Probiotics and its health benefits

Deepak Kumar1 and Himanshu Kumar Singh1

1Research Scholar

Dairy Technology Division, ICAR-National Dairy Research Institute Karnal.

**ABSTRACT**

Probiotics is a broad term used for a very large number of microorganisms, Probiotics are ‘a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance and the required suggestive concentration of probiotic bacteria is 106 cfu/g of a product to provide health benefits**.** Incorporation of probiotics into various food products has led to the popularization of a novel category of functional foods known as 'probiotic foods.*“Let food be thy medicine and medicine be thy food”,* famous statement given by Hippocrates, embodies the fundamental philosophy embraced by health-conscious individuals. Bacteria belonging to the *Lactobacillus* and *Bifidobacterium* genera are commonly recognized as probiotics as they help in improving gut health. Some other lactic acid bacteria (LAB) encompassed within the probiotic family are *Streptococcus, Lactococcus, Enterococcus, Leuconostoc, Propionibacterium*, and *Pediococcus.* To show health benefits in host body, probiotic organisms must withstand the challenges posed during their passage through the gastric system, including resistance to bile, gastric acid and gastric enzymes, followed by effective adherence and colonization within the intestinal epithelium. Probiotics helps in managing diarrhea. Probiotics products are beneficial in fighting with several diseases such as Hepatic disease, Inflammation/ arthritis, allergies and eczema. It also boosts immune system. Several studies reported it helps in controling blood cholesterol, hyperlipidemia and colon cancer. Now-a-days there is a new concept of probiotics is becoming popular i.e Pshycobiotics these are those probiotics which help in improving mental health condition like anxiety, depression, stress.

### PROBIOTIC

Hippocrates famous statement, *“Let food be thy medicine and medicine be thy food”,*' embodies the fundamental philosophy embraced by health-conscious individuals. The term 'probiotic' finds its origin in the Greek language, derived from 'pro' (for) and 'biotic' (life), signifying its relation to promoting life. In the late 19th century, microbiologists made noteworthy observations regarding the disparities in gut flora between healthy and diseased individuals. Nobel laureate Elie Metchnikoff reported the advantageous role of selective bacteria in the gastrointestinal tract. Probiotic organisms play a pivotal role in enhancing the body's natural defense mechanisms (Getahun *et al.,* 2017). The term 'Probiotics' was initially introduced by Vergin, who was investigating the detrimental effects of antibiotics and similar agents on gut microflora. He observed that 'probiotika' exhibited favorable attributes for the gut microbiota. Fuller (1989) postulated that "probiotics" encompass live microbial feed supplements that exert advantageous effects on the host through the amelioration of its microbial equilibrium. The Food and Agriculture Organization (FAO) presents the official elucidation of 'probiotic' as living microorganisms that, when ingested in certain quantities, confer advantageous effects on the health of the consumer (FAO/WHO, 2002). For a food product to be classified as 'probiotic,' it is essential to maintain a microbial count of ≥6 log CFU (Colony Forming Units) per milliliter or gram at the end of its shelf-life to ensure the claimed health benefits (Ganguly *et al.,* 2019). Recent recommendations propose that a concentration of 8 log CFU/g of product is necessary to claim health-promoting effects on the host (ICMR-DBT, 2011). The utilization of probiotics for disease management and the preservation and enhancement of health is not a novel concept. Nonetheless, there has been a resurgence in interest regarding their application as biotherapeutic agents, primarily driven by consumer demand and media influence. Subsequent research in this domain has unveiled that regular consumption of these beneficial bacteria can impart a diverse array of health advantages.

In the era of the fast-growing population, changing lifestyle, consumers are now looking for products that provide value addition and meet health requirements beyond nutrition which can also be used as a value addition to food product of specific health benefits like reducing the risk of developing diseases, overall well-being along with ample of health benefits (Singh *et al*., 2021; Patwadi *et al*., 2021). Incorporation of probiotics into various food products has led to the popularization of a novel category of functional foods known as 'probiotic foods.' Bacteria belonging to the *Lactobacillus* and *Bifidobacterium* genera are commonly recognized as probiotics (Doron *et al.,* 2006) and are considered safe, exerting beneficial effects on gut health (Kimoto-Nira *et al.,* 2007). Among the frequently utilized *lactobacilli* strains are *L. acidophilus, L. casei, L. rhamnosus, L. reuteri, L. delbrueckii ssp. bulgaricus, L. plantarum, L. johnsonii, L. brevis, L. cellobiosus, L. fermentum,* and *L. gasseri* (Meurman and Stamatova, 2007). The employed *bifidobacteria* species include *Bifidobacterium* *breve, B. animalis subsp lactis*, formerly known as *B. lactis* (Masco *et al.,* 2004), as well as *B. longum* *biotypes infantis* and *longum* (Masco *et al.,* 2005). Additionally, other lactic acid bacteria (LAB) encompassed within the probiotic family are *Streptococcus, Lactococcus, Enterococcus, Leuconostoc, Propionibacterium*, and *Pediococcus* (Power *et al.,* 2008). Some non-related microbial species, have been identified as probiotics, comprises of non-pathogenic *Escherichia coli strain Nissle* 1917 and *Clostridium butyricum* (Harish and Varghese, 2006), yeast species (*Saccharomyces boulardii*), filamentous fungi (*Aspergillus oryzae*), and certain spore-forming *bacilli* (Gibson *et al.,* 2003). To confer health benefits to the host, probiotic organisms must successfully withstand the challenges posed during their passage through the gastric system, including resistance to gastric acid, bile, and gastric enzymes, followed by effective adherence and colonization within the intestinal epithelium (Huang and Adams, 2004).

Foods with an appropriate pH and high buffering capacity can elevate the gastric pH, thereby promoting the stability of probiotics. The viable count of probiotic bacteria present in a food product at the time of consumption is a critical factor influencing their efficacy. The survival of probiotics in food products is influenced by various factors, such as post-acidification during storage (in the case of fermented products), product pH, susceptibility to oxygen toxicity, storage temperatures, among others (Shah, 2000).

