2.1. INTRODUCTION
The term food means a substance consumed to provide nutritional support to the body and a healthy food contains carbohydrates, protein, fat, fibres, vitamins and minerals. As long as human beings have walked across the globe, they have searched for food and the means to produce it and efforts to improve food resources are likely to continue as long as life exists. There has been an increasing interest in probiotic foods in recent years for health promoting effects beyond traditional nutrients. In this review the importance and applications of probiotics and nutraceuticals is discussed in detail. The major emphasis is to discuss the history of probiotics and their health benefits, mode of action of probiotics, lactic acid bacteria as probiotics, characteristics of microorganisms to be used as probiotic, development of dairy based probiotic products using free and microencapsulated cultures and supplementation of probiotic dairy based products with fruit nutraceuticals.

2.2. HISTORY AND CONCEPT OF PROBIOTICS
The term probiotic comes from Greek word probios which means for life (Gismondo et al., 1999; Guarner et al., 2005). In 1907 Metchnikoff proposed that the acid producing bacteria in fermented milk products could prevent fouling in the large intestine and if consumed regularly it leads to a long and healthier life. According to him Bulgarians are hypothesized as healthy and long lived because of the consumption of fermented milk products containing rod shaped bacteria (Lactobacillus spp.). These bacteria affect the gut microflora positively and decrease the microbial toxic activity (Gismondo et al., 1999; Chuayana et al., 2003).

In early 1930’s, in Japan, Minoru Shirota developed Yakult, a probiotic yogurt like product by fermenting a mixture of skimmed milk with a special strain of Lactobacillus casei Shirota (Gilliland, 1990). The term probiotic was first used in 1965 by Lilly and Stillwell to describe substances which stimulate the growth of other microorganisms and after this the term was used in different context according to its mechanism and the effects on human health.

Probiotics are defined as substances and organisms which contribute to intestinal microbial balance (Parker, 1974). Fuller (1989) redefined the word as a live microbial feed supplement which beneficially affects the host animal by
improving its intestinal balance. Havenaar and Huisin't Veld (1992) defined probiotic as viable mixed culture of bacteria which when applied to animal or human beneficially affects the host by improving the properties of the indigenous flora. According to definitions probiotics are live microorganisms which when consumed in adequate amounts confer a health effect on the host (Guarner and Schaafsma, 1998) and a preparation or a product containing viable microorganisms in sufficient numbers alter the microflora in a compartment of the host and exert beneficial health effects in his host (Schrezenmeir and de Vrese, 2001). FAO/WHO (2001) has adopted the definition of probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Two groups of bacteria generally used in probiotic products and associated with microbial balance of intestine are *Lactobacillus* and *Bifidobacteria* (Cebeci and Gurakan, 2003).

There are a large number of probiotics currently used and available in dairy fermented foods, especially in yogurts. Some selected strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and *Saccharomyces* have been promoted in food products for their reputed health benefits (Dimer and Gibson, 1998; Sanders, 1998; Fuller, 1991; Ouwehand *et al.*, 1999; Puupponen-Pimia *et al.*, 2002).

More than 400 bacterial species exist in human digestive tract and is an enormously complex ecosystem that includes both facultative anaerobic and anaerobic microorganisms (Naidu *et al.*, 1999). The numbers of genera is nearly steady, because each group is having its own growth niche (Fooks *et al.*, 1999). The composition of the gut microflora is constant and can be affected by some factors viz. age, diet, environment, stress and medication (Fuller, 2007). The oral consumption of probiotic microorganisms produces a protective effect on the gut flora. A lot of studies suggest that probiotics have beneficial effects on microbial disorders of the gut, but it is very difficult to prove the clinical validity of such products. However, the probiotic preparations used for traveller’s diarrhoea, antibiotic associated diarrhoea and acute diarrhoea have shown positive therapeutic effects (Gismondo *et al.*, 1999; Cakir, 2003; Ouwehand *et al.*, 1999). Different types of microorganisms used as probiotics are shown in Table 2.1.
Table 2.1. Different types of microorganisms recognised as probiotics

| **Lactobacillus spp.** | L. acidophilus, L. rhamnosus  
| | L. gasseri, L. casei  
| | L. reuteri, L. delbrueckii subsp. bulgaricus  
| | L. plantarum, L. salivarius  
| | L. johnsonii, L. gallinarum  
| | L. plantarum, L. fermentum  
| | L. helveticus, L. brevis  
| | L. murinus, L. crispatus  
| | L. amylovorus  
| **Bifidobacterium spp.** | B. infantis, B. longum  
| | B. lactis, B. adolescens  
| | B. bifidum, B. animalis  
| | B. breve, B. thermophilum  
| | B. pseudolongum  
| **Other microorganisms** | B. subtilis, B. licheniformis  
| | Enterococcus faecalis, Enterococcus faecium  
| | Lactococcus lactis subsp. lactis  
| | Lactococcus lactis subsp. cremoris  
| | Leuconostoc mesenteroides  
| | Leuconostoc lactis, Leuconostoc citreum  
| | Pediococcus acidilactici, Pediococcus pentosaceus  
| | Propionibacterium freudenreichii  
| | Streptococcus salivarius subsp. thermophilus  
| | Streptococcus infantarius  
| | Saccharomyces bouardii, Saccharomyces lactis  
| | Saccharomyces carlsbergensis  
| | Kluyveromyces marxianus  

(Fuller, 1991; Klein et al., 1998; Puupponen-Pimia et al., 2002; Kumura et al., 2004; Soccol et al., 2010; Mozzi et al., 2015)

Lactic acid bacteria have widespread use in fermented food production (Azcarate-Peril, 2001) and are considered as generally recognized as safe (GRAS) organisms and can be safely used for medical and veterinary applications (Fuller, 1989).

### 2.3. LACTIC ACID BACTERIA

Lactic acid bacteria (LAB) consist of a number of bacterial genera viz. *Enterococcus, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc,*
Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragnococcus, Vagococcus, Weissella, Aerococcus and Carnobacterium within the phylum firmicutes (Jay, 2000; Ercolini et al., 2001; Holzapfel et al., 2001; Rattanachaikunsopon and Phumkhachorn, 2010). Members of LAB share the property of being Gram-positive bacteria (Fooks et al., 1999) that ferment carbohydrates into energy and lactic acid (Jay, 2000). LAB were first isolated from milk (Sandine et al., 1972; Metchnikoff, 1908; Carr et al., 2002) and have since been found in fermented foods e.g. meat, milk products, vegetables, beverages and bakery products (Jay, 2000; Lonvaud-Funel, 2001; O’Sullivan et al., 2002; Liu, 2003).

Lactic acid bacteria constitute a diverse group of organisms providing considerable benefits to mankind, some as natural inhabitants of the intestinal tract and others as fermentative organisms in food industry and impart flavor, texture and preservative properties to the foods (Sanders, 2001). Beside this, some species e.g. Lactobacillus spp. are administered to humans as live microbial supplements, which positively influence health by improving the composition of intestinal microbiota and several strains possesses probiotic properties (Shah, 2007).

The bacteriocins produced by the LAB can be used as food additives and for instance, nisin a bacteriocin from LAB is commercially prepared in a partially purified form (Schnurer and Magnusson, 2005) and a marketed preparation of nisin with the pediocin PA-1 (AcH) producer is also available (Rodriguez et al., 2000). Several studies have shown that LAB starter cultures or co-cultures are able to produce their bacteriocins in food matrices, and display their inhibitory activity towards sensitive food spoilage or pathogenic bacteria. The latter trait has mainly been documented for fermented sausage, fermented vegetables, olives, and dairy products (De Vuyst et al., 2004).

Lactobacilli are characterized as Gram-positive facultative bacteria, nonspore forming, catalase negative and non-flagellated rods (Hammes and Vogel, 1995). These microorganisms are strictly fermentative, so they have the ability to ferment lactose and other monosaccharides to lactic acid predominantly by the homofermenters and to lactic acid with carbon dioxide and ethanol by the heterofermenters. Examples of probiotics Lactobacillus spp. include L. acidophilus, L. delbrueckii subsp. bulgaricus, L. casei, L. fermentum, L. plantarum and L. reuteri (Klein et al., 1998).
2.4. PROBIOTIC CHARACTERISTICS

Most of the probiotics are related to the *Lactobacillus* and *Bifidobacterium* genera (Reid *et al.*, 2003; Guarner *et al.*, 2005). However, to use a strain as probiotics it should survive the upper digestive tract and should be capable of surviving and growing in the intestine (acid and bile resistant), safe for human consumption, produce antimicrobial substances (bacteriocins) to reduce pathogenic microorganisms, able to adhere to the epithelial cells and should also be able to withstand the stress of commercial manufacturing, processing and distribution (Morelli, 2000; Guarner *et al.*, 2005).

