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Role of Probiotics in Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC), the third most common form of cancer, is treated by surgery, adjuvant chemotherapy and radiotherapy. Probiotics have been proposed as an option for combating CRC. There are several possible mechanisms that might explain how probiotic bacteria protect against CRC. The strongest evidences for the anticancer effects of probiotics come from animal studies; however, fragmentary evidences are available in case of human volunteers. Various mechanisms which have been attributed to the anti-carcinogenic potential of probiotics are binding and degradation of carcinogens, prevention of DNA damage, stimulation of protective enzymes, augmentation in immune response, alterations in metabolic activities of intestinal microflora and physicochemical conditions of the colon, and production of anti-tumorigenic/anti-mutagenic compounds. In the present review, these mechanisms have been precisely addressed keeping in view the role of probiotics.

Keywords: probiotic, colon, cancer, anti-genotoxicity
Abbreviations: CRC, colorectal cancer; **GIT**, gastrointestinal tract; **LAB**, lactic acid bacteria; **4-NQO**, 4-nitroquinoline-1-oxide; **MNNG**, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; **DMH**, 1,2-dimethylhydrazine

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INTRODUCTION

Cancer is the leading cause of deaths in western and developed countries (Jemal *et al*. 2008; Karim-Kos *et al.* 2008). On worldwide basis, colon cancer ranks amongst the first five cancers by incidence rates (World Health Organization 1987). Colorectal cancer (CRC) is the third most prevalent form of cancer in men with a survival rate of 10 % in patients with metastatic disease (Goldberg 2005). The etiology of colorectal cancer is complex which involves interplay of environmental and genetic factors. Life style factors, especially dietary intake, affect the risk of CRC development (Correa Lima and Gomes da Silva 2005). Diet rich in fat, especially of animal origin has been correlated with high incidence of colon cancer (Meyerhardt *et al*. 2007). Surgery is the most feasible treatment option available in colon cancer. Adjuvant chemotherapy is usually recommended for patients in whom residual cancer remains are suspected in the body after removal of primary tumor. Even if the tumor has been completely removed, tiny cancer cells may remain in the body and grow, causing relapse after surgery. This is most likely in patients who have positive lymph nodes i.e. Stage III of the disease. In such patients, chemotherapy can prevent the relapse and prolong survival. Like chemotherapy, radiation therapy may also be helpful for patients who are at high risk of recurrence. Radiation therapy may also be useful in treating advance stages of the disease especially in metastasis, particularly if it is painful (Braendengen *et al*. 2008). Inspite of surgical removal followed by chemo and radio therapy, the success rate of CRC treatment is still variable with high mortality rates (Liong 2008). Therefore, new strategies are needed in order to avoid the emergence of CRC.

One of the novel approaches in combating colon cancer involves consumption of probiotics. The FAO/WHO (2001) defines probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit to the host'. Probiotics include *Bifidobacteria*, lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *L. casei* subsp. *rhamnosus* (*Lactobacillus* GG), *L. bulgaricus*, *L. acidophilus*, *Enterococcus faecium*, *Lactococcus lactis*, *Streptococcus thermophilus* and non lactic acid bacteria such as *Bacillus subtilis*, *Escherichia coli* strain nissle and yeasts like *Saccharomyces cerevisiae* and *Sachharomyces boulardii* (Robertson *et al*. 2000; Verschuere *et al*. 2000; Frece *et al*. 2005; Kanwar *et al.* 2008; Szabo *et al.* 2009; Sourabh *et al.* 2010). Probiotics in particular have been accredited with various functional properties, such as improvement of

