Review of Literature
2. REVIEW OF LITERATURE

2.1 Human Gastrointestinal Tract (GIT) Ecology

2.1.1 The human being is actually a walking bioreactor:

We all carry out about $10^{14}$ bacteria in our intestinal tract (ten times more than total cells making up the human body), as per Beng (1996). Bacterial numbers and composition vary considerably along the human GIT. The total bacterial count in gastric contents is usually below $10^3$ per g, with numbers in the small intestine ranging from about $10^4$ per mL of contents to about $10^6$-$10^7$ at the terminal ileum (Gorbach et al., 1967). The human large intestine is an intensely populated microbial ecosystem. Several hundred species of bacteria are usually present, with typical numbers of about $10^{11}$-$10^{12}$/g. Most bacteria growing in colon are non-sporing anaerobes and include members of genera *Bacteroides*, *Bifidobacterium* and *Eubacteria* among many others. The genus *Lactobacillus* contains many species that occur in the gut of most warm-blooded animals. The large gut microflora is acquired at birth. Initially, facultatively anaerobic strains dominate. Thereafter, differences exist in the species composition that develops and this is largely controlled by the type of diet.

The principal role of the intestinal microflora is to retrieve energy from carbohydrate not digested in the upper gut, through fermentation. The major substrates for fermentation and dietary carbohydrates that have escaped digestion in the upper GIT, these include starch, cellulose, hemicelluloses, pectin’s and gums. Other carbohydrate sources available for fermentation are non-digestible oligosaccharides, various sugars and sugar alcohols (Cummings and Macfarlane, 1997). In addition to its role in fermentation the large-intestinal microflora contributes towards health in number of ways.
The availability of non-digestible oligosaccharides is able to alter the composition of human gut flora towards a predominance of beneficial organisms such as lactobacilli and bifidobacteria by selectively stimulating the growth and/or activity of these bacteria. It is obvious that the gut flora composition could be modulated by the supplementation of a food containing beneficially live microorganism and non-digestible carbohydrates (Pimia et al., 2002; fig. 2.1).

**Fig.2.1**: Reaction of various food ingredients with colonic microflora with respect to their health effects (Pimia et al., 2002).

Due to their perceived health benefits probiotic bacteria have been increasingly included in yoghurts and fermented milk during past two decades (fig.2.2). *Lactobacillus acidophilus* and bifidobacteria
are the most common example of probiotic organisms (Daly and Davis, 1998). A major development in functional food pertains to foods containing living microorganism (probiotics) and non-digestible carbohydrates (prebiotics), which enhance health promoting microbial flora in the intestine (Liong, 2008).

There is growing scientific evidence to support the concept that the development and maintenance of healthy gut microflora may provide protection against GIT disorders including GIT infections (e.g. diarrheas), inflammatory bowel diseases and even cancer (Mitsuoka, 1982; Salminen et al., 1998b). Healthy gut flora may also promote beneficial immune functions or prevent atopic disease (Kalliomaki and Isolauri, 2001). Before a probiotic can benefit human health it must fulfil several criteria. It must have good technological properties so that it can be manufactured and incorporated into food products without losing viability and functionality (Mattila et al., 2002).

2.2 Background on Probiotics

The LAB associated with fermented milk products, were advocated by Metchnikoff (1908) for their health benefits. It was probably Vergio (1954) who first introduced the term “probiotic”, when he compared in his manuscript “Anti- und Probiotika” the detrimental effects of antibiotics and other antimicrobial substances on the gut microbial population, with factors (“Probiotika”) favourable to the gut microflora. Lilly and Stillwell (1965) referred to probiotics as “microorganisms promoting the growth of other microorganisms”. Presently, there is general agreement that a “probiotic” refers to viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the GIT (Holzapfel et al., 2001; Holzapfel et al., 1998). Such microorganisms may not necessarily be constant inhabitants of the GIT, but they should have a beneficial
effect on the general and health status of man and animal (Fuller, 1989; Havenaar et al., 1992; Salminen et al., 1998b).

2.2.1 History of probiotic organism

The history recording the beneficial properties of live microbial food supplements such as fermented milks dates back many centuries. Their use in treatment of body ailments has been mentioned even in Biblical scriptures. Known scientist in early ages, such as Hippocrates and others considered fermented milk not only a food product but a medicine as well. They prescribed sour milks for curing disorders of the stomach and intestines (Oberman, 1985). A beneficial association of “lactic acid producing” microorganisms with the human host has been suggested more than 100 years ago (Doderlein, 1982) for vaginal bacteria and, more particularly for LAB in gut ecology studies conducted by Moro (1900), Beijerinck (1901) and Cahn (1901).

2.2.2 Definition of probiotics

Early efforts to classify probiotics were not generally accepted (Lilley and Stillwell, 1965; Sperti, 1971). Parker (1974) proposed one early definition as organism and substances that influence intestinal microbial balance. This definition was subsequently modified when fuller (1989) redefined probiotics by removing the reference to “substances”, like microbial stimulants- which later became prebiotics (Gibson and Roberfroid, 1995). Fuller’s revised definition was: “A live microbial feed supplement, which beneficially affects the host (animal) by improving its intestinal microbial balance. A up to date formal definition of probiotics was agreed by a working party of European scientist is given as “a live microbial feed supplement that is beneficial to health” (Salminen et al., 1998c).
Fig. 2.2: Proposed health benefits stemming from probiotic consumption (Saarela et al., 2002)

### 2.2.3 Development of the probiotic concept

The first significant introduction of probiotic concept was by Metchnikoff at the beginning of the 1900s. He believed that the
complex microbial population in the colon was adversely affecting the host through so-called 'autointoxication' and reported that Bulgarian peasants, who consumed large quantities of fermented milk, experienced longevity (Metchnikoff). This was attributed to the health promoting values of the live organisms. He therefore abandoned his practice of surgical removal of the colon and began modification of the activity of the colonic microflora by the ingestion of soured milks.

A Gram-positive rod, which he called the Bulgarian bacillus and later *Bacillus bulgaricus*, is likely to be the organism later known as *Lactobacillus bulgaricus*. This now called *L. delbrueckii* subsp. *bulgaricus*, which together with *Streptococcus salivarius* subsp. *thermophilus* is responsible for the fermentation of milk to form traditional yoghurt.

