3. Review of the literature

Probiotics can be defined as dietary supplements containing potentially beneficial bacteria. According to the currently adopted definition by FAO/WHO (2002), probiotics are the live microorganisms which when administered in an adequate amount confer health benefit to the host. Probiotics are the bacterial cultures intended to assist the body's naturally occurring gut flora to establish themselves. In certain circumstances like the use of antibiotics, drugs, excess alcohol, stress, disease or exposure to toxic substances could disturb the balance maintained inside the body. In such abnormal conditions the intake of probiotic foods will retain the ecological balance of the body and aids for better health of the host. For centuries, folklore suggested that the fermented food containing probiotics were healthful. Recent scientific investigations also supported the traditional views, suggesting the important role of probiotics in bringing better health to the host. The gastrointestinal tract serves as the bridge between inside and outside the body. Normal microbial inhabitants of the gastrointestinal tract re-force the barrier function of intestinal lining, decreasing the passage of bacteria or antigen from the intestine into the blood stream.

Some of the criteria that should be followed to choose bacteria/microorganisms as probiotics include, they should be isolated from the same species as it is intended host. Secondly, they should demonstrate beneficial effect on its host and should show stability against bile acids, enzymes and oxygen, they should be nonpathogenic and able to produce antimicrobial substances. Further, they should able to colonize, survive and transit through the gastrointestinal tract. Additionally, large number of these viable bacteria must able to survive for prolonged periods on storage and should be safe (Kailaspathy and Chin 2000, Harish and Varghese 2006).

3.1 History of probiotics

Probiotics and its phenomenon was discovered by Mietchnikoff in 1908 (Tomasik and Tomasik 2003). The word probiotic stems from the Greek word 'pro' 'bios' (for life) a term traditionally used to describe the use of live microorganisms that benefit to the host by improving its intestinal microbial balance (Falony and Devuyst 2007). The beneficial association of microorganisms with humans was first described by Albert Dolderllin in 1892 (Falony and
Devuyst 2007). They observed that the vaginal bacteria producing lactic acid which in turn prevented the growth of pathogenic bacteria. Later, in 1906 Frenchman Henry Tissier followed by Russian Elias Metchnikoff and Retteger and Cheplin in initial stages contributed various findings on the association of microorganisms with the host. Dr. Minoru Shirita (Japan) brought an idea of manufacturing a dairy drink named yakult (Falony and Devuyst 2007). Now a day there are numerous probiotic products are in market both as food supplements and medicines.

3.2 Probiotic species

Saccharomyces cerevisiae, S. boulardii, Aspergillus oryzae are commonly used fungi as a probiotics (Vibhute et al., 2011). The use of blue green algae, Cyanobacteria, micro algae as a probiotic in fisheries are in progress. Some of the species of Bacillus viz., B. subtilis, B. licheniformis, B. polymyxa, B. laterosporus, B. circulans, B. coagulans and some spores of Bacillus are being used as probiotics (Casula and Cutting 2002, Ziaei-Nejad et al., 2006). Streptococcus phocae, Enterococcus faecium, Carnobacterium divergens are also being used as probiotics (Kanmani et al., 2010). However, Lactic acid bacteria are most commonly used probiotics.

3.3 Commonly used Lactic acid bacteria

Salminen and Wright (1998) suggested that the use of lactic acid in food had long history of safe food preservation and use of members of the genera Lactococcus and Lactobacillus in addition to the genera Streptococcus and Enterococcus and other genera of lactic acid bacteria for food preservation. Further, Marteau (2001) suggested that the use of probiotic strains assured with health benefits and safe in animals and humans except in immuno compromised individuals. It has been estimated that atleast 500 different microbial species exists in gastrointestinal tract on a quantitative basis, of which about 20 genera predominates. These may include, Bacteriodes, Lactobacillus, Clostridium, Fusobacterium, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Escherichia and Veillonella (Harish and Varghese 2006). Among the probiotic lactic acid bacteria (LAB), Lactobacillus acidophilus, L. casei, L. lactis, L. helviticus, L. salivarius, L. plantarum, L. bulgaricus, L. rhamnosus, L. johnsonii, L. reuteri, L. fermentum, L. delbrueckii, Streptococcus thermophilus, Enterococcus
faecium, *E. faecalis*, *Bifidobacterium bifidum*, *B. breve*, *B. longum* and *Saccharomyces boulardii* (Suvarna and Body 2005, Gayathri et al., 2011) are most commonly used.

### 3.4 Beneficial effects of probiotics

Lactic acid bacteria convert lactose mainly into lactic acid, in addition to this certain carbohydrates, starches, fibers, oligosaccharides and certain types of sugars will also get fermented. Characteristic features of probiotics in the intestine include the release of antimicrobial substances and the formation of a biofilm to protect the intestinal mucosal membrane. Probiotics maintains the dynamic equilibrium of the intestinal microflora. Use of probiotics may lower the nitrogen excretion by increasing digestibility and desorption. Probiotics supports the intestinal microflora by means of specific metabolic activities and by stimulating the host's immune system (Tomasik and Tomasik 2003). Undesirable microorganisms are thus reduced and protection is given against colonization or attachment of harmful microorganisms and probiotics in turn bring immunomodulation. Probiotics therefore contribute to averting any disruption of the intestinal microflora in the human colon. The probiotic microflora also highly abundant, diversified and responsible for digestion of dietary components which have been previously degraded in upper part of digestive tract.

Beneficial effects of probiotics have been recorded in human system and food industry. Further, the probiotics are reportedly useful in controlling inflammation, prevention of lactose intolerance, antitumor, anticholesterolemic effects, prevention of infections and allergy (Rijkers et al., 2010), respiratory diseases (Vanderhoof 2001, Suvarna and Body 2005), reduction in the adverse effect of pelvic radiotherapy (Salminen and Wright 1998), prevention of all types of diarrhea (Thomas and Greer 2010), reduction of irritable bowel syndrome (IBS), prevention of ulcerative colitis, Crohn's disease, Pouchitis, constipation and in controlling liver diseases, several types of cancers (Harish and Varghese 2006, Kailaspathy and Chin 2000, Ajmal and Ahmed 2009). Antimicrobial compounds such as bacteriocins released by potential probiotic species were beneficial in preventing intestinal pathogens. Many researchers proved that the beneficial effect by utilizing probiotic products in improving pedigree, in increase in body weight, enhancing immunity and anticancer effects (Harish and Varghese 2006).
3.4.1 Probiotics in controlling inflammation

Interactions between the commensal microflora and the intestinal mucosa stimulate the inflammatory activity (Guamer and Malagelada 2003). Probiotics are believed to alleviate allergic and inflammatory skin disorders. Oral treatment with the \textit{L. casei} alone alleviated antigen-specific skin inflammation mediated by either protein-specific CD4\(^+\) T cells or hapten-specific CD8\(^+\) T cells. \textit{Lactobacillus casei} treatment enhanced the frequency of FoxP3\(^+\) Treg in the skin and increased the production of IL-10 by CD4\(^+\)CD25\(^+\) regulatory T cells in skin draining lymph nodes of hapten-sensitized female C57Bl/6 mice. Therefore it has been demonstrated that orally administered \textit{L. casei} efficiently alleviate T cell-mediated skin inflammation without causing immune suppression, via mechanisms that include control of CD8\(^+\) effector T cells and involve regulatory CD4\(^+\) T cells and further prevented cell-mediated allergic skin diseases in human (Hacini-Rachinel et al., 2009).

\textit{Lactobacillus acidophilus/L. rhamnosus} treatment of differentiated Caco-2 monolayers increased (three fold) multidrug resistant \textit{1/P-glycoprotein} mRNA and protein levels in dextran sulfate sodium induced mice. \textit{Lactobacillus acidophilus} or \textit{L. rhamnosus} stimulated P-glycoprotein activity through phosphoinositide 3-kinase and ERK1/2 MAPK pathways. Further, myeloperoxidase activity was reduced by the expression of multidrug resistant \textit{1/P-glycoprotein} mRNA and protein in the distal colon. Thus, these results intended to provide new mechanism involving P-glycoprotein up regulation in beneficial effects of probiotics in intestinal inflammatory disorders (Saksena et al., 2011).

3.4.2 Probiotics in prevention of lactose intolerance

Intolerance to lactose containing foods (primarily dairy products) is the most common disorder of intestinal carbohydrate digestion with the prevalence ranging from 7 to 20 percent in Caucasians, 50% to 85% in African –Asian and from 90% to 100% in Asians (Scrimshaw and Murray 1988). Lactose maldigestion occurs frequently, especially in adults and in persons with bowel resection or enteritis (secondary lactose maldigestion). It is well established that persons with lactose maldigestion experience better digestion and tolerance of the lactose contained in yogurt than that of contained milk (de Vrese et al., 2001). Digestion of lactose in the gut lumen by the lactase contained in the yogurt bacteria and slower intestinal
delivery or transit time of yogurt compared with milk (de Vrese et al., 2001). Lactose is converted to lactic acid, pH of the gut decreases, i.e. it becomes acidic favouring enhanced absorption of calcium. If probiotics are fed to lactose intolerance patients, then milk lactose is hydrolysed by probiotic strains and lactose is assimilated and calcium absorption is also favoured (Suvarna and Body 2005).