2.4.1. Bile and acid tolerance

Probiotic strains need to be resistant to the stressful conditions of the stomach (pH 1.5-3.0) and upper intestine which contain bile (Chou and Weimer, 1999; Cakir, 2003). To show probiotic potential, microorganisms should reach to the lower intestinal tract and maintain themselves as acid and bile resistant microorganisms. Bile acids are synthesized in the liver from cholesterol and sent to the gall bladder and finally secreted into the duodenum in the conjugated form (500-700 ml/day). In the large intestine this acid undergoes some chemical modifications (deconjugation, dehydroxylation, dehydrogenation and deglucuronidation) due to the microbial activity. Conjugated and deconjugated bile acids show antimicrobial activity especially on *E. coli*, *Klebsiella* spp., and *Enterococcus* spp. in *in vitro* conditions and deconjugated acid forms are more effective on Gram positive bacteria (Dunne *et al.*, 2001; Cakir, 2003).

Bile salt resistance is one of the criteria for any strain to be used as probiotic culture as bile in intestine affects the viability of Lactic acid bacteria. Gilliland (1979) and Patel *et al.* (2004) reported that lactobacilli isolated from animal intestines showed high tolerance to bile salt than the isolates from milk products. Gilliland *et al.* (1984) observed a great variability among *L. acidophilus* strains isolated from calf intestinal contents to grow *in vitro* in the presence of bile salts. Garriga *et al.* (1998) reported the selected LAB strains with resistant to 4 % bile salts. The resistance variability among lactobacilli is due to the presence of bile salt hydrolase (BSH), an enzyme that reduces toxic effects by conjugating bile (Du Toit *et al.*, 1998). Although the bile concentration of the human
gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3 % w/v with staying time of 4 h (Prasad et al., 1998).

The LAB survival in low pH of stomach is very important for bearing the initial acid stress. Idoui et al. (2007) showed resistance of L. plantarum BJ0021 to pH 3. Idoui (2012) showed that in comparison to the Lactobacillus isolated from the gastrointestinal tracts of the human, the strains of L. fermentum, L. gasseri and L.delbrueckii ssp. bulgaricus were having better acid tolerance. Mishra and Sharma (2014) reported that the ability of a isolate to grow at a pH 4 shows that the isolate is quite stable in acidic conditions and this property is attributed to its ability to ferment lactose and produce lactic acid. Jacobsen et al. (1999) also reported acid tolerance of Lactobacillus and reported that 4 out of 8 cultures were capable of survival at pH 2.5 for 2 h.

2.4.2. Antimicrobial activity

One of the major properties of an isolate to be used as probiotic is its ability to inhibit microbial pathogens. Antimicrobial effects of lactic acid bacteria are due to production of substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins (Cakir, 2003; Quwehand and Vesterlund, 2004). Garriga et al. (1998) reported inhibition of one or more enteric indicator strains (E. coli, Salmonella Enteritidis) by Lactobacillus strains. L. paracasei subsp. paracasei and L. acidophilus strains isolated from infant faeces had weak antibacterial activity on E.coli and Yersinia enterocolitica (Xanthopoulous et al., 2000). Daeschel (1989) reported that the antimicrobial effect of lactic acid bacteria is due to the production of lactic acid and reduction of pH, acetic acid, diacetyl, fatty acids, aldehydes and other compounds during growth. The antimicrobial action is due to the potential of LAB to produce lactic acid, bacteriocines and peptides having inhibitory properties (Strus et al., 2001).

There are reports on antimicrobial activity of Lactobacillus against Shigella flexneri, Shigella dysenteriae, Vibrio cholerae and Salmonella typhi, Staphylococcus epidermidis, S. flexneri, S. dysenteriae, V. cholerae, S. typhi, and Pseudomonas spp. (Diba et al., 2013; Mishra and Sharma, 2014). This indicates the presence of bioactive molecules in the Lactobacillus culture and has been
attributed to production of organic acids or hydrogen peroxide or bacteriocins or any other inhibitory substances by the bacterial cell (Sieladie et al., 2011).

Some milk products were used to isolate potential probiotic bacteria with antimicrobial activities and *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens* and *Candida albicans* were used as indicator microorganisms in this study (Chuayana et al., 2003).

### 2.4.3. Antibiotic susceptibility

The susceptibility to antibiotics is a common feature of all probiotic bacteria. Several studies have confirmed that *Lactobacillus* spp. are generally susceptible to a large number of antibiotics viz. chloramphenicol, erythromycin, clindamycin and tetracycline which inhibits the synthesis of proteins (Coppola et al., 2005; D’Aimmo et al., 2007). In general, glycopeptide, aminoglycoside and sulfamethoxazole resistance has been described in lactic acid bacteria, and in all cases it has been associated with their natural and intrinsic resistance due to membrane impermeability, probably complemented by potential efflux mechanism resistance (Elkins and Mullis, 2004). Intrinsic resistance is not horizontally transferable and poses no risk in non-pathogenic bacteria (Mathur and Singh, 2005).

The determination of antibiotic susceptibility of a bacterial strain is an important prerequisite to consider it safe for human and animal consumption. Probiotics should be resistant to certain antibiotics so as to survive in the gastrointestinal tract. However, this opinion is controversial and probiotics containing resistance traits may have negative consequences on the human health. The presence of antibiotic-resistance genes in many LAB and the transfer of plasmids and conjugative transposons to and from LAB have been reported in *Lactobacillus* species (Yoshiyuki et al., 2009). The resistance to chloramphenicol, kanamycin, erythromycin, gentamycin, streptomycin and tetracycline have shown to be plasmid borne in some probiotic cultures (Moubareck et al., 2005; Hummel et al., 2007). Thus, there exists risk of potential transfer of antibiotic resistance from probiotic strains to other bacteria residing commensally in intestine or to the pathogens. Hummel et al. (2007) demonstrated that resistant genes might be present in probiotic strains but are silent and further the genetic basis and
associated resistance mechanisms of probiotics towards some antibiotics are still unknown.

2.4.4. Adherence to epithelial cells
The ability of microbe to adhere to intestinal mucosa is an important selection criterion for its probiotic use. Indeed, the ability of probiotic to adhere to the intestinal epithelium is regarded as a prerequisite to colonize the human gastrointestinal tract (GIT) for beneficial exclusion of enteropathogenic bacteria (Collado et al., 2005; Marco et al., 2006). Idoui (2012) reported the adherence specificity of \textit{L. fermentum} HG3 and \textit{L. gasseri} HG8, adherence specificity to chicken intestinal epithelium. Jamaly et al. (2011) reported that all the tested probiotic strains were able to adhere to the rat ileum epithelial cells and \textit{Lactobacillus} isolates with more than 15 cells adhered per epithelial cell were considered positive. In general the adhesion capability depends upon the strain origins as well as their surface properties (de Ambrosini et al., 1998).

2.5. IDENTIFICATIONS OF THE NEW STRAINS
Identification of a new strain of bacteria is traditionally done by phenotypic characterization by elucidation of its morphological and biochemical properties (Woo et al., 2002). Further, the comparison of the gene sequences of bacterial species revealed that the 16S rDNA gene is highly conserved within a species and among different species and hence can be used as new “gold standard” for determination of the species of bacteria (Olsen, 1992, 1993). The 16S rDNA gene sequencing was firstly proposed by Woese (1987) and 16S rDNA gene represents variable regions and the general structure is highly conserved therefore the probes used for its identification have the broadest specificity ranging from universal to species specific. The 16S rDNA gene sequencing helps to study phylogenetic relationships between microorganisms and identify them more accurately (Charteris et al., 1997; Cakir, 2003). Tannock (1999) identified \textit{Lactobacillus} and \textit{Bifidobacteria} using 16S rDNA sequencing. Similarly, identification of \textit{Lactobacillus salivarius} was done using 16S rDNA gene sequencing by Woo et al. (2002). Identification of \textit{Lactobacillus plantarum}, \textit{Lactobacillus pentosus} and \textit{L. fermentum} was done using 16S rDNA sequencing method as reported by Tajabadi et al. (2011).
2.6. MECHANISM OF ACTION OF PROBIOTICS
Probiotic bacteria beneficially affect the individual by improving the properties of the indigenous microflora and its microintestinal balance (Saarela et al., 2000; Betoret et al., 2003) and compete with disease causing bacteria for villi attachment sites and nutrients absorption (Chen and Yao, 2002). Probiotic bacterial cultures encourage growth of beneficial microorganisms and crowd out potentially harmful bacteria thereby, reinforcing the body’s natural defense 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, inactivation of pathogen, modification of dietary proteins by intestinal microflora, modification of bacterial enzyme activity, influencing gut mucosal permeability, and regulation of the immune system (Betoret et al., 2003; Krasaekoopt et al., 2003).

