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Lactobacillus sp. as probiotics for human health with special emphasis on colorectal cancer

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Abstract

Probiotics are live microorganisms when administered in adequate amount confer health benefit to the host. Extensive usage of probiotics in dairy as a starter in preparation of cheese and other dairy products has become popular in the recent past. The representatives of probiotics mainly include Lactic Acid bacteria such as *Lactobacillus acidophilus, L. plantarum, L. johnsonii, L. gasseri, L. casei, L. rhamnosus* and *Bifidobacterium longum, B. breve, B. infantis, B. thermophillum, B. pseudolongum* and others. A number of animal studies conducted by various research groups indicated that the probiotics possess potential anticancer effects. Colorectal cancer (CRC) is one of the most devastating diseases causing high morbidity and mortality among human in most of the urban, eastern and western parts of the world. Oral intake of large number of substances among human perhaps act as mutagenic compounds resulting in change of native microbiota of intestine and could induce cancer. In the present review, the beneficial effects of probiotics in reducing the risk of colorectal cancer (CRC) have been discussed drawing observations from an ongoing related research work in our laboratory. The present article also focuses to describe the beneficial applications of *Lactobacillus* spp. particularly in human.

Keywords: Probiotics, Lactic acid bacteria, *Lactobacillus,* Colorectal cancer.

Introduction

Probiotics are dietary supplements containing potentially beneficial bacteria needed for the betterment of gastrointestinal tract (FAO, 2002). Probiotics can also be defined as the preparations or product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or by colonization in the host and exert beneficial health implications on their host. While, prebiotics are the non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon. Prebiotics are also known as colonic foods and mainly are the symbiotic make up of fructooligosaccharide molecules and short- chain sugar molecules containing fructose molecules. Prebiotics derived from insoluble fiber and fructosaccharide sugars, are often found in fruit and honey (Thomas & Greer 2010)

Probiotics convert sugars and other carbohydrates into lactic acid and produce a characteristic sour taste to fermented dairy foods and in fortified foods. Some of the probiotic bacteria act as preservative by lowering the pH of the medium and reduce opportunities for spoilage organisms to grow. Harish & Varghese (2006) reported that the fermented dairy products and freeze-dried bacteria are most popular vehicles for delivering these organisms to the gastrointestinal tract of human and animal system. Further, they have also been used in preparation of feed for poultry, dairy and fisheries to maintain better health in recent times.

Probiotic bacteria

Gastrointestinal tract of human have been inhabited by a variety of microorganisms. Physiological balance of this microbiota is greatly influenced by intestinal environment. Among the numerous intestinal bacteria that beneficially affect the host intestine, some could be recognized as probiotics (Ishibashi & Yamazaki 2001).

More than eighty species of microorganisms have been recorded as probiotics from various sources. Among them, the most used *Lactobacillus* sp. are present in raw milk, dairy products as well as infant, children and adult faeces (Coeuret *et al*., 2003). The representative species of probiotics *viz*., *Lactobacillus* and *Bifidobacterium* include *Lactobacillus acidophilus, L. plantarum, L. johnsonii, L. gasseri, L. casei, L. rhamnosus, Bifidobacterium longum, B. breve, B. infantis, B. thermophillum, B. infantis, B. pseudolongum* and others. In addition to these, dairy product comprising *L. bulgaricus, Streptococcus thermophilus* and *Leuconostoc* could be used as probiotics.

However, *Lactococcus* which is not in the group of probiotics help in intestinal microbial balance (Ishibashi & Yamazaki, 2001). The extensive usage of *Lactobacillus, Leuconostoc* and *Pediococcus* in food processing has been recorded in the history of mankind. However, safety of consumption of these probiotics have not been questioned until now, although, absence of pathogenicity and infectivity are the prerequisites in them. In addition to this, factors to be addressed for evaluation of probiotics include virulence as well as intrinsic properties. Epidemiological studies or post marketing surveillance,

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are the criteria that are recommended to assess the safety of probiotics. In addition, efficacy and health promoting effects of probiotics have been considered for specific strains (Salminen, 2001). Marteau (2001) had proposed studies on intrinsic factors, pharmacokinetics and interaction between hosts and probiotics to assess the safety of probiotics.