Subsequent research looked to confirm that the consumption of LAB was having a beneficial effect on health. For example, preparations containing *Lactobacillus acidophilus* were used to alleviate constipation (Rettger *et al.* 1935), whilst concomitantly, in Japan Shirota selected beneficial strains of LAB which could survive passage through the intestine and subsequently used them to develop fermented milk drinks (Shortt, 1999). It was soon established that there were many species of LAB in the intestine and these have subsequently being incorporated into many probiotic preparations. Common examples of microbial species used as probiotics include *Lactobacillus, Bifidobacterium, Streptococcus* and *Saccharomyces*. *Lactobacillus* and *Bifidobacterium* species have accomplished recognition in the manufacture of probiotic products because of their possession of GRAS status (Salminen *et al.*, 1998d). *Lactobacillus* and Bifidobacteria are the most frequently used genera (Fooks and Gibson, 2002).
2.2.4 Lactobacilli as probiotics

The LAB strains inhabit the human oral cavity, the intestinal tract and vagina and may beneficially influence human ecosystems (Sun et al., 2007). This explains why they are considered as “ideal” candidates for application as probiotics. In their pioneering work, Reuter and co-workers (Reuter, 1965a, 1965b, 1969) have described the typical lactobacilli associated with the human GIT. The “yoghurt-type” products was prepared primarily with strains of L. acidophilus, Lactobacillus crispatus, Lactobacillus johnsonii, Lactobacillus casei/paracasei and Bifidobacterium species. The longest history of proved health benefits and “safe-use” may probably be documented for L. casei strain “Shirota” (Shirota et al., 1966) and some strains of the L. acidophilus group. The functional properties and safety of particular strains of L. casei, Lactobacillus rhamnosus, L. acidophilus and L. johnsonii have extensively been studied and well documented (Salminen et al., 1998d).

Since at least 40 years in Japan and more than 20 years in Germany, LAB cultures of human origin are applied in the manufacture of fermented milk products. Viable strains of especially “L. acidophilus” and Bifidobacterium bifidum were introduced in Germany during the late 1960s into dairy products because of their expected adaptation to the intestine and the sensory benefits for producing mildly acidified yoghurts (Schuler-Malyoth et al., 1968). In Germany, such products first became known as mild yoghurts or “biyogurths”, whilst in the USA, acidophilus milk was developed.

In a survey by Schillinger (1999) on various mild yoghurts and novel probiotic yoghurt-type dairy products, 26 Lactobacillus strains were isolated and identified by DNA hybridisation methods. The species present were found to be L. acidophilus, L. johnsonii, L. crispatus, L. casei, L. paracasei and L. rhamnosus. These
identifications revealed that some strains had been misclassified. Three strains designated as *L. acidophilus* (*L. acidophilus* LA-1, *L. acidophilus* ATCC 43121 and the *Lactobacillus* strain from Biogarde1 culture) were found to belong to *L. johnsonii* and *L. acidophilus* L1 to be *L. crispatus*. Strains designated as *L. casei* were shown to be members of *L. casei*, *L. paracasei* or *L. rhamnosus*. Viable numbers of lactobacilli in mild and probiotic yoghurts varied greatly, whilst a few products contained only low *Lactobacillus* numbers (Schillinger, 1999). This was followed up by a recent study. The most frequently used probiotics found in dairy-based food products are lactobacilli and Bifidobacteria. Since these belong to the indigenous human microflora, they have a long history of safe use and there are evidences to support their positive roles, some of these have been designated as GRAS by the Food and Drug Administration’s (FDA) due to their long history of use in food fermentations (Teitelbaum and Walker, 2002).

A) Lactobacilli – General Characteristics

*Lactobacillus*, first identified by Pasteur, was the first genus of bacteria suspected to have health benefits, rather than to agents of disease. Genus *Lactobacillus* is the largest group among the *Lactobacteriaceae*, family and contains over 100 species (Dellaglio *et al.*, 2005; Satokari *et al.*, 2003). Lactobacilli are a part of the normal flora of human and animal oral cavity, vaginal and GIT (Klaenhammer and Russell, 2000) and found in dairy products, grain products, meat and fish products, water sewage, beer, wine fruit and fruit juices, pickled vegetables, sauerkraut, silage, sour dough and mash.

Members of lactobacilli are fairly large non-sporing and gram positive rods. They are straight or curved rods of varying length and thickness with parallel sides arranged singly or in chains, sometimes filamentous or pleomorphic, without branching, clubbing. They have
complex nutritional requirements. Growth favored by anaerobic or microaerophilic conditions and by CO₂. They are catalase, oxidase and indole negative and lack nitrate reduction. Gelatin not liquefied. Most of the strains have cell-bound proteinase and peptidase. Lactobacilli change carbohydrates and poly alcohols by homofermentation to lactic acid or by heterofermentation to lactic acids, alcohols and carbon dioxide.

Many lactobacilli are unusual in that they operate using homofermentative metabolism (i.e. they produce only lactic acid from sugars) and are aerotolerant despite the complete absence of a respiratory chain. According to metabolism, Lactobacillus species can be divided into three major groups:

Obligatory homofermentative (Group I)

- Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus helveticus, Lactobacillus salivarius

  Facultatively heterofermentative (Group II)

- Lactobacillus casei, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus sakei

  Obligatory heterofermentative (Group III)

- Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus fermentum, Lactobacillus reuteri

The growth temperature range for lactobacilli is 2-53°C and the optimum growth temperature is 30 to 40°C. Optimum pH is 5.5 to 6.2. Lactobacilli and bifidobacteria are most common organisms, which are primarily responsible for the maintenance of balance among intestinal microflora. Such bacteria are also thought to play an important role in the maintenance of the colonisation resistance and prevention of overgrowth of enteric pathogens (Wells et al., 1987).
Increasing evidence indicates that consumption of live probiotics can help to maintain such a favourable microbial profile and results in several therapeutic benefits (Lourens-Hattingh et al., 2001).

In recent years, probiotics have increasingly been incorporated into foods as dietary adjuncts. The concept of probiotic foods is based on the fact that the micro flora in GIT are having significant role in the health status of an individual, which is influenced by a diet consisting of the organisms (Guerin et al., 1998), which upon ingestion in certain numbers exert health benefits over intrinsic nutritional significance (Guarner and Schaafsma, 1998).

2.3 Selection of a Probiotic Strain

The main basis for selection of probiotic microorganisms include technological safety and functional aspects (i.e. viability, attachment adherence, production of antagonistic substances prevention of pathogens and immune stimulation) and technological details such as growth in milk based food, sensory qualities, resistance to phage and viability (Havenaar and Huis int Veld, 1992; Lee and Salminen, 1995; Salminen et al., 1998b; Mishra and Prasad, 2000; Sabikhi and Mathur, 2001; Mishra et al., 2008). Mostly, strains used as probiotic should be of human origin. This is based on the observation that only human origin strains can be adhesive and persists longer in the intestinal tract and thus has better possibilities of showing metabolic effects i.e. antimicrobial. One of the most important desired characteristics for a probiotic strain is that it must be non-pathogenic and should possess GRAS status. Before considering an organism to a probiotic strain some desirable technical features and factors related to health promotion or health sustaining, serve as important criteria for their selection (Holzapfel et al., 1998).
The functional requirements of probiotics should be established by using in vitro methods and the results of these studies should be reflected in controlled human studies. While selecting a preferable probiotic strain following aspects should be taken into account as key criteria for selection of an appropriate strain (Matttila et al., 2002);

(a) Acid tolerance and tolerance to human gastric juice.
(b) Bile tolerance (an important property for survival in the small bowel).
(c) Adherence to epithelial surfaces and persistence in the human GIT.
(d) Antagonistic activity against pathogens.
(e) Retain good viability during formulation and storage.