According to the World Health Organization's 2018 study, more than 300 million people worldwide suffer from depression and other mental health issues. Anxiety and depression are the two most prevalent mental health issues in the world. People who suffer from mental illness experience a decline in their health and in the quality of their lives. Recently, there has been an increase in scientific interest in the idea of focusing on the gut microbiome to determine how it positively affects our brain and behaviour. The use of psychobiotics in the treatment of anxiety and depression is thought to be very promising.

#### Probiotic organisms

Numerous strains have been recognized as probiotic microorganisms exhibiting advantageous health effects, primarily characterized by their capacity to naturally reside within the human gastrointestinal (GI) tract. Essential prerequisites for a probiotic strain include its ability to endure the challenging conditions of the gastric environment, such as stomach acidity, and to withstand the presence of bile within the small intestine, thus enabling the exertion of favorable influences on the host.

##### Lactic acid bacteria as probiotic

Lactic acid bacteria (LAB) include a diverse array of genera, comprising a substantial number of species. These bacteria are Gram-positive and typically lack catalase activity. They thrive in environments ranging from micro-aerophilic to strictly anaerobic conditions, and they do not produce spores. The most notable genera among LAB include *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Weissella, Carnobacterium, Tetragenococcus,* and *Bifidobacterium*. Various species from these genera can be found in the gastrointestinal tracts of both humans and animals, as well as in fermented foods. Notably, strains utilized as probiotics are predominantly affiliated with the genera *Lactobacillus*, *Enterococcus*, and *Bifidobacterium*.

The metabolic attributes of these LAB are primarily chemo-organotrophic, and they ferment carbohydrates into lactic acid as a major end product. Key physiological characteristics that render them suitable as probiotics include their capacity to survive in the gastrointestinal tract, resistance to low pH and/or bile, and their temperature growth ranges (Fuller, 1989). For taxonomic considerations, the most significant physiological and biochemical features are related to carbohydrate fermentation patterns, resistance to different salt concentrations, growth on various nutrient media, growth at defined temperatures, and resistance to antibiotics. These traits are essential for identifying and classifying LAB species accurately.

In various ecosystems, LAB exert beneficial effects by hindering the growth of pathogenic bacteria through the production of antimicrobial substances, such as organic acids, hydrogen peroxide, and bacteriocins (Reid *et al.,* 2001). Bacteriocins are small proteins that possess bactericidal or bacteriostatic activity (Klaenhammer *et al.,* 1988). Bacteriocin-producing *Lactobacilli*, for instance, can serve as protective cultures to enhance the microbial safety of food products (Olasupo *et al.,* 1997). Moreover, bacteriocins exhibit antagonistic effects against food spoilage flora (Barbuti *et al.,* 2008), primarily targeting microorganisms such as *Pseudomonas, Staphylococcus aureus, Salmonella sp*., and *Listeria monocytogenes* (Chinang *et al.,* 2000). Consequently, bacteriocins hold considerable potential as bio-preservatives for food (Arokiyamary and Sivakumar, 2011).

##### Genus *Bifidobacterium*

In 1899-1990, Tisser isolated Y-shaped bacteria belonging to the *Bifidobacterium* genus from infant feces, which he named *Bacillus bifidus*. Prior to this discovery, *bifidobacteria* were classified as subspecies of *Lactobacillus*, but in 1924, they were reclassified as a separate genus, *Bifidobacterium*. These microorganisms are characterized as obligate anaerobes, displaying Gram-positive properties, lacking spore formation, being catalase negative, and non-motile. Some *bifidobacteria* can tolerate oxygen as such or in the presence of carbon dioxide (Talwalkar and Kailasapathy, 2004). Morphologically, they exhibit pleomorphic shapes, appearing as short, curved rods, club-shaped rods, and bifurcated Y-shaped rods. Numerous species of *Bifidobacterium* have been identified and isolated from various sources, including humans, animals, and insects. The optimal growth conditions for these organisms include a temperature range of 37-41°C and a pH range of 6-7, with growth being completely suppressed below pH 4.5 and above pH 8.0.

##### Genus *Lactobacillus*

Genus Lactobacilli comprises non-flagellated rods or coccobacilli with characteristics of being gram-positive, catalase-negative, non-sporing, microaerophilic or anaerobic, and strictly fermentative (Salvetti *et al.,* 2012). Presently, approximately 70 species of *lactobacilli* have been characterized. Among these, the most commonly employed *lactobacilli* in dietary formulations are *L. acidophilus, L. casei, L. paracasei, L. plantarum,* and *L. rhamnosus* (Tannock, 2002). For commercial food products, particularly in yogurt production, commonly used *lactobacilli* strains include *L. acidophilus La-5* (Christian Hansen) and *L. acidophilus* LAFTI L10 (DSM Food Specialties) (Talwalkar and Kailasapathy, 2004). *Lactobacilli* possess several essential probiotic features, such as the production of digestive enzymes, synthesis of vitamins, breakdown of bile salts, enhancement of immunity, and inhibition of pro-inflammatory mediators. The regular consumption of exogenous *Lactobacillus spp*. through the diet can establish colonization in the intestine, in addition to the indigenous *Lactobacillus spp*. Figure 1.1 illustrates common lactobacillus species used as probiotics. Among the various *lactobacillus* species, *Lactobacillus acidophilus* stands out as a prevalent dietary supplement due to its inherent probiotic potential. *L. acidophilus* is a non-spore forming, gram-positive microorganism occurring as non-flagellated rods singly, in pairs, and in short chains. It predominantly thrives in a microaerophilic environment and anaerobic conditions support its growth on suitable media. The optimal growth temperature for *L. acidophilus* ranges from 35°C to 40°C, although it can tolerate temperatures as high as 45°C. The optimum pH range for growth of *Lactobacillus acidophilus* is between 5.0 and 5.6, and it exhibits a tolerance to acidity ranging from 0.3% to 1.9% titratable acidity. Liong and Shah (2005a) have demonstrated that *L. acidophilus* 33200 displays superior resistance to bile salts and acidic conditions compared to several other organisms. Additionally, this strain effectively facilitates the removal of cholesterol from fermentation broths (Liong and Shah, 2005b). Various strains of *L. acidophilus* are commonly incorporated into food products such as yogurt and buttermilk. These strains include MUH-41, O-61, L-1, 43121, and La-5 (Nighswonger *et al.,* 1996). Moreover, in commercial food products, other frequently used *L. acidophilus* strains are La-5 (Christian Hansen) and *L. acidophilus* LAFTI L10 (DSM Food Specialties) (Talwalkar and Kailasapathy, 2004).