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digestion and intestinal transit, competitive exclusion of harmful microflora, immunomodulatory activity, antiallergic effects and reduction in irritable bowel syndrome, small bowel bacterial overgrowth, lactose intolerance, incidence of diarrhoea and side effects from antibiotic therapy and anticarcinogenic activity (Rolfe 2000; Tuohy *et al*. 2003; Geier *et al.* 2007; Wagar *et al.* 2009; Foligne *et al.* 2010). LAB play an important role in retarding colon carcinogenesis possibly by influencing metabolic, immunologic, and protective functions in the colon (Roberfoid *et al*. 1995). In animals, probiotic ingestion has been shown to prevent carcinogen-induced pre-neoplastic lesions and tumors (Rowland *et al*. 1998). The mechanisms that produce these protective effects of probiotics are less known. It is expected, however, that probiotics or their metabolites may prevent the carcinogens from inducing genotoxic effects. It has been hypothesized (Parvez *et al.* 2006) that probiotic cultures might decrease the exposure to chemical carcinogens by several mechanisms which are as below:

(i) detoxification of ingested carcinogens;
(ii) reduction in population or metabolic activities of bacteria that generate carcinogenic compounds;
(iii) production of metabolic products which improve apoptosis;
(iv) stimulation of immune system; or
(v) production of compounds that inhibit the growth of tumour cells

The antimutagenic and anti-genotoxic properties of LAB strains belonging to different species (*Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. gasseri*, *L. confusus*, *L. longum*, *L. brevis*, etc.) have been demonstrated in animals and under *in vitro* studies (Pool-Zobel *et al*. 1996; Lankaputhra and Shah 1998; Burns and Rowland 2000; Cenci *et al*. 2002; Orrhage *et al.* 2002; Caldini *et al.* 2005). Consequently, antimutagenicity and anti-genotoxicity are now considered as new parameters in characterizing the functional properties of probiotics (Suvarna and Boby 2005). The main purpose of this review is to compile information related to mechanisms of anticarcinogenic effects of probiotics, especially in CRC.

MECHANISMS OF ANTICARCINOGENICITY OF PROBIOTICS

Binding and degradation of carcinogens

The bacterial cell wall may be an important factor in determining the ratio of bound to free (bioavailable) toxins in the intestine. Mutagenic compounds, commonly found in the diet, can bind to LAB *in vitro* (Wollowski *et al*. 2001). The main elements responsible for binding mutagens are cell wall polysaccharides and peptidoglycan (Morotomi and Mutai 1986; Tanabe *et al*. 1991; Zhang and Ohta 1991; Rajendran and Ohta 1998). The extent of binding is correlated with the reduction in mutagenicity observed after exposure to the bacterial strains (Orrhage *et al.* 1994). Simple physical binding followed by subsequent degradation by probiotics of potential dietary carcinogens, may be responsible for their anticarcinogenic action, and thereby reducing the bioavailability of carcinogens in the gastrointestinal tract (GIT) (Geier *et al.* 2006; Fotiadis *et al*. 2008; Verbeke *et al*. 2008).There are large number of reports describing the adsorption or binding of mutagens and pro-mutagens such as 4-nitroquinoline 1-oxide, 2-nitro uorene, benzopyrene, heterocyclic amine, 2-amino-3,4-dimethyl-3*H*-imidazol(4,5-*f*) quinoline etc. as well as food-borne carcinogens to LAB under *in vitro* conditions (Ayebo *et al*. 1982; Zhang and Ohta 1991; Orrhage *et al.* 1994; Bolognani *et al.* 1997). In several of these studies, a concomitant decrease in mutagenicity has been reported where extent of binding is dependent on the mutagen and bacterial strain. In general, highest binding has been seen with the heterocyclic amines and the least with Aflatoxin B1 and AF2. Haskard *et al*. (2001) reported that binding of aflatoxin B_1 is predominantly extracellular in viable and non viable (heat-treated) bacteria. However, acid treatment results in intracellular binding which is of reversible nature but, the stability of the complex depends upon bacterial strain, type of treatment, and available physical conditions. The viable and non viable (heat- and acid-treated) cells of well known probiotics *Lactobacillus* GG and *L. rhamnosus* LC-705 (DSM 7061) have been reported to bind aflatoxin B_1 effectively (El-Nezami *et al*. 1998). It seems that this property of binding mutagens with non-viable bacteria may be important under *in vivo* conditions where these organisms encounter hostile environment of the stomach. Similar type of binding ability for mutagen has been reported with viable and non viable bacteria by various other workers (Zhang and Ohta 1990; Orrhage *et al*. 1994; Thyagaraja and Hosono 1994). It is suggested (Haskard *et al.* 2001) that both cell wall components (polysaccharide and peptidoglycan) are expected to be greatly affected by heat and acid treatments. Heat treatment results in protein denaturation or the formation of Maillard reaction products between polysaccharides and peptides/proteins, while acid treatment breaks down the peptidoglycan structure, resulting in disturbing structural integrity. The overall process results in decrease in thickness, reduction in cross-linkages, and/or increase in pore size of cell wall. These changes in the bacterial cell allow mutagen(s) to bind to cell wall and plasma membrane constituents that were not available when the cell was intact. Thus, the effective removal of mutagen by nonviable bacteria is through their binding rather than metabolism. Apart from bacteria, probiotic yeast *Saccharomyces boulardii has* also been shown to inhibit genotoxicity induced by well-known mutagen 4-nitroquinoline-1-oxide (4-NQO) and by some antibacterial drugs (Toma *et al.* 2005). Probiotic microorganisms such as *Saccharomyces cerevisiae*, *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus LC705* are known to inhibit aflatoxicosis by binding toxins or metabolically transforming them into non-toxic degradation products (Nada *et al.* 2010).