3.4.3 Probiotics in prevention of cholesterol

Probiotic strains, especially lactic acid bacteria have a major role in cholesterol lowering mechanism. As the cholesterol level keeps increasing in the serum, it leads to cardiac diseases. These cholesterol levels can be brought down using probiotics (Fuller 1989). Probiotic strains assimilate the cholesterol for their own metabolism. Probiotic strains can get bound to the cholesterol molecule, and they are capable of degrading cholesterol to its catabolic products. The cholesterol level can be reduced indirectly by deconjugating the cholesterol to bile acids, thereby reducing in total body pool.

Grunewald (1982) observed a significant reduction in the serum cholesterol in rats. Rats were fed with fermented milk to study the growth response and lipid profiles. Rats were divided into three groups, viz. water (control); water + 10% milk, and water + 10% fermented milk. Fermented milk was prepared with probiotic strain \textit{L. acidophilus}. There was a drastic reduction in serum cholesterol of fermented milk-fed rats, indicating that cholesterol levels in serum can be reduced by consumption of probiotics. In an another study, pigs were fed with high cholesterol diet followed by feeding with probiotic strains of \textit{L. acidophilus} for ten days and observed the significant decrease in cholesterol level (Gillilland et al., 1985).

Studies examining the efficacy of probiotics in reducing cholesterol often do not sufficiently address the mechanisms by which probiotics modulate hypocholesterolemic effects and the optimum dose, frequency, and duration of treatment for different probiotic strains. Several mechanisms have been hypothesized, which may include enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics (Lambert et al., 2008), assimilation of cholesterol by probiotics (Pereira and Gibson 2002), co-precipitation of cholesterol with deconjugated bile (Liong and Shah 2006), cholesterol binding to cell walls of probiotics (Liong and Shah 2005), incorporation of cholesterol into the cellular membranes of probiotics during growth (Lye et al.,
2010), conversion of cholesterol into coprostanol (Lye et al., 2010) and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics (De Preter et al., 2007).

3.4.4 Probiotics in prevention of allergy

Common infectious diseases continue to be a major cause of death among preschool children in developing countries. Probiotics have been shown to reduce the incidence of childhood eczema by half, compared to placebo, when administered during pregnancy and up to 6 months postnatal period (Kalliomäki et al., 2001). In addition, probiotics exert a beneficial effect on allergic reaction by improving mucosal barrier function. In addition, probiotics consumption by young children beneficially affected immune system development. Probiotics such as *L. rhamnosus* was helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein (Majamaa and Isolauri 1997). Heat killed *B. breve* and *S. thermophilus* were fed to the infants with family history of atopy during first month of their life were found to decrease the incidence of respiratory allergy (Morisset et al., 2011). Probiotics consumption may thus be a means for primary prevention of allergy in susceptible individuals. Perinatal administration of the probiotic *L. rhamnosus* strain, reduces incidence of atopic eczema in at-risk children during the first 2 years of life (infancy) has been reported (Kalliomäki et al., 2003). A further study (Rosenfeldt et al. 2003) in infants and children (1–13 years) with established atopic dermatitis treated with *L. rhamnosus* and *L. plantarum* for 6 weeks has shown no difference in the score for severity of atopic dermatitis when compared with treatment with a placebo. It has also been demonstrated that children with IgE-mediated atopic dermatitis induced by cow’s milk allergy have a reduced interferon-γ response that can be markedly increased by treatment with *L. rhamnosus*, thereby providing a strengthened cytokine response that could potentially reduce the Th2-mediated allergic potential in these individuals (Pohjavuori et al. 2004). Management of allergy through probiotics has also been demonstrated in infants, using Lactobacilli to control atopic eczema and cow’s milk allergy (Furrie 2005).

3.4.5 Probiotics in controlling respiratory diseases

Respiratory allergies include allergic rhinitis, sinusitis and asthma. Increasing attention on pathogenesis of allergic airway diseases has given rise to *atopic march* hypothesis (clinical features of atopic eczema occur first and precede the development of asthma and
allergic rhinitis) (Sazawa et al. 2010). Children of 1–3 years of age were fed with $10^7$ cfu/day of probiotic *B. lactis* in milk for one year and observed that the reduction of incidence of pneumonia (24%) and severe acute lower respiratory infection by 35% (Sazawa et al. 2010).

*Lactobacillus rhamnosus* (LGG) fed at a dose of $10^9$ cfu/100 ml of a fermented milk product to children for seven days observed the reduction in the risk for developing nosocomial gastrointestinal and respiratory tract infections (Hojsak et al., 2010). Children fed with LGG group at a dose of $10^9$ cfu/100 ml of a fermented milk product for three months significantly reduced the risk of upper respiratory tract infections (Hojsak et al., 2010).

### 3.4.6 Probiotics in controlling diarrhea

The most studied gastrointestinal condition treated by probiotics is acute infantile diarrhea. Rotavirus is one of the leading causes of infantile diarrhea worldwide and rapid oral rehydration is the primary treatment. Researchers have conducted several double-blinded placebo controlled trials involving Finnish infants and young children with rotaviral diarrhea, which showed significant reduction of disease duration in those receiving different LAB compared with placebo groups (Kaila et al., 1992, Kaila et al., 1995). Treatment of probiotics results in reduction in the duration of diarrhea by about 17 to 30 hours in children (Van Niel et al., 2002, Allen et al., 2004). Oberhelman et al., (1999) evaluated prophylactic effect of *L. rhamnosus* in preventing diarrhea in children. A lower incidence of diarrheal disease with the regular administration of a daily dose of *Lactobacillus*, 6 day a week for 15 months was evident but only in non breast fed infants. Probiotic treatments have been proven for the treatment of antibiotic associated diarrhea, *Clostridium difficile* associated diarrhea, radiation induced diarrhea and traveler’s diarrhea (Harish and Varghese 2006). Tyndalized *L. acidophilus* does not shown any significant beneficial effects in reduction of rota viral diarrhea on children fed with tyndalized *L. acidophilus* with ORS for three days (Khanna et al., 2005).

### 3.4.7 Probiotics in reduction of irritable bowel syndrome (IBS)

Irritable bowel syndrome as defined by the Rome II criteria, is a syndrome lasting for more than three months and may present with either constipation or diarrhea associated with abdominal pain (Harish and Varghese 2006, Kailaspathy and Chin 2000, Ajmal and Ahmed
In an experimental condition, patients were randomly assigned to receive the active preparation containing *L. plantarum/B. breve*, both at a concentration of $5 \times 10^9$ CFU/ml, or placebo powder containing starch for 4 weeks. The severity score of characteristic inflammatory bowel disease (IBD) symptoms significantly decreased in probiotic group versus placebo group after 14 days that is, 49.6% versus 9.9% (Saggioro and Alfredo 2004) was recorded. Several studies (Whorwell et al., 2006, Niedzielin et al., 2001, Guyonnet et al., 2007) showed interesting effects of probiotics on inflammatory bowel disease in animals. In addition, intracolonic administration of *L. reuteri* to rats with acetic acid-induced colitis significantly decreased the disease (Fabia et al., 1993).

### 3.4.8 Probiotics in prevention of *Helicobacter pylori* infection

*Helicobacter pylori* is a highly prevalent pathogen considered as an aetiologial factor for gastroduodenal ulcers, and a risk factor for gastric adenocarcinoma and lymphoma in humans. Most subjects colonized by this microorganism are asymptomatic and remain untreated. In symptomatic patients, the antibiotic treatment has a high cost and is not 100% effective because of resistance to antibiotics and to moderate patient compliance. Some strains of *Lactobacillus* and *Bifidobacterium* are able to inhibit *H. pylori* growth through the release of bacteriocins or organic acids, and may also decrease its adhesion to epithelial cells (Bhatia et al., 1989). In addition, probiotics have a possible role in the stabilization of the gastric barrier function and decrease of mucosal inflammation. Other aspects that are considered are the contribution of probiotics to the healing of the gastric mucosa linked to their antioxidant and anti-inflammatory properties.