Probiotic bacteria are found to be potentially useful in managing lactose intolerance, lowering of cholesterol and blood pressure, *Helicobacter pylori* infection, antibiotic associated diarrhea, inflammation, irritable bowel syndrome, colitis, gastro-enteritis, rotavirus gastro-enteritis in children, ulcerative colitis, Crohn's disease, pouchitis, intestinal cancer, mammary gland cancers and in colorectal cancer (Harish & Varghese, 2006).

Isolation of probiotic lactobacilli

Coeuret *et al*. (2003) reported a number of culture media for *Lactobacilli* which include resuspension medium enriched with phosphate, cysteine, antifoaming agents and sugar before plating on de Man, Rogosa, Sharpe (MRS) medium, MRS medium has been routinely employed for the isolation and enumeration of lactic acid bacteria (LAB) in most food (fermented) products (Shah, 2000; Mirlohi *et al*., 2008). Coeuret *et al*. (2003) reviewed the application of MRS with vancomycin and bromocresol green, a new anaerobic media for the enumeration of *Lactobacilli* from faeces and various dairy products. Furthermore, they described the X- glu agar being selective for *L. acidophilus* from yogurt and related milk products. Presently, widely used MRS medium is considered to be an ideal one for the cultivation of lactic acid bacteria.

Phenotypic identification of lactobacillus

Lactobacillus is a non-motile, non-spore forming, Gram-positive bacteria and the cell morphology varies widely from long, straight or slightly crescent shaped bacilli to coryneform *coccobacilli.* Classical phenotypic tests for identification of *Lactobacilli* are based on physiological characteristics such as respiratory type, motility, growth, temperature sensitivity as well as growth in NaCl. *Lactobacilli* are typically chemo-organotrophic and ferment carbohydrates producing lactic acid as a major end product (Kandler & Weiss 1986). Protein analysis such as protein finger printing or multi-locus enzyme electrophoresis are advanced phenotypic methods used in identification of *Lactobacillus* spp (Coeuret *et al*., 2003).

Molecular methods for identification of lactobacilli

A number of probiotic research groups have described molecular methods for the identification of species through taxonomic analysis by DNA/DNA hybridization, sequencing, polymerase chain reaction (PCR), ribotyping. Polymorphism analysis permit strain differentiation by the use of techniques such as, restriction enzyme assay (REA), randomly amplified ploymorphic DNA (RAPD), repeated sequence extragenic palindromic PCR (REP-PCR), amplified fragment length polymorphism (AFLP), plasmid profiling and pulsed field gel electrophoresis (PFGE) (Gilliland *et al*., 1975; Collins *et al*., 1987; Williams *et al*., 1990; Janssen *et al*., 1996; Brandt *et al*., 2000; De-Angelis *et al*., 2001; Coeuret *et al.,* 2003).

Furthermore, Pot *et al*. (1993) described the partial sequence analysis of 23S rRNA genes of *L. acidophilus, L. johnsonii, L. gasseri* and showed that the DNA probe Lbg was proven to be specific for the strains belonging to *L. johnsonii*. The combined use of differential plating and molecular strain typing methodologies provided food and medical microbiologists a best tool for the enumeration and identification of *Lactobacillus* groups.

Ribotyping proved to be more effective, although some strains appeared to have identical ribotype and chemotype patterns. Therefore, Zhong *et al*. (1998) reported that more specific identification methods such as the use of repetitive sequence PCR probes, are most preferred. The taxonomy and physiology of probiotic LAB can only be understood by using polyphasic taxonomy combining morphological, biochemical and physiological characteristics with molecular based phenotype and genotype techniques (Klein *et al*., 1998). Screening new primers in RAPD analysis and using other restriction enzymes in ribotyping could possibly increase specificity for strain typing (Tynkkynen *et al*., 1999). In addition, Torriani *et al*. (2001) differentiated a *L. plantarum* through phylogenetic analysis of partial rec A gene sequence.

Dimitrov (2009) identified *Lactobacillus* and *Bifidobacterium* at species level by the combination of ARDRA (amplified ribosomal DNA restriction analysis) (by using enzymes Hae 111, Msp 1 and Eco RI) and SDS-PAGE. Stoyancheva *et al*., (2009) performed ARDRA analysis for the identification of *L. delbrueckii, L. helviticus* and *L. acidophilus* and other thirteen isolates from dairy foods.