Although binding represents a plausible mechanism for the inhibition of genotoxicity by probiotics under *in vitro* conditions, its impact under *in vivo* conditions needs thorough investigations. Bolognani *et al.* (1997) demonstrated that simultaneous administration of LAB along with various carcinogens to mice had no effect on absorption of the compounds from the gastrointestinal tract, as well as on mutagenicity of the carcinogens in the liver. On the contrary, Zhang and Ohta (1993) reported that co-administration of freeze-dried LAB and food mutagen (Trp-P-1) to rat resulted in significant reduction in absorption of the mutagen by small intestine accompanied by decreased levels of this mutagen in blood. Recently, a well known probiotic bacterium i.e. *Lactobacillus rhamnosus* GG has been reported to be successful in protecting against genotoxicity induced by a common food mutagen Ochratoxin A which is carcinogenic, genotoxic, and hepatonephrotoxic to humans and animals (Farag *et al.* 2010). Plenty of reports are available on binding/alteration of mutagens to probiotic bacteria under *in vitro* conditions whereas, concrete evidences are lacking under *in vivo* conditions to reach to any final conclusion. Therefore, more studies are required under *in vivo* conditions to substantiate this mechanism.

Prevention of DNA damage

Chronic inflammation in the colonic mucosa caused by increased and continuous exposure to reactive oxygen species (ROS) promotes oxidative DNA damage of the epithelial cells, thus triggering the appearance of genetic mutations and initiating colorectal cancer (Ribero *et al*. 2008). An antimutagenic effect of fermented milks has been detected against a range of mutagens and promutagens in various test systems based on microbial and mammalian cells. Using the technique of single cell microgel electrophoresis (Comet assay), Pool-Zobel *et al.* (1996) investigated the ability of range of species of LAB to inhibit DNA damage in the colon mucosa of rats treated with carcinogens MNNG (*N*-