When *L. salivarius* and *L. casei* administered to *H. pylori* colonized mice, induced a significant decrease in the counts of the bacteria in the body and antral mucosa compared with untreated animals, and resulted in a concomitant reduction in the associated gastric inflammation (Oh et al., 2002). A traditional yogurt originating from Asia was evaluated for its bactericidal activity against *H. pylori* (Oh et al., 2002). Both the yeasts (*Kluyveromyces lactis* and *Issatchenkia orientalis*) and Lactobacilli (*L. crispatus* and *L. kefiri*) were present in the product exerted independently an anti-*H. pylori* activity. This yogurt had high levels of lactic acid and formic acid but only the latter was found to have a consistent inhibitory effect against *H. pylori*. 
3.5 Probiotic in food industry

"Let food be thy medicine and medicine be the food" as quoted by Hippocrates, probiotics can be obtained as a diet. Probiotic food is the food that contains live bacteria, which is considered as beneficial and not harmful to humans. The first example of probiotic food was the introduction of acidophilus to milk, which in some cases helped people who had difficulty in digesting milk to be able to tolerate milk better. The specific bacteria used in probiotic food particularly acidophilus milk was *L. acidophilus*. Such milk was available in the early 1920s, and yogurt predates it, but was not specifically supplemented for probiotic effects. This may also include probiotic food like yogurt, primarily, *L. bulgaricus*, *L. rhamnosus GG*, and variants of *Bifidobacterium*.

A range of probiotic food choices includes miso soup, some soft cheeses, yogurt products like kefir, sauerkraut and many pickles. There are several food products such as, Kefir and Kumis (popular in Central Asia), yoghurt, tempeh (Indonesian fermented product), Choucroute (French fermented dish) curd, butter milk and fermented milk (Popular in India), sauerkraut and kimchi (Korean famous fermented product), kombucha, tsukemono, natto and miso (Japanese traditionally used fermented drink) are available which are enriched with probiotic health boosters. Prebiotic foods are also found to be beneficial and they do not generally contain bacteria but appears to help healthy bacteria grow in the intestines. Prebiotic foods include Jerusalem and regular artichokes, oats, honey, many fruits, and goat’s milk. Human breast milk is also thought to have prebiotic properties and hence benefits the human newborn (Food and Agriculture Organization 2006).

Probiotic bacteria can also be consumed in the form of capsule. However, it is not been clear that how well probiotic bacteria work. *Lactobacillus rhamnosus* GG, one of the newest probiotics, has shown that it could survive processing by the gut and be especially beneficial to the colon. Not all probiotics have shown evidence that they promote better intestinal health. Some studies do show that eating yogurt during antibiotic treatment may help prevent diarrhea, and is beneficial in reducing risk of yeast infections in women. However, in most cases, probiotic food is well tolerated, and may provide the better intestinal health. Eating prebiotic foods may also be a good way to promote regularity, and certain prebiotic foods like...
oats have other benefits, like increasing daily fiber and lowering cholesterol in host system (Saikali et al., 2004, Ooi and Liong 2010)

3.6 Probiotics in dairy

Dairy products containing live microbial cultures are called bio-dairy products. It would contain cultures about 2 million bacteria in 1cm$^3$ and decreases at the end of the recommended storage period. In addition, about $10^8$-$10^9$ cm$^3$ of titer of probiotic bacteria must be present in fermented drinks but its titer value decreases on storage. Food containing probiotics and fiber were put into a group of products called neutraceticals (Tomasik and Tomasik 2003). Although, there were no such reports on Lactobacillemia in connection with probiotic consumption, some of the side effects like systemic infections, deleterious metabolic activities, excessive immune stimulation and gene transfer are associated with probiotic consumption. Some of the risk factors are also depends on age, pregnancy or immunodeficiency and the formation of digestive lesions were recorded (Marteau 2001).

Probiotics have been used in dairy to yield better milk production and productivity in dairy animals. Lactobacillus rhamnosus and B. lactis applied in dairy products, showed inhibitory effects against Brevibacterium linens when tested using the spot-on-lawn assay (Knox et al., 2005). It has been observed that the early establishment of microbial flora in digestive tract of ruminants has a beneficial effect on their health condition and productivity.

Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. lactis and L. rhamnosus isolated from milk sample of buffalo, cow and goat have shown antagonistic activity against E. coli, Enterobacter aerogenes, K. pneumoniae, P. vulgaris and S. typhi (Tambekar et al., 2009) and also been found that some of the isolates were resistant to ampicillin, nalidixic acid, norfloxacin, cephalothin and co-trimoxazole antibiotics and there fore could be used for both preventive and therapeutic purposes in controlling intestinal infections.

Lactobacillus plantarum isolated from sauerkraut showed anti-listerial activity and inhibited Listeria monocytogenes, L. innocua and E. coli (Wilson et al., 2005). Probiotics mixed with cattle feeds would increase the efficiency of milk production and better survivality of cattle by preventing pathogen infections. Live cultures of L. acidophilus and Propionibacterium
*freudenreichii* strain in feedlot increased the beef production. But the efficiency was varied with the dose of probiotics (Vasconcelos et al., 2008) and the inhibitory compound was suggested as lactic acid. Daily consumption of a probiotic dairy product Actimel (probiotic dairy product, Actimel, Groupe Danone, Paris, France) in elderly person increase the antibody levels against the three influenza strains and also raised seroprotection against H1N1, H3N2 strains and the seroconversion of the strains in turn was prevented (Aubin et al., 2008). It has been observed that daily weight gain and reduction in the diarrhea cases have been documented when probiotic mixture were fed to the young Holstein calves (Aldana et al., 2009). Male Holstein calves were given a basal diet containing a combination of whole pasteurized milk and reconstituted milk replacer. In another group commercial culture of *L. acidophilus* and *L. plantarum* was added to the basal diet. A significant increase in serum Ig G concentration was recorded in the probiotic-supplemented groups, which also showed a significant increase in body weight at 5 weeks and during the entire experimental period (Al-Saiady 2010).

### 3.7 Probiotics in fisheries

Probiotics were used in fisheries to increase the egg production, development and to increase the survival rate of commercially important fishes. Potential probiotic strain *L. rhamnosus* was fed (10⁹ and 10¹² CFU/g) to the rainbow trout for 51 days. Sixteen days after the start of *Lactobacillus* feeding, fishes were challenged with *Aeromonas salmonicida* ssp *salmonicida*, causative agent furunculosis. It was found that the motility rate in probiotic fed group were reduced by 18.9% and 46.3% in 10⁹ and 10¹² CFU/g feed group respectively and probiotic feeding found to reduce the infection in fishes (Nikoskelainen et al., 2001). Additionally, some of the probiotics were isolated from the gut of the common clownfish, *Amphiprion percula* showed antagonism towards aquatic pathogens such as, *Aeromonas hydrophila* and *V. alginolyticus* (Vine et al., 2004). A rainbow trout (*Oncorhynchus mykiss*) was fed with 2 × 10⁸ cells of probiotic *Bacillus* sp. and *Aeromonas sobria* for 14 days were observed to control the infections caused by *A. salmonicida*, *Lactococcus garvieae*, *Streptococcus iniae*, *V. anguillarum*, *V. ordalii* and *Yersinia ruckeri* (Brunt et al., 2007). However, probiotic application prior and after the packing improved the survival, growth, resistance and post-transportation growth of Indian major carp *Catla catla* (Raj et al., 2008).
Commercial aquaculture probiotic and a mixture of fish gut antagonistic bacterial flora such as *Lactobacillus* sp. and *Bacillus* sp. significantly increased the wet weight in ornamental fishes *Carassius auratus* and *Xiphophorus helleri* and exhibited the protection against *Pseudomonas fluorescens* infection (Abraham et al., 2008). Cell free extracts of Lactic acid bacteria (LAB) viz. *L. acidophilus*, *S. cremoris* and *L. bulgaricus* inhibited the growth of *V. alginolyticus* in nutrient broth. Further, Juveniles of *Penaeus indicus* was orally administered with a moist feed base containing $5 \times 10^6$ cells/g of for a period of *L. acidophilus*, *S. cremoris* and *L. bulgaricus* for four weeks showed better survival when challenged with *V. alginolyticus* by intra-muscular injection. Further, inhibition of *V. alginolyticus* by LAB and stimulation of the non-specific immune response resulting in resistance to disease in the shrimp fed on LAB incorporated diets (Ajitha et al., 2004).

Abundance of luminous *Vibrio* strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm in the Philippines, which had been devastated by luminous *Vibrio* disease (*Vibrio harveyi*) while using heavy doses of antibiotics in feed, achieved survival of 80-100% of shrimp (*Penaeus monodon*) in all ponds treated with probiotics (Moriarty 1999). The probiotic bacterium *Streptococcus phocae* and *Enterococcus faecium* inhibited fish pathogens such as, *V. parahaemolyticus*, *V. anguillarum*, *V. monocytogenes* and *E.coli* and also showed highest bacteriocins activity (Kanmani et al., 2010). *Lactobacillus plantarum*, *L. rhamnosus*, *L. lactis*, *B. licheniformis*, *B. subtilis* and *E. faecium* were used as probiotic to restrain the bacterial pathogen in fish and shrimp hatchery (Panigrahi et al., 2005, Balcazar et al., 2009).