The probiotic effect of microorganisms is accredited to its 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 and Yao, 2002). Lactic acid bacteria inhibit growth of pathogenic microorganisms by producing short chain fatty acids viz., acetic, propionic, butyric, lactic and formic acids which reduces intestinal pH (Krasaekoopt et al., 2003). A general mechanism of action of probiotic microorganisms is discussed in the following sections:

2.6.1. Inhibition of pathogenic bacteria
The gastrointestinal environment contains a wide range of contents including harmless beneficial dietary components, microbial flora and harmful pathogenic bacteria. Probiotics fights against pathogenic bacteria through various ways viz. blocking pathogenic bacteria effects by producing bactericidal substances, competing with pathogens and toxins for adherence to the intestinal epithelium (Corcionivoschi et al., 2010). The mode of action of probiotics is based on their ability to bind to intestinal epithelial tissue and remove microbial pathogens (Louvard et al., 1992). Inhibitory action of probiotics includes the production of lactic acid which decreases the pH, interaction with the toxins produced by
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pathogens, production of hydrogen peroxide and bacteriocin synthesis (Brashears et al., 1998). Production of certain metabolites e.g. lactic acid lowers the pH and further inhibits the development of pathogenic bacteria. But there are also reports where pathogen inhibition (Shigella) is not due to low pH but by some antibacterial substances secreted by the lactic acid bacteria (Apella et al., 1992). Probiotic bacteria can modify the composition of the local microenvironment in two ways. First, probiotic bacteria mediate antimicrobial effects that directly inhibit pathogenic bacteria; secondly, they enhance the richness and diversity of more beneficial components of the gut microbiota. Probiotics have been shown to suppress pathogen growth through the release of a variety of antimicrobial factors viz., defensins, bacteriocins, hydrogen peroxide, nitric oxide, and short chain fatty acids, such as lactic acid, which reduce the pH of the lumen (Penner et al., 2005).

2.6.2. Immunomodulation

Probiotics can influence the immune system by production of metabolites, cell-wall components (peptidoglycans, lipopolysaccharides) and DNA. Probiotic products are recognized by sensitivity of host cells for these components with their recognition receptors (Vanderpool et al., 2008). Probiotics shows immunomodulatory properties through the inhibition of bacterial translocation, stimulation of phagocytes/macrophages and natural killer cells, increased proliferation in organs of the immune system (Peyer’s patches spleen) and increased release of cytokines (de Verse and Schrezenmeir, 2008).

2.6.3. Enhancement of barrier function

Several probiotic bacteria preserve epithelial barrier function and prevent and repair mucosal damage triggered by food antigens, drugs, enteric pathogens, and proinflammatory cytokines (Rosenfeldt et al., 2004). These protective effects are mediated by a number of mechanisms which include the induction of mucin secretion, maintenance or enhancement of cytoskeletal and tight junction protein phosphorylation, restoration of chloride secretion, and augmentation of transepithelial resistance (Chichlowski et al., 2007; Ng et al., 2009). Several clinical conditions like enteric infections, celiac diseases and infection bowl disease causes disruption of epithelial barrier (Ng et al., 2009). Enhancement of epithelial barrier is an important mechanism by which probiotic bacteria can
prevent the host from these disease conditions (Ng et al., 2009). The enhancement of epithelial barrier by the probiotics is accomplished by two major mechanisms i.e. production of thick blanket of mucus by entertocytes and second secretion by goblet cells which are dispersed throughout the luminal epithelium of the intestine (Chichlowski et al., 2007).

2.7. HEALTH BENEFITS OF PROBIOTICS
There are a lot of reports on the health benefits of fermented foods and probiotics. In most of studies researchers did not use sufficient test subjects or the microorganisms were not identified and a number of reported effects have only been partially established, whereas, some reports can be regarded as well established and clinically well documented for specific strains. The health-related effects of probiotics are reduction of allergic symptoms, enhanced mineral metabolism, improved bone density and stability, reduction of *Helicobacter pylori* infection, suppression of pathogenic microorganisms (antimicrobial effect), prevention of urogenital infections, improving immune system, prevention of colon cancer, lowering of blood pressure, cholesterol and reduced inflammation etc. (Dugas et al., 1999; Scherezenmeir and De Vrese 2001; Dunne et al., 2001). Some probiotics with health benefits are shown in Table 2.2.

2.7.1. Lactose intolerance
The major health benefit of probiotics is the enhancement of lactose digestion and minimization of intolerance symptoms. Probiotics survives in stomach and are deposited in the small intestine to support lactose hydrolysis by its own enzymes (de Verse and Schrezenmeir, 2008). Some probiotic cultures in yogurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) are able to produce lactase, and when consumed with dairy products can improve lactose digestion (Kolars et al., 1984). Probiotic bacteria contain high levels of lactase, which is released within intestinal lumen after the lysis by the bile secretions and lactase acts on the ingested lactose thereby decreasing maldigestion symptoms (Tuohy et al., 2003). Some probiotic strains have shown beneficial effects on lactose digestion and symptoms in lactase deficient persons (Hiele, 1988; Lin, 1993; Rabot, 2010).
### Table 2.2. Some probiotics with beneficial effects on human health

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Effect on human health</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>B. bifidum</strong></td>
<td>Necrotizing enterocolitis</td>
<td>Deshpande <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><strong>B. longum B. infantis</strong></td>
<td>Pouchitis</td>
<td>Gionchetti <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><strong>Bacillus subtilis,</strong></td>
<td>Reduce stress, control pathogenic bacteria and their virulence, stimulate the immune</td>
<td>Decamp <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><strong>Bacillus licheniformis</strong></td>
<td>system, improve gut flora, substitute antibiotics, improve growth</td>
<td>Perez <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><strong>Bifidobacterium animalis</strong></td>
<td>Normalizes the intestinal motility of obstipated subjects</td>
<td>Vasiljevic and Shah, 2008</td>
</tr>
<tr>
<td></td>
<td>Reduces the risk of acute diarrhoea</td>
<td>de Verse and Schrezenmeir, 2008</td>
</tr>
<tr>
<td><strong>Bifidobacterium bifidum</strong></td>
<td>Compete successfully for space and nutrients against pathogenic bacteria</td>
<td>Pariyaporn <em>et al.</em>, 2003</td>
</tr>
<tr>
<td></td>
<td>Increase antibody response and reduces the incidence of diarrhoea</td>
<td>Gill and Prasad, 2008</td>
</tr>
<tr>
<td><strong>Bifidobacterium breve</strong></td>
<td>Activates the humoral immune system by augmenting anti rotavirus IgA production or</td>
<td>Kaur <em>et al.</em>, 2002</td>
</tr>
<tr>
<td></td>
<td>anti-influenza virus</td>
<td></td>
</tr>
<tr>
<td><strong>Bifidobacterium longum</strong></td>
<td>Produce antimicrobial substances against pathogens such as <em>Campylobacter</em></td>
<td>Gionchetti <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><strong>Enterococcus faecalis,</strong></td>
<td>Bacteriocin-like inhibitory substances, antimicrobial activity against Gram +ve bacteria, colonize transiently</td>
<td>Strompfova <em>et al.</em>, 2004</td>
</tr>
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<td><strong>E. faecium</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Lactobacillus acidophilus</strong></td>
<td>Significant decrease of diarrhoea in patients receiving pelvic irradiation</td>
<td>Gorbach <em>et al.</em>, 1987</td>
</tr>
<tr>
<td></td>
<td>Decreased polyps, adenomas and colon cancer in experimental animals</td>
<td>Marteau <em>et al.</em>, 2001</td>
</tr>
<tr>
<td></td>
<td>Prevented urogenital infection with subsequent exposure to three pathogens</td>
<td>Sanders <em>et al.</em>, 2001</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa</em></td>
<td></td>
</tr>
<tr>
<td><strong>Lactobacillus casei</strong></td>
<td>Reduces the severity and duration of diarrhoea.</td>
<td>Kaur <em>et al.</em>, 2002</td>
</tr>
<tr>
<td><strong>Shirota</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Lactobacillus casei</strong></td>
<td>Reduces nasal symptoms medication, strong human health efficacy for management of lactose malabsorption, rotaviral diarrhoea, and antibiotic associated diarrhoea.</td>
<td>Shah, 2007</td>
</tr>
<tr>
<td>Shirota</td>
<td></td>
<td>de Verse and Schrezenmeir, 2008</td>
</tr>
<tr>
<td><strong>Lactobacillus delbrueckii</strong></td>
<td>Pouchitis</td>
<td>Gionchetti <em>et al.</em>, 2005</td>
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Table 2.2