2.4 Proposed Mechanism of Action of Probiotics

A set of requirements have been identified for a microorganism to be defined as an effective probiotic (Salminen et al., 1996). These include the ability to: (a) adhere to cells; (b) exclude or reduce pathogenic adherence; (c) persist and multiply; (d) produce acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth; (e) be safe, non-invasive, noncarcinogenic and non-pathogenic; and (f) coaggregate to form a normal balanced flora.

Promising evidences have shown that prevention of GIT colonization by a variety of pathogens is a primary mechanism of beneficial effects mediated by probiotics (Lu and Walker, 2001; Forestier et al., 2001). Probiotic bacteria attach to enterocytes and thus inhibit the binding of enteric pathogens to the intestinal mucosa by production of inhibitory substances (competitive exclusion of pathogens) (Nemcova, 1997; Kopp Hoolihan, 2001). These inhibitory substances include bacteriocins, lactic acid and toxic oxygen metabolites (Fig.2.3). Of the toxic oxygen metabolites, the production
of hydrogen peroxide is of prime importance as, in combination with lactoperoxidase-thiocyanate milk system, it exerts a bactericidal effect on most pathogens.

**Fig. 2.3: Proposed mechanisms of action of probiotics (Kaur et al., 2002)**

Therefore, inclusion of probiotic bacteria in fermented dairy products enhances their value as better therapeutic functional foods (Kailasapathy and Chin, 2000). Attachment of probiotic bacteria to cell surface receptors of enterocytes also initiates signalling events that result in the synthesis of cytokines. Furthermore, the production of butyric acid by some probiotic bacteria affects the turnover of enterocytes and neutralizes the activity of dietary carcinogens, such as nitrosamines, the latter being generated by the metabolic activity of commensal bacteria in subjects consuming a high-protein diet (Wollowski et al., 2001).
2.5 Suitability of Lactobacilli as Probiotics: Selection Criteria

2.5.1 Acid and Bile Salt Tolerance

The GIT of a healthy human is a harsh environment because it contains gastric juices, digestive enzymes and bile acids. These conditions impose a significant threat to probiotic strains (Oh \textit{et al.}, 2000). Before reaching the intestinal tract, probiotic bacteria must first survive transit through the stomach where the pH can be low as 1.5 to 2.0 (Dunne \textit{et al.}, 2001).

The 2% oxgall (bile salts) used for testing \textit{Lactobacillus} strains represents the extreme concentration obtained in animal or human intestine during the first hour of digestion (Gotcheva \textit{et al.}, 2002). Afterwards the normal level of bile salts in intestine is around 0.3%. Therefore, the survival of organism in low pH and high concentration of bile salt is thought to be an important criterion for selection of probiotic organism.

A number of studies had been accomplished to evaluate different strains of lactobacilli for their acid and bile salt tolerance, for their fitness to be used as probiotics. Several investigators have studied the survival of \textit{L. acidophilus} and \textit{Bifidobacterium} species in the presence of acid and bile salts (Berrada \textit{et al.}, 1991; Conway \textit{et al.}, 1987; Kim \textit{et al.}, 1988; Ibrahim \textit{et al.}, 1993).

Holocomb and Frank (1991) reported that the viability of \textit{L. acidophilus} and \textit{B. bifidum} was unaffected in bile concentrations ranging from 0.15 to 0.45%. This may have been due to the lower concentrations of bile used in their study. Conway \textit{et al.} (1987) found survivability of lactobacilli to be slightly less when Phosphate buffer saline (PBS) was used instead of gastric juice. This observation
validates the in vitro saline assay system used in present study, to screen strains for their tolerance to low pH. Although, in the stomach, pH can be as low as 1.0, in most in vitro assays pH 3.0 has been preferred (Garriga et al., 1998; Suskovic et al., 1997). This is due to the fact that a substantial decrease in the viability of strains is often observed at pH 2.0 or below, (Gupta et al., 1996; Lankaputhra and Shah, 1995; Hood and Zottola, 1988).

In order to determine the suitability of the strains of Bifidobacterium spp. for use as dietary adjuncts in fermented dairy products, Lankaputhra and Shah (1995) evaluated the tolerance of six strains of L. acidophilus under acidic conditions which is commonly exist in the human stomach (3.0, 2.5, 2.0 and 1.5) and bile concentrations (1 and 1.5%). Samples were taken every hour for 3h and the viable number of L. acidophilus were enumerated by pour plate counts of all samples using 10 fold serial dilutions prepared in 0.1% peptone water. All the six strains of L. acidophilus studied survived well at pH 3.0 or above and the viable counts remained >10^7 cfu/g after 3h incubation. L. acidophilus strains 2404 and 2415 survived best in bile. Prasad et al. (1998) studied the survivability of lactobacilli at pH 3.0 for 0, 1, 2 and 3h and obtained a survival rate of more than 80% after 3h exposure to pH 3.0. This organism not only survived up to 1% (w/v) bile concentrations, but also showed normal growth at this bile concentration. This property may provide these strains with an advantage in vivo.

Mourad and Nour-Eddine (2006) screened eleven strains of L. plantarum for in vitro resistance to low pH and tolerance to bile. For acid tolerance, strains were exposed to pH 1, 2 and 3 for 2, 4 and 6h respectively. Strains were cultivated in MRS broth enriched with 2% (w/v) of oxgall at 37°C for 24h. All tested strains survived an incubation period of 2h to 6h at pH 2.0 and pH 3.0 with decrease in
survival percentage when the exposure time progress for strains. For
tolerance to bile salts, strains demonstrated variable susceptibility to
2% oxgall concentration. *L. plantarum* OL9 and *L. plantarum* OL36
were more sensitive strains with survival percentage of 11 and 18%,
respectively. Correspondingly, *L. plantarum* OL16 and OL15 *L.
plantarum* strain showed the highest tolerance (65 and 59%). The
other strains have showed variable survival percentage ranged
between 22 and 43%.

2.5.2 Antimicrobial Action

Antimicrobial activity is to be an important means for probiotic
bacteria to competitively exclude or inhibit activities of harmful or
pathogenic intestinal microbes (Kaur et al., 2002). Antimicrobial
compounds produced by probiotic bacteria include organic acids
(lactic and acetic acid), hydrogen peroxide, diacetyl, β-
hydroxypropionaldehyde or bactericidal or bacteriostatic peptides and
proteins (De Vuyst and Vandamme, 1994).