In addition, *L. plantarum* fed to the one day old turbot larvae, *Scophthalmus maximus*, showed a significant limitation of larval mortality rate when the larvae was challenged with the pathogen *Vibrio* (Gatesoupe 1994). *Pseudomonas fluorescens* a probiotic strain when fed to rainbow trout (*Oncorhynchus mykiss* Walbaum) has been found to be antagonistic to *V. anguillarum* and mortality rate was found to be decreasing (Gram et al., 1999). Probiotic strain *B. subtilis* isolated from the intestine of *Cirrhinus mrigala* (Hamilton) was incorporated in fish feed at different concentrations ($5 \times 10^8$ cells g$^{-2}$, $5 \times 10^6$ cells g$^{-1}$ and $5 \times 10^5$ cells g$^{-1}$) and fed to four species of live bearing ornamental fish, *Poecilia reticulata*, *P. sphenops*, *Xiphophorus helleri* and *X. maculatus* for one year duration and observed to increase
the gonadosomatic index, fecundity and fry production of female brood stock and length and weight of fry in all the four species of the fishes (Ghosh et al., 2007). Overall, a beneficial effect in weight gain and disease resistance was documented in aquaculture sector.

3.8 Probiotics in poultry

Probiotics have been used in poultry to increase the egg production, improvement in the meat production and for the better survival rate of the poultry animal. *Lactobacillus fermentum* isolated from the canine and orally administered 2-d-old Japanese quail (*Coturnix Coturnix japonica*) found significant reduction of *E. coli*, count in the feces with the increase of lactic acid without change in the intestinal pH (Strompfova et al., 2005). Further, *L. animalis* a potential strain was found to be effective in reducing the attachment of *Salmonella gallinarum, S. pullorum* and *S. enteritidis* to host-specific epithelial cells while, *L. fermentum* was able to reduce the attachment of *S. gallinarum* and *S. pullorum* in chicks (Gusils et al., 2006). While, *L. fermentum* isolated from swine and poultry showed better adhesion in chicken and *L. fermentum* suspension showed antagonistic effect against *E. coli*, *Salmonella* sp, *Shigella sonnei* and some enterotoxigenic *Staphylococcus aureus* (Lin et al., 2007).

Major antimicrobial activity of *Lactobacillus* broth cultures have been observed against *Clostridium perfringens* and lower antimicrobial activity were observed against *E. coli* and *Salmonella enteritidis* in chicks (Kizerwetter – Swida and Binek 2005) and also found to prevent necrotic enteritidis caused by *C. perfringens*. *Lactobacillus acidophilus* isolated from chicken intestinal mucus showed extensive adhesion ability to mucus and antibacterial activity against some of *C. perfringens* strains (Kizerwetter – Swida and Binek 2006). Further, Kizerwetter – Swida and Binek (2009) isolated potent bacterium *L. salivarius* from chicken feces which prevented *S. enteritidis, Campylobacter jejuni* and *C. perfringens* attachment in one day old chicken. A total of 12 *Lactobacillus* sp. were isolated from mucosa of 3-5 week old weaned pigs and found to show a significant antimicrobial activity against common porcine pathogens such as, *S. aureus, B. cereus, E. coli* and *C. perfringens* in healthy weaned pigs (Hacin et al., 2008) and identified as *L. crispatus* or *L. amylovorus*. It was also found that these *Lactobacillus* sp. to produce hydrogen peroxide and metabolites other than organic acids.
*Lactobacillus animalis* and *L. fermentum* sub sp. *cellobiosus* isolated from chickens were also found to be great adhesion to the croup (a thin-walled expanded portion of the alimentary tract), small and large intestine by inhibiting the binding sites for *Salmonella* adhesion (Gusils et al., 1999). In their study, *L. fermentum* subsp. *cellobiosus* was found to be lowest and *L. animalis* was found to be highest adhesive ability. Carbohydrate fermentation of poultry feeds reduced the pH levels by *L. salivarius* and *L. plantarum* which further prevented the growth of *E. coli, S. typhimurium* and *C. perfringens* in chickens (Murry et al., 2004). *Lactobacillus casei* and *L. cellobiosus* in combination with organic acid had found to reduce the *Salmonella* infection in turkey houses and the risk of *Salmonella* cross contamination in the processing plant (Vicente et al., 2007).

*Lactobacillus* strains have been isolated from chicken intestine were found to inhibit the growth of *S. enteritidis, S. pullorum, S. typhimurium, S. blockley,* and *E. coli* (Jin et al., 1996). *Lactobacillus acidophilus, L. fermentum, L. crispatus* and *L. brevis* isolated from chicken intestine fed to one day old Arbor Acres broiler chicks were found to increase in the growth performance (Jin et al., 1998). *Lactobacillus fermentum* and *L. brevis* isolated from caecum were found to be most effective in preventing serotypes of *E. coli* and *Salmonella* sp. A rifampin resistant *L. salivarius* was fed together with *S. enteritidis* directly into the proventriculus in one day old leghorn chickens were found to improve the survival rate (Pascual et al., 1999). Single feed administration of *L. animalis* and *L. salivarius* in ducks for a period of 18 to 22 days showed aggregation, co-aggregation, cell surface hydrophobicity and adhesion activities on poultry crop cells and human Hep2-cells (Ehrmann et al., 2002).

Better feed conversion in 21 days-old male broiler chicks from Cobb strain fed with probiotic like *Bacillus subtilis, L. acidophilus, L. casei, Streptococcus lactis, S. faecium* and *B. bifidum* along with few prebiotics have been documented (Pelicano et al., 2004). It has also been found that more than one dose of probiotic would be necessary to ensure the presence of probiotic and freeze-drying and freezing with glycerol or skim milk as cryoprotective agents were suitable methods to preserve the probiotic strain. Significant increase in liver mass and weight gain has been observed in female broiler chicks (*Gallus gallus domesticus*) and ducks (*Anas platyrhynchos domestica*) when fed with fecal sample containing *Lactobacillus* sp, *Bacteroidetes* and *Firmicutes* (Angelakis and Raoult 2010).
Above evidences indicates that the usage of probiotics prevent intestinal pathogens in addition to improvement of poultry animals.

3.9 Probiotics in controlling intestinal pathogens

A large member of intestinal pathogens adopts different methods to colonize, penetrate the mucosal barrier and invade the host system. *Salmonella enterica typhimurium* interacts with the physiological receptor for epidermal growth factor to co-opt the receptor’s signal transduction mechanisms. Intestinal pathogenic *E. coli* secretes a receptor (type III secretion) into the microvillus surface of enterocytes that disrupts the microvillus and alters its actin structure to form a dome like anchoring site. *Shigella* attaches to M cells use their endocytotic properties to enter the cell (Lu and Walker 2001). Once inside the cell, the organism lyses the endocytic vacuole and co opts actin and myosin to form propelling tail for further penetration of epithelium through the basolateral surface. Probiotics can protect the intestine by competing with pathogens for attachment, strengthening tight junctions between enterocytes and enhancing the mucosal immune response to pathogens (Lu and Walker 2001).

*Lactobacillus acidophilus* and *L. casei* isolated from feces of healthy children and found to be inhibiting *Shigella sonnei* which causes shigellosis (Apella et al., 1992). *Lactobacillus reuteri* isolated from healthy vaginal ecosystem was effectively inhibited *Staphylococcus aureus, E. coli, K. pneumoniae, S. typhi* and *S. typhimurium* (Voravuthikunchai et al., 2006). Probiotic lactic acid bacteria such as, *L. casei, L. acidophilus* and *L. paracasei* found to be antagonistic against *E. coli, S. typhimurium, S. sonnei, Enterobacter cloacae* and *Citrobacter freundii*. The antagonistic property has also been observed in different sources like galactose, sorbitol, gal-sorbitol, lactitol, erythritol, gal-erythritol, xylitol and gal-xylitol (Azizpour and Esmaili 2008). While, in the presence of erythritol and xylitol antagonistic activity was absent. Carbohydrate source also plays important role in antagonistic activity. *Lactobacillus lactis* strain isolated from marine environment showed maximum antibacterial activity against some of the major food borne pathogens such as, *Bacillus subtilis,* *Staphylococcus aureus, Enterococcus faecalis* and *Pseudomonas aeruginosa,* minimum antibacterial activity against *S. boydii* and average antibacterial activity against *Shigella shiga, B. megaterium* and *E. coli* (Rajaram et al., 2010).
Human isolate *L. casei* subsp. *rhamnosus* in intestinal Coco-2 cell lines prevented the adhesion of intestinal pathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and *Klebsiella pneumoniae* (Forestier et al., 2001). *Lactobacillus crispatus* found to inhibit the adhesion of diarrheagenic *E. coli* adhering to basement membrane via competition with laminin molecules for binding sites (Horie et al., 2002). Several in vitro studies also showed that the antagonistic activity of *Lactobacillus* sp. against intestinal pathogens. Certain strains of LAB such as, *L. rhamnosus*, *B. lactis*, *L. pentosus* and *E. faecium* were found to inhibit the adhesion of selected canine zoonotic pathogens such as *Staphylococcus intermedius*, *Salmonella typhimurium*, *Clostridium perfringens* and *Campylobacter jejuni* in human, chicken and pigs (Rinkinen et al., 2003).