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<thead>
<tr>
<th><strong>Lactobacillus plantarum</strong></th>
<th>Produce antimicrobial substances against pathogens such as <em>Campylobacter</em></th>
<th>Santini <em>et al.</em>, 2010</th>
</tr>
</thead>
</table>
| **Lactobacillus reuteri**   | Mass gain, antimicrobial activity against *E. coli* and *S. aureus*     | Shornikova *et al.*, 1997  
|                             |                                                                         | Marteau *et al.*, 2001  
|                             |                                                                         | Pancheniak *et al.*, 2005 |
| **Lactobacillus rhamnosus** | Decreased duration of erythromycin induced diarrhoea                    | Tomioka *et al.*, 1992  
|                             | subjects, infant diarrhoea                                              | Allen *et al.*, 2004  
|                             |                                                                         | Tong *et al.*, 2007  
|                             |                                                                         | Prado *et al.*, 2008 |
| **Pediococcus acidilactici**| Improves performance, reduces serum cholesterol                        | Alkhal *et al.*, 2010 |
| **Pediococcus acidilactici**| Stimulate animal growth, reduce coliform counts by the production of    | Perez *et al.*, 2007 |
|                             | antimicrobial metabolites                                               |                      |
| **S. salivarius subsp.**    | Necrotizing enterocolitis                                               | Kim and Gilliland, 1983 |
| thermophilus, B.            |                                                                         | Kolars *et al.*, 1984  
| bifidum                     |                                                                         | Savaiano *et al.*, 1984 |
|                             |                                                                         | Deshpande *et al.*, 2007 |
| **Saccharomyces cerevesiae**| Prevent traveller’s diarrhoea                                           | Pant *et al.*, 1996  
| **boulardii**               | Reduces the risk and duration of antibiotic associated diarrhoea        | Raza *et al.*, 1995  
|                             |                                                                         | Shornikova *et al.*, 1997 |
|                             |                                                                         | Guandalini *et al.*, 2000 |
|                             |                                                                         | Armuzzi *et al.*, 2001  
|                             |                                                                         | Cremonini *et al.*, 2002 |
|                             |                                                                         | Allen *et al.*, 2003  
|                             |                                                                         | Fric, 2007            |

(Parvez *et al.*, 2006; Sanders *et al.*, 2008; Carlos *et al.*, 2010; Gonzalez *et al.*, 2011; Arora *et al.*, 2013)
2.7.2. Antimicrobial activity

Probiotic microorganisms produce antimicrobial peptides and bacteriocins which induce increased permeability of the cytoplasmic membrane which leads to the release of small cytoplasmic particles, depolarization of the membrane potential and eventually leads to the cell death (Simova et al., 2009). The lowering of pH by lactic acid has bactericidal and bacteriostatic effects. The production of H$_2$O$_2$ by the probiotic culture may result in an antimicrobial effect due to the oxidation of sulfhydryl groups causing denaturing of a number of enzymes and peroxidation of membrane lipids thus increasing the membrane permeability (Ammor et al., 2006). The role of antimicrobial prophylactic components produced by probiotic strains in opposition to enteric infections is crucial and is well reported (Kos, 2008; Frece, 2009; Saulnier, 2009).

2.7.3. Reduction of symptoms of food allergy

Probiotics are helpful in reducing the symptoms of food allergy as they enhance gut defence by non-immunologic and immunologic mode of action. Non-immunologic action is carried out by normalizing the gut microflora and decreasing membrane permeability whereas, immunologic action involves the enhancement of immunological defense system of host by boosting the IgA action. This leads to enhanced degradation of food antigens and reduced food allergy (Kirjavainen et al., 2001; Castellazzi et al., 2013).

2.7.4. Inflammatory bowel syndrome prevention

Inflammatory bowel disorder is characterized by inflammation, ulceration resulting in abdominal pain, diarrhoea and gastrointestinal bleeding (Vasiljevic and Shah, 2008; Agrawal et al., 2009). Probiotics enhances barrier function to prevent the invasion of tight junctions or modulation of gut microbiota composition and this activity might bring relief in inflammatory bowel syndrome or maintain remission from symptoms (Santosa et al., 2006).

2.7.5. Reduction in risk factor for colon cancer

Some strains of LAB (Lactobacillus delbrueckii subsp. bulgaricus) have shown anti-mutagenic effects because of their ability to bind to heterocyclic amines which are carcinogenic (Wollowski et al., 2001). Some human trials suggest some LAB
to be anti-carcinogenic due to ability to decrease the activity of β-glucuronidase enzyme, which can produce cancer producing substances in the digestive system. During population studies it was observed that the incidence of colon cancer in people consuming dairy products is low as compared to the others (Brady et al., 2000).

2.7.6. Prevention of diarrhoea

The incidence of antibiotic-associated diarrhoea (AAD) ranges in between 5 % and 30 % (Szymanski, 2006) in a population. Antibiotic therapy causes mild and severe outbreaks of diarrhoea as normal microflora is disturbed during the antibiotic therapy. The change of microflora may also encourage the resistant strains e.g. Clostridium difficile which is mainly involved in antibiotic associated diarrhoea. There is significant reduction of AAD when antibiotic therapy is associated with probiotic treatment (Cremonini, 2002; D’Souza, 2002; Szajewska, 2006). Saccharomyces boulardii appears to be the most effective microorganism against AAD (Lewis, 1988; Arvola, 1999). Lactobacillus acidophilus LB1, Bifidobacterium lactis and Lactobacillus reuteri also have significant effect on the reduction of diarrhoea (Salminen et al., 1998).

2.7.7. Cholesterol reduction

Probiotic microorganisms exhibits cholesterol reduction effects and there are two hypotheses to explain this mechanism. According to first mechanism, bacteria may bind or incorporate cholesterol directly into the cell membrane and the other mechanism exhibits that bile salt hydrolysis enzymes deconjugate the bile salts resulting in increased cholesterol breakdown (Jones, 2004). A study showed that Lactobacillus reuteri CRL 1098 decreased total cholesterol by 38 % when it is given to mice for 7 days at a rate of $10^4$ cells/day and caused a 40 % reduction in triglycerides and 20 % increase in ratio of high density lipoprotein to low density lipoprotein without translocation of native microflora into the spleen and liver (Kaur et al., 2002). Kieling et al. (2002) performed a randomized, crossover, and placebo-controlled design trial consisting of 29 women to test the hypocholesterolemic effect of yogurt containing L. acidophilus and B. longum. This cross over study was performed for 21 weeks using 300 g/day yogurt and resulted in significant increase of high density lipoprotein (HDL). Some studies
reported probiotics to be beneficial in lowering hypertension by decreasing blood cholesterol level and increasing resistance of low density lipoprotein (LDL) to oxidation (Kieling et al., 2002; Sindhu and Khetarpaul, 2003; Goel et al., 2006).

2.7.8. Reduction in upper respiratory tract infection
Upper respiratory tract (URT) infection is generally represented by laryngitis, tracheal inflammation and common cold and analgesics and antipyretics are most frequently used to treat headache, pain and fever (Hao et al., 2011). Fermented food containing probiotics are found to reduce the duration of URT infections, risk and incidence of respiratory tract infection in the children of 3-5 years age (Ouwehand et al., 2008). Probiotics reduce the severity of respiratory tract infections, by enhancement in IgA-secreting cells in the bronchial mucosa (Perdigon, 1999).

2.8. MICROENCAPSULATION OF MICROBIAL CULTURES
Microencapsulation is a physicochemical or mechanical process used for entrapment of microorganisms by coating them with proper hydrocolloids to protect microbial cells from the surrounding environment in a way that results in appropriate cell release in the intestinal medium (Sultana et al., 2000; Krasaekoopt et al., 2003; Picot and Lacroix, 2003; Chen et al., 2007).