Lactobacilli have been found to produce metabolic products that
play important role in controlling undesirable microflora in the gut
(Itoh et al., 1995) in both in vivo and in vitro conditions, including
*Salmonella, Shigella, Clostridium, Bacillus cereus, Staphylococcus
aureus, Candida albicans* and *Campylobacter jejuni* (Anand et al.,
1984; Tojo et al., 1987; Tomoda et al., 1988; Reddy et al., 2006). In
another study *Lactobacillus* spp. obtained from chicken caecum had
been found to display antagonistic effect against other bacteria such as
*Escherichia coli* and *Salmonella* spp. Lactic acid was found to be the
metabolic product responsible for the inhibition of other bacteria (Jin
et al., 1996).

Gopal et al. (2001) investigated the inhibitory effect of *L.
rhamnosus* DR20, *L.acidophilus* HNO17 and *Bifidobacterium lactis*
DR10 against the intestinal cell monolayer colonization by a known enterotoxigenic strain of *E. coli* (strain O157:H7). Pre-treatment of *E. coli* O157:H7 with 2.5-fold concentrated cell-free culture supernatants from the probiotics reduced the culturable *E. coli* numbers on nutrient plates and also reduced the invasiveness and cell association characteristics of this toxic strain.

Ehrmann *et al.* (2002) reported the inhibition of faecal strains (*E. coli* CTC1028, *Salmonella enteritidis* CTC039 and *Salmonella typhimurium* CTC1037) by lactobacilli isolated from crops and intestine of ducks.

Eduardo *et al.* (2003) were isolated probiotic organisms from different commercially prepared milk products (Yakult Drink, Ski D’ Lite Yoghurt, Nestle Yoghurt, Gain Powered Milk and Neslac Powdered Milk) and tested for their antimicrobial activity for *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens* and *C. albicans* using the agar overlay method. The spectrum of their antimicrobial activity varied, Yakult and Ski D’ Lite probiotics inhibited the growth of all the isolate tested against them.

Oyetayo (2004) assessed the inhibitory potential of *Lactobacillus* isolates from different sources against some spoilage and pathogenic bacteria (*B. cereus* NCIB6349, *E. coli* NCIB86, *P. aeruginosa* NCIB 950, *Klebsiella pneumonia* NCIB14070, *S. aureus* NCIB8588 and *Shigella dysenteriae* clinical isolate). *L. acidophilus* isolated from milk was found to display a higher antagonistic effect with zone of inhibition of 6 and 15mm against *E. coli* and *P. aeruginosa* respectively.

Reddy *et al.* (2006) investigated antibacterial activity of *L. acidophilus* (NCDC11, NCDC13, NCDC14 and NCDC16) against *E. coli* NCDC134, *S. typhi* NCDC113, *S. dysenteriae* NCDC107 and *Yersinia enterocolitica* NCDC258, using agar well assay technique. All
Lactobacillus species were found active against the tested indicator strains with diameter (dia) of zone of inhibition from 10.5 to 16.25mm, S. typhimurium NCDC113 and E. coli NCDC134 exhibited 10.75 to 14.50; 10.75 to 13.75mm dia of zone of inhibition respectively.

Osuntoki et al. (2008) studied the antagonistic effects of lactobacilli isolated from fermented dairy products on enteropathogens (Enterotoxigenic E. coli, S. typhimurium and Listeria monocytogenes). Six of the 12 isolates (Five from indigenous products and one from commercial Yoghurt) showed antimicrobial activity by inhibiting the growth of an indicator organism. Four of the isolate inhibited the growth of L. monocytogenes while S. typhimurium and ETEC were inhibited by two organisms each.

2.5.3 Adherence Properties

The adhesion of lactobacilli to human intestine is thought to be an important characteristic of probiotics that show initial colonization and later proliferation in the human intestinal tract (Saito et al., 2004) as well inhibition of the growth of pathogen through the production of antimicrobial components such as organic acid-lactic acid, acetyl, hydrogen peroxide and bacteriocins (Jin et al., 1996).

The microbial surface properties have been widely studied in order to understand the interactions between bacteria and interfaces resulting in the formation of biofilms (Bellon-Fontaine, 1996; Briandet et al., 2001; Strevett and Chen, 2003; Van der Mei et al., 2000) and set up of a successful colonisation in human intestinal tract (Saito et al., 2004). The physical and chemical characteristics of the cell surface have been determined mainly by determining surface hydrophobicity and electrical mobility (Busscher et al., 1993; Geertsema-Doornbusch et al., 1993; Gilbert et al., 1993; Crow et al., 1995).
For *in vitro* demonstration of these phenomenon, the hydrophobicity of bacterial cells surface can be measured in various hydrocarbons (n-hexadecane, toluene and xylene) using as a hydrophobic marker. The hydrophobicity of bacteria can explain and enable to predict the affinity of microorganism for polar compounds (Bouchez-Naitali *et al.*, 2001; Bruinsma *et al.*, 2001; Ly *et al.*, 2006; Pascual *et al.*, 2000). The partitioning of cells between water and hexadecane depends on hydrophobic interactions between microorganisms and the hydrocarbon (Ly *et al.*, 2008). Furthermore, the mechanisms of adhesion of various pathogenic bacteria by fimbriae or flagella have been extensively investigated (Simpson *et al.*, 1992; Purushothaman *et al.*, 2001). There have been a few reports on the mechanisms of adhesion of non-pathogenic bacteria such as probiotic LAB (Saito *et al.*, 2004).

It has been proposed that the lectin-like components in surface layered proteins (SLP) of lactobacilli play an important role in the adhesion to receptors, such as sugar chains of glycolipids (Yamamoto *et al.*, 1996) or glycoproteins (Gusils *et al.*, 1999), on the surfaces of intestinal epithelium cells. Gopal *et al.* (2001) studied the *in vitro* adherence properties of *L. rhamnosus* DR20, *L. acidophilus* HN017 and *B. lactis* DR10 using the differentiated human intestinal cells including HT-29, Caco-2 and HT-29-MTX. All these three strains showed strong adhesion with the human intestinal cell lines *in vitro*, which were comparable to the adhesion indices of two commercial probiotic strains *L. acidophilus* LA-1 and *L. rhamnosus* GG.

### 2.6 The Concept of Prebiotics

As the viability of live bacteria in food products and during transit through the GIT may be variable, the prebiotic concept has been developed. Here, a selective growth of indigenous gut bacteria
through the diet is required. “A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health” (Gibson and Roberfroid, 1995; Liong, 2008). Thus, the prebiotic approach advocates administration of non-viable entities and aims to overcome survival problems in the upper GIT. Certain oligosaccharides which cannot be digested, except through bacterial activity, are prebiotics. Those that contain fructose (e.g. inulin) are able to alter the composition of the human gut flora towards a predominance of bifidobacteria. Prebiotics exploit selective enzyme production by those gut microorganisms that may impart health benefits to the host. While some peptides, proteins and certain lipids are potential prebiotics, non-digestible carbohydrates, oligo- and poly- saccharides occur naturally and meet the criteria of prebiotics (Ziener and Gibson, 1998). Criteria which allow the classification of a food ingredient as a prebiotic include:

(1) It must be neither hydrolysed, nor absorbed in the upper part of the gastro-intestinal tract.