*Bifidobacterium breve* and *B. infantis* were shown to inhibit the cell association of enterotoxigenic, intestinal pathogenic, *E. coli* and *S. typhimurium* strains to enterocytic Caco-2 cells in a concentration–dependent manner (Bernet et al., 1993). The cell free supernatant of the strain were able to prevent enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella typhimurium*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Clostridium difficile* in-vitro. *Lactobacillus rhamnosus*, *L. gasseri*, *L. casei* and *L. plantarum* were also shown potential for adhering to the colon epithelial cell line and found to prevent the enterohemorrhagic *E. coli* (EHEC) colonization in human colon epithelial cell line (Hirano et al., 2003). It has also been found that *L. crispatus* reconstitute basement membrane preparation called matrigel by which it would prevent the attachment of pathogens. Probiotics such as *Streptococcus thermophilus* and *L. acidophilus* when applied to human intestinal epithelial cell lines (Caco-2) and exposed to enteroinvasive *E. coli*, and increased transepithelial resistance contrasting markedly with the fall in resistance evoked by enteroinvasive *E. coli* infection (Resta-Lenert and Barrett 2003).

*Lactobacillus acidophilus*, *L. gasseri* and *L. jensenii* isolated from the vagina of healthy premenopausal women have been found to inhibit *E. coli* and *Streptococcus agalactiae* (Boris et al., 1998). It has also been found that the proteins, lipoprotein and glycolipids produced by probiotics were found to adhere to epithelial vaginal cells. *Lactobacillus brevis*, *Enterococcus faecium* and *Pediococcus acidilactici* isolated from feces of children have shown antibacterial activity against some of the uropathogenic *E. coli* isolated from urine of the...
urinary tract infected patients in addition to Staphylococcus saprophyticus, Citrobacter freundii, Proteus vulgaris, Enterobacter cloacae, P. aeruginosa and B. anthracis (Lim et al., 2009).

3.9.1 Bacteriocins from Lactobacillus sp.

Probiotics mainly Lactobacillus sp. have been found to inhibit the pathogens by the production of antimicrobial substances like bacteriocins. Lactobacillus GG isolated from the feces of the normal person showed potent inhibitory activity against members of Enterobacteriaceae family, Pseudomonas sp, Staphylococcus sp and Streptococcus sp (Silva 1987) and found that the inhibitory substance was distinct from lactic and acetic acids and supposed to be having low molecular weight and considered as microcin. Lactobacillus acidophilus isolated from yoghurt sold in America were found to inhibit the enteric and food-borne microbial pathogens by the production of bacteriocins (Kilic et al., 1996). Among 20 yogurt products, 11 were found to contain lysogens that spontaneously released a large number of virulent phages that could attack Lactobacillus starter cultures of other yogurts. This indicated that phages could be a factor which inhibited yogurt starter cultures. Lactobacillus lactis sub sp. lactis isolated from boza (Bulgarian traditional drink) and its bacteriocin was obtained and its antimicrobial activity have been observed against food borne E. coli (Ivanova et al., 2000).

Lactobacillus lactis isolated from jben was found to be inhibiting food borne pathogen Listeria monocytogenes (Benkerroum et al., 2003) and the inhibitory substance has been detected as bacteriocins. A bacteriocin of 6 KDa was obtained from the L. acidophilus strain of poultry origin which produced antimicrobial activity against some of the test microorganisms and has showed extensive adhesive nature to the intestinal mucus (Kizerwetter – Swida and Binek 2006). Lactobacillus pentosus isolated from boza found to inhibit E. coli, P. aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, L. curvatus and L. casei (Todorov and Dicks 2007). Further, bacteriocin of 14.0 KDa was obtained and the maximum activity was found to be maximum in MRS medium.

Lactic acid producing bacteria were isolated from digestive tract of the tilapia Oreochromis mossambicus and used as a probiotic in fresh water tilapia, against the most common fish pathogen Aeromonas hydrophila and high antimicrobial activity was recorded
(Vijayabaskar and Somasundaram 2008). It has also been observed that the antagonistic activity of extracellular protein (ECP) or bacteriocin was produced higher antagonistic activity when compared to the intracellular protein (ICP). The use of LAB also found to enhance the production rate in rotifers, which act as biocarriers of probiotics and when fed to fish, they showed increased growth rate and weight of the animal.

It has been reported that the *L. lactis* strain isolated from marine environment produced bacteriocin of 94 KDa and its maximum activity has been observed against some of the major food borne pathogens such as, *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis* and *Pseudomonas aeruginosa* and minimum antibacterial activity against *S. boydii* and average antibacterial activity against *Shigella shiga, B. megaterium* and *E. coli*. The bacteriocin showed their maximum inhibition at 30°C, pH 6.0, 1.5% sodium chloride solution and at 30 h incubation time (Rajaram et al., 2010). While, Karthikeyan and Santosh (2009) isolated bacteriocin of molecular weight 2.5 KDa from *L. plantarum* isolated from marine shrimp (*Penaeus monodon*) gut and its maximum activity was observed at 50°C, pH 4 and 0.9% sodium chloride solution against food spoilage bacteria such as, *B. subtilis, S. aureus, S. typhimurium, S. paratyphi ‘B’, E. coli, Klebsiella sps, Serratia marcscens, P. aeruginosa* and *V. cholerae*. These reports indicate the diversity of bacteriocins in their molecular weight and antibacterial spectrum of activity.

3.10 Probiotics in enhancing immunity

The main functions of the immune system are to eliminate invading viruses and other foreign microorganisms, to relieve the body of damaged tissue, and to destroy neoplasms in the body. Healthy humans have two immune mechanisms: acquired (specific) immunity, which responds to specific stimuli (antigens) and is enhanced by repeated exposure; and innate (nonspecific) immunity, which does not require stimulation and is not enhanced by repeated exposure. Innate immune mechanisms consist of physical barriers, such as mucous membranes, and the phagocytic and cytotoxic function of neutrophils, monocytes, macrophages, and lymphatic cells (NK cells). Acquired immunity can be classified into two types on the basis of the components of the immune system that mediate the response, ie, humoral immunity and cell-mediated immunity (Kuby 1994).
Enhancement of host innate and acquired immunity is one of the dynamic features of probiotic microorganisms. There are different mechanisms by which probiotics triggers the host immune system. Probiotic lactic acid bacteria reported to enhance phagocytic activity, cytokine production, antibody production, immunoglobulin E-mediated hypersensitivity have been reported (Meydani and Ha 2000). The ability to perform phagocytosis and kill microorganisms, including bacterial pathogens, is a major effector function of macrophages. These properties of macrophages are particularly important for host defense against facultative intracellular organisms, which can replicate within macrophages. Measurement of phagocytic activity of macrophages was among the earliest technique for evaluating the immunologic effect of LAB. It has been proposed that the LAB that survive through the gastrointestinal tract, whether interact or modified, can bind to the limina surface of M cells. LAB-bound M cells reaching the dome region of phagocytic cells and stimulates local immune response, resulting in production of IFN-γ by γδ T cells. This may increase the M cell population with subsequent rapid amplification of bacterial translocation, which can further activate the local immune system, resulting in stimulation of the local and the systemic immune response (Meydani and Ha 2000).

The combinational effect of *L. acidophilus* and *B. bifidum* was studied by Schiffrin et al (1995) and showed increased phagocytic activity by monocytes and phagocytic activity of blood leukocytes, particularly granulocytes. In addition, increased counts of *L. acidophilus* and *B. bifidum* in stool sample counts have been observed in healthy volunteers. They also reported that the increment in phagocytosis was coincident with fecal colonization by lactic acid bacteria and persisted for 6 weeks after the consumption. *Lactobacillus acidophilus* and *B. bifidum* fed to the volunteers tested for lymphocyte subsets and leukocyte phagocytic activity in blood showed enhanced phagocytosis of *E. coli* (Schiffrin et al., 1997).

Children were found to show a gradual increase in weight and significant rise in mean TNF-α, TNF-γ and IN-10 levels. But there was a slight fall in mean IL-4 levels. The study indicated that the stimulation of inflammatory cells such as, lymphocytes and NK cells by the consumption of probiotics (Meydani and Ha 2000). In similar kind of study it has been observed (Arunachalam et al., 2000) that there was an increased level of TNF-α, phagocytic
and bactericidal activity in *L. lactis* fed 60 to 83 years old healthy individuals regularly. Increased antibody responses and receptor expression on blood neutrophils in healthy volunteers vaccinated with *Salmonella typhi* also have been observed in *L. rhamnosus* or *L. lactis* consumed individuals (He et al., 2000).

