CHAPTER 1:
INTRODUCTION
Introduction

A probiotic is a live microorganism which, when administered in adequate amount, confers a health benefit to the host [1]. Probiotics are the names given collectively to all the beneficial bacteria in the human colon. These friendly bacteria utilize the prebiotic substances for their growth. Prebiotics are basically any non-digestible food ingredients that can benefit the human body by stimulating the growth and activity of one or more species of probiotics in the large intestine and produce short chain fatty acids (SCFAs) resulting in a decrease of the colon pH [2]. Lowering the pH to definite extent of the colon assists in the reduction of pathogenic bacteria and viruses which causes gastro-intestinal diseases. The activity of the beneficial bacteria and other various substances they produce in the large intestine contribute to numerous health benefits ranging from prevention of chronic diseases to improvement of immune system as well as improving the absorption of minerals and vitamins into the body. Therapeutic actions of these bacteria also include anti-microbial activities and anticancer properties [3]. Some of the common probiotics include species Lactobacillus such as L. acidophilus etc. and Bifidobacterium (Jamaly et al, 2011) [4] which are collectively called lactic acid bacteria (LAB). Some more LAB include L. plantarum, L. Casei, L. delbruckii, L. bulgaricus, L.fermentum, L. renteri, B. infantis, B. adelocentis, B. longum, B. breve, Enterococcus faecalis, etc.. Other genera which are also very common are Lactococcus, Carnobacterium, Enterococcus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Melissosoccus, weissella, etc (Dallaglio et al, 1981) [5]. Dairy products remain the most important vehicle of administration of probiotics to the consumer. Most of the dairy products, especially, yogurt and curd are good sources of probiotics [2]. Scanning Electron Microscopy pictures of two probiotics, viz., Pediococcus acidilactici and Lactobacillus casei are shown in Figure 1.1.
On the other hand, prebiotics are usually carbohydrates such as oligosaccharides. However, this may also include non-carbohydrates. These prebiotics provide the food for the beneficial bacteria or probiotics. Prebiotics are naturally classed as soluble fibres. These include various short chain fructo-oligosaccharide (such as inulin), gums and various lactose derivatives (such as tagatose, malto-oligosaccharide, polydextrose, resistant starches) and many others [2]. According to Roberfroid [6], only two particular fructo-oligosaccharides, namely, oligofructose and inulin fully meet the definition of prebiotics. Prebiotics may also be categorized as short-chain, long-chain and full spectrum ones. Short-chain prebiotics, e.g., oligofructose, contain 2-8 links per saccharide molecule, typically get fermented more quickly in the right side of the colon providing nourishment to the bacteria in that area. Longer chain prebiotics, e.g., inulin contain 9-64 links per saccharide molecules and tend to be fermented more slowly, nourishing bacteria predominantly in the left side of the colon. Full-spectrum prebiotics, e.g., oligofructose-enriched inulin (OEI) provide the full range of molecular link lengths from 2-64 links per molecule and nourish bacteria throughout the colon. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of β-(2-1) fructosyl-fructose links [6]. Inulin producing plant species are found in several monocotyledonous and dicotyledonous families, including Liliaceae, Amaryllidaceae, Gramineae, and Compositae. However, only one inulin-containing plant species (Chicory. Chichorium intybus) is used to produce inulin industrially. In chicory inulin, both Gpy and Fn (α– D-glucopyranosyl-[β-Dfructofuranosyl]n-1β-D-fructofuranoside) compounds are considered to be included under the same nomenclature and the number of fructose units varies from 2 to >70.
Native inulin is processed by the food industry to produce either short chain fructans, especially oligofructose (degree of polymerization: 2-10; average: 5) as a result of partial enzymatic hydrolysis (inulinase) or long chain fructans by applying an industrial physical separation technique. Prebiotic carbohydrates are also found naturally in fruits and vegetables like bananas, berries, asparagus, garlic, wheat, oatmeal, barley (other whole grains), flaxseed, tomatoes, Jerusalem-artichoke, onion, greens (especially dandelion greens but also spinach, collard greens, chard, kale mustard greens and others) and legumes (lentils, kidney beans, chickpeas, navy beans, black beans) [6]. Inulin was found to support larger total bacterial counts but lower populations of bifidobacteria and lactobacilli than soybean oligosaccharides (SOS), isomalto-oligosaccharides (IMO) or lactulose. Fructo-oligosachcharides (FOS) resulted in higher populations of streptococci compared to galactooligosaccharide (GOS) or SOS [6]. The largest increase in bifidobacteria was seen on xylooligosaccharides and lactulose, the largest increase in lactobacilli was on FOS and the largest decrease in clostridia was on GOS. Generally, galactose-containing oligosaccharides proved to support higher populations of bifidobacteria, higher levels of lactate and lower levels of gas compared to fructose-containing oligosaccharides. These prebiotics may be taken as a supplement or added to foods, to help probiotics to naturally re-establish normal population in the colon over-time. Figure 1.2 shows photographs of some of the natural prebiotic sources.