(2) Selective fermentation by potentially beneficial bacteria in the colon.

(3) Alteration in the composition of the colonic microbiota towards a healthier composition.

(4) Preferably, induce effects which are beneficial to the host health. Following physiological and health claims have been made about prebiotics (Salminen et al., 1998a);

- Non-digestibility and low energy value (<9kJ/g).
- Production of short-chain fatty acids (SCFA), lactate, CO₂ and H₂ through fermentation in the large bowel
• Modulation the gut flora promoting, bifidobacteria and lactobacilli populations.
• Suppression in clostridia growth.
• Prevention of intestinal disorders and infections, including diarrhea.
• Modulation of immune response.
• Prevention of colon carcinogenesis.
• Reduction in serum levels of triacylglycerols and cholesterol.
• Improved bioavailability of minerals (Ca, Mg).

Walker et al. (1998) reviewed the health benefits of prebiotic and probiotic in clinical, in vivo and in vitro studies (Table 2.1). Any foodstuff that reaches the colon, e.g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a candidate prebiotic. Certain non-digestible carbohydrates seem authentic prebiotics. FOS is β-d-fructans with various degrees of polymerisation. A number of other non-digestible oligosaccharides have now been developed, for which there is some evidence of their prebiotic effect. These include gluco-oligosaccharides (GOS), galacto-oligosaccharides, transgalacto-oligosaccharides (TOS), isomalto-oligosaccharides, xylo-oligosaccharides (XOS) and soybean-oligosaccharides (Gibson et al., 1999; Hayakawa et al., 1990; Ito et al., 1990; Ito et al., 1993; Imaizumi et al., 1991; Saito et al., 1992). Of all the possible prebiotics, the inulin type fructans have been the most thoroughly investigated. The fermentability of various dietary components has been studied in vitro using mixed faecal culture, with the predominant culturable bacterial groups, including bacteroides, clostridia, lactobacilli and bifidobacteria being enumerated (Wang & Gibson, 1993).
Table 2.1: Selected studies showing beneficial effects of prebiotics and probiotics (Walker et al., 1998).

<table>
<thead>
<tr>
<th>Type</th>
<th>Main Supplement</th>
<th>Study</th>
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<tr>
<td>Probiotic</td>
<td><em>B. bifidum</em></td>
<td>Clinical</td>
<td>Lactose digestion was improved in irritable bowel adolescents and adult volunteers</td>
<td>Duffy et al., 1992</td>
</tr>
<tr>
<td>Probiotic</td>
<td><em>B. bifidum</em></td>
<td>Clinical</td>
<td>Reduced severity of rotavirus infection in children and stimulated rotavirus-specific antibody response</td>
<td>Saavedra et al., 1994</td>
</tr>
<tr>
<td>Probiotic</td>
<td><em>L. casei</em> strain GG</td>
<td>Clinical</td>
<td>Shortened the diarrheal phase in children with rotavirus infection and kept urease activity constant</td>
<td>Isolauri et al., 1994</td>
</tr>
<tr>
<td>Probiotic</td>
<td><em>L. plantarum</em></td>
<td><em>In vivo</em></td>
<td>Reduced severity of enterocolitis</td>
<td>Mao et al., 1996</td>
</tr>
<tr>
<td>Probiotic</td>
<td><em>L. acidophilus</em> La1 and <em>B. bifidum Bb12</em></td>
<td>Clinical</td>
<td>No modification in lymphocyte subsets, but enhanced leukocyte phagocytosis of <em>E. coli</em></td>
<td>Schiffrin et al., 1997</td>
</tr>
<tr>
<td>Probiotic</td>
<td><em>L. salivarius</em></td>
<td><em>In vivo</em></td>
<td>Inhibited colonization by <em>Helicobacter pylori</em> in the stomach</td>
<td>Kabir et al., 1997</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Oligofructose and inulin</td>
<td><em>In vitro</em></td>
<td>Had stimulatory effect on <em>Bifidobacteria</em>, while maintaining <em>E.coli</em> and <em>Clostridium</em> populations at relatively low levels</td>
<td>Gibson and Wang, 1994</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Fructo-oligosaccharides (FOS)</td>
<td><em>In vivo</em></td>
<td>Improved recovery from anemia and increased absorption of iron (Fe), calcium and Magnesium, in Fe-deficient anemic rats</td>
<td>Ohta et al., 1995</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Lactulose</td>
<td><em>In vivo</em></td>
<td>Oral lactulose treatment prior to surgical trauma reduced bacterial translocation to mesenteric lymph nodes and portal venous blood</td>
<td>Ozcelik et al., 1996</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Lactulose (and calcium)</td>
<td><em>In vivo</em></td>
<td>Combination of dietary lactulose and calcium phosphate were protective against <em>Salmonella</em> infection.</td>
<td>Bovee-Oudenhoven and Van der Meer, 1997</td>
</tr>
</tbody>
</table>
2.6.1 Galacto-oligosaccharides

Galacto-oligosaccharides consists of a number of β- (1-6) linked galactopyranosyl units linked to a terminal glucopyranosyl residue via α-(1-4) glycosidic bond. They are produced commercially from lactose using the glycosyltranferase activity of the enzyme β- galactosidase. Major companies involved in galacto-oligosaccharides are Yakult Honsha, Nissin Sugar Manufacturing Company and Snow Brand Milk Products. Snow Brand produced galacto-oligosaccharides to incorporate into their infant milk formulæ, but do not sell them outside their organization. GOS have as yet not been extensively investigated. Although they are thought to be bifidogenic, one study fed gnotobiotic rats a diet of 40 g/d of GOS and found little effect on the bacterial groups. However, they did modify certain glycolytic activities (Djouzi and Andrieux, 1997).

2.6.2 Lactulose

Lactulose is produced in the largest quantity of any oligosaccharide. Like galacto-oligosaccharides, it is manufactured from lactose. Evaporated milk contains 0.4 to 0.9% of lactulose. Evaporated milk had been used for infant feeding and, therefore, lactulose had been administered to infants without recognition. The glucose moiety of lactose can easily be isomerizes to fructose to yield the disaccharide referred to as lactulose (4-O-β-D-galactopyranosyl-D-fructose). Commercially, it is produced by heating, using borate as a catalyst (Mizota et al., 1987). The significance of lactulose as bifidogenic factors have been recognized as early as 1957 (Harju, 1991). Lactulose plays the role of an energy source to stimulate the growth of bifidobacteria, a beneficial intestinal microbe, selectively because it is not metabolized by humans or animals due to the absence of enzymatic activity to split the galactose-fructose bond.
2.6.3 Fructo-oligosaccharides

FOS represents one of the major classes of bifidogenic oligosaccharides in terms of their production volume. Numerous studies have explored the effects of FOS on human gut bacteria. Chemically, FOS is short and medium length chains of β-D fructans in which fructosyl units are bound by a β-2-1 osidic linkage. Depending on the chain length, as defined by the number of osyl units called degree of polymerization (DP), FOS are named oligofructose (DP<9, average DP=4.8) or inulin (DP up to 60, average DP=12). The inulin extracted from chicory roots contains some fructo-oligosaccharides in addition to polysaccharides (Gibson and Roberfroid, 1995). The Belgian company ORAFTI markets their inulin products under the trade name Raftiline. It contains fructose chains ranging in size from DP 3 to larger than DP 50 (Crittenden and Playne, 1996).