2.8.1. Methods of microencapsulation
A brief description of different methods used for microencapsulation of microbial cultures is given below:

2.8.1.1. Spray drying
In this method a solution containing probiotic cells is taken and dissolved in polymer matrix. The polymer matrices are generally gum arabic and starch because they tend to form spherical microparticles during the drying process (Chen et al., 2007; Kailasapathy, 2009). This technique is cost effective but one disadvantage of this technique is use of high temperature which can effect microorganism survival.
2.8.1.2. Emulsification

Emulsification is a chemical technique for microencapsulation of probiotic microorganisms and use hydrocolloids (alginate, carrageenan etc.) as encapsulating materials. The principle of this technique is based on the relationship between the discontinuous and the continuous phases. For encapsulation in an emulsion, an emulsifier and a surfactant are needed. A solidifying agent (calcium chloride) is then added to the emulsion (Chen et al., 2007; Kailasapathy, 2009; De Vos et al., 2010). The emulsion technique is easy to operate and gives a high survival rate of the bacteria (Chen et al., 2007).

2.8.1.3. Extrusion method

Extrusion is a physical technique to encapsulate probiotic microorganisms by using hydrocolloids (alginate and carrageenan) as encapsulating materials. In this method the probiotic microorganisms are dropped in a solution through a nozzle at high pressure. Extrusion is a simple and cheap method that uses a gentle operation which causes no damage to probiotic microorganisms and gives high probiotic viability (Krasaekoopt et al., 2003).

2.8.2. Microencapsulation of probiotic cultures

Microencapsulation technique can be used in food industry for controlling oxidative reaction, masking flavors, colors and odors, providing sustained and controlled release of microorganisms and extending shelf life of products (Champagne and Kailasapathy, 2008; Zuidam and Shimon, 2009). To confer health benefit, probiotics bacteria must arrive in intestines alive in sufficient numbers at $10^6$-$10^7$ CFU/g products (Fuller, 1989; Kailasapathy and Chin, 2000; Krasaekoopt et al., 2003).

However, significant reduction of the number of viable cells occurs in a food product inevitably due to the processing conditions of food carriers, especially low pH and low temperature as well as the transportation and storage conditions in fermented and frozen products (Iwana et al., 1993; Shah et al., 1995). A lot of reports suggested that viability and survivability of probiotics in gastrointestinal tract and food products (dairy fermented products) decreases due to their exposure to environmental factors such as organic acids, hydrogen ions and antibacterial components (Shah et al., 1995; Dave and Shah, 1997; Kailasapathy...
and Rybka, 1997; Vinderola et al., 2000; Sultana et al., 2000). The various factors affecting the stability of probiotics are shown in Figure 2.1.

![Factors influencing the stability of probiotics during food production](Lacroix and Yildirim, 2007)

Viability loss of probiotics in food products and acidic conditions of gastrointestinal tract has always encouraged researchers to find new methods to improve the viability of microorganisms. To overcome this problem microencapsulation is an efficient technique to improve the viability of probiotic microorganisms by protecting the cells against an adverse environment (Mortazavian and Sohrabvandi, 2007; Champagne and Kailasapathy, 2008; Zuidam and Shimoni, 2009). There are two common techniques applied for the microencapsulation of probiotic bacteria: the extrusion (droplet) method (Audet et al., 1989) and emulsion (two-phase) system method (Sheu et al., 1993; Desmond et al., 2002). Out of these two methods the extrusion method is simpler and cheaper (KrasaeKoopt et al., 2003). Different steps involved in microencapsulation are described in Fig. 2.2.
After their consumption probiotic cells are exposed to various hydrolytic enzymes, acidic conditions of the stomach, and bile salts in the gastrointestinal tract (Sultana et al., 2000). Various approaches viz. addition of different growth promoters (Dave and Shah, 1997; Gomes and Malcata, 1999), manipulation of fermentation and storage conditions of the food carriers (Sheu et al., 1993; Shah, 2000), careful selection of the culture organisms according to their interrelationships and acid-bile resistance (Shah, 2000) and microencapsulation technology to reduce the cell loss (O’Riordan et al., 2001; Chandramouli et al., 2004) have been investigated to improve the survival rates of viable cells arriving in the intestine.

Microencapsulation of probiotics in hydrocolloid beads has been found to improve the viability of probiotics in food products and intestinal tract by segregating them from adverse environmental conditions (Kearney et al., 1990; Krasaekoopt et al., 2003).
2.8.2.1. Microencapsulation in alginate

Alginate belongs to family of natural polysaccharides, which is widely used in industry and medicine for many applications, such as immobilization and cell entrapment systems (Poncelet et al., 1992; Strand, 2000). Alginates are produced by the marine brown algae *Macrocystis, Laminaria, Ascophyllum, Fucus, Eklonia* and *Pelvetia* (Guiseley, 1989). Alginates are a family of linear binary copolymers of 1, 4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) of varying composition and sequence. Mannuronic and guluronic acid residues are arranged in homopolimeric regions, blocks of guluronic and mannuronic residues and regions of alternating guluronic and mannuronic residues (Grasdalen, 1981, 1983).

Fig. 2.3. Structure of alginate used for microencapsulation (Atkins, 1970; 1971)

Alginate is the most commonly used hydrocolloid for the entrapment of cells due to its simple and low cost gelling mechanism, excellent biocompatibility and reversibility of the immobilization (Bordelius and Nilsson, 1980; Prevost and Divies, 1992). Different materials are used to encapsulate probiotic cells viz. alginate, k-carrageenan, starch, gelatin, chitosan, milk proteins, gallan gum and xanthan gum. Alginate is a naturally derived polysaccharide extracted from various species of algae and composed of D-mannuronic and L-guluronic acids. Alginate hydrogels are extensively used in cell encapsulation (Rowley et al., 1999) and calcium alginate is preferred for encapsulating probiotics because of its simplicity, non-toxicity, biocompatibility and low cost (Krasaekoopt et al., 2003). There are reports on microencapsulation of probiotics with alginate or other gels which improve the survival of probiotics in food products and digestive system.
2.8.2.2. Microencapsulation in carrageenan
Carrageenan is a natural carbohydrate (polysaccharide) obtained from edible red seaweeds. The name Carrageenan is originated from the *Chondrus crispus* species of seaweed known as Carrageen Moss or Irish Moss in England and Carraigin in Ireland. Carraigin has been used in Ireland since 400 AD as a gelatin and as a home remedy to cure coughs and colds (Necas and Bartosikova, 2013). It is a hydrocolloid composed of α-d-1, 3 and β-d-1, 4 galactose residues that are sulphated up to 40% of the total weight; strong negative charge over normal pH range; associated with ammonium, calcium, magnesium, potassium, or sodium salts. It is mostly used in various dietetic formulations, infant formula, toothpaste, cosmetics, skin preparations, pesticides and laxatives (Van de Velde *et al*., 2002).

![k-carrageenan](image)

**Fig. 2.4. Structure of k-carrageenan used for microencapsulation (Kadaji and Betageri, 2011)**

Carrageenan is a natural polymer which is commonly used in the food industry at 40-50°C at which the cells are added to the polymer solution. By cooling the mixture to room temperature the gelation occurs and the microparticles are stabilised by adding potassium ions (Kraskaekoopt *et al*., 2003). The encapsulation of probiotic cells in k-carrageenan beads keeps the bacteria in a viable state (Dinakar and Mistry, 1994). Kebary *et al.* (1998) reported that entrapment of *Bifidobacterium bifidum* and *B. infantis* in alginate or k-carrageenan beads significantly improved their survival in frozen ice milk over 10 weeks of storage at -20°C and the *Bifidobacterium* survived better in beads prepared from alginate than those prepared from k-carrageenan. Adhikari *et al.* (2000) reported significant difference between the viable counts of microencapsulated *B. longum* as
compared to free cells in set yogurt over a storage period of 30 days at 4.4°C. Shue et al. (1993) reported 40% more survival of lactobacilli during freezing of ice milk when they were entrapped as compared with free cells. Shah and Ravula (2000) also reported the encapsulation of probiotic bacteria in carrageenan (Khalida et al., 2000).