![Figure 1.2: Photograph showing different natural prebiotic sources](image-url)
Table 1.1 presents a few sources of probiotics and prebiotics from natural sources.

Table 1.1 Sources of probiotics and prebiotics

<table>
<thead>
<tr>
<th>Sources of Probiotic</th>
<th>Yogurt</th>
<th>Curd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sources of Prebiotics</td>
<td>Fruits and vegetables</td>
<td>Banana, Berries, Asparagus, Garlic, Onion, Tomatoes, Chicory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole grains</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat, Oatmeal, Flaxseed, Barley</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinach, Collard greens, Mustard greens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Legumes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lentils, Kidney beans, Chickpeas, Navy beans, Black beans</td>
</tr>
</tbody>
</table>

1.1 Use of probiotics

- Probiotics are widely used to prepare fermented dairy products such as yogurt or freeze-dried cultures.
- Bacteriocinogenic probiotics are useful to control the frequent development of pathogens and spoiling microorganisms in food and feed.
- Probiotics are effective to reduce the excessive use of therapeutic antibiotics probiotics are effective.
- Probiotics prevents chronic diseases in some cases and thus improve the immune system as well as providing better absorption of minerals and vitamins into the body.
- Therapeutic action of probiotics also include anti-microbial activities and anti cancer properties in some instances.

1.2 Use of prebiotics

Multipurpose uses of prebiotics are shown schematically in Figure 1.3.
1.3 Combined effect of synbiotics on the health of human body

Effect of presence of probiotics and prebiotics technically known as synbiotics on the health of host body is illustrated in Figure 1.4.

1.4 Nutraceutical and pharmaceutical importance of prebiotics in food additive and starter culture

1.4.1 Prebiotics as food additive and starter culture

Inulin plays a major role as an important prebiotic due its multipurpose use in various industries such as bakery, dairy, fermented food etc.. It is observed that, soluble fibers of inulin influence dough and bread quality [7]. Inulin, as a prebiotic is used to improve the quality of skim milk fermented by pure cultures of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus* and *Bifidobacterium lactis, Streptococcus thermophilus*. Inulin supplementation, lowered the generation
time of *S. thermophilus* and *L. acidophilus* [8]. Addition of prebiotic in probiotic cheese manufacturing helps to produce better quality product with lower atherogenicity index [9]. FOS and maltodextrin supplementation of soy milk increased the $\alpha$-galactosidase activity of probiotics viz. *Lactobacillus acidophilus* and *Bifidobacterium longum*. The increased activity of the enzyme enhances the hydrolysis of substrate prebiotics resulting in increased production of fructose and glucose, which in turn promotes the growth of the probiotic cells [10].