Figure 1. 1 *Lactobacillus* species used as probiotics

Source: Sanders and Klaenhammer (2001)

#### Selection criteria for probiotic organisms

The probiotic microorganism must possess the following desirable properties:

1) High acid and bile salt tolerance to ensure its viability and survival in the gastrointestinal tract.

2) Ability to attach itself to mucosal and epithelial surfaces, thereby facilitating the provision of health benefits to the host.

3) Exhibiting antimicrobial activity against harmful organisms, thereby contributing to the maintenance of gut health.

4) Displaying bile salt hydrolase activity, which aids in the breakdown of bile salts and supports digestive processes.

5) Demonstrating specific cell surface properties that promote probiotic adhesion to host tissues, including cell surface hydrophobicity, aggregation, and adhesion mechanisms.

##### Role of probiotic in human health

##### Synthesis and bioavailability of nutrients

Probiotic microorganisms have been reported to exert beneficial effects on nutrient availability and digestibility during the fermentation and absorption processes of fermented foods. Fermented dairy products, such as yogurt, serve as a source of folic acid. Additionally, research indicates that yogurt, following fermentation, exhibits higher levels of niacin and riboflavin compared to non-fermented counterparts (Alm, 1982). The release and synthesis of diverse enzymes and vitamins into the intestinal lumen play a crucial role in digestion, leading to the amelioration of malabsorption issues (Mack *et al.,* 1999). Probiotic bacteria produce enzymes that facilitate the hydrolysis of proteins and fats, consequently enhancing the bioavailability of these essential nutrients (Fernandes *et al.,* 1987). Furthermore, the fermentation process increases the production of functional components, such as free amino acids, short-chain fatty acids (SCFA), lactic acid, propionic acid, among others. In the context of lactose intolerance, the presence of lactase, produced by milk *Lactobacilli* during fermentation, aids in the degradation of lactose, thus preventing the occurrence of symptoms associated with lactose intolerance (Martini *et al.,* 1991).

##### Management of diarrhea

Lactic acid, a metabolic product synthesized by probiotic organisms, exerts a regulatory effect on the intestinal pH, leading to the inhibition of growth for invasive pathogens, including *Salmonella spp*. and various strains of *Escherichia coli* (Mack *et al.,* 1999). Probiotics are widely recognized for their efficacy in the prevention and management of acute viral and bacterial diarrhea, as well as their ability to mitigate antibiotic-associated diarrhea. Several specific probiotic strains have been documented to provide substantial benefits in the treatment of diarrhea, notable examples being *Lactobacillus GG, Lactobacillus reuteri, Saccharomyces boulardii,* and various *Bifidobacteria spp*. (Benchimol and Mack, 2004). These strains have shown particular effectiveness in managing rotavirus-induced diarrhea in young children (Vanderhoof, 2000). Noteworthy *lactobacilli* species, such as *L. reuteri, L. casei, S. boulardii, B. bifidum, and S. thermophilus,* are established for their therapeutic potential in the treatment of diarrheal diseases among children (Gorbach, 2000; Solga and Diehl, 2003). The ingestion of probiotics represents a promising approach for addressing antibiotic-associated diarrhea and *Clostridium* difficile infections (McFarland, 2009).

The potential mechanism underlying the regulation of diarrhea involves the secretion of diverse enzymes into the intestinal lumen, promoting effective digestion and reducing malabsorption. In the field of pediatrics, probiotics have been observed to confer benefits in cases of viral diarrhea. This is achieved through an augmented production of secretory IgA, which results in decreased viral shedding, thereby enhancing the body's immune response. Additionally, probiotics exert a preventive effect against infections by competitively excluding pathogens from accessing food and binding sites on the epithelial cells of the intestines. Moreover, probiotics stimulate the production of intestinal mucin, further inhibiting the attachment of enteropathogenic organisms.

##### Alleviation of lactose intolerance

Probiotics have been demonstrated to alleviate the symptoms of lactose intolerance in individuals with lactase deficiency. This beneficial effect is attributed to the potential of certain probiotic bacteria to enhance lactase activity within the small intestine (Marteau *et al.,* 1990), thereby improving lactose digestion and alleviating intolerance symptoms (Sanders, 1993).

Milk is renowned for its high calcium content, which is vital for fulfilling the body's calcium requirements. Researchers have verified that lactose deficiency can lead to impaired calcium absorption, potentially resulting in osteoporosis. The mechanism behind calcium malabsorption may involve the avoidance of milk consumption due to complications arising from lactose intolerance (Lonkar *et al.,* 2022). Notably, higher calcium absorption occurs in an acidic environment; therefore, the presence of lactic acid leads to a decrease in gut pH, favoring calcium absorption.

In individuals afflicted with lactose intolerance, the supplementation of probiotics stimulates the enzymatic breakdown of milk lactose by probiotic species, thereby facilitating efficient lactose assimilation and calcium absorption. This mechanism effectively counteracts calcium malabsorption, consequently contributing to the fulfillment of the body's calcium demands.

##### Hepatic disease

Hepatic encephalopathy (HE) represents a severe liver disorder, yet its precise patho genesis remains still unknown. Probiotic microorganisms, including *Bifidobacteria, L. plantarum, L. casei, L. acidophilus,* have demonstrated diverse preventative and therapeutic properties, exerting their effects through various mechanisms to impede the pathogenesis of Hepatic disease (Solga, 2003).

##### Inflammation/arthritis

Probiotic microorganisms have demonstrated utility in mitigating inflammation by regulating inflammatory mediators, including cytokines. The inflammatory responses within the gastrointestinal (GI) tract can be modulated through the regulation of immune markers. The utilization of probiotics has shown potential in the modulation of rheumatoid arthritis (Marteau *et al.,* 2001).