methyl-*N*'-nitro-*N*-nitrosoguanidine) or 1,2-dimethylhydrazine (DMH). It was found that strains of *L. acidophilus* (isolated from a yoghurt), *Lactobacillus gasseri*, *L. confusus*, *Bifidobacterium breve* and *B. longum*, prevented MNNG-induced DNA damage when administered at a dose of 10^{10} cells/kg body weight, 8 hours before the administration of carcinogen. In most cases, the DNA damage was reduced to a level similar to that in untreated rats. This protective effect was dose dependent and lower doses were found to be less effective in reducing MNNG-induced DNA damage. Importantly, heat-treatment of *L. acidophilus* abolished its antigenotoxic potential indicating the importance of viable cells. Similar results were obtained when the LAB strains were administered to rats fed with DMH as DNA damaging agent. On the contrary, Corsetti *et al*. (2008) reported complete reduction in antigenotoxicity when genotoxins such as 4-nitroquinoline-1-oxide and MNNG were co-incubated with dead cells, instead of live cells. Antigenotoxic activity depends upon the type of strain used as it was observed in case of *S. thermophilus*, where two strains were ineffective and one provided protection against DNA damage (Burns and Rowland 2000). In one such study, *Lactobacilli* and *Bifidobacteria* were strongly found to inhibit DNA damage in the colon mucosa, whereas *S. thermophilus* was less effective (Pool-Zobel *et al.* 1996). Similarly, Corsetti *et al*. (2008) showed that antigenotoxic activity is strain and genotoxic compound dependent and is not influenced by viable cell concentration up to the range of 10^5–10^9 CFU g^{-1}. On the contrary, cell-density dependent reduction of faecal water genotoxicity was reported by Burns and Rowland (2004) in case of probiotic strains of *Bifidobacterium* spp. and *L. plantarum*.

Lactic acid bacteria isolated from dairy products (yoghurt and fermented milk) have extensively been characterized for anti-genotoxicity (Pool-Zobel *et al.* 1996; Lankaputhra and Shah 1998; Orrhage *et al*. 2002), but many non-starter *Lactobacilli* isolated from cheeses have also been subjected to antigenotoxic analysis (Caldini *et al*. 2008). Apart from strain dependent antigenotoxic effect of probiotics, it has also been shown to be dependent upon structure/spectroscopic modification of genotoxins (MNNG and NQO) in some cases (Caldini *et al*. 2008) where consistent shift in λmax values has always been associated with more than 50 % genotoxicity inhibition.

Stimulation of protective enzymes

Many of the food-borne carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons are known to be conjugated to glutathione which results in their inactivation. The enzyme involved in this process is glutathione (GSH) transferase, which is found in the liver and in other tissues including the gut. If not conjugated to GSH, the ileal mucosa (Venitt 1988) as well as the colonic mucosa (Fang and Strobel 1978) has the capacity to absorb mutagenic compounds from the intestinal lumen and pass on these compounds into the bloodstream, either unchanged or as metabolites responsible for genotoxicity. LAB have been shown to increase colonic NADPH-cytochrome P-450 reductase activity (Pool-Zobel *et al.* 1996) and glutathione *S*-transferase (GST) levels (Challa *et al*. 1997) which are involved in the metabolism of carcinogens in rats. Challa *et al.* (1997) studied the effect of *B. longum* and lactulose on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in the colon, and reported an inverse relationship between the activity of GSH in the colonic mucosa and ACF numbers. Such a mechanism of protection may be effective against a wide range of dietary carcinogens.