LAB that resides in gastrointestinal tract may bind to the surface of M cells which further produced TNF-α, interferon-γ, TNF-γ, IL-10 and IL-4 in addition to an increase in secondary immune response (Dewan et al., 2009). In their study, they conducted a study on children aged between 12 to 60 months for 15 days by feeding curd sample containing $10^8$ CFU each *L. bulgaricus* and *S. thermophilus* along with few antibiotics with limited food supply.

Lactic acid bacteria were also found to stimulate non-specific immunity. The effects of LAB on human peripheral blood mononuclear leucocytes and found that certain strains of *B. longum*, *L. rhamnosus*, *L. acidophilus* and *L. lactis* sub sp. *cremoris* showed a trend of better inducer of TNF-α, IL-6 and IL-10 release after 24 hours of induction. They also found that the live bacteria stimulated better than glutaraldehyde fixed bacteria (Miettinen et al., 1996). *Lactobacillus casei*, *L. acidophilus*, *L. fermentum*, *Bifidobacterium bifidum*, *B. animalis* and *Enterococcus faecalis* orally administered to the BALB/c mice indigenous ($10^4$ cells day) and exogenous ($10^7$ cells day) Bifidobacteria and Lactobacilli for 5 consecutive days. This dose of administration activated the systemic and intestinal mucosal immune response in a strain-specific way, independently whether the strain was indigenous or exogenous in relation to the host. It is also observed that the increase in immunoglobulin (Ig)A, TNFα, IL-6 and IL-10 in mice. Cell wall related proteins are presumed to induce immunopotentiating capacity of LAB (Vinderola et al., 2004).

To measure secretory immunoglobulin A (IgA) *L. casei* bacterium was orally administered to BALB/c mice, mononuclear cells from Peyer's patches, have increased the CD-206 and TLR-2 compared to control and an increase in the T population or in the IL-5-positive cells. IgA+ cells and IL-6-producing cells increased after 7 days of *L. casei* administration but a specific antibody against *L. casei* was not recorded (Galdeano and Perdigón 2006). Further, in mice fed with *B. longum* for more than 8 weeks, an antibody response was detected for crop lamina cell wall. On the other hand, in mice fed with *L. acidophilus* for more than 6 weeks, an
antibody response was detected to both the cytoplasm and cell wall. Moreover, feeding each organism for 2 weeks enhanced the proliferative response of Peyer's patches cells to the cell fraction against which the serum antibody was detected and not found in spleen cells (Takahashi et al., 1993). These results suggested that mucosal stimulation by lactic acid bacteria would inhibit a systemic immune response in them. However, intravenous injection of \textit{L. casei} to the BALB/c mice stimulated the production of peritoneal macrophages or fixed macrophages in liver and inhibited the growth of \textit{Listeria monocytogenes} (Hashimoto et al., 1984).

\textit{Lactobacillus lactis} and \textit{Streptococcus gordonii} bacterial vectors are capable of delivering antigen to mucosal and systemic immune response which generated specific antibody response (Oyetayo and Oyetayo 2005). The immunoregulatory properties of \textit{L. casei} was extensively studied using \textit{in vitro} and \textit{in vivo} murine macrophages to secrete IL-12, which induced T cells to produce IFN-\(\gamma\) and also promoted the differentiation of immature CD4+ T cells. Administration of \textit{L. casei} to mice enhanced the production of IL-12, TNF-\(\gamma\) and augmented NK cell activity lead to the prevention of influenza virus infection and cancer (Shida et al., 2006). It has been suggested that yoghurt and other fermented products may have immunostimulatory properties related to their bacterial components. Oral administration of milk containing \textit{L. bulgaricus} or \textit{L. casei} to mice have been shown to activate the lymphocytes and macrophages (Perdigon et al., 2001). While, \textit{L. crispatus} found to increase the toll like receptor (TLR2 and TLR4) in mice and pre exposure has found to enhance TLR-2 mediated IL-10 up regulation (Voltan et al., 2007).

Lactic acid bacteria could improve mucosal immunity by triggering the activity of cytokine IFN-\(\gamma\) which were able to increase the immune response against tumor by inducing the macrophages or CD8+ T cells. It had also brought cellular apoptosis induction by enhancing the release of cytokines (Mishra et al., 2008). Probiotic bacteria stimulated cell mediated immunity by enhancing the production of IFN-\(\gamma\) by polymorphonuclear leukocytes (PMN) and to a lesser extent of monocytes and enhanced expression of complement receptor on PMN's (Word 2001). Anti-inflammatory effects of the \textit{L. plantarum} was studied by treating it with intestinal epithelial cell and macrophage or primary culture murine dendritic cells (Petrof et al., 2009). It has also been found that the \textit{L. plantarum} was able to inhibit NF-\(\kappa\)B

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binding activity, degradation of 1 κBα and the chymotrypsin-like activity of the proteasome. This would give a novel strategy for the treatment of intestinal inflammation which can eliminate the risk of bacteremia. *Lactobacillus lactis* ssp *lactis*, *L. sakei* and *Leuconostoc mesenteroides* fed brown trout (*Salmo trutta*) showed the greater complement activity and immunoglobulin in the serum and elevated level of lysozyme activity (Balcazar et al., 2007). Overall probiotics bring defense to the host by activating non specific, local, systemic, secondary, mucosal and both cell and humoral mediated immune response.

3.11 Colorectal cancer

Globally, colorectal cancer is the third leading cause of cancer in males and fourth leading cause of cancer in females. Colon and rectal cancers are often referred as colorectal cancer. Colorectal cancer mortality rates among whites generally tend to be lower in Western states, with the exception of Nevada, and higher in some Southern and many Midwestern states. However, colorectal cancer mortality rates are substantially higher among African Americans compared to whites; the highest age-adjusted state mortality rate among African American men is 39.1 per 100,000 (Iowa), compared to 25.3 (West Virginia) among white men (American cancer society 2011).

Development of colorectal cancer represents a sequence of events. First step is an initiation step, in which a carcinogen produces an alteration in the DNA leads to the metabolic activation of a precursor to produce the carcinogen. It is believed that several mutations must occur for a tumor to develop. Further, overgrowth in colonic crypts can be seen morphologically as aberrant crypts. Aberrant crypts have a serpentine growth pattern may occur singly or as in groups within a single focus that will progress to polyps and eventually tumors (Brady et al., 2000).

Development of colorectal cancer may be in different stages. Initially, in stage 0 and the cancer is very early and observed only in the innermost lining of the colon and the rectum. The next stage is stage 1, the cancer involves more of the inner wall of the colon or the rectum. It is followed by stage 2, the cancer spread out side the colon or the rectum to nearby tissue but not with the lymph nodes. Further, in stage 3, cancer cells spread to other parts of the body. Colorectal cancer tends to spread to liver and lungs. Finally, stage 4, where the cancer
has spread to nearby lymph nodes, but not to other parts of the body. In the latter stages cancer will spread to the entire organs of the body (Brady et al., 2000).

Change in bowel habits, diarrhea, constipation, appearance of either bright red or very dark in the stool, narrower stools than usual, general abdominal discomfort such as, pain, bloating fullness, cramps, weight loss with no known reason, tiredness and vomiting are some of the symptoms associated with colorectal cancer (http://www.cancer.gov/cancertopics).

3.11.1 Diagnostic tests for colorectal cancer

There are several diagnostic methods are available for the detection colorectal cancer such as, FOBT (A fecal occult blood test): It is to check for hidden blood in the stool. Sometimes cancers or polyps can bleed, and FOBT is used to detect small amount of bleeding. FOBT, aims to detect subtle blood loss in the gastrointestinal tract, anywhere from the mouth to the colon. Positive tests may result from either upper gastrointestinal bleeding or lower gastrointestinal bleeding and warrant further investigation for peptic ulcers or a malignancy (such as colorectal cancer or gastric cancer). The test does not directly detect colon cancer but is often used in clinical screening for that disease, but it can also be used to look for active occult blood loss in anemia (Harewood and Ahlquist 2000) or when there are gastrointestinal symptoms (Bardhan et al., 2000).