2.9. MILK BASED PROBIOTIC FOOD PRODUCTS

Milk is a prime source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrums, which carries the mother’s antibodies to the baby and can reduce the risk of many diseases in the baby and it also contains many other nutrients (Pehrsson et al., 2000). Milk is a key contributor in improving nutrition and food security particularly in developing countries. Fermentation was the first technique employed by humans for food preservation and it has played many important roles in human nutrition. The fermented milk products vary considerably in composition flavour and texture, depending on the nature of fermenting organisms and the type of manufacturing process used (Rasic and Kurman, 1978; Tamime and Deeth, 1980).

Milk and milk products provide an excellent carrier for probiotic organisms and most of them can readily utilize lactose as an energy source for their growth. Besides this milk protein also provides important protection to the probiotic bacteria during passage through the stomach (Charteris et al., 1998). Several probiotic LAB strains are available to the consumers in both traditional fermented foods and in supplemented form and the majority of probiotics are incorporated into dairy products viz. milk powders, yogurt, semi-hard and hard cheeses and ice cream (Desmond et al., 2002) as these products offer a suitable environment for probiotic viability and growth (Ozer et al., 2009). Some probiotic milk based products are discussed in the following sections:

2.9.1. Probiotic milk and buttermilk (lassi)

Probiotics can be found in a wide variety of commercial dairy products including sour, fresh milk, yogurt and cheese etc. Dairy products play important role in delivering probiotic bacteria to human as these products provide a suitable environment for probiotic bacteria and support their growth and viability (Gardiner et al., 1999; Phillips et al., 2006). Probiotic dairy drinks were the first
commercialized products that are still consumed in larger quantities than other probiotic beverages. Today, a wide range of dairy beverages containing probiotic bacteria is available for consumers in the market and includes acidophilus milk, sweet acidophilus milk, bifidus milk, acidophilus buttermilk, yakult and procult drink etc. (Ozer et al., 2009). Raileanu and Rotaru (2009) developed a probiotic drink with milk and fruit juice addition which combines the milk components with fruit juices rich in amino acids, vitamins with health benefits of probiotic culture. Junaid et al. (2013) developed a probiotic acidophilus milk with different flavours and high acceptability.

The growing concern for health and nutrition among the consumers has increased the market potential for functional foods. Fermented dairy products especially lassi containing probiotics and herbs can serve the needs of health conscious consumers. Hussain et al. (2014) have developed an Aloe barbadensis Miller supplemented probiotic lassi (APL) and reported the decrease in pH during storage at 5°C. They also reported decrease in probiotic cells count from 8.4 log CFU/ml on initial day to 8.0 log CFU/ml on 12th day.

2.9.2. Probiotic yogurt

Yogurt is a dairy product produced by the fermentation of milk. The bacteria used to prepare yogurt are known as yogurt cultures and include L. delbrueckii subsp. bulgaricus and Streptococcus thermophilus. These bacteria produce lactic acid by fermentation of lactose which acts on milk protein to give yogurt its texture. Yogurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12 (da Mota et al., 2000; Wall et al., 2006). Lactose intolerant individuals can sometimes tolerate yogurt better than any other dairy product as lactose in milk is converted to glucose and galactose and is also partially fermented to lactic acid by the bacterial culture (Kolars et al., 1984). Yogurt can be prepared in various forms viz. set, stirred, drinking, frozen, concentrated and flavoured form and the flavoured yogurt is commercially more preferred as compared to the plain yogurts. Flavoured yogurt is known for its nutritional value and health benefits and common additives are fruit or berries, usually as a puree or as whole fruit in syrup.

Yogurt has been widely used as probiotic food but most commercial yogurt products have low viable cells at the consumption time (Shah, 2000; Donkor et al., 2006). Viability of probiotics in yogurt depends on the availability of nutrients,
growth, concentration of solutes, inoculation level, incubation temperature, fermentation time and storage temperature and strain used. The main factor for loss of viability of probiotic microorganisms have been attributed to the decrease in the pH of the medium and accumulation of organic acids as a result of growth and fermentation (Donkor et al., 2006). The presence of probiotic was found to affect some characteristics of yogurt viz. acidity, texture, flavor, and appearance (Aryana and McGrew, 2007). However, encapsulation in plain alginate beads and in chitosan coated alginate could improve the viability and stability of probiotics in yogurt (Kailasapathy, 2007; Olivera et al., 2007; Paseephon and Sherkat, 2009).

Yogurt with orange and strawberry juice was developed by Hossain et al. (2012). Cakmakci et al. (2012) developed a probiotic banana yogurt which retained probiotic values of $10^6$ log CFU/g up to 7 days of storage at 4°C. In another study microencapsulation enhanced the survival of probiotic cultures as compared to free cells and sensory analysis of the yogurts showed that addition of probiotic capsules did not alter the appearance, colour, acidity, flavour and after taste attributes of the yogurts (Kailasapathy, 2002).

2.9.3. Other dairy products

Other dairy products including chocolate mousse, frozen fermented dairy desserts, sour cream, and ice cream can be good vehicles for probiotic products. Quark was tested with two probiotic cultures to improve its nutrition characteristics and the results showed the high utilization of fat, protein and lactose in skimmed milk (Duric et al., 2004). Chocolate mousse with probiotic and prebiotic ingredients were developed by supplementing with *L. paracasei* subsp. *paracasei* LBC 82 and showed good results (Aragon-Alegro et al., 2007). Ice-creams are the other food products with high potential for use as probiotic vehicles (Cruz et al., 2009). Some commercial probiotic products available in market are listed in Table 2.3.
Table 2.3. Some commercial probiotic food products available in the market

<table>
<thead>
<tr>
<th>Strain</th>
<th>Product Name</th>
<th>Product Type</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus casei</em> Shirota</td>
<td>Yakult</td>
<td>Drink</td>
<td>Yakult, Japan</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> CL1285</td>
<td>Bio K+</td>
<td>Capsule</td>
<td>Bio K+ International, Canada</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em> Lbc80r</td>
<td>LC1</td>
<td>Drink</td>
<td>Nestle, India</td>
</tr>
<tr>
<td><em>Lactobacillus johnsonii</em> La1 (Lj1)</td>
<td>FemDophilus</td>
<td>Capsule</td>
<td>Chr. Hansen, France</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> GR-1</td>
<td>A’Biotica</td>
<td>Capsule</td>
<td>Institut Rosell, Canada</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> RC14</td>
<td>A’Biotica</td>
<td>Capsule</td>
<td>Institut Rosell, Canada</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em> DN 173 010</td>
<td>Activia</td>
<td>Yogurt</td>
<td>Danone, Europe</td>
</tr>
<tr>
<td><em>Bifidobacterium breve</em> Yakult</td>
<td>Bifiene</td>
<td>Drink</td>
<td>Yakult, Japan</td>
</tr>
<tr>
<td><em>Lactobacillus GG</em> (“LGG”)</td>
<td>Tablets</td>
<td>Tablets</td>
<td>Biogagia, Sweden</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em> DN-144 001 <em>Lactobacillus immunitas</em></td>
<td>DanActiva fermented milk</td>
<td>Drink</td>
<td>Danone, Europe</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> 299V</td>
<td>Juice</td>
<td>Juice</td>
<td>Probi AB; NextFoods, America</td>
</tr>
</tbody>
</table>

(Guarner et al., 2008; Carlos et al., 2010; Bhadoria et al., 2011; Gitika and Tiwari, 2013)

2.10. FOOD BORNE PATHOGENS IN DAIRY PRODUCTS

Milk and milk based products can be a source of food borne pathogens. The major reason of presence of food borne pathogens in milk is contact with contaminated sources in the dairy farm and infected animals. More than 200 known diseases are communicated through food by bacteria, fungi, viruses, and parasites. According to public health and food safety experts, every year millions of illnesses in the United States and throughout the world can be traced to food borne pathogens. Most
prevalent food borne pathogens includes *Campylobacter jejuni, Escherichia coli, Salmonella Typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* (Oliver et al., 2005; Gwida et al., 2013). The pathogen linked to the milk products depends upon the environment of the farm and the natural raw milk collected from mammary glands of animal is usually less contaminated. *E. coli* is normally present in intestine of animals and humans but if present in food may lead to gastrointestinal disturbance (Soomro et al., 2002). Milk is also an excellent media for the growth and multiplication of *Staphylococcus aureus* and this organism is responsible for approximately 30-40% of all mastitis cases (Asperger, 2003). Unhygienic milk processing methods, contaminated equipments, *S. aureus* infected mammary gland and contaminated hands of milkers during handling and processing of raw milk are considered the main cause of milk contamination (Scherrer et al., 2004). *S. aureus* is considered the most important cause of food borne illnesses all over the world (Asperger, 2003). Van Kessel *et al.* (2004) reported the prevalence of *Salmonella, L. monocytogenes*, and fecal coliforms in bulk tank milk in the United States.