Culture independent method of identification involves the extraction of nucleic acid DNA or RNA from raw samples by using probes for hybridization and primers for denaturing gradient gel electrophoresis (DGGE), temperature gradient gel and by linear temperature gradient, and a single strand conformation polymorphism (SSCP) which detect the variable region of the 16S r-RNA gene and used neutral non- denaturing polyacrylamide gels (Coeuret *et al*., 2003). They further reported that the analysis of *Lactobacilli* in dairy product is very complicated and use of molecular based phenotypic or genotypic technique would give more accurate results**,** indicating the diversity of *Lactobacillus* sp.

Probiotics for human health

Various investigations have projected numerous therapeutics and control measures for gastrointestinal disorders, asthma in addition to several types of cancers

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using probiotics. Native microorganisms from yogurt would prevent infections of gastrointestinal tract by influencing the microbial ecosystem. The inhibitory mechanisms of LAB against pathogenic bacteria are primarily due to the production of organic acid and bacteriocins (Gorbach *et al*., 1987; Kim 1988). Kaila *et al.* (1992) have recorded that, in children using oral microbial feeding with LAB has prevented acute rotavirus associated diarrhea and antibiotic induced gastrointestinal disorders. Further, they reported that LAB augmented the local immune defense by increasing the number of immunoglobin secreting cells and perhaps improve the gastrointestinal system of children with acute rota virus associated diarrhoea. Harish & Varghese (2006) also reported that probiotics have been proposed to be a novel approach in the management of allergic diseases especially in infants.

In addition, oral intake of *Bifidobacterium longum* in yogurt with erythromycin have been observed to reduce the frequency of gastrointestinal disorders. Thus, consumption of LAB would reduce antibiotic induced changes of the intestinal ecosystem (Kaila *et al*., 1992). Audin *et al*., (2008) suggested that the daily consumption of Acimel, a probiotic fermented dairy product significantly improved seroprotection against H1N1 strain observed in elderly women although the mechanism of protection is yet to be resolved. Several animal and studies in human have shown beneficial effects of LAB in yogurt consumption as it aids in building resistance to gastrointestinal pathogens. Recent report reveals that probiotics play precise role in preventing all types of diarrhea, gastroenteritis (Thomas & Greer 2010), inflammatory bowel diseases, ulcerative colitis, Crohn's disease, pouchitis, irritable bowel syndrome (IBS), lactose intolerance, colon cancer, constipation (Ajmal & Ahmed, 2009), infection and allergy (Rijkers *et al*., 2010), peptic ulcer, stimulation of immune system, antifungal actions and preservation of food (Masood *et al.,* 2010).

Immunological aspects of probiotics

It has been found that phagocytic activity of macrophages was significantly higher in mice fed with milk containing *B. bifidum, L. acidophilus, L. casei, L. helveticus* than in control mice fed with unfermented milk (Perdigon *et al*., 1988). Furthermore, they showed that feeding milk fermented with *L. casei and L. acidophilus* increased *in vitro* and *in vivo* phagocytic activity of peritonial macrophages against sheep RBC in Swiss mice. In addition, *L. acidophilus* induced the production of interferon (INF)-α and INF-β in murine peritoneal macrophage cell culture (Kitazawa *et al*., 1992). Also, dose dependent orally administered LAB and yogurt increased secretory IgA producing cells in the small intestine in an experimental animal (Perdigon *et al*., 1988).

Later, Puri *et al*., (1996) reported that serum IgA concentration in yogurt fed mice were significantly higher than the milk fed mice after *Salmonella* challenge. Earlier, Takahashi *et al*., (1993) reported that specific IgG and IgA production in mice administered with LAB when compared to control non administered group. These studies perhaps indicated that the IgA secreted by the intestinal B cell enter the circulation and raise the serum IgA concentration. The influence of a yogurt-supplemented diet on the immuno competence and survival of animals subsequently infected with *Salmonella typhimurium* suggested that live LAB would enhance local and systemic immune response (De Simone *et al*., 1988). Additionally, increased phagocytic activity of human blood cells particularly granulocytes after the injection of fermented milk with *L. acidophilus* and *B. bifidum* have also been reported (Schiffrin *et al*., 1997 & Schiffrin *et al.,* 1995).