Increase in immune responses

It has been observed that decreased intestinal microflora increases antigen transport across gastrointestinal mucosa, which is the primary interface between the external environment and the immune system. This suggests that the normal gut microflora is important in maintaining gut defenses. The beneficial probiotic bacteria have been found to interact with gut epithelial cells, the M cells in the Peyer's patches and allied immune cells to initiate immune responses. In addition to regulating immunoglobulin production, these bacteria are also involved in increasing the profiles of some cytokines (TNF-α, IFN-γ, IL-10) which are known to regulate the immune responses and to maintain intestinal homeostasis (Gupta and Garg 2009). Moreover, these bacteria also stimulate the activity of Natural Killer (NK) cells, which are directly involved in daily fight against transformed cells (Watzl 2008). Probiotics induce the production of antimicrobial peptide, human beta-defensin 2 (HBD-2) in the intestinal epithelial cells via NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) leading to increased barrier function in the gut (Wehkamp *et al*. 2004; Schlee *et al*. 2008). These peptides recognize the conserved bacterial products or bacteria by a class of proteins known as Toll-like receptors (TLRs) expressed on them and result in activation of the immune response (Paolillo *et al*. 2009). There are many studies that suggest that lactic acid bacteria play an important function in the host's immunoprotective system by increasing IgA secreting cells and CD4+ T lymphocytes to have an anti-tumor effect (Aso *et al.* 1995; Schiffrin *et al*. 1995). In human subjects, consumption of probiotics has been reported to modulate immune system (Marteau *et al.* 1997) by increasing phagocytic activity of monocytes, granulocytes and levels of antibody secreting cells. However, significance of these changes in relation to tumor development has not been properly established. *Lactobacillus casei* Shirota (LcS) has been shown to exert potent antitumour and antimetastatic effects on transplantable tumour cells and suppress chemically induced carcinogenesis in rodents (Matsuzaki 1998). In tumor bearing mice, the intrapleural administration of *Lactobacillus casei* Shirota has resulted in the production of several cytokines, such as IFN-γ, IL-1 and TNF-α, which inhibit the growth of tumour and thus prolong the survival. These findings suggest that treatment with LcS has the potential to ameliorate or prevent tumorigenesis through the modulation of host's immune responses, specifically the cellular immune responses Similar results have been reported with strains of *L. acidophilus* SNUL, *L. casei* YIT9029 and *B. longum* HY8001 by Lee *et al*. (2004). A cell component like peptidoglycan of *Lactobacillus* species reduced the growth of CT26 colon cancer cells in BALB/c mice in a dose-dependent manner by increasing level of cell apoptosis (Sun *et al.* 2005). Interestingly, peptidoglycan had no effect on tumor cell apoptosis *in vitro*, indicating thereby that *in vivo* antitumorigenic effect may be mediated by the modulation of immune response. In addition to these studies, probiotics have been found not only to be effective against Caco-2 colonic adenocarcinoma (Ghoneum *et al.* 2005), but also against a breast cancer cell line (Ghoneum and Gollapudi 2004), suggesting that probiotic therapeutic interventions may not necessarily be restricted to cancers affecting the gastrointestinal system.

Alteration of the metabolic activities of intestinal microflora

Certain mutagenic compounds, after absorption, are detoxified in the liver by conjugation with glucuronic acid and are released/secreted again into the intestine as glucuronide conjugates. In the GI tract, certain bacteria cause regeneration (release) of toxic mutagenic aglycones from these conjugates by secreting enzymes like β-glucuronidase, nitroreductase and azoreductase. In general, species of anaerobic bacteria of GI tract possess high activities of these enzymes which are important in carcinogenesis (Saito *et al.* 1992). Apart from above enzymes, another bacterial enzyme i.e. β-glycosidase is known to hydrolyze the plant glycoside cycasin to a carcinogen in the gut. Therefore, bacteria liberating/secreting such harmful enzymes are responsible for catalyzing reactions which yield carcinogenic compounds

(Fotiadis *et al.* 2008). In contrary, certain probiotic bacteria such as *Lactobacilli* and *Bifidobacteria* lower the concentration and activity of these enzymes, as well as reduce the level of preneoplastic lesion or tumour in GI tract of carcinogen treated rats (Burns and Rowland 2000; Wollowski *et al*. 2001; Fotiadis *et al*. 2008). Thus, it can be suggested that one of the mechanisms for anticarcinogenecity of probiotics may be due to inactivation of these enzymes involved in synthesis or activation of carcinogens, genotoxins and tumour promoters (Geier *et al*. 2006; Liong 2008). Consumption of fermented milk containing *L. acidophilus* has been shown to reduce significantly the counts of faecal putrefactive bacteria and increase the levels of *Lactobacilli* in the intestine (Ayebo *et al*. 1980; Shahani and Ayebo 1980) suggesting that supplementing *L. acidophilus* may have a beneficial effect on the intestinal microecology by suppressing the putrefactive organisms that are possibly involved in the production of tumour promoters and putative pre-carcinogens.

