Role of Probiotics in Colorectal Cancer

Aditi Sourabh • Sohini Walia • S. S. Kanwar

Department of Microbiology, College of Basic Sciences, CSK Himachal Pradesh Agricultural University, Palampur, H.P., 176062 India

Corresponding author: sskanwar1956@gmail.com

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

CONTENTS

INTRODUCTION................................................................................................................... ....................................................................... 1
MECHANISMS OF ANTICARCINOGENICITY OF PROBIOTICS ................................................................. .................................................................................. 2
Binding and degradation of carcinogens................................................................. 2
Prevention of DNA damage .............................................................................. 2
Stimulation of protective enzymes .................................................................. 3
Increase in immune responses ........................................................................... 3
Alteration of the metabolic activities of intestinal microflora........................... 3
Alteration of physicochemical conditions in the colon......................................... 4
Production of short chain fatty acids ................................................................. 4
Production of anti-tumorigenic or anti-mutagenic compounds............................ 4
CONCLUSIONS.................................................................................................................... ........................................................................ 5
REFERENCES..................................................................................................................... ........................................................................ 5

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; Verschuer e 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
digestion and intestinal transit, competitive exclusion of harmful microflora, immunomodulatory activity, antiinflam-
matic effects and reduction in irritable bowel syndrome, small
bowel bacterial overgrowth, lactose intolerance, incidence of
diarrhoea and side effects from antibiotic therapy and anti-
carcinogenic 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 carcinogene-
sis, possibly by influencing metabolic, immunologic, and
protective functions in the colon (Roberfoid et al. 1995). In
animals, probiotic ingestion has been shown to prevent car-
cinogen-induced pre-neoplastic lesions and tumors (Row-
land et al. 1998). The mechanisms that produce these pro-
tective 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 bac-
teria that generate carcinogenic compounds;
(iii) production of metabolic products which improve apop-
tosis;
(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 ani-
mals and under in vitro studies (Pool-Zobel et al. 1996;
Lankaputhra and Shah 1998; Burns and Rowland 2000;
Cenci et al. 2002; Orhange et al. 2002; Celdini 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 infor-
mation 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 deter-
mining 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 cor-
related with the reduction in mutagenicity observed after
exposure to the bacterial strains (Orhage et al. 1994). Sim-
ple physical binding followed by subsequent degradation by
protease of peptidoglycan, or competitive exclusion, may be respon-
sible for their anticarcinogenic action, and thereby reducing
the bioavailability of carcinogens in the gastrointestinal
tract (GIT) (Geier et al. 2006; Fotiakis 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 urea, benzopy-
rene, heterocyclic amine, 2-amino-3,4-dimethyl-3H-imida-
zol[4,5-f] quinoline etc. as well as food-borne mutagens may be responsi-
ble for their binding properties under in vitro conditions
(Ayebo et al. 1982; Zhang and Ohta 1991; Orhage et al. 1994;
Bolognani et al. 1997). In several of these studies, a concomitant decrease in muta-
genicity 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 B1 is predomi-
nantly 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 Lact-
obacillus GG and L. rhamnosus LC-705 (DSM 7061) have been reported to bind aflatoxin B1 effectively (El-Nezami et al. 1998). It seems that this property of binding mutagens to probiotics may be important under in vivo conditions where these organisms encounter hostile envi-
ronment of the stomach. Similar type of binding ability for
mutagens has been reported with viable and non viable bac-
teria by various other workers (Zhang and Ohta 1990; Or-
hrage et al. 1994; Thyagaraja and Hosono 1994). It is sug-
gested (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 peptidogly-
can 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, pro-
biotic 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 transform-
ing 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 thoro-
gough investigations. Bolognani et al. (1997) demonstrated
that simultaneous administration of LAB along with various
carcinogens to mice had no effect on absorption of the com-
pounds from the gastrointestinal tract, as well as on muta-
genicity 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 result-
ed 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 bac-
terium 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 carcino-
genic, 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 con-
clusion. 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 in-
creased and continuous exposure to reactive oxygen species
lack an important role in 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-

International Journal of Biomedical and Pharmaceutical Sciences 5 (1), 1-6 ©2011 Global Science Books
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. con- fusus*, *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-N-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, *Lactobacillus“ 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 \(10^7–10^{10}\) 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; Lan- kaphutra 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 \(\lambda_{max}\) 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 the 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. The genotoxic effects of these metabolites are mediated by the modulation of the 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.

**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 regul- late the immune responses and to maintain intestinal home-ostasis (Gupta and Ganguly 2008). 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 conserva- tion of 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. *Lactococcus casei* Shirota (LcS) has been shown to exert potent antitumour and antimetastatic effects on transplantable tumour cells and suppress chemically induced carci- nogenesis in rodents (Matsuzaki 1998). In tumor bearing mice, the intraperitoneal administration of *Lactococcus 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 consequently 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-depend- ent 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 anti-tumour effect might be mediated by the modulation of the 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.
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 anticarcinogenicity of probiotics may be due to inactivation of these enzymes involved in proliferation of preneoplastic cell colonies, growth of 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 azo-reductase 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 activities 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 azo-reductase 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 β-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 (Bagley 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 faecal 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 effects their 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 Butyribiother fibrisolvens MDT-1 producing high amounts of butyrate has been investigated by Okhawara 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. 1980). 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. Antitumorigenic 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. acidophilus have shown to have antimutagenic activity against mutagens and promutagens like 2-nitrofluorene, aflatoxin-B2 and 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (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) (Mutar 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 comes from animal studies, however, fragmentary evidence is 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.

REFERENCES
Geier MS, Butler RN, Giffard PM, Howarth GS (2007) Lactobacillus fermentu BR1, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulphate sodium (DSS) in rats. International Journal of Food Microbiology 114, 267-274
Geke MS, Butler RN, Giffard PM, Howarth GS (2007) Lactobacillus fermentu BR1, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulphate sodium (DSS) in rats. International Journal of Food Microbiology 114, 267-274
Goldin BR, Gorbach SL, (1984a) Alterations of the intestinal microflora by diet, oral antibiotics, and Lactobacillus. Decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. Journal of the National Cancer Institute 73, 689-695

Microbial Ecology in Health and Disease 4, 81-88
Thyagarajan N, Hosono A (1994) Binding properties of lactic acid bacteria from ‘Idly’ towards food-borne mutagens. Food and Chemical Toxicology 32, 805-809
Vince A, Killingley M, Wrong OM (1978) Effect of lactulose on ammonia production in the fecal incubation system. Gastroenterology 74, 544-549
Robertson PAW, Owod W, Williams P, Austin B (2000) Use of Carnobacterium sp. as a probiotic for Atlantic salmon (Salmo salar) and rainbow trout, Oncorhyncus mykiss (Walbaum). Aquaculture 185, 235-243