Inulin is prepared by hot water extraction of chicory roots and oligo-fructose is obtained by serial enzymatic hydrolysis of inulin under strictly controlled conditions (Gibson and Roberfroid, 1995). Generally, feeding of FOS increases bifidobacteria and lactobacilli, increases SCFA concentrations and decreases clostridia and bacteroides and pH (Gibson et al., 1995, 1996; Fuller and Gibson, 1997). The ingestion doses of FOS, which have elicited a bifidogenic effect in human studies, ranged from 4 to 15 g d⁻¹ (Gibson et al., 1996; Roberfroid, 1996). It is always preferable to use possible lowest dose that gives a demonstrable effect.

2.6.4 Lactitol

Chemically, lactitol (4-O-β-D-galactopyranosyl-D-glucitol) is a sugar alcohol which can be prepared by a variety of techniques such as electrolytic reduction, reduction with sodium bicarbonate or hydrogenation with nickel catalyst (Harju, 1988).
Use of lactitol as a bifidogenic factor was recommended by Petuely (1966). Similarly, lactulose it also stimulates the growth of bifidobacteria. The rate of hydrolysis is very slow in the small intestine (Harju, 1988). Majority of it delivered to the large intestine, where it is utilized by intestinal flora to produce SCFA (Booy, 1987). These acids are in turn absorbed and serve as sources of energy for the host. Lactitol also appears to have some positive benefits in terms of reducing serum cholesterol (Sugimoto, 1976) and altering the metabolism of cholic acids in the intestine (Booy, 1987). Granby (1989) reported that Lactitol has a low level of carcinogenicity and therefore, it can be used in dietetic sweeteners.

2.6.5 Xylo-oligosaccharides

At present, XOS represent only a small proportion of the total oligosaccharide market. All land plants contain a group of polysaccharides known as D-xylans which form a polymer-homologous series such as xylobiose, xylotriose, up to xyloheptaose. Xylobiose (4-O-β-D-xylopyranosyl-D-xylopyranose) has been isolated from a variety of sources (corn cobs, aspen and wheat straw) using concentrated acid hydrolysis and endoxylanases or xylans. The raw material for XOS synthesis is the poly saccharides xylan extracted mainly from corncobs. The xylan is hydrolysed to XOS by the controlled activity of the enzyme endo-1, 4-β- xylanase. Only few data are available on the properties of xylobiose or its application as a bifidogenic factor; however, work published by Okazaki et al. (1990) found the XOS, principally xylobiose to be effective as bifidobacterial factors when administered at 1 or 2 g daily.

2.6.6 Trans-galactosylated oligosaccharides

TOS are manufactured from lactose by transglycosylation reactions and consist of galactosyl derivatives of lactose with β(1-3) and β(1-6) linkages. Bifidobacterial numbers were significantly
increased in the faeces of rats fed TOS (Djouzi and Andrieux, 1997), confirming data from earlier experiments (Tanaka et al., 1983; Mitsuoka, 1990). One study used transgalactosylated disaccharides in a human volunteer trial to determine their effects on the faecal flora. This showed that the prebiotic increased bifidobacteria and Lactobacillus numbers, whilst decreasing Bacteroides spp. and Candida spp. (Ito et al., 1993). The bifidogenic nature of TOS has been related to a linkage specificity of the Bifidobacterium β-galactosidase, which cleaves β(1-3) and β(1-6) linkages, instead of β(1-4) linkages (Dumortier et al., 1994).

TOS compounds are available commercially from two Japanese suppliers: Ensuiiko Ltd. produces lactosucrose, while Yakult offers a commercial preparation containing approximately 51% (w/w) tri-saccharides, 35% tetra-saccharides and 13% penta- and hexa-saccharides (Wijsman et al., 1989). Several experimental data also indicate that oligosaccharides might modulate the immune system and contribute to the improvement of the protective properties of infant formulas (Fanaero and Vigi, 2008). A prebiotic mixture from galacto-oligosaccharides and FOS has been used to mimic the effect of human milk oligosaccharides.

It has been demonstrated that such a mixture significantly increases the number of bifidobacteria in a dose-related way (maximum effect at 0.8 g/d) and reduces the number of pathogens in term as well as in preterm infants when compared with a group of infants fed a non-supplemented formula (Moreno, 2008).

### 2.7 Inulin as a Prebiotic

An ingredient typically extracted from chicory root, inulin is finally being recognized by the U.S. dairy industries for its healthful benefits. Benefits include nourishing beneficial intestinal bacteria while inhibiting the growth of harmful bacteria, reducing the severity
of incidence of diarrhea, relieving constipation and improving the resorption of minerals such as calcium. According to Gibson (1999) any food or its ingredient that reaches the colon such as non-digestible carbohydrates, some peptides and proteins in addition to certain lipids is a prebiotic candidate. Non-digestible but fermentable carbohydrates, especially FOS are authentic prebiotics.

As inulin is not digestible in the small intestine and reach the large intestine almost intact. This attribute contribute them as being ideal for fermentation in the colon by the saccharolytic resident microbiota. Inulin is legally classified as a food or food ingredient in all countries where it is used. It is well accepted for food use without limitations (Coussémente, 1999). A variety of products containing inulin formulations, claiming to have beneficial effects on gut health and general well being, are starting to become prevalent in the market.

2.7.1 Composition of Inulin

The inulin extracted from chicory roots contains some FOS in addition to polysaccharides (Gibson and Roberfroid, 1995). Chemically, inulin is a FOS in which short and medium length chains of β-D fructans in which fructosyl units are bound by a β-2-1 osidic linkage. Depending on the chain length, as defined by the number of osyl units called the DP, FOS are named inulin (DP up to 60, average DP=12). The Belgian company ORAFTI markets their inulin products under the trade name Raftilin. It contains fructose chains ranging in size from DP =3 to larger than DP=50 (Crittenden and Playne, 1996).

2.7.2 In vitro and in vivo prebiotic assessments

The effects of inulin on the human intestinal microflora have been extensively studied both in vivo and in vitro and the majority of studies report selective fermentation by the beneficial flora, namely,
bifidobacteria and to lesser extent lactobacilli (Kolida et al., 2002). Lactobacilli possess hydrolytic enzymes known as inulases, which cleave fructo-oligosaccharides in to simpler forms.