Colonoscopy is the endoscopic examination of the colon and the distal part of the small bowel with a CCD camera or a fiber optic camera on a flexible tube passed through the anus. It may provide a visual diagnosis (e.g. ulceration, polyps) and grants the opportunity for biopsy or removal of suspected lesions. Colonoscopy can remove polyps as small as one millimetre or less. Once polyps are removed, they can be studied with the aid of a microscope to determine if they are precancerous or not. Colonoscopy is similar to, but not the same as, sigmoidoscopy. The difference being related to which parts of the colon each can examine. A colonoscopy allows an examination of the entire colon (measuring four to five feet in length). A sigmoidoscopy allows an examination of the distal portion (final two feet) of the colon, which may be sufficient because benefits to colonoscopy (cancer survival) have been limited to the distal portion of the colon (Baxter et al., 2009, Singh et al., 2010, Brenner et al., 2010). A sigmoidoscopy is often used as a screening procedure for a full colonoscopy, often
done in conjunction with a fecal occult blood test (FOBT). About 5% of these screened patients are referred to colonoscopy (Atkin et al., 2010).

DCBE (A double contrast barium enema) is an advanced method where series of X-rays of colon and rectum has been taken and observed. The patients were given an enema with a solution that contains barium solution and air is introduced into the colon which outlines the colon and rectum on X-rays. It detects about 30 to 50 percent of the cancers that can be found with standard colonoscopy (Cancer. Gov/clinical trial), PDQ ®, NCI’s database http://cancer.gov). DRE (A digital rectal exam) insertion of a lubricated, gloved finger in to the rectum to feel the abnormal areas. X-Ray is the most common method where X-rays of large intestine will reveal the polyps or other changes. DRE allows examination of only the lower part of the rectum. It is often performed as part of a routine physical examination.

3.11.2 Treatments for colorectal cancer

There are various treatments strategies are available to cure colorectal cancer depends on size, location and extent of the tumor and on the patients general health. Gastroenterologist, medical oncologist and radiation oncologist are the experts giving treatment to colorectal cancer (Beck et al., 2007). Surgery is the most common treatment involving the removal of tumors. Generally in surgery removal of the tumor along with the parts of the healthy colon or rectum and nearby lymph node may takes place. The removed healthy parts can be reconnected. In case of inability to reconnect, a new surgical opening (stoma) can be made through the wall of abdomen to provide a new pathway for waste material. Patients need to carry a temporary bag to collect waste material from the body (Tjandra et al., 2006).

Chemotherapy is another common treatment where anticancer drugs are used to kill the cancer cells. It may be used to destroy any cancerous cells, to control the development of the tumor or to relieve the symptoms of the diseases. Systemic chemotherapy, where the drugs enter the blood stream and travel through the body. Most of them are given by injection directly in to the vein (IV) or by the means of catheter, or in the form of a pill. (Cancer. Gov/clinical trial, PDQ ®, NCI’s database http://cancer.gov). Radiation therapy involves the use of high energy X-rays to kill cancer cells. Radiation therapy is the local therapy where it affects
to the cancer cells only in the treated area and often used before surgery. It is from external radiation, come from machine or is called an implant. A small container of radioactive material is placed directly in or near the tumor is called internal radiation (Cancer. Gov/ clinical trial, PDQ®, NCI’s database http://cancer.gov). Biological therapy is also called immunotherapy. It uses the body’s immune system to fight cancer. They are used to repair, stimulate or to enhance the immune system. It is being used after surgery, either in combination with chemotherapy or with radiation therapy. Most of the biological therapies are given by injection in to vein IVR (Cancer. Gov/ clinical trial), PDQ®, NCI’s database http://cancer.gov).

Most of the side effects are temporary; there will be short term pain and tenderness in the area of operation. It may cause temporary constipation or diarrhea. Irritation of the skin around stoma is the common side effect associated with the surgery. Enterostomal therapist teaches the patients how to clean the area to prevent irritation. Chemotherapy can also affect normal cells. Nausea, vomiting, hair loss, mouth sores, diarrhea and fatigue, less often serious side effects may occur, such as infection or bleeding. Common side effects from radiotherapy are fatigue, skin diarrhea. Some times it can cause bleeding through the rectum (bloody stools). Even the biological therapy causes the side effects like symptoms, such as, chills, fever, weakness and nausea (Cancer. Gov/ clinical trial), PDQ®, NCI’s database http://cancer.gov). Although, these treatments are available, it is expensive and would not guarantee in curing the condition. Therefore, prophylactic measures are better in preventing this condition.

3.12 Probiotics in controlling cancer

Cancer is an important global public health problem. Cancer is a disease where abnormal cells divide without control and form a lump (tumor) as their numbers increase. Cancer cells can invade nearby tissues and can spread through the bloodstream and lymphatic systems to other parts of the body. Cancer incidence and mortality have been steadily rising throughout the world since last century. Cancers are classified by the type of cell that the tumor resembles and is therefore presumed to be the origin of the tumor. Based on these cancers are usually named carcinoma (cancers derived from epithelial cells), sarcoma or blastoma (cancers derived from connective tissue), lymphoma and leukemia (cancers derived from hematopoietic cells), germ cell tumors (cancers derived from pluripotent cells) and blastoma (cancers derived...
from immune precursor cells or embryonic tissue). Although, breast, prostate, lung, pancreas and colon cancers are the most leading carcinoma cancers, among these lung, colon and breast cancer causing the most deaths each year in the world. There are more than 200 types of cancers were known up to date (Cancer. Gov/ clinical trial, PDQ ®, NCI’s database http://cancer.gov).

A number of studies conducted by various research groups indicated that the potential effect of probiotics in controlling cancer. *Lactobacillus acidophilus* reduced the tumor in rats challenged with DMH (Dimethyl hydrazine hydrochloride) (McInosh et al., 1999). *Lactobacillus rhamnosus* GG was found to inhibit the MGH human bladder cancer cell lines proliferation and it was cytotoxic to RT112 cells. *Lactobacillus casei* or *L. rhamnosus* strain GG (1 x 10⁷ and 1 x 10⁸ cfu) or BCG (Bacillus Calmette-Guerin) (1 x 10⁷ cfu) was found to be cytotoxic to the mammalian cell lines. BCG had a similar cytotoxic effect in MGH cells as *Lactobacillus* sp but was not as effective in RT112 cells. Streptomycin abrogated (abolish) the cytotoxic effect of *Lactobacillus* sp but not that of BCG. Cytotoxic activity was not found in Lactobacilli culture supernates but it was induced in the presence of mammalian cells. Unlike BCG, it requires bacterial protein synthesis. Like BCG, *L. casei* induced cell death primarily via necrosis *Lactobacillus rhamnosus* and *L. casei* can be used in the treatment of bladder cancer (Seow et al., 2002). In addition, *Lactobacillus* strains were found to be protective against chemically induced tumors in rats and also found to be reducing the formation of superficial bladder tumors in humans. Oral administration of *L. casei* (3 g/day) preparation and biolactis powder (BLP) for 350 days have been found to be preventive on the recurrence after transurethral resection of the bladder tumor (TUR-Bt) in bladder cancer patients (Aso and Akazan 1992).

*Enterococcus faecium, E. durams, E. avium* and *L. reuteri* isolated from human feces were found to be having capacity to transfer 2-amino 1- methyl-1,6 phenylimidazo (4,5-6) pyridine [PhIP] a dietary carcinogen formed in meat products during cooking into its microbial food derivative 7-hydroxy-5 methyl-3 phenyl-1,6,7,8,9- tetrahydropyrido (3^{12}^{1}-4,5)imidazo (1,2-α) pyridine-5 chloride [PhIP-M1] which was a non toxic compound (Vanhaecke et al., 2008). The symbiotic combination of *L. rhamnosus* GG and *B. lactis* as a probiotic, oligofructose enriched inulin as a prebiotic has reduced the capacity of fecal water to
induce necrosis in colonic cells and showed improved epithelial barrier function in polypectomized patients (Liong 2008). There are several studies were in progress in the prevention of lung, breast as well as colorectal cancer by probiotic therapy. It has been observed that ingestion of L. acidophilus in humans significantly reduced the excretion of mutagens following consumption of meat heavily browned or burnt by cooking at high-temperature (Lidbeck et al., 1992).

Lyophilized cultures of B. longum found to reduce the percentage of tumors in colon, small intestine, liver and lesser in mammary gland in 2- amino 3- methylimidazo {4,5-6} quinoline induced carcinogenic male and female rats by altering the physiological conditions which effect the metabolic condition of intestine and bile acid produced by bacteria (Reddy and Rivenson 1993).

3.13 Probiotics in controlling colorectal cancer (CRC)

Probiotic strains were proved to be excellent chemotherapeutic agents. Dietary administration of lyophilized cultures of B. longum resulted in significant suppression of colon tumor incidence and tumor multiplicity and also reduced the tumor volume in male azoxymethane induced rats (Singh et al., 1997). It was also been observed that a reduced the cell proliferation, ODC (ornithine decarboxylase) activity and expression of ras-p21 oncoprotein. Freeze dried Lactobacillus sp/samples from the dairy products had been found to attach strongly to human Caco-2 cell line. While L. acidophilus and L. bulgaricus showed no adhesion to the cell line and the strains of Bifidobacterium showed very weak adhesion to the Caco-2 cell line (Elo et al., 1991). The adhering properties of probiotics mainly inhibited the attachment of carcinogen sites to the cell receptors and therefore bring about anticancer effects.

