotic cultures is still on going. There are also concerns about the negative effects of probiotics on sensitive consumers but there is insufficient evidence to support the raised concerns. Prebiotics and synbiotics are alternative mechanisms for increasing the levels of beneficial microorganisms in the gut.

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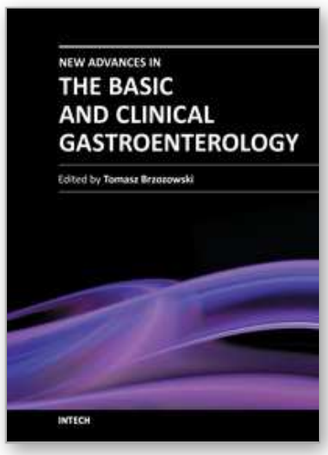

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The purpose of this book was to present the integrative, basic and clinical approaches based on recent developments in the field of gastroenterology. The most important advances in the pathophysiology and treatment of gastrointestinal disorders are discussed including; gastroesophageal reflux disease (GERD), peptic ulcer disease, irritable bowel disease (IBD), NSAIDs-induced gastroenteropathy and pancreatitis. Special focus was addressed to microbial aspects in the gut including recent achievements in the understanding of function of probiotic bacteria, their interaction with gastrointestinal epithelium and usefulness in the treatment of human disorders. We hope that this book will provide relevant new information useful to clinicians and basic scientists as well as to medical students, all looking for new advancements in the field of gastroenterology.

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