### 2.11. FOOD NUTRACEUTICALS

The term nutraceutical was coined by Stephen DeFelice (1989) in context to food or a part of food that provides medical or health benefits, including the prevention and treatment of disease. Nutraceutical products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods and herbal products (DeFelice, 1995; Pandey *et al.*, 2010). Foods and nutrients play a vital role in the normal functioning of the body and help to maintain the health of the individual and reduce the risk of various diseases. A majority of the nutraceuticals claim to possess multiple therapeutic benefits although lack substantial evidence for the benefits as well as unwanted effects (Rajasekaran, 2008) and are generally grouped in two types, as established nutraceuticals and potential nutraceuticals. Established nutraceuticals have a sufficient clinical data to demonstrate the promised or labeled benefit, however a potential nutraceutical holds a promise of a particular health or medical benefit and is considered nutraceutical only after there is sufficient clinical data to demonstrate such a benefit (DeFelice, 1995).
Phytochemicals and antioxidants are two specific types of nutraceuticals and research has proved that foods with phytochemicals may help to provide protection from diseases viz. cancer, diabetes, heart disease, and hypertension. Nutraceuticals may be traditional and non-traditional nutraceuticals.

Traditional nutraceuticals are simply natural ingredients with no changes to the food. Food contains several natural components that deliver benefits beyond nutrition, such as lycopene in tomatoes and saponins in soy. They are grouped on the basis of chemical constituents, probiotic microorganisms and nutraceutical enzymes.

Chemical constituents constitute three components viz. nutrients, herbals and phytochemicals. The most commonly known nutrients are antioxidants, vitamins and essential minerals. Antioxidants are substances, which retard or prevent deterioration, damage or destruction caused by oxidation. Antioxidants form an integral part of the nutraceutical market (Devi and Rehman, 2002; Sagar et al., 2004). Various nutraceuticals are present in medicinal herbs as key components (Ravi, 2007). A great attention has been given to discover the link between dietary nutrients and disease prevention. Large number of herbs, which are in use since ancient time, has been shown to play a crucial role in the prevention of disease. In addition to the macro and micro nutrients for normal metabolism, a plant based diet contains numerous non-nutritive phyto-constituents which may play an important role in health enhancement (Dulloo et al., 1999; Bell and Goodrick, 2002).

Phytochemicals are classified on the basis of chemical properties. Carotenoids found in various fruits, vegetables and egg yolk, are anti-carcinogenic, boost natural killer immune cells and protect cornea against UV light. Legumes, grains, palm oil contain non-carotenoids, which remove cholesterol and are anti-carcinogenic. Flavonoid, polyphenolics are found in berries, fruits, vegetables, and legumes, which are potent antioxidants, whereas phytoestrogens prevent breast cancer, prostate cancer and control diabetes (Sirtori and Galli, 2002).

Probiotics are friendly bacteria that promote healthy digestion and absorption of some nutrients. They act to crowd out pathogens, such as yeasts, other bacteria and viruses that may otherwise cause disease and develop a mutually advantageous symbiosis with the human gastrointestinal tract (Holzapfel et al., 2001). Enzymes are an essential part of life, without which our vital metabolic
activities would cease to function. Individuals who are suffering from medical conditions e.g. hypoglycaemia, blood sugar disorders, digestive problems and obesity, can recover the symptoms by enzyme supplements to their diet (Singh et al., 2012). Non-traditional nutraceuticals includes the artificial foods prepared with the help of biotechnology. They are arranged into two groups viz., fortified nutraceuticals and recombinant nutraceuticals. Fortified nutraceuticals includes fortified food from agricultural breeding, added nutrients and/or ingredients e.g. orange juice fortified with calcium, cereals with added vitamins and minerals and flour with added folic acid. Other example is milk fortified with cholecalciferol used in vitamin D deficiency (Casey et al., 2010). Recombinant nutraceuticals are energy-providing foods, such as bread, alcohol, fermented starch, yogurt, cheese, vinegar, and other products produced with the help of biotechnological processes (Singh et al., 2012).

2.12. ANTIOXIDANTS

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules (Sies, 1996). Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent and oxidation reactions can produce free radicals, which starts a chain reactions that damages cells. Antioxidants on the other hand terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions by oxidizing themselves and acting as reducing agents due to presence of thiols, ascorbic acid and polyphenols (Sies, 1997). Free radicals are short lived, with half life in milli, micro or nanoseconds and are related with many human diseases and also with ageing (Harman, 1956; Halliwell and Gutteridge, 1997). The process of oxidation plays an important role in defence against infections and body damage (Herbert et al., 2001). Oxygen is essential for survival and about 5% of its inhaled part is converted to reactive oxygen species (ROS) viz., O₂, H₂O₂ and OH plays an important role as cellular secondary messengers, regulators or signaling molecules with nitric oxide (Kimura et al., 2005; Liu et al., 2010). Whereas oxygen is a highly reactive atom and is capable of becoming part of potentially damaging molecules called as free radicals which are capable of attacking the healthy cells and causing them to lose their structure and function. When production of free radicals exceeds the body's antioxidant defense system, it results in oxidative stress (OS) and leads to increase in oxidant generation, decrease in antioxidant protection and failure in
repair of oxidative damage (Prakash et al., 2009). Antioxidants are capable of stabilizing and deactivating free radicals before they attack cells. Antioxidants, neutralizes free radicals or their actions and act at different stages. The first level of antioxidant activity is prevention, which stop the formation of reactive oxygen species and the second level includes scavenging of free radicals. However, at repair and reconstitution level, mainly repair enzymes are involved (Sies, 1996; Cadenas and Packer, 1996; Dizdaroglu, 1993). Different types of antioxidants are shown in Table 2.4.

Table 2.4. Various types of antioxidants

<table>
<thead>
<tr>
<th>Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endogenous antioxidants</strong></td>
</tr>
<tr>
<td>Enzymatic antioxidants</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD): enzyme detoxifying superoxide radical</td>
</tr>
<tr>
<td>Glutathione reductase enzyme involved in the regeneration of glutathione</td>
</tr>
<tr>
<td>Iron dependent catalase</td>
</tr>
<tr>
<td>Selenium dependent glutathione peroxidise</td>
</tr>
<tr>
<td>Thioredoxin reductase enzyme involved in the protection against protein oxidation</td>
</tr>
<tr>
<td>Non-enzymatic antioxidants</td>
</tr>
<tr>
<td>Glutathione (GSH), uric acid, lipoic acid, NADPH, coenzyme Q, albumin, bilirubin</td>
</tr>
<tr>
<td><strong>Dietary antioxidants</strong></td>
</tr>
<tr>
<td>Vitamins</td>
</tr>
<tr>
<td>vitamin C, vitamin E, vitamin B</td>
</tr>
<tr>
<td>Antioxidants minerals</td>
</tr>
<tr>
<td>Carbohydrates, selenium, copper, iron, zinc, manganese</td>
</tr>
<tr>
<td>Phenolic acids</td>
</tr>
<tr>
<td>chlorogenic acids, gallic acid, caffeic acid, etc</td>
</tr>
<tr>
<td>Phytochemicals</td>
</tr>
<tr>
<td>Flavonoids e.g vegetable fruits, seed leaves, flowers.</td>
</tr>
<tr>
<td>Beta carotene e.g carrot</td>
</tr>
<tr>
<td>Trace elements</td>
</tr>
<tr>
<td>zinc, selenium</td>
</tr>
<tr>
<td>Carotenoids</td>
</tr>
<tr>
<td>β carotene, lycopene, lutein, zeaxanthin</td>
</tr>
<tr>
<td><strong>Metal binding proteins</strong></td>
</tr>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>Metallothionein</td>
</tr>
<tr>
<td>Ferritin</td>
</tr>
<tr>
<td>Myoglobin</td>
</tr>
<tr>
<td>Transferrin</td>
</tr>
</tbody>
</table>

(Percival et al., 1998; Hamid et al., 2010; Bouayed and Bohn, 2012)
2.12.1. Oxidative stress versus antioxidants

The relation between free radicals and disease can be explained by the concept of oxidative stress (Sies, 1986). Oxidative stress is a disturbance in the prooxidant–antioxidant balance in favour of the prooxidant which leads to potential damage (Sies, 1993). Oxidative stress can damage Na+/K+ ATPase (Andreoli, 1993; Matalon, 2003). Oxidative stress damaging DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of cancer, pulmonary disorders and heart disease.