##### Allergies/eczema

Probiotics exert a beneficial effect on allergic reactions by enhancing the function of the mucosal barrier and stimulating the immune system (MacFarlane and Cummings, 2002). Hypersensitivity reactions, often associated with inflammation in individuals with atopic eczema, and food allergies, can be effectively managed through the administration of probiotic bacteria (Isolauri, 2004). The introduction of *Lactobacillus* *rhamnosus GG* in infants at risk of eczema resulted in a significant reduction of eczema occurrence by half (Isolauri *et al.,* 2000). In the early stages of life, the colonization of probiotic organisms in the previously sterile gut of newborns appears to have long-term effects on immunoregulation and the development of atopic disorders. Probiotics play a crucial role in enhancing the endogenous barrier mechanisms of the gut, thus mitigating intestinal inflammation. Consequently, they represent a valuable therapeutic approach in the treatment of food allergies (MacFarlane and Cummings, 2002). Furthermore, probiotic organisms have shown efficacy in alleviating symptoms associated with milk protein-related food allergies. This effect is attributed to the breakdown of milk proteins into smaller peptides and amino acids, which subsequently leads to a reduction in symptoms of atopic dermatitis (Majamaa and Isolauri, 1997).

##### HIV and immune function

The utilization of probiotics represents a promising approach for improving the immune system in individuals with compromised immunity, as they exhibit the potential to modulate immune responses. Consuming a combination of probiotic organisms in synergy may further enhance the immune response (Cunningham-Rundles *et al.,* 2000). Accumulating evidence from studies conducted in vitro, in vivo, and on humans suggests that probiotics have the capacity to augment both specific and nonspecific immune responses (MacFarlane and Cummings, 2002; McNaught *et al.,* 2005). These beneficial effects are believed to be mediated through various mechanisms, including the activation of macrophages, elevation of cytokine levels, enhancement of natural killer cell activity, and increased secretion of immunoglobulins (Ouwehand *et al.,* 2002).

##### Immuno-modulatory activity

Zoumpopoulou et al. (2009) demonstrated in various experimental colitis models that probiotic microorganisms manifest inhibitory actions on inflammatory responses. These probiotic organisms hold potential for application in the treatment of chronic bacterial infections, as indicated by the same study. The stimulation of gut immunity by probiotic organisms involves crucial mechanisms, including the clonal expansion of immunoglobulins, augmentation of the immune response, and induction of either up- or down-regulation of the innate response. Galdeano et al. (2007) discussed that these processes collectively contribute to the preservation of intestinal homeostasis. Furthermore, the impact of probiotic organisms on cytokine production also plays a significant role in modulating immune responses. It should be noted that the effects of probiotic organisms on the immune system may exhibit strain-specific characteristics and have the potential for synergistic interactions.

##### Control of blood cholesterol and hyperlipidemia

Cholesterol is important biomolecule in human physiology, serving as a vital precursor to various hormones and vitamins. Additionally, it constitutes an integral part of cell membranes and the nervous system. Elevated levels of total blood cholesterol have been linked to an increased risk of coronary heart disease. The regulation of serum cholesterol levels is heavily influenced by dietary factors. The consumption of probiotics has been associated with favorable effects on blood lipid profiles. In a hypercholesterolemic murine model, the administration of a low dose of *L. reuteri* for a duration of 7 days resulted in a significant reduction of total cholesterol and triglyceride levels by 38% and 40%, respectively. Furthermore, this treatment led to a notable increase in the ratio of high-density lipoprotein (HDL) to low-density lipoprotein (LDL) by 20% (Taranto *et al.,* 1998).

##### Helicobacter pylori infections

High levels of lactic acid produced by *Lactobacillus salivarius* were shown to effectively inhibit the growth of *Helicobacter pylori* in laboratory experiments (Ai-ba *et al.,* 1998). Conversely, *Lactobacillus acidophilus, Lactobacillus casei,* and *Lactobacillus salivarius* exhibited poor colonization and growth in the stomach, leading to lower lactic acid production and an inability to inhibit the growth of *H. pylori* (Bazzoli *et al.,* 1992). Probiotic bacteria have been found to inhibit the colonization and activity of *H. pylori*, as observed through the administration of probiotics in connection with gastric or peptic ulcers. Both in vitro and in vivo studies have demonstrated the ability of *L. salivarius* to inhibit the colonization of *H. pylori* (Aiba *et al.,* 1998; MacFarlane and Cummings, 2002). Considering the potential benefits, the administration of probiotics has been suggested as a strategy to improve eradication rates, increase tolerability, and reduce the need for multiple antibiotic treatments in *H. pylori* infections (Bazzoli *et al.,* 1992). Additionally, the consumption of *Lactobacillus johnsonii* has also been shown to exhibit inhibitory effects against *H. pylori* (Marteau *et al.,* 2001).

##### Inflammatory bowel disease (IBD)

Certain strains of *lactobacilli* have demonstrated beneficial effects in alleviating symptoms associated with Inflammatory Bowel Disease (IBD), pouchitis, and ulcerative colitis (Schultz and Sartor, 2000). Probiotic bacteria have shown the potential to improve intestinal motility and alleviate constipation, likely achieved through a reduction in gut pH (Sanders and Klaenhammer, 2001). Combining probiotic therapies has been reported to be advantageous for patients with IBD (Schultz and Sartor, 2000; Shanahan, 2001). Both *S. boulardii* and *LGG* have been observed to increase secretory IgA levels in the gastrointestinal tract, thereby providing benefits in cases of IBD.

##### Irritable bowel syndrome (IBS)

Probiotic therapy has demonstrated beneficial effects in the management of inflammatory and functional bowel disorders. A clinical study investigated the impact of *L. plantarum* 299 v and DSM 9843 strains on irritable bowel syndrome (IBS) patients and found that they effectively reduced symptoms such as abdominal pain, bloating, flatulence, and constipation (MacFarlane and Cummings, 2002). Another probiotic, *Saccharomyces boulardii*, was found to be effective in reducing diarrhea in IBS, but did not show significant control over other symptoms associated with the syndrome (Marteau *et al.,* 2001). Recent research has shed light on the potential role of intestinal microbiota in reducing inflammation in both the intestines and joints. The interaction between intestinal microbes and the gastrointestinal (GI) tissues plays a crucial role in normal gut physiology, influencing processes such as motility, absorption, secretion, and intestinal permeability (Verdu and Collins, 2005).