An interesting report has been made on innate acquired immune influence and observed the expression of innate immune response of CV206, TLR2, IgA^+, $CD4^+$, $CD8^+$ cells as well as cytokines which increased upon *L. casei* treatment in the small intestine of mice. In addition, the consumption of yogurt containing viable LAB was shown to increase INF-β, IL(interleukin)-6, IL-10, INF-γ and TNF (Tumor necrosis factor)-α production (Halpern *et al*., 1991, Miettinen *et al*., 1996; Aattouri & Lemonnier 1997). Human and animal studies indicated that oral intake of LAB yogurt would stimulate the immune response with the higher production of cytokine, macrophage activity leading to better cell mediated immune response. Further, Crittenden *et al*., (2005) reviewed the intestinal microbial ecosystem and interaction between gut bacteria, diet and health of the human host.

Tao *et al*., (2006) investigated that the probiotic *Lactobacilli* induced heat shock in intestinal epithelial cell in a time and concentration dependent manner in conditioned media. They observed that the effects were mediated by low molecular weight peptide which was an acid and heat stable. This induced the expression of cryoprotective proteins in gut epithelial cells and activated signal transduction pathway, thus, inducing beneficial clinical effects.

Very recently, it has been found that, curd enriched with *L. bulgaricus* and *S. thermophilus* increased the levels of cytokine (INF-α, INF-λ, IL-10, IL-4) there by increased secondary immune response (Dewan *et al.*, 2009), perhaps a boon for malnourished children. A probiotic *Bacillus substilis natto* increased serum IgG and IFN-λ levels and increased general performance by improving the average daily weight gain in Holstein male calves (Sun *et al.*, 2010). van Hemert *et al.,* (2010) isolated *L. plantarum* from human source and identified the genes responsible for anti inflammatory cytokine IL-10 and secretion of pro- inflammatory cytokine IL-12. Also, *L. fermentum* was found to induce immunological response by reducing pro- inflammatory cytokine interleukin- 10 in the liver without typhoid nodule in mice

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challenged with *S. typhimurium* (Truusalu *et al.,* 2010). Probiotic curd containing *L. acidophilus* and *L. casei* suppressed ovalbumin specific IgE and lymphocyte in ovalbumin induced allergy in mice (Jain *et al.,* 2010) indicating antiallergic potential of *Lactobacillus* spp.

Probiotics in controlling cancer

Rats challenged with carcinogen DMH fed with *L. acidophilus* developed fewer tumors than animals treated with other LAB **(**McIntosh *et al*. 1999). In addition, *Lactobacillus* strains were found to protect against chemically induced tumors in rats and reduce the formation of superficial bladder tumors in humans (Aso & Akazan 1992; Yamazaki *et al*., 2000).

Seow *et al.* (2002) investigated the high growth inhibition in human bladder cancer cell lines using *Lactobacillus* and compared with the effect of *Mycobacterium bovis* (BCG) on human bladder cell lines. They reported that *Lactobacillus* spp induced cytotoxic effects in bladder cancer cell and stated the use of *L. rhamnosus* and *L. casei* bacterial species in treating bladder cancer was encouraging.

Enterococcus faecium, E. durans, E. avium and *E. reuteri* isolated from human feces had the capacity to transfer 2- amino 1- methyl 1-6 phenylimidazo{4,5-6} pyridine (PhlP) a dietary carcinogen into its non toxic form (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).

Colon cancer risk in India and in the world

Colon cancer rates in India are lower than those seen in western countries. Colon cancer rates vary from 4.7 in male and 3.2 in female per 1,00,000 population in India to 40.6 in male, 30.70 in female in United States (Sinha *et al.,* 2003). Colorectal cancer (CRC) is the fourth most common cancer in men and third most common cancer in women worldwide. IARC (International Agency for Research on Cancer) found that in Slovenia, CRC incidence increased 70% among men and 28% among women. In Miyagi, Japan rates are 92% among men and 47% among women. Substantial regional variations of CRC incidence have been observed in the countries such as, Japan, Israel and Singapore. Recent reports states that, the United States was the only country where CRC incidence rates declined in both in males and females (Centere *et al*., 2009; Umar & Greenwald, 2009)

Probiotics in controlling colorectal cancer

Colorectal cancer is one of the most devastating diseases causing high morbidity and mortality among human in most of the urban, eastern and western countries. Change of life style, food would perhaps attribute to change of native microbiota of intestine and act as mutagen in inducing cancer.