### 1.4.2 Gut health maintenance, colitis and constipation prevention

Prebiotics have a positive influence on the gut-associated lymphoid tissues (GALT). They also influence the maintenance of gut health, colitis and constipation. Inulin, FOS, mannoooligosaccharides, and arabinogalactans are therapeutic nutritional preparations used for the optimum gut function for favouring the proliferation of normal bacterial flora and impeding the growth of pathogenic organisms. The consumption of prebiotics can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation [11]. The growth of intestinal bacterial community is accelerated due to administration of probiotics and prebiotics. It is evident that probiotics decrease the incidence of NEC [12]. Prebiotic supplemented formula increases stool colony counts of bifidobacteria and lactobacilli in preterm neonates without adversely affecting weight gain [13]. FOS is being rapidly included in food products and infant formulae due to their laxative effect. Their consumption increases faecal bolus and the frequency of depositions, reducing instances of constipation which are considered as one of the growing problems associated with inadequate fiber diet consumption in the modern society and neonates [14]. Prebiotic mixture improves the infant formulae. Acute diarrhoea is a major cause of child morbidity. The conventional ORS is not capable of reducing the duration and severity of the acute disease. It is observed that, the use of fortified ORS with zinc and prebiotics (FOS and XOS) treats the diarrhoea in children more efficiently.

It is also observed that, the combination of zinc and the prebiotics decreases the duration of diarrhoea in patients by stimulating water and electrolyte absorption across gut mucosa and inhibiting the pathogens, respectively. Hence, it can be said that, the therapeutic efficacy is attributed to the synergistic relation between the additives.
Arabinoxylan-oligosaccharides (AXOS)- enriched diet has been reported to reduce the occurrence of preneoplastic lesions in the colon of rats treated with the carcinogen 1, 2-dimethylhydrazine [15]. Conversion of lactose to epilactose is effectively done by Cellobiose 2-epimerase from Ruminococcus albus. Consumption of epilactose increases cecal contents and decreases its pH which in turn enhances the population of bifidobacteria and lactobacilli and suppresses clostridia in Wistar-ST rats. The conversion of primary bile acids to secondary bile acids which is the promoter of colon cancer is inhibited by the prebiotic epilactose [16]. The Short Chain Fatty Acid (SCFA) obtained from fermentation of GOS is known to stimulate apoptosis. Propionate has anti-inflammatory effect with respect to colon cancer cells [17], butyrate suppresses the expression of transcription factor NF-jB in HT-29 cell lines, whereas acetate is known to increase the peripheral blood antibody production and NK activity in cancer patients [18]. The specific prebiotic oligosaccharides viz. GOS, FOS and pectin-derived acidic oligosaccharides have immunemodulatory effect during the early phase of a murine immune response [19]. Prebiotics may reduce the incidence of degenerative diseases, such as neoplasias, diabetes, coronary diseases and infections. They also seem to promote a positive modulation of the immune system [20]. It is observed that, a prebiotic mixture of short-chain GOS, long-chain FOS and pectin-derived acidic oligosaccharides, resembling the composition of oligosaccharides in human milk, promote T Helper 1 (Th1) and regulatory T cell (Treg)-dependent immune responses and induce down regulation of IgE-mediated allergic responses. Additionally, the prebiotic administration does not interfere with the desired vaccine specific serum antibody responses in healthy term infants [21].

1.4.3 Cholesterol removal, reduction of cardiovascular disease and prevention of obesity

Synbiotic effect of P.acidilactici with sorbitol for 1 month lowers the plasma cholesterol level of Swiss albino mice [22]. The risk of coronary heart disease and vascular instances has been reduced due to consistent ingestion of resistant carbohydrate rich whole grains (CHD) [23]. Reason for the health benefits reported for dietary fiber in cardiovascular diseases is due to higher plasma ferulic acid concentration. Increase in free ferulic acid from the enzyme-treated prebiotic durum wheat results in higher plasma ferulic acid concentration [24]. Supplementation of fermentable dietary fibres in food as
shortchain FOS is effective to induce satiety and thus prevent obesity [25]. It is observed that intake of soy-fortified prebiotic reduces low-density lipoprotein-cholesterol and increases high-density lipoprotein [26].