Numerous probiotics have been investigated for their effectiveness in managing IBS, and among them, *Bifidobacterium infantis* has been established as particularly efficacious (Brenner *et al.,* 2009; Niedzielin *et al.,* 2001).

##### Colon cancer

Cancer represents the predominant cause of mortality in industrialized nations and ranks as the second leading cause of death in developing countries, as reported by the World Health Organization in 2008. According to Jemal et al. (2011), colorectal cancer (CRC) is globally the third most common cancer in men and the second most common in women. The development of colon cancer exhibits a strong correlation with dietary patterns and environmental factors. Huycke and Gaskins (2004) observed that significant colorectal neoplasms develop sporadically, originating from adenomatous polyps due to the gradual accumulation of mutations in oncogenes such as APC, K-ras, and TP53. The current management of CRC entails a combination of surgical interventions, chemotherapy, and radiation therapy, but these approaches often lead to a notable decline in the patient's quality of life. In recent studies, probiotic therapy has shown considerable promise in the context of colorectal cancer, as outlined in Table 1. 1.

The mechanisms underlying the preventive effects of CRC encompass several aspects:

1. The modulation of intestinal micro-ecology,
2. A reduction in the production of carcinogenic enzymes within the fecal matter
3. The attenuation of toxigenic and mutagenic reactions within the gut, iv
4. Safeguarding against DNA damage through the production of short-chain fatty

acids (SCFA), as reported by Park et al. in 2005.

### HUMAN GASTROINTESTINAL ECOLOGY

The majority of the microbial population inhabits the large intestine or colon of the gastrointestinal (GI) tract in humans. This colonic microflora serves various functions in digestion. It is estimated that a single individual's intestine contains approximately 100 trillion viable bacteria belonging to more than 100 bacterial species (Mitsuoka, 1982).Microbial colonization of the human intestine begins immediately after birth (Gronlund *et al.,* 2000). The GI tract is composed of a diverse microbial community that includes both facultative and obligate anaerobic microorganisms (Tannock, 2001). The primary source of bacteria that colonize a baby's intestine is the vaginal and intestinal flora of the mother. Initially, the colon of a newborn is mainly populated by *Enterobacteriaceae* and enteric *streptococci*. Subsequently, microorganisms such as *Lactobacillus, Clostridium, Bacteroides*, and *Bifidobacterium* genera appear within the first week of life. *Bifidobacteria* play a significant role in colonizing the infant's gut and start to establish within the first 4-7 days after birth, reaching concentrations of 10 billion colony-forming units per gram of feces in breastfed infants. This colonization becomes stable after the first 6-10 months of infancy. These organisms have a profound influence on stimulating the normal growth of gut microbiota and gut maturation (Saarela *et al.,* 2000). The indigenous gut flora provides protection to the host against exogenous pathogenic infections and opportunistic bacteria in the gut (Guarner, 2006; Mitsuoka, 1992). Certain inherent colonic bacteria, such as *lactobacilli* and *Bifidobacteria*, are considered beneficial to health. The colonic microflora exerts health-promoting effects through several actions, including colonization, facilitation of digestion, production of short-chain fatty acids (SCFA), anti-toxigenic activity, and stimulation of the immune system (Guarner and Malagelada, 2003).

Table 1. 1 Application of probiotics in colorectal cancer

|  |  |  |
| --- | --- | --- |
| **Diseases** | **Function** | **References** |
| Colon Cancer | Antimutagenic effects | Hirayama and  Rafter, 2000;  Marotta *et al.,* 2003 |
| Colon Cancer | Reduction in the risk of colon cancer by suppressing carcinogen-induced DNA damage | Pool-Zobel *et al.,* 1996 |
| Antimutagenic | Activation of immune cells after oral *L.*  *casei* administration to mice | Galdeano and  Perdigon (2006) |
| Colon carcinogenesis | Probiotics *Lactobacillus rhamnosus GG* and *Lactobacillus acidophilus* inhibit DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in initial colon carcinogenesis in Sprague-Dawley rats. | Verma and Shukla, 2013 |
| Colon Cancer | Probiotic yoghurt reduces the risk of large adenomas in colon human  intervention studies | Burns and Rowland, 2000 |

#### Therapeutic modulation of gut flora

The normal gut microbiota constitutes a significant and metabolically active component of the intestinal defense system (Isolauri *et al.,* 2001). This gut flora actively prevents the proliferation of potential pathogenic microorganisms within the gastrointestinal tract (Ziemer and Gibson, 1998). Consumption of probiotic foods can introduce novel microbial species into the gastrointestinal tract, leading to the proper maintenance and modulation of the gut microbiota (Kajander *et al.,* 2005). Specifically, the introduction of probiotic strains like *Bifidobacterium* and *Lactobacillus spp*. into the gastrointestinal tract holds considerable potential in promoting human health (Salminen and Gueimonde, 2004). Moreover, incorporating probiotic foods into the diet has been shown to reduce the incidence of abnormal and dysregulated gut microecology, enhance immunological barrier functions, and mitigate intestinal inflammatory responses (Saarela *et al.,* 2000).

**1.2.2 Psychobiotics: Probiotics for mental health**

Psychobiotics are defined as probiotics that upon ingestion in ample amounts, shows positive influence on mental health. Psychobiotics have an effect on the microbiota-gut-brain axis (MGBA), which regulates bidirectional communication between the gut microbiota and the central nervous system (CNS). A solid animal evidence base now supports the idea that the MGBA's modulatory effect on brain chemistry and function occurs through changes in parameters such as intestinal permeability and inflammation, or in the levels of neurotransmitters and other neurotrophic factors such as serotonin or brain-derived neurotrophic factor (BDNF). (Cryan & Dinan, 2012)

Psychobiotics are probiotics that can produce and deliver neuroactive substances like gamma-aminobutyric acid (GABA) and serotonin (the "happy" chemical) that act across the brain-gut axis. It can also lower cortisol levels (the "stress" hormone) while increasing oxytocin levels. Use of probiotics as adjunct therapy in the management of depression was first suggested by Logan and Katzman. Probiotics act as carriers for neuroactive compounds and have the potential to be psychotropic agents. As a result, it is hypothesized that a wide variety of bacteria produce and secrete neuro-chemicals. Lactobacillus and Bifidobacterium strains secrete gamma-aminobutyric acid (GABA). This is the main inhibitory neurotransmitter in the brain, regulating many physiological and psychological processes, and system dysfunction is linked to anxiety and depression. In this article we are going to discuss about psychobiotic strains, mechanisms of action and its effect on neuropsychological disorders (by using some studies conducted on animal model). (Barbosa *et al.,* 2020)