LAB have been reported to reduce the specific activities of fecal enzymes β-glucuronidase, nitroreductase, and azoreductase in human volunteers (Goldin and Gorbach 1984a). Feeding of *L. acidophilus* strains NCFM and N-2 to 21 healthy volunteers caused a significant decline in the specific activity of these enzymes in all subjects after 10 days of feeding (Goldin and Gorbach 1984b). However, this trend was reversed within 30 days of stopping *Lactobacillus* feeding; suggesting that continuous consumption of probiotics is essential to maintain the protective effect. Human studies have demonstrated that the capacity of probiotics to decrease the activity of bacterial enzymes is strain specific. It has been demonstrated that LcS and *L. acidophilus* significantly decreased β-glucuronidase activity in healthy subjects (Goldin *et al*. 1980; Spanhaak *et al*. 1998) whereas *L. plantarum* 299V and *L. rhamnosus* DR20 could not decrease this activity (Tannock *et al.* 2000; Goossens *et al*. 2003). To achieve a decrease in enzymatic activity, a continual intake of LAB is obligatory. Martaeu *et al*. (1990) reported a decrease in the fecal activity of nitroreductase, but an increase in β-glucosidase activity and no change in activities of β-glucuronidase and azoreductase in 9 subjects who consumed *L. acidophilus* (1×10^9 colony-forming-units/day) and *Bifidobacterium bifidum* (1×10^{10} colony-forming units/day) for 3 weeks. An increase in β-glucosidase might be advantageous to health by releasing flavonoids having antimutagenic, antioxidative, anti-carcinogenic, and immunostimulatory effects (Stoner and Mukhtar 1995; Cai *et al*. 1998). Recently, Strojney *et al*. (2011) demonstrated significant reduction in activities of β-glucuronidase and α-glucosidase enzymes which provided protection against DMH induced colon cancer in *Lactobacillus plantarum* fed rats.

Alteration of physicochemical conditions in the colon

One of the hypotheses regarding colon carcinogenesis postulates that secondary bile acids in the aqueous phase of faeces exert cytotoxic effect on colonic epithelium which results in increased proliferation of intestinal cells (Bruce 1987). This phenomenon may be mediated by increased level of secondary bile acids in the colon, produced by the action of bacterial 7α-dehydroxylase on primary bile acids (Begley *et al*. 2006). Administration of *L. acidophilus* fermented milk supplements to colon cancer patients for six weeks resulted in lowering concentrations of soluble bile acids in faeces as observed by Lidbeck *et al*. (1991).

It has been suggested that large bowel cancer could be influenced directly by reducing intestinal pH (Modler *et al*. 1990), which effects the growth of putrefying bacteria. Administration of diet containing probiotic *B. longum* and inulin has been reported to increase caecal weight and β-glucosidase enzyme activity along with reduction in caecal pH (Rowland *et al*. 1998). In another study, administration of *L. acidophilus* together with *B. bifidum* to patients with colonic adenomas resulted in significant decrease in fecal pH which affected the proliferative activity in the upper colonic crypts (Biasco *et al.* 1991). Thus, it seems that lowering of soluble bile acids and intestinal pH are two important protective mechanisms in colon carcinogenesis.