Prebiotics are useful tool for controlling food intake and glucose homeostasis. Prebiotic treatment resulting in loss in appetite is responsible for increase in postprandial plasma gut peptide concentration [27].

1.4.4 Restoration of vaginal ecosystem

Anaerobic pathogens tend to dominate the vaginal microbiota in post-menopausal women. Neutraceuticals (Prebiotic-probiotic conjugates) have abilities to restore vaginal ecosystem. Encapsulated pectinate-hyaluronic acid microparticles are used to design a vaginal bio-adhesive delivery system [28].

1.4.5 Effect of neutraceuticals on bacteriocin production

Prebiotic sorbitol has a positive influence on bacteriocin produced by *Pediococcus acidilactici*, isolated from vacuum-packed fermented meat product [29]. Vamanu and Vamanu (2010) [30] studied the effect of prebiotics viz. inulin from chicory and dahlia, raffinose and lactulose on the synthesis of bacteriocins from *Lactobacillus paracasei* CMGB16 strain. The inhibition of *E.coli* as determined by agar well diffusion method confirmed bacteriocin production.

1.4.6 Effect of prebiotics in poultry, fishery, pig, cattle feed

Use of eco-friendly alternatives of antibiotics in poultry, fishery, pig, cattle feed is increasing day by day. *Salmonella enteritidis* and *Salmonella typhimurium* infections are world-wide problem as major etiological agent of food borne illness in humans and commercial poultry. Phagocytic activity and *Salmonella*-killing activity of macrophages in chickens are achieved by the treatment with prebiotic b-1, 4mannobiose (MNB) [31]. Enhanced growth performance and digestive enzyme activities of the allogynogenetic crucian carp, Carassius auratus gibelio are observed with prebiotic xylooligosaccharides [32].

The probiotic-prebiotic conjugate has different applications in salmonid aquaculture, resulting in elevated health status, improved disease resistance, growth performance,
body composition, reduced malformations, improved gut morphology and microbial balance [33].

It is observed that with the increase in supplementation level of inulin, enzymes and white blood cell count increased significantly in fishery and poultry [34]. Holstein heifer calves, when given prebiotic supplement, showed more *Lactobacilli* in their faeces [35]. Prebiotic potentiality of brown *Ascophyllum nodosum* algae in weaned piglet feed material improves the gut flora, an important index of the gastrointestinal health status [36]. Oral administration of prebiotic chicory root to pigs increased the mRNA and protein expression of Cytochrome P450 1A2 and 2A, the enzymes having crucial role in metabolism of drugs and endogenous compounds [37]. The prebiotic, Celmanax TM formulated with a non-living yeast cell walls or MOS, acts as an antiadhesive for Shiga toxin producing *E. coli* O157:H7 colonization and a mycotoxin in vitro. The Celmanax TM also improves milk production and feed conversion efficiencies in dairy cattle [38].

### 1.4.7 Prebiotic production from food industry wastes

Production of prebiotics from various food industry-wastes is schematically shown in Figure 1.5

### 1.5 Commercial prebiotics

Nowadays consumers are becoming more interested in foods with health promoting features as they gain more awareness of the links between food and health. Among the functional products, probiotic products are showing promising trends worldwide [40]. They have beneficial effects on human health and well-being through production of...
short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of pathogenic bacteria, reduction of colon cancer risk, improving the immune system and lowering blood cholesterol levels [41].

In commercial products, various probiotic lactobacilli and bifidobacteria show a decline in their viability during storage of product [42]. Some factors are responsible for the viability of these organisms e.g. the strains used, culture conditions, antagonism among cultures present, storage time and temperature, initial counts, hydrogen peroxide and oxygen contents in the medium, and the amount of organic acids in the product [42, 43]. Probiotic bacteria grow slowly in milk due to their lack of proteolytic activity, thus requiring the incorporation of essential growth factors such as peptides [44]. Co-culturing with proteolytic yogurt bacteria i.e. *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST) also increases the growth and viability of probiotics and helps to reduce fermentation time [45].