**Table: - Pyscobiotics used in different neurological conditions.**

|  |  |  |
| --- | --- | --- |
| **Neurological**  **Condition** | **Gut microbes** | **Psychobiotic Strains** |
| Anxiety | *Lactobacillus* spp | *Lactobacillus casei* spp *shirota* |
| *L. helveticus* ROO52 |
| *Bifidobacterium* spp | *B. longum* 1714 |
| *B. breve* 1205 |
| Depression | *Lactobacillus* spp | *L. acidophilus* W37 |
| *L. brevis* W63 |
| *Lactococcus* spp | *L. lactis* W19 |
| *L.lactis* W58 |
| *Bifidobacterium* spp | *B. bifidum* W23 |
| *B. lactis* W52 |
| Stress | *Lactobacillus s*pp | *L. plantarum* PS128 |
| *L. rhamnosus* |
| *Bifidobacterium* spp | *B. infantis* |
| *B. longum* R0175 |

**Conclusion**

Here we discussed about various aspects of probiotics, including their history, classification, benefits, and potential applications in human health. It highlights the role of probiotics in promoting gut health, improving digestion, managing various health conditions such as diarrhea, lactose intolerance, allergies, immune function, and even their potential use in mental health (psychobiotics). It delves into the selection criteria for probiotic organisms, which includes attributes like acid and bile tolerance, ability to adhere to mucosal surfaces, antimicrobial activity, bile salt hydrolase activity, and specific cell surface properties. It also emphasizes the importance of probiotics' immunomodulatory activity and their role in maintaining intestinal homeostasis. The chapter also mentions the significant role of the gut microbiota in human health and digestion. It explains how gut flora develops from birth and how introducing probiotics into the diet can help modulate and maintain a healthy gut microbiome. Additionally, the concept of psychobiotics is introduced, where certain probiotics are suggested to have positive effects on mental health by influencing the gut-brain axis and affecting neurotransmitter levels. Overall, we can say that probiotic bacteria are beneficial for gut health and also helps to improve immunity and boosts health.

**References**

Aiba, Y., Suzuki, N., Kabir, A. M., Takagi, A. and Koga, Y. (1998). Lactic acidmediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. *The American Journal of Gastroenterology*, ***93*(11)**, 2097-2101.

Alm, L. (1982). Effect of fermentation on lactose, glucose, and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals. *Journal of Dairy Science*, ***65*(3),** 346-352.

Arokiyamary, A., and Sivakumar, P. K. (2011). Antibacterial activity of Bacterocin producing *Lactobacillus sp.,* isolated from traditional milk products. *Current Botany,* **2**(3).

Barbosa, M. D. T., Romero, A. H., Amezquita, L. E. G. and Cayuela, T. G. 2020. Psychobiotics: mechanisms of action, evaluation methods and effectiveness in applications with food products. *Nutrients* **12:** 1-31.

Barrett, E., Ross, R. P., O’Toole, P. W., Fitzgerald, G. F. and C. Stanton. 2012. Y-Aminobutyric acid production by culturable bacteria from the human intestine. *J. Appl. Microbiol.* **113**:411–17.

Bazzoli, F., Zagari, R. M., Pozzato, P., Fossi, S., Alampi, G., Scottili, S., Simoni, P., Roda, A. and Roda, E. (1994). 13C-Urea-breath-test to quantify Helicobacter pylori colonization of gastric mucosa and association with severity of inflammation. *Gastroenterology*, **106**, A48.

Benchimol, E. I., and Mack, D. R. (2004). Probiotics in relapsing and chronic diarrhoea. *Journal of Paediatric Haematology/Oncology*. ***26*(8)**, 515-517.

[Burns, A. J.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Burns%20AJ%5BAuthor%5D&cauthor=true&cauthor_uid=11709850), and [Rowland, I. R.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Rowland%20IR%5BAuthor%5D&cauthor=true&cauthor_uid=11709850) (2000). Anti-carcinogenicity of probiotics and prebiotics. *Current Issues in Intestinal Microbiology*, 1(1), 13-24.

Chinang, B. L., Sheih, Y. B., Wang, L. H., Lino, C. K., and Gill, H. S. (2000). Enhancing immunity by dietary optimization and definition of cellular immune responses. *European Journal of Clinical Nutrition*, **54**(11), 849855.

Cryan, J. F., and Dinan, T. G. 2012. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci*. **13**:701–712.

Cunningham-Rundles, S., Ahrne, S., Bengmark, S., Johann-Liang, R., Marshall, F., Metakis, L. and Cervia, J. (2000). Probiotics and immune response. *The American Journal of Gastro-enterology*, ***95*(1),** S22-S25.

Davis, D. J., Doerr, H. M., Grzelak, A. K., Busi, S. B., Jasarevic, E., Ericsson, A. C. and Bryda, E. C. 2016. *Lactobacillus plantarum* attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. *Scientific Reports* :1-11.

Doron, S. and Gorbach, S. L. (2006). Probiotics: their role in the treatment and prevention of disease. *Expert Review of Anti-Infective Therapy***, 4**(2),261-275.

FAO/WHO. (2002). Guidelines for evaluation of probiotics in food. London, Ontario: Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report Pp:1-34.

from rationale to clinical use. *Current Opinion in Gastroenterology,* **21**(6), 697-701.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology,* **66,** 365–78.

Galdeano, C. M., De Leblanc, A. D. M., Vinderola, G., Bonet, M. B. and Perdigon, G. (2007). Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. *Clinical and Vaccine Immunology*. ***14*(5),** 485-492.

Ganguly, S., Sabikhi, L., and Singh, A. K. (2019). Effect of whey-pearl milletbarley based probiotic beverage on Shigella-induced pathogenicity in murine model. *Journal of Functional Foods,* **54**, 498-505.