The FOS are fermented by colonic microbiota has been demonstrated in vitro using mixed human fecal bacteria. Like other carbohydrates such as glucose, fructose sucrose, starch and pectin, FOS induces a decrease in pH of the culture medium during anaerobic fermentation (Wang and Gibson, 1993). Hopkins et al. (1998) observed the ability of seven Bifidobacterium isolates to utilize a selection of fifteen different carbohydrate sources in 48h batch culture experiments. In a continuous culture study Sghir et al. (1998) demonstrated through molecular techniques that inulin and oligofructose were selectively fermented not only by bifidobacteria but also by lactobacilli. Oligofructose and galacto-oligosaccharides preferentially supported growth of the test organisms.

Karppinen et al. (2000) documented that inulin was most rapidly fermented of the test substrates by human faecal bacteria giving most butyrate production and the largest decrease in pH. Kaplan and Hutkins (2000) screened a selection of 28 LAB and bifidobacteria for their ability to ferment inulin and oligofructose on MRS agar. Twelve of 16 Lactobacillus strains and seven of eight Bifidobacterium strains tested were able to ferment the substrates.

Gibson and Roberfroid, 1995) reported the selective stimulation of bifidobacteria by inulin and oligofructose in a 45d study of eight healthy male human subjects. Both oligofructose and inulin caused significant increases in faecal bifidobacteria. Bacteroides, clostridia and fusobacteria all decreased during oligofructose supplementation and Gram-positive cocci were reduced during inulin supplementation. Total bacterial levels remained unaffected, while little change was observed in faecal SCFA and breath CH₄.
The effect of dietary supplementation of inulin and lactose on faecal flora, microbial activity and bowel habit in 35 elderly constipated patients was studied by Kleessen et al. (1997). A significant increase was observed in bifidobacterial levels in the inulin group, while a decrease in enterococci numbers and enterobacteria occurred. Lactose had no effect on bifidobacteria, while it increased enterococci counts and decreased lactobacilli levels. A better laxative effect was reported with inulin. In two studies, the effect of inulin on dextran sulphate sodium (DSS) induced colitis rats was reported (Videla et al., 1998; Videla, 1999). It was established that dietary inulin promoted growth of lactobacilli in the rat colon, reduced the severity of DSS induced colitis and reduced the luminal pH in a wide area extending from left to right colon.

Reddy (1999) evaluated inulin (raftiline) and oligofructose (raffinose) for their potential inhibitory properties against the development of colonic aberrant crypt foci (ACF) in rats. The results of this study indicated that the dietary administration of oligofructose and inulin inhibits the development of angiotensin converting enzyme (ACE) in the colon, suggesting the potential colon tumor inhibitory properties of chicory fructans.

Den Hond et al. (2000) investigated the effect of high performance inulin on constipation in six healthy humans with a low stool frequency in a double-blind placebo control crossover study. A significant increase in stool frequency and faecal bulk was observed with inulin administration. Langlands et al. (2000) investigated a human feeding study to investigate the effect of inulin and oligofructose on mucosal microflora. Fifteen healthy subjects were selected from a colonoscopy waiting list and supplemented their usual diet with 15 g/d of an inulin + oligofructose mixture for 2 weeks before their colonoscopy. The effect on the mucosal flora was to
increase considerably both bifidobacterial and lactobacilli counts on the epithelium, while counts of bacteriodes, clostridia and enterobacteria were unaffected.

2.8 Gum Acacia as a Prebiotic

The term “dietary fibre” was first introduced between 1972 and 1976 by Burkitt et al., (1972), Trowell (1972) and Painter (1975), although Hipsley (1953) first applied the term as shorthand for nondigestible constituents of food. It was used to describe the remnants of plant components that are resistant to hydrolysis by human alimentary enzymes. It was, therefore, a physiological-botanical description, with plant cell walls being the major source of digestion-resistant material. Trowell et al. (1976) extended this definition to include all indigestible plant polysaccharides. This broadened definition includes cellulose, hemicellulose, lignin, gums, modified celluloses, mucilages, oligosaccharides, pectins and associated minor substances such as waxes, cutin and suberin. These were included because they have the functional properties of dietary fibre but could not necessarily be chemically identified as having their origins in the cell wall.

Gum acacia is a natural polysaccharide exudate from the stems and branches of sub-Saharan of Acacia senegal and Acacia seyal trees, produced naturally as large nodules during a process called gummosis to seal wounds in the bark of the tree (Maslin et al., 2003). Both species are acceptable gum for use in food and medicine (Dondain and Phillips, 1988). Gum acacia is traditionally utilised by Indian and African populations (Cherbut et al., 2003) to prevent and treat intestinal disorders. There is evidence that gum feeding can improve intestinal transit and provide digestive comfort (Cherbut et al., 2003). Such traditional health promoting characteristics of dietary fibre have been used and accepted.
2.8.1. Chemical composition of Gum acacia

In chemical terms these are arabinogalactan proteins (AGP), the component sugars are D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl glucuronic acid and the proportions vary in A. seyal and A. senegal. Approximately 50% of the proteinaceous matter found in gum arabic consists of hydroxyproline, serine and proline. There are variations in protein content for various gums of the acacia species ranging from 0.13% to 10.4%. In the polysaccharide chain there are uniform blocks of (1-3)-linked D-galactopyranosyl residues (Stephen and Churms, 1995). The point of attachment of the gum acacia to the protein chain is probably via hydroxyproline. Both A. seyal and A. senegal are polydisperse in character. These gums are not a discrete chemical species and are complex polysaccharides. The gum consists of three broad molecular fractions, which differ principally in their size and protein contents (Osman et al., 1993; Randall et al., 1988).

2.8.2 In vitro and in vivo prebiotic evaluations

As the gum acacia is not digestible in the small intestine and is therefore a potential substrate for the colonic microflora. Its fermentation by the intestinal microflora has been studied both in vitro (Bourquin et al., 1993; May et al., 1994) and in vivo (Wyatt et al., 1986; Tulung et al., 1987; McLeanRoss et al., 1983; McLean Ross et al., 1984; Topping et al., 1985; Annison et al., 1995; Walter et al., 1986; Walter et al., 1988) although conclusions have been equivocal. For example, this gum has been demonstrated as highly fermented in vivo both in humans (McLean Ross et al., 1983) and in rats (McLean Ross et al., 1984) and in vitro using either human faecal flora (Bourquin et al., 1993) or pig faecal flora (May et al., 1994). Conversely, Topping et al. (1985) and Annison et al. (1995) observed low faecal SCFA concentration when rats were fed gum acacia.
discrepancies could stem from differences in the biochemical composition of the gums (Annison et al., 1995) and/or in administration time between studies.