The products containing a combination of prebiotics and probiotics are recognized as synbiotics. The most studied prebiotics are fructan-based inulins and oligofructoses. Prebiotics such as oligofructose and inulin provide some beneficial effects, namely improved bioavailability of minerals such as calcium, magnesium and iron; increased activity of beneficial live active cultures; and inhibition of harmful bacteria in the digestive tract. Inulin helps in digestion of high-protein diets, and decreases fat absorption [46]. Other effects of inulin include providing a sense of fullness, lowering serum cholesterol and decreasing incidence of colon cancer [47].

### 1.6 Biosynthesized nano prebiotics

Over the past decades, nanoparticles have been the subject of enormous interest due to their potential applications in industrial, biomedical and electronic applications. Presently there is a growing need to develop environmentally benign nanoparticle synthesis process, which does not use toxic chemicals in the synthesis protocols.

Among the different bio-synthesis process, use of plants is of particular interest in synthesis of prebiotic metal nanoparticles because of its advantages over other environmentally benign biological process. The principal advantage of using plants is the elimination of the elaborative process of maintaining cell cultures [48, 49 and 50].
Plant mediated synthesis of nanoparticles is gaining importance due to its simplicity and eco friendliness. Synthesis of nanoparticles using microorganisms makes nanoparticles more biocompatible [51]. Furthermore, overall material and energy consumption in biological methods are extremely lower, offering a low cost green alternative.

Now-a-days silver nanoparticles have greater attentions due to their antimicrobial properties [52]. Controlling the size, shape and structure of metal nanoparticles is technologically important because of strong correlation between these parameters and optical, electrical and catalytic properties. Silver nanoparticles produced by biosynthesis has large number of applications such as in non linear optics, spectrally selective coating for solar energy absorption, bio-labelling, intercalation materials for electrical batteries, as optical receptors, catalyst in chemical reactions and as antibacterial capacities [53].

Plants like Aloe barbadensis, Azadirachta indica, Camellia sinensis, Capsicum annum, Cinnamomum camphora, Cinnamom zeylanicum, Carica papaya, Coriandrum sativum, Jatropha curcas Cymbopogon flexuosus, Diopyros kaki, Emblica officinalis, Gliricidia sepium, natural rubber, Pelargonium graveolens, Psidium guajava, Tamarindus indica, Hibiscus rosa sinensis, Ocimum sanctum, Musa paradisiaca, Ocimum sanctum, Cochlospermum gossypium and Rosa rugosa have been reported to form gold, palladium and platinum metal nanoparticles [54].

1.7 Probiotic bacteria-genus Pediococcus

Pediococcus is a genus of Gram-positive lactic acid bacteria, belonging to the family of Lactobacillacea. The genus Pediococcus consists of the following species: P. acidilactici, P. pentosaceus, P. damnosus, P. parvulus, P. inopinatus, P. halophilus, P. dextrinicus, and P. urinaeequi [55]. They often referred to as P. cerevisiae, which is currently designated as P. damnosus. Strains formerly known as P. cerevisiae now are distributed among P. damnosus, P. acidilactici and P. pentosaceus [55-59]. Pediococcus spp. cells are spherical and arranged in tetrads, however, pairs are not uncommon in liquid cultures. They divide along two planes of symmetry, as do the other lactic acid cocci genera of Tetragenococcus and Aerococcus. They are
facultative anaerobes, non motile and non sporulating [60]. The genus is paraphyletic and *P. dextrinicus* is only distantly related to the other species. *Pediococci* are cultivated successfully in rich media [61]. Various species and strains differ in tolerance to oxygen, pH, temperature and NaCl [59]. They are homo fermentative, although carbohydrate assimilation patterns and fermentation may differ among species and strains. Glucose is always fermented to racemic DL-lactate by the Embden-Meyerhof-Parnas (EMP) pathway [62]. Metabolic end products vary according to the conditions provided.