It has been estimated that dietary increase in antioxidant vitamins may reduce the risk of heart disease by 20-30% (Gaziano and Hennikens, 1996). Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk (Block et al., 1992). Oxidative stress is responsible for many pathological conditions viz. cancer, neurological disorders (Toshniwal, 1992; Lyras, 1997; Sayre, 2001; Jenner, 2003), atherosclerosis, hypertension, ischemia/perfusion (Kukreja, 1992; Kerr, 1999; Dhalla, 2000; Kasparova, 2005).

Antioxidants are substances which are present in low amount and significantly delays or prevents the oxidation of oxidizable substrate (Kohen, 2002). Antioxidants are very effective against oxidative stress because they can donate their own electrons to ROS and reduce the adverse effect (Kojo, 2004). The antioxidants exert their protective effect by decreasing oxidative damage to DNA by decreasing abnormal increases in cell division.

Studies have reported that reduction in cancer risk is associated with a diet high in Vitamin C, vitamin E, and beta carotene supplementation (Bendich, 1995; Hatch et al., 1995). Some evidence suggests glutathione and N-acetyl cysteine (precursor to glutathione) is helpful in protecting against pulmonary damage (Bland, 1995). Sulfur containing phytochemicals, such as the allyl sulfides found in the allium family (garlic, onions, and leeks) and isothiocyanates and sulforaphane in cabbage, broccoli, and cauliflower have been shown to inhibit various steps in tumor development in animal and in vitro studies (Milner, 1994).
2.13. WILD FRUITS AS SOURCE OF NUTRACEUTICALS AND ANTIOXIDANTS

Wild fruits are generally rich in nutraceutical and antioxidant properties and a large number of fruits grows wild in nature and are rich in nutrition and other phytochemical constituents. Himachal Pradesh (H.P.) is known as the fruit bowl of India and is one of the major producers of fruits and vegetables and various fruit species occurs wild in H.P. Among different wild fruits, jamun, wild apricot, wild raspberries, damsun plum etc. occupy an important place and are highly rich in antioxidants and nutrients (Wang et al., 1996; Schmitzer et al., 2011; Belapurkar and Goel, 2014). These fruits are still underutilized in the state and as a result of this a lot of natural produce goes as waste. The Geographical location of Himachal Pradesh is 30' 22' 40" North to 33' 12' 40" North latitude and its longitudinal extent is 75' 45' 55" East to 79' 04' 20" East. According to India state of forest report (2009) the recorded forest area of Himachal Pradesh is 37,033 km². Some wild fruits rich in nutraceutical and antioxidant properties found wild in H.P. are discussed in the following sections:

2.13.1. Syzygium cumini

Common Name: Jamun, Jambul, Black Plum

Fig. 2.5 Fruits of Syzygium cumini (Jamun) found wild in Himachal Pradesh

Jamun (Syzygium cumini) fruit is an important member of the family Myrtaceae. It is a tropical evergreen plant with its origin in India. Jamun fruit seeds and pulp have been reported to serve various purposes in diabetic patients, such as lowering blood glucose levels and delaying diabetic complications including neuropathy and
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cataracts (Helmstadter, 2008). Jamun is a good source of iron and used as an effective medicine against diabetes, heart and liver trouble (Patil et al., 2007). The bark of Jamun contains tannins and carbohydrates, accounting for its long term use as an astringent to combat ailments like dysentery (Namasivayam et al., 2008). A glycoside jamboline in the seed is considered to have antidiabetic properties (Ratsimamanga et al., 1973). The antioxidant activity of Himalayan Syzygium cumini was reported by Belapurkar and Goel (2014).

2.13.2. *Prunus domestica*

**Common Name:** Plum, Prune, Damsun Plum

![Plum](image)

**Fig. 2.6. Fruits of Prunus domestica (Plum) found wild in Himachal Pradesh**

Plum (*Prunus domestica*) belongs to family Rosaceae and is known for its nutritional values and therapeuticals properties. It contains a high amount of natural phytochemicals e.g. phenolic acids, flavonoids and anthocyanins which constitute a large amount of valuable components of dietary and medicinal value (Dorota, 2008; Kristl, 2011). Health benefits of plum ranges from reduction of food poisoning (Lee et al., 2003), nitrite scavenging inhibition (Ahn et al., 2007) and higher antioxidant potential (4.4 times higher) as compared to apples (Wang et al., 1996). In addition, it acts as a scavenger against oxygen derived free radicals e.g. hydroxyl radicals (Murcia et al., 2001).
2.13.3. *Prunus armeniaca*

**Common Name:** Apricot

![Image of Apricot Fruits](image1)

**Fig. 2.7. Fruits of *Prunus armeniaca* (Apricot) found wild in Himachal Pradesh**

Apricot (*Prunus armeniaca*) belongs to the family Rosaceae and grows as a hard tree bearing stone fruit. It is cultivated mostly in hot temperate regions. This plant is used as a traditional medicine for treatment of fever, cold, cough, asthma, bronchitis, laryngitis, constipation, anemia, haemorrhages and also for the treatment of tumor and infertility (Fazlin *et al.*, 2002). Bark of apricot is also used as astringent to treat irritated skin (Lily and Metzger, 1980). Apricots are excellent sources of vitamin-A, carotene, vitamin C and antioxidants (Ruiz *et al.*, 2005; Schmitzer *et al.*, 2011; Leccese *et al.*, 2011).

2.13.4. *Rubus ellipticus*

**Common Name:** Raspberries

![Image of Raspberry Fruits](image2)

**Fig. 2.8. Fruits of *Rubus ellipticus* (Raspberries) found wild in Himachal Pradesh**
Raspberry (*Rubus ellipticus*) belongs to the family Rosaceae and is a wild edible fruit of Himalayan range and few members of genus *Rubus* (family Rosaceae) are considered as an important constituent of the traditional diets of the indigenous population. The *Rubus* species are collectively known as brambles (including blackberries and raspberries) and are widely distributed across the globe from North Temperate Zone to the tropics (Kalkman, 2004; Hummer, 2010). Raspberry is consumed globally because of its good taste and as a rich source of natural pharmaceuticals e.g. phenolics, tannins and flavonoids (Finn, 2008; Quideau, 2009; Vasco *et al.*, 2009; Rao and Snyder, 2010; Lee *et al.*, 2012). Raspberry also exhibits a wide range of biological effects viz. antioxidant, anti-carcinogenic, anti-inflammatory and antibacterial activities due to high content of phenolics and flavonoids present in its fruits (Nohynek *et al.*, 2006; Pantelidis *et al.*, 2007; Bobinaite *et al.*, 2012). Besides this, the leaves and roots of raspberry have traditionally been used as medicinal agents for muscle spasms, morning sickness, sour throats and diarrhea. (Ryan *et al.*, 2001; Venskutonis *et al.*, 2007).

### 2.14. FRUIT SUPPLEMENTED MILK BASED PRODUCTS

Dairy products provide an excellent carrier for the probiotic organisms (Dinakar and Mistry, 1994; Desmond *et al.*, 2002). There has been increasing trends to fortify the dairy product with fruits and fruit parts (Ghadge *et al.*, 2008) to improve their nutritional value and the taste (Kailaspathy *et al.*, 2008).

Vahedi *et al.* (2008) and Hossain *et al.* (2012) have developed fruit fortified yogurts. Raileanu and Rotaru (2009) developed probiotic drink with milk and fruit juice addition which combines the milk components with fruit juices rich in amino acids, vitamins with health benefits of probiotic culture. Junaid *et al.* (2013) developed a strawberry, pineapple and mango flavored probiotic acidophilus milk using probiotic starter culture *Lactobacillus acidophilus*. Cakmakci *et al.* (2012) reported preparation and studies on probiotic banana yogurt.

### 2.15. CONCLUSION

In conclusion supplementation of probiotic culture with dairy product such as yogurt is a useful and widely accepted approach. In addition, incorporation of antioxidant rich fruit pulps from wild fruits is a value addition to the finished milk based products. The review of literature emphasized the importance of functional
food development using probiotics and nutraceuticals as food additive. A large number of wild fruits are still underutilized and as a result of this a lot of natural produce goes as waste. Therefore, these wild fruits can be used for the development of antioxidant rich fruit nutraceutical supplemented probiotic products to meet the healthy food demands of the population.