Getahun, L., Tesfaye, A. and Muleta, D. (2017). Investigation of the Potential Benefits and Risks of Probiotics and Prebiotics and their Synergy in Fermented Foods. *Singapore Journal of Chemical Biology*, ***6*,** 1-16.

Gibson, G. R., Rastall, R. A., & Fuller, R. (2003). The health benefits of probiotics and prebiotics. *Gut flora, nutrition, immunity and health*, 52-76.

Gorbach, S. L. (2000). Probiotics and gastrointestinal health. *The American Journal of Gastroenterology*. ***95***(1), S2-S4.

Gronlund, M. M., Arvilommi, H., Kero, P., Lehtonen, O. P., & Isolauri, E. (2000). Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, **83**(3), F186-F192.

Guarner, F. and Malagelada, J. R. (2003). Gut flora in health and disease. *The Lancet*. **361**(9356), 512-519.

Guarner, F., Bourdet-Sicard, R., Brandtzaeg, P., Gill, H. S., McGuirk, P., Van Eden, W., & Rook, G. A. (2006). Mechanisms of disease: the hygiene hypothesis revisited. *Nature Reviews Gastroenterology and Hepatology*, *3*(5), 275.

Harish, K. and Varghese, T. (2006). Probiotics in humans–evidence-based review. *Calicut Medical Journal.* **4**(4),e3.

Hirayama, K. and Rafter, J. (2000). The role of probiotic bacteria in cancer prevention. *Microbes and Infection*, **2**(6), 681-686.

Huang, Y. and Adams, M. C. (2004). In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. *International Journal of Food Microbiology*, **91**(3)**,** 253-260.

Huycke, M. M. and Gaskins, H. R. (2004). Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. *Experiment Biological Medicine (Maywood)*, **229**, 586-97.

Isolauri, E. T. Y. E. S., Arvola, T., Sütas, Y., Moilanen, E. and Salminen, S. (2000). Probiotics in the management of atopic eczema. *Clinical and Experimental Allergy*, **30**(11), 1605-1610.

Isolauri, E., Salminen, S. and Ouwehand, A. C. (2004). Probiotics. *Best Practice and Research Clinical Gastroenterology*, **18**(2), 299-313.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. (2011). Global cancer statistics. *A Cancer Journal for Clinicians*, **61**(2), 69-90.

Kajander, K., Hatakka, K., Poussa, T., Färkkilä, M., & Korpela, R. (2005). A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6month intervention. *Alimentary Pharmacology and Therapeutics*, ***22***(5), 387-394.

Kimoto-Nira, H., Mizumachi, K., Nomura, M., Kobayashi, M., Fujita, Y., Okamoto, T. and Ohmomo, S. (2007). *Lactococcus sp*. as potential probiotic lactic acid bacteria. *Japan Agricultural Research Quarterly,* **41**(3), 181-189.

Klaenhammer, T. R. (1988). Bacteriocins of lactic acid bacteria. *Biochimie*, **70**(3), 337-349.

Liong, M. T. and Shah, N. P. (2005b). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *Journal of Dairy Science*, **88**(1), 55-66.

Lonkar, S. A., Khatkar, A. B., Chandla, N. K., Singh, P. K., Kumar, S., Sain, M., & Khatkar, S. K. (2022). Lactose Intolerance: A Review for facts and fictions. *Environment Conservation Journal*, *23*(3), 479-485.

Macfarlane, G. T. and Cummings, J. H. (2002). Probiotics, infection and immunity. *Current Opinion in Infectious Diseases,* **15**(5), 501-506.

Mack, D. R., Michail, S., Wei, S., McDougall, L. and Hollingsworth, M. A. (1999). Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *American Journal of PhysiologyGastrointestinal and Liver Physiology*, **276**(4), G941-G950.

Majamaa, H., and Isolauri, E. (1997). Probiotics: a novel approach in the management of food allergy. *Journal of Allergy and Clinical Immunology*, **99**(2), 179-185.

Marotta, F., Naito, Y., Minelli, E., Tajiri, H., Bertuccelli, J., Wu, C.C., Min, C.H., Hotten, P. and Fesce, E. (2003). Chemoprotective effect of a probiotic preparation on development of preneoplastic and neoplastic colonic lesions: an experimental study. *Hepato-gastroenterology*, **50** (54), 19141918.

Marteau, P. R., Vrese, M. D., Cellier, C. J. and Schrezenmeir, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. *The American Journal of Clinical Nutrition*, **73**(2), 430s-436s.

Marteau, P., Pochart, P., Flourie, B., Pellier, P., Santos, L., Desjeux, J. F. and Rambaud, J. C. (1990). Effect of chronic ingestion of a fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. *The American Journal of Clinical Nutrition*, **52**(4), 685-688.

Martini, M. C., Lerebours, E. C., Lin, W. J., Harlander, S. K., Berrada, N. M., Antoine, J. M., and Savaiano, D. A. (1991). Strains and species of lactic acid bacteria in fermented milks (yogurts): effect on in vivo lactose digestion. *The American Journal of Clinical Nutrition*, **54**(6), 1041-1046.

Martini, M. C., Lerebours, E. C., Lin, W. J., Harlander, S. K., Berrada, N. M., Antoine, J. M., and Savaiano, D. A. (1991). Strains and species of lactic acid bacteria in fermented milks (yogurts): effect on in vivo lactose digestion. *The American Journal of Clinical Nutrition*, **54**(6), 1041-1046.

Masco, L., Ventura, M., Zink, R., Huys, G. and Swings, J. (2004). Polyphasic taxonomic analysis of *Bifidobacterium animalis* and *Bifidobacterium lactis* reveals relatedness at the subspecies level: reclassification of *Bifidobacterium animalis* as *Bifidobacterium animalis subsp. animalis subsp. nov.* and *Bifidobacterium lactis* as *Bifidobacterium animalis subsp. lactis subsp. nov.* *International Journal of Systematic and Evolutionary Microbiology,* **54**(4), 1137-1143.

McFarland, L. V. (2009). Evidence-based review of probiotics for antibioticassociated diarrhea and Clostridium difficile infections. *Anaerobe*, **15**(6),274-280.

McNaught, C. E., Woodcock, N. P., Anderson, A. D. and MacFie, J. (2005). A prospective randomised trial of probiotics in critically ill patients. *Clinical Nutrition*, **24**(2), 211-219.