Among the known *Pediococcus* strains, *P. acidilactici*, *P. pentosaceus*, and *P. halophilus* are mostly associated with food fermentations. *P. acidilactici* and *P. pentosaceus*, take place in food fermentations either as indigenous microflora or in starters and both have been used in natural and controlled fermentations of vegetables and sausages [63 and 64]. Since pediococci typically are unable to ferment lactose [56], their applications in milk fermentations are restricted. There are however, a number of reports [65-67] which indicate that non starter and adjunct *Pediococcus* spp. impart desirable attributes to cheese suggesting that they may be good dairy starters if they possess the ability to utilize the particular sugar [68]. The ability of *P. acidilactici* and *P. pentosaceus* to produce antimicrobial peptides has attracted the interest for the use of either the cultures or their products as protective cultures or biopreservatives, respectively, in many foods. Both *P. acidilactici* and *P. pentosaceus* have also been used in silage fermentation, in the fermentation of dough and fruit juices, while several commercial probiotic feeds containing either species are currently available in the market. *P. halophilus* (also known as *Tetragenococcus halophila*) plays an important role in the fermentation of miso and soy sauce [63] and it is known as the soy *Pediococcus* [69, 70]. The soy sauce mash or moromi has been the source for isolation of *P. halophilus* strains. The soy pediococci, salt-tolerant and homo-fermentative lactic acid bacteria, metabolize citrate and malate during lactic acid fermentation of soy sauce brewing. Citrate and malate are the acids that lactic acid bacteria most often encounter in their food environments and in the manufacture of fermented dairy products, it is desirable that they are able to metabolize the two acids into especially, acetoin and diacetyl, both regarded as being favourable in
enriching the flavours of cheese, butter and other products. Several strains however, have been described as non-citrate-metabolizing strains [71].

*P. damnosus* occurs in wine and cider and is found in brewery environments. It occurs as primary contaminant in pitching yeast and it is among the most prevalent spoilage microorganisms [72]. *P. damnosus* is sensitive to bacteriocins nisin of *Lactococcus lactis* [73] and pediocin AcH of *P. acidilactici* H [59], while it has been reported to produce a pediocin [74].

The various *Pediococcus* species exhibit different physiological characteristics which can be used for identification purposes. However, there are strains within defined species that are different from the type strains. Various genetic tools have been used to discriminate between strains in the genus *Pediococcus*. These include the use of specific DNA target probes [75-77], ribotyping [78-80], total DNA-DNA hybridization [81], 16S rRNA gene sequencing [80, 82]. Simpson et al. [83] studied the genomic diversity within the genus *Pediococcus* by randomly amplified polymorphic DNA PCR and pulse-field gel electrophoresis. Specific DNA fragments within the NotI and AscI macrorestriction patterns for each of the 33 examined strains from six species were observed that allowed 27 of the 33 strains to be assigned to their proposed species. Following digestion with AscI, all *P. parvulus* strains were characterized by two DNA fragments (220 kb and 700–800 kb).

While the synbiotic effect of inulin as prebiotic on different probiotic organisms including *Pediococcus acidilactici* is investigated by many researchers, there is a wide variation of composition of growth media. Determination of the cell growth dynamics is also inadvertently ignored in majority of these reported investigations. However, systematic study and to some extent economic viability of the cell growth process of any probiotic greatly depends on selecting media formulation and its micro environment. Therefore, a thorough study on the selection of media composition including its micro environment along with the knowledge of cell dynamics will lead to a commercially viable technology for the production and application of synbiotics neutraceuticals containing probiotic-prebiotic mixture and for the production of bio molecules like Lactic acid and pediocin.
References


[27] Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with