In fact, several studies have recommended that an adaptation of the fermentation activity occurs when the duration of the gum administration is prolonged (Wyatt et al., 1986; Tulung et al., 1987 Walter et al., 1986; Walter et al., 1988). Some of the above-mentioned studies suggest that fermentation of gum acacia could induce microbial changes, as demonstrated by specific increase of gum acacia utilizing micro-organisms among which bacteriodes and Bifidobacterium spp. have been identified (Wyatt et al., 1986). In addition, the ability of gum acacia to support bifidobacterial growth has been demonstrated (Macfarlane et al., 1994; Salyers et al., 1978) particularly for Bifidobacterium longum and Bifidobacterium adolescentis (Crociani et al., 1994).

Michel et al. (1998) investigated that the fermentation of two gum acacia of different biochemical characteristics by both unadapted and adapted human intestinal flora, using batch incubations and continuous culture operated at pH 5.8 and pH 6.5. Continuous cultures, once specific adaptation was achieved, were also used to characterize the effects of the gums onto the microbial populations. It has been demonstrated that, similarly to FOS, gum acacia can exert putatively beneficial effects on host health through both the improvement of the composition of the large intestine microflora and SCFA formation.

2.9 Synbiotics

A further possibility in microflora management procedures is the use of synbiotics, where probiotics and prebiotics can be used in combination. The concept of prebiotics has become very popular since
its introduction in 1995 (Gibson and Roberfroid, 1995). The combination of suitable prebiotics with probiotic/s has been found to enhance the survival and activity of the organism, both in vitro and in vivo experiments, for example a fructooligosaccharides in conjunction with a Bifidobacterium strain or lactitol in conjunction with Lactobacillus (Gibson et al., 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 (Mattila et al., 2002; Fig 2.4).

**PROBIOTICS**
- Exogenous Strain
- Known health properties
- Immunomodulation
- Colonisation

**PREBIOTICS**
- Proliferation of endogenous strain
- Effects on the microbiota’s metabolic activity as well as population dynamics

**SYNBOTICS**
- Synergy between probiotic and prebiotic effects in the GI-tract
- Limited specificity of prebiotic for added probiotic strain

**INTEGRATED SYNBOTICS**
- Specific synergies between probiotic and prebiotic ingredients in the GI-tract
- Prebiotics additionally protect probiotic during manufacture, storage, formulation and intestinal transit.
- Controlled site specific release of probiotics in the GI-tract
- Larger, more slowly fermentable prebiotics with SCFA production more distally in the colon

**EVOLUTION OF DEVELOPMENTS IN PROBIOTIC/PREBIOTIC TECHNOLOGIES**

**Fig. 2.4: Evolution in developments of probiotic/prebiotic technologies (Mattila et al., 2002).**

The combination of Bifidobacterium and oligofructose synergistically retarded colon carcinogenesis in rats compared to when both were given individually (Gallaher et al., 1999). Interaction
between the probiotic and prebiotic in vivo might be favored by an adaption of the probiotic to the prebiotic substrate prior to consumption (Sandholm et al., 2002). The commercial application of synbiotic products is in its infancy but offers the potential to develop prebiotics targeted at specific probiotic strains to optimize health benefits (Rastall, 2006). Recently in a study, conducted by Liong (2008) concluded that the combination of probiotic and prebiotic (Synbiotic) has a synergistic effect in improving colon carcinogenesis as compared to both, when they were used individually. Probiotic strains can be successfully manufactured and incorporated into highly acceptable food products where they can retain their viability and functionality (Sandholm et al., 2002).

Use of inulin and oligofructose in fruit yoghurts, milk-based drinks, milk, spreads, cheese and ice cream has also been reported (Coussen et al., 1996). Some synbiotic dairy products e.g., Symbalance, mixture of Lactobacillus reuteri, L. acidophilus and L. casei along with RAFTILINE, an inulin and John après Jour a UHT skimmed milk with ACTILIGHT, etc. have also been marketed in Europe (Young, 1998), probiotic plus oligofructose (yoghurt), two Lactobacillus strains plus inulin, Actimel (cholesterol control yoghurt), L. acidophilus plus oligofructose, Fysiq (dairy drink), L. acidophilus plus inulin (Kolida et al., 2002).

The most important benefits of synbiotics are believed to be increased persistence of the probiotic in the GIT. A synbiotic preparation of L. acidophilus (probiotic strain 74-2) and FOS has been studied in an in vitro model of the human gut (Gmeiner et al., 2000). The model used was the SHIME reactor and the synbiotic resulted in higher levels of lactobacilli (an increase of 0.89 log) in the vessel corresponding to the ascending colon. An increase in bifidobacteria was seen in the vessels corresponding to the ascending colon (1.27 log) transverse (0.9 log) and descending (0.47 log) colon, presumably due to the prebiotic component.
of the synbiotic. Increases were also seen in levels of propionate and butyrate and in β-galactosidase. A decrease was seen in β-glucuronidase levels.

When combining both a probiotic and prebiotic in a single food, the expected benefits are, an improved survival during the passage of the probiotic bacteria through the upper intestinal tract and a more efficient implantation in the colonic microbiota together with a stimulating effect of the prebiotic on the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (Lactobacilli, bifidobacteria etc.) Without specifically referring to it as a ‘synbiotic approach’, Bouhnik et al. (1996) have assessed in healthy humans, the effects of prolonged ingestion of Bifidobacterium spp. fermented milk with or without inulin (equivalent to 18g/day) on faecal bifidobacteria. They concluded that the Bifidobacterium spp. fermented milk substantially increased the number of bifidobacteria colony-forming units (cfu), but that the concurrent administration of inulin did not enhance the effect. This observation is not surprising in view of the fact that the number of bifidobacterial cfu in the faeces of probiotic-fed volunteers was already so much increased (from 10^7 to 10^9) that they could hardly be additionally increased by the prebiotic. In an another study, Gibson and Roberfroid (1995) reported that a substantial effect on the composition of faecal flora on feeding a group of volunteers a everyday supplement of a synbiotic composed of 125mL of Lactobacillus fermented milk containing 2.75g oligofructose for 7 weeks.

In a study conducted by Martin (1996) deliberated the effects of bifidogenic growth factors on survival of B. longum, Bifidobacterium infantis and B. adolescentis in various dairy products. They supplemented 10% solids skim milk containing B. longum or B. infantis with 0.5% FOS, 0.5% lactulose and 0.5% units/mL of oxyrase. Their data point out that the level of FOS and lactulose used (0.5%) does not significantly affect
numbers of *B. longum* or *B. infantis* in skim milk during incubation at 37°C for 48h. Spray-drying probiotic lactobacilli in conjunction with soluble fibre, gum acacia, increased *L. paracasei* NFBC 338 viability during powder storage at both 15 and 30°C compared with RSM control (Desmond *et al.*, 2002). A new study has reported that probiotic bacteria used in synbiotic ice cream had 30% higher survivability in storage when encapsulated in calcium alginate than 'free' probiotics (Mandal *et al.*, 2006; Homayouni *et al.*, 2008).