Development of colorectal cancer (CRC) involves a sequence of events although, partially being understood. During initiation step, mutagen causes alteration in the native DNA, followed by metabolic activation of a precursor to produce the carcinogen. Next step is involved with changes in signal transduction pathway followed by overgrowth in colonic crypts or aberrant crypt. These aberrant crypts were considered to be pre-neoplastic structures. They are enlarged and elevated than normal crypts and show cell lines growth patterns. Aberrant crypt would occur singly or in groups and certain fractions of these aberrant crypts would progress to polyps to become tumors. Brady *et al*. (2000) suggested that pro-biotics with or without pre-biotics have an inhibitory effect on the development of aberrant crypts and reported that it is difficult to analyze the efficacy of probiotics to control tumor development at initiation stage or post initiation stage.

Rafter *et al*. (2007) investigated on dietary symbiotics that would reduce cancer risk factors in polyectomised and colon cancer patients. Animal studies suggested that pre and probiotics exert protective effects against tumor development in the colon. The prebiotic concept as induced by a synbiotic preparation - oligofructose enriched inulin and *L. rhamnosus, Bifidobacterium lactis* to reduce the risk of colon cancer in human. It was found that synbiotic intervention resulted in significant changes in faecal microbiota that is, *Bifidobacterium* and *Lactobacillus* increased while *Clostridium perfringens* decreased. This intervention reduced colorectal cancer proliferation and the ability of faecal water to induce necrosis in colonic cells and improved epithelial barrier function in polypectomised patients and increased production of INF-λ in cancer patients. They further reported that several colorectal biomarkers can be altered by synbiotic intervention.

Oligofructose and inulin, selective fermentable chicory fructans, have been shown to stimulate the growth of *Bifidobacteria* (Reddy, 1999) and evaluated inulin and oligofructose for their potential inhibitory properties against the development of colonic aberrant crypt foci in rats. Their results indicated that dietary administration of oligofructose and inulin inhibited the development of ACF in the colon and showed anitumor activities. Further, the degree of ACF inhibition was more pronounced in animals given inulin than those fed oligofructose. And, reasoned that probiotics selectively stimulated the growth of bifidobacteria, ornithine decarboxylase activities, *ras*-p21 ontoprotein expressions and tumor inhibitory activity of lyophilized cultures of *Bifidobacteria longum* against chemically induced colon and mammary carcinigenesis and against colonic tumor cell proliferation. However, human studies in this line perhaps broaden the application of pre and probiotics.

Further, Reddy & Rivenson (1993) evaluated the inhibitory effect of *B. longum* on colon, mammary and

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liver carcinogenesis induced by 2-amino-3-methylimidazol [4,5-f] quinoline (IQ), a food mutagen in rats. Their results indicated that dietary *B. longum* significantly inhibited the IQ induced incidence of tumors in colon, and liver and in female rats mammary carcinogenesis was suppressed to 50%. Thus usage of *B. longum* significantly reduced the risk of colon cancer and considerably the mammary cancer in rats.

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. They found that when compared to native Africans, African American consume meat, saturated fat and cholesterol along with more calcium, vitamin A and C with fiber intake. 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. 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. Thus they hypothesized that CRC risk was determined by interaction between external- dietary and internal- bacterial environments. In addition to this, most recently Saraf *et al.,* (2010) reported that the beneficial uses of probiotics in reducing HIV infection and cancers including colorectal carcinomas.

Therefore, the consumption of probiotic and prebiotic would decrease the risk of colon cancer particularly in producing 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 is scarce. In addition, human clinical trials would record significant observations on the beneficial effects of probiotics. Further, characterization of highly potential strains of probiotics having specific anti-cancerous effects and their mechanisms need to be addressed.

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