Meurman, J. H. and Stamatova, I. (2007). Probiotics: contributions to oral health. *Oral Diseases*, **13**(5), 443-451.

Mitsuoka, T. (1982). Recent trends in research on intestinal flora. *Bifidobacteria and Microflora*, **1**(1), 3-24.

Niedzielin, K., Kordecki, H. and Birkenfeld, B. A. (2001). Controlled, doubleblind, randomized study on the efficacy of *Lacobacillusplantarum* 299V in patients with irritable bowel syndrome. *European Journal of Gastroenterology and Hepatology,* **13**(10), 1143-1147.

Olasupo, N. A., Olukoya, D. K., & Odunfa, S. A. (1997). Assessment of a bacteriocin-producing Lactobacillus strain in the control of spoilage of a cereal-based African fermented food. *Folia microbiologica*, **42**(1), 31.

Ouwehand, A. C., Salminen, S. and Isolauri, E. (2002). Probiotics: An overview of beneficial effects. *Antonie Van Leeuwenhoek,* **82**(1-4), 279–289.

Park, Y., Hunter, D.J. and Spiegelman, D. (2005). Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. *Journal of American Medical Association,* **294** (22), 2849–2857.

Pool-Zobel, B.L., Neudecker, C., Domizlaff, I., et al. (1996). *Lactobacillus*- and *Bifidobacterium*-mediated antigenotoxicity in the colon of rats. *Nutrition and Cancer,* **26**, 365–80.

Power, D. A., Burton, J. P., Chilcott, C. N., Dawes, P. J., & Tagg, J. R. (2008). Preliminary investigations of the colonisation of upper respiratory tract tissues of infants using a paediatric formulation of the oral probiotic Streptococcus salivarius K12. *European Journal of Clinical Microbiology and Infectious Diseases*, **27**(12), 1261.

Reid, G. (1999). The Scientific Basis for Probiotic Strains of *Lactobacillus*. *Applied and Environmental Microbiology,* **65** (9), 3763–3766.

Reid, G. (2001). Probiotic agents to protect the urogenital tract against infection. *American* *Journal of Clinical Nutrition*, **73**, 437S - 443S

S Patwadi, P., Puranik, D. B. ., & Sain, . M. (2021). Study of effect of functional ingredients on sensory attributes of ready-to-use (RTU) and ready-to-reconstitute (RTR) health beverages for diabetic population. *Environment Conservation Journal*, *22*(1&amp;2), 191–198. https://doi.org/10.36953/ECJ.2021.221227.

Saarela, M., Mogensen, G., Fonden, R., Matto, J. and Mattilia-Sandholm, T. (2000) Probiotic bacteria: Safety functional and technological properties. *Journal of Biotechnology,* **84** (3), 197-215.

Salminen, S. and Gueimonde, M. (2004). Human studies on probiotics: what is scientifically proven. *Journal of Food Science*, **69**(5), M137-M140.

Salminen, S., and Von Wright, A. (2004). *Lactic acid bacteria: microbiological and functional aspects*. CRC Press.

Salvetti, E., Torriani, S. and Felis, G. E. (2012). The genus Lactobacillus: a taxonomic update. *Probiotics and Antimicrobial Proteins,* **4**(4),217-226.

Sanders, M. E. (1993). Effect of consumption of lactic cultures on human health. *Advances in Food and Nutrition Research*,**37**, 67-130.

Sanders, M. E. and Klaenhammer, T. R. (2001). Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *Journal of Dairy Science*, **84**(2),319-331.

Schultz, M. and Sartor, R. B. (2000). Probiotics and inflammatory bowel diseases. *The American Journal of Gastroenterology*, **95**(1)**,** S19-S21.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science*, **83**(4), 894-907.

Singh, H.K., Puranik, D.B., Poornima and Sain, M. (2021) Process optimisation for the production of papaya leaf extract based therapeutic whey beverage. *Environment Conservation Journal*, **22** (1&2): 153-158.

Solga, S. F. and Diehl, A. M. (2003). Non-alcoholic fatty liver disease: lumenliver interactions and possible role for probiotics. *Journal of Hepatology*, **38**(5), 681-687.

Talwalkar, A. and Kailasapathy, K. (2004). A review of oxygen toxicity in probiotic yogurts: Influence on the survival of probiotic bacteria and protective techniques. *Comprehensive Reviews in Food Science and Food Safety*, **3**(3),117-124.

Tannock, G. W. (2001). Molecular assessment of intestinal microflora. *The American Journal of Clinical Nutrition*, **73**(2), 410s-414s.

Tannock, G. W. (2002). The bifidobacterial and Lactobacillus microflora of humans. *Clinical Reviews in Allergy and Immunology*. **22**(3), 231-253.

Taranto, M. P., Medici, M., Perdigon, G., Holgado, A. R. and Valdez, G. F. (1998). Evidence for hypocholesterolemic effect of Lactobacillus reuteri in hypercholesterolemic mice. *Journal of Dairy Science*, **81**(9), 23362340.

Tisser, H. (1906). Traitement des infections intestinales par la methode de la flore bacterienne de l’intestin. C.R. Soc. Biol, 60, 359-361.

Vanderhoof, J. A. (2000). Probiotics and intestinal inflammatory disorders in infants and children. *Journal of Pediatric Gastroenterology and Nutrition*, **30,** S34-S38.

Verdu, E. F., & Collins, S. M. (2005). Irritable bowel syndrome and probiotics:

Verma, A. and Shukla, G. (2013). Administration of prebiotic inulin suppresses 1, 2 dimethylhydrazine dihydrochloride induced procarcinogenic biomarkers faecal enzymes and preneoplastic lesions in early colon carcinogenesis in Sprague Dawley rats. *Journal of Functional Foods,* **5**(2), 991-996.

Ziemer, C. J. and Gibson, G. R. (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal*, ***8***(5-6), 473-479.

Zoumpopoulou, G., Tsakalidou, E., Dewulf, J., Pot, B., & Grangette, C. (2009). Differential crosstalk between epithelial cells, dendritic cells and bacteria in a co-culture model. *International Journal of Food Microbiology*, **131**(1), 40-51.