The risk of colorectal cancer is determined by interactions between the external (dietary) and internal (bacterial) environments. O'Keefe et al., (2007) reported that the incidence of CRC was dramatically higher in African Americans than in native Africans. They conducted a study to analyze the difference in incidence by examining interaction between diet and colonic bacterial biota using healthy middle aged group of humans. Diet was measured by 3-d recall and colonic metabolism by breath hydrogen and methane responses to oral lactulose.
On culturing faecal samples, 7-α dehydroxylating bacteria and *L. plantarum* were recovered. Further, colonoscopic mucosal biopsies were used to measure the proliferation rates, and reported that when compared to native Africans, African American consume meat, saturated fat and cholesterol along with more calcium, vitamin A and C with fiber. Breath hydrogen was higher and methane was lower in African American and faecal colony counts of 7-α dehydroxylating bacteria were higher and while *Lactobacilli* were lower. Further, it was also observed that colonic crypt cell proliferation rates were dramatically higher in African Americans and proposed that higher CRC risk in African Americans than native Africans and they hypothesized that CRC risk was determined by interaction between - dietary and bacterial environments in the intestine.

Oligofructose and inulin, naturally occurring fermentable chicory fructans, was shown to stimulate the growth of Bifidobacteria which were regarded as beneficial strains in the colon and inhibit preneoplastic lesions such as aberrant crypt foci (ACF) formation in colon carcinogenesis azoxymethane induced male rats. The feeding of oligofructose or inulin significantly inhibited the ACF formation and crypt multiplicity in the colon (Reddy et al., 1997). Further, prebiotics such as, oligofructose, inulin along with lyophilized cultures of *B. longum* were found to inhibit the formation of preneoplastic lesions in the colon (Reddy 1999). While, *B. longum* in combination with inulin resulted in decrease in β-glucoronidase activity and ammonia concentration in caecal content in azoxymethane induced male Sprague-Dawley rats. Furthermore, the combined administration significantly decreased the incidence of large ACF in experimental animals (Rowland et al., 1998). These results suggested that consumption of *B. longum* or inulin was associated with potentially beneficial changes in caecal physiology and bacterial metabolic activity in relation to tumor risk and in the incidence of putative preneoplastic lesion in the colon.

It has been observed that synbiotic combination of oligofructose enriched inulin, *L. rhamnosus* GG and *B. lactis* have been found to reduce the colorectal proliferation particularly by preventing the increased production of interleukin 2 by peripheral blood mononuclear cells in polypectomized patients and increased production of INF γ in cancer patients in fecal water induced carcinogenesis (Rafter et al., 2007). Synbiotic consumption prevented the increased secretion of IL-2 by peripheral blood mononuclear cells in the
polypectomized patients and increased the production of INF γ in the cancer patients. Prebiotics such as fructans, and probiotics such as, *B. lactis* and *L. rhamnosus* or the combination reduced the malignant tumors and found to protect the rats against azoxymethane induced colon cancer in male rats (Femia et al., 2002). Lyophilized cultures of *B. longum* was fed to the azoxymethane induced male rats along with the basal diet for six weeks. It has been observed that the feeding of lyophilized cultures of *B. longum* significantly inhibited the ACF formation and crypt multiplicity in the colon. It has also been observed a significant decrease in the fecal bacterial beta-glucuronidase in the probiotic fed animal group (Kulkarni and Reddy 1994). The large ACF (those comprising four or more aberrant crypts per focus) had significantly decreased in azoxymethane induced rats which had consumed the *L. casei* strain Shirota diet. The number of rats with colon cancers and the number of colon cancers per rat, were significantly decreased in the rats which had consumed the *L. casei* diet. *Lactobacillus casei* strain inhibited chemically-induced colon carcinogenesis in rats (Yamazaki et al., 2000).

Therefore, the consumption of probiotic would decrease the risk of colon cancer particularly in production of aberrant crypt in tumor development. The results of these could be beneficial in reducing the risk of CRC in animal models that can be extrapolated to human system. Majority of evidence for anticancer effects of probiotics are gathered from animal studies, while the evidence from human studies are scarce.

3.14 Catharanthus roseus

*Catharanthus roseus* belongs to the family Apocynaceae commonly called periwinkle, Nityakalyani, sadabahar, Sadaphul etc has its native from Madagascar. Abundantly naturalized in many regions, particularly in arid costal locations. Grown commercially for its medical uses in Australia, Africa, India and Southern Europe. Its leaf, flower and root extracts has more than 400 alkaloids approved as antineoplastic agents to cure leukemia, Hodgkin’s diseases, malignant lymphoma, neuroblastoma, rhabdomyosarcoma, Wilm’s tumor and other cancers (Nayak and Pereira, 2006). Vincristine and vinblastin are the two important alkaloids are used in several types of cancers. One tone of its leaves yield 50 grams of vincristine sulfate in pure form.
Catharanthus roseus flower extract showed wound healing property and antimicrobial activity against *Pseudomonas aeruginosa*, Beta-hemolytic *Streptococci*, *E. agglomerans* and *S. aureus* increased tensile strength, increased hydroxyproline content in healthy inbred gender-matched Sprague Dawley rats (Nayak and Pereira, 2006). Indole alkaloids from the plant *C. roseus* strengthened immune system and showed antimicrobial activity against *S. typhimurium*, *S. aureus*, *B. subtilis*, *P. aeruginosa* (Patil and Ghosh 2010).

Vincristine and vinblastin are the two complex indole alkaloids used in the treatment of diabetes and several types of cancer in ayurveda (Noble 1990, Garodia et al., 2007). Vincristine (VCR) in combination with betulinic acid (BA), a pentacyclic triterpene, showed to be effective inhibition of metastasis of tumor cells in the lung and bring enhancement of the chemotherapeutic effect on malignant melanoma (Sawada et al., 2004). Where as, combined use of VCR and indomethacin have been found to be useful in modulating therapy for advanced lung cancer tested in all cell lines such as colon carcinoma, melanoma and tetracarcinoma (Kobayashi et al., 1998). Combination of vincristine with topotecan resulted in significantly greater response in tumor lines such as, neuroblastoma, brain tumors and in rhabdomyosarcomas in CBA/CaJ mice implanted with tumor cells. Vincristine induced complete responses in all mice bearing two rhabdomyosarcomas and relatively few responses in other tumours (Thompson et al., 1999).

Synergistic supra additive combinations of vincristine (VCR) with methotrexate (MTX) sensitive human neoplasm were found to reduce the human acute lymphoblastic leukemia cell lines. Simultaneously, VCR with daunorubicin, 1-β-D-arabinofuranosylcytosine or bleomycin also had protective effects. VCR with daunorubicin, VCR with 1-β-D-arabinofuranosylcytosine and VCR with vinblastin or L-asparaginase were found to be having sub additive and protective effects in human acute lymphoblastic leukemia cell line (MOLT-3) (Kano et al., 1988). Further, (Junior et al 2009) evaluated the effects of vincristine on gastrointestinal motility of awake rats and found that the increase in colon weight. Chronic vincristine treatments with total cumulative doses of at least 250 μg/kg significantly decreased gastric emptying (GE) by 31-59% and gastrointestinal (GI) transit by 55-93%. Colon weight
was found to increase after 2 and 5 doses of vincristine. Fecal output has found to decrease up to 48 h after the fifth dose of vincristine.

Although, several treatments available for CRC such as, chemotherapy, adjuvant therapy, gene transfer therapy ultimately organ transplantation for curing colorectal cancer, they are very painful, expensive and burden to the lower sections of the society who are in need. To overcome the side effects of the available treatments, the cost effective one treatment has to be established. Probiotic application in controlling colorectal cancer would be choice by regular practice the CRC incidence could be reduced or prevented or postponed. By enhancing the normal intestinal flora through the diet will maintain the equilibrium by which it may prevent the development of colorectal cancer. In addition, several chemotherapeutic drugs along with such probiotics will give more additional benefits. Vincristine is one of such chemotherapeutic drug which is used in the treatment of Hodkin lymphoma, neuroblastoma, non- Hodgkin lymphoma (NHL), rhabdomyosarcoma, Wilm’s tumor and colorectal cancer in these days (http://www.cancer.gov/cancertopics). Probiotics along with vincristine the extract of the plant C. roseus would provide an additional beneficial effect in controlling colorectal cancer.

Further, probiotic treatment and probiotic along with vincristine would give a new idea of synbiotic combination in control the colorectal cancer. Complete curing perhaps does not takes place with available therapeutic measures in addition to being expensive. However, it is always safe to avoid the CRC condition prophylactically. Therefore, the present research program was undertaken to study the synergistic impact of Lactobacillus sp. with vincristine in controlling colorectal cancer.