Production of short chain fatty acids

Short chain fatty acids (SCFAs) are organic fatty acids with 1 to 6 carbon atoms and are the principal anions which arise from bacterial fermentation of polysaccharides, oligosaccharides, proteins, peptides and glycoprotein precursors in the colon (Miller and Wolin 1979; Cummings and MacFarlane 1991). Increase in SCFAs results in decrease of pH which indirectly influences the composition of colonic microflora, decreases solubility of bile acids, increases absorption of minerals, and reduces ammonia absorption by protonic dissociation of ammonia and other amines (Vince *et al.* 1978; Jackson 1983; Jenkins *et al.* 1987). It has been observed that anaerobic breakdown of prebiotics and their subsequent fermentation by probiotics not only enhances the growth of probiotics but also leads to the production of SCFAs like butyrate, acetate and propionate as byproducts of fermentation. These SCFAs decrease the pH of colonic contents, which contribute towards their anticancer action (Wollowski *et al*. 2001). Out of these SCFAs, butyrate has been most extensively studied and is known to inhibit cancer cell proliferation and promote apoptosis *in vitro* (Pool-Zobel 2005). Butyrate administration in animal models of CRC has produced varying results (Sengupta *et al*. 2006). Laminar delivery of butyrate has been shown to reduce aberrant crypt foci (ACF) by 45% compared to untreated rats (Wong *et al*. 2005). In the context of CRC treatment, the bacterial strain *Butyrivibrio fibrisolvens* MDT-1 producing high amounts of butyrate has been investigated by Ohkawara *et al.* (2005). In a mouse model of colon cancer, administration of MDT-1 led to a significant decrease in ACF and number of mice having an increased proportion of ACF, indicating the role of butyrate in inhibition of tumour progression. MDT-1 also reduced β-glucuronidase activity and increased the immune response as reflected by an increase in NK cell numbers. Similar effects have been observed with propionate and acetate producing probiotic i.e. *Propionibacterium acidipropionici* (Jan *et al*. 2002). It has been suggested that short chain fatty acid delivery through probiotic ingestion may be an exciting treatment option for CRC (Geier *et al.* 2006).

Production of anti-tumorigenic or anti-mutagenic compounds

Beneficial intestinal microflora can result in the generation of potential anti-carcinogenic and anti-mutagenic substances in the form of flavonoids such as quercetin by glycoside hydrolysis (Rowland 1995). It has been suggested that lactic acid bacteria or soluble compounds produced by these bacteria may interact directly with tumor cells in culture and inhibit their growth (Reddy *et al.* 1983). Milk fermented with *B. infantis*, *B. bifidum*, *B. animalis*, *L. acidophilus* and *L. paracasei* exhibited inhibition in the growth of MCF7 breast cancer cell line (Biffi *et al*. 1997). This antiproliferative effect was due to the presence of bacterial products. Antitumorogenic and antimutagenic compounds produced by probiotic bacteria may be organic acids and peptides. Organic acids produced by probiotic bacteria such as *L. acidophilus* and *B. bifidum* have shown to exhibit antimutagenic activity against mutagens and promutagens like 2-nitroflourene, aflatoxin-B and 2-amino-3-methyl-3H-imidazoquinoline (Lankaputhra and Shah 1998). Production of antimutagenic compounds in milk during fermentation by *L. helveticus*, and the release of peptides are considered to be one of the possible contributing mechanisms for inhibitory effect on carcinogen 4-nitroquinoline-1-oxide (4-NQO) (Matar *et al.* 1997) as milk fermented by a non-proteolytic variant of the same strain did not show inhibitory effect.

CONCLUSIONS

Apart from anticancer attribute, many other health-promoting attributes of probiotics have adequate scientific support available in the literature. As discussed above in this review, there are several possible mechanisms that might explain how probiotic bacteria protect against CRC. The strongest evidence for the anticancer effects of probiotics come from animal studies, however, fragmentary evidences are available on human volunteers. Clearly there is a need to have carefully controlled intervention studies in human subjects using biomarkers of cancer risk. An important goal for the future is to carefully design human clinical trials to corroborate with the information generated through experimental studies.

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