Journal of Medical Microbiology (2013), **62**, 1107–1123 DOI 10.1099/jmm.0.048975-0

Review

Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives

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Received 2 June 2012
Accepted 1 April 2013

Colorectal cancer (CRC) is the third most common form of cancer. Diverse therapies such as chemotherapy, immunotherapy and radiation have shown beneficial effects, but are limited because of their safety and toxicity. Probiotic formulations have shown great promise in CRC as preventive and early stage therapeutics. This review highlights the importance of a balanced intestinal microbiota and summarizes the recent developments in probiotics for treating CRC. Specifically, this report describes evidence of the role of probiotics in modulating the microbiota, in improving the physico-chemical conditions of the gut and in reducing oxidative stress. It also discusses the mechanisms of probiotics in inhibiting tumour progression, in producing anticancer compounds and in modulating the host immune response. Even though some of these effects were observed in several clinical trials, when probiotic formulations were used as a supplement to CRC therapies, the application of probiotics as biotherapeutics against CRC still needs further investigation.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in western countries (Kumar *et al.*, 2010). According to the Canadian Cancer Society and the National Cancer Institute, in 2012, an estimated 23 300 Canadians and 143 460 Americans were diagnosed with CRC and 9200 and 51 690, respectively, died of it. CRC develops by the accumulation of mutations, starting in stem cells at the base of the crypts (Barker *et al.*, 2009), and usually begins as a non-cancerous polyp (Jemal *et al.*, 2011). CRC incidence can be associated with a number of genetic factors such as germline mutations in the mismatch repair genes (Silva *et al.*, 2009) and adenomatous polyposis coli (APC) gene (de Miranda *et al.*, 2009). In addition to genetic predispositions, environmental factors such as lifestyle and diet play an important role in CRC risk (Steffensen *et al.*, 1997). Researchers agree that a diet rich in red meat and processed food with a low consumption of fruits and vegetables increases CRC incidence (Ahmed, 2007; Kono, 2011). This lifestyle and diet also leads to disturbances in the intestinal environment, including the luminal content and microbiota (Zhu *et al.*, 2011). The microbiota plays a role in generating biochemical and physiological conditions that may increase the number of colonic pre-neoplastic lesions (Rowland, 2009; Uronis *et al.*, 2009). Interestingly, consumption of beneficial bacteria can modulate the micro-organisms of the gastrointestinal (GI) system (Prakash *et al.*, 2011). Modulation of the unbalanced gut microbiota can provide a therapeutic and preventive effect by downgrading carcinogenic stimulating events in the colon (Rafter, 2001).

Probiotics are 'live microorganisms which, when administered in an adequate amount, confer a beneficial heath effect to the host' (Ochmański & Barabasz, 1999; FAO/WHO, 2006). Although probiotics have been used to manage a number of GI disorders such as diarrhoea, infection and inflammation (Ehlers & Kaufmann, 2010), their role in preventing and treating CRC is still under extensive investigation. In this context, probiotic bacteria should have potential features relevant to the development of CRC biotherapeutics. For example, lactic acid bacteria (LAB) have shown protective effects against CRC by reinforcing and modulating the host's natural defence mechanisms (Klaenhammer *et al.*, 2012). LAB may also modify luminal secretions, reinforce the mucosal barrier (Tlaskalová-Hogenová *et al.*, 2011), affect epithelial cell proliferation (Grimoud *et al.*, 2010) and reduce the exposure to toxic and carcinogenic compounds in the colon (Olejnik *et al.*, 2010).

This review will highlight the effects of probiotics on the modulation of the gut microbiota, the reinforcement of gut integrity and the physico-chemical conditions. It will

describe the relevance of probiotics in preventing neoplastic formation within the large intestine by decreasing the generation and levels of carcinogens and by the production of anti-carcinogenic and antioxidant metabolites. The review will describe the relationship between the mechanisms of actions of probiotics and the attenuation of CRC risk factors (Table 1). It will subsequently discuss recent studies of the mechanisms of action of probiotics and the efficacy of the different doses and treatments used in preclinical and clinical studies. Then, it will discuss recent findings about immunoregulatory properties of probiotics. Finally, the use of probiotic treatments as supplements to reduce the complications associated with conventional CRC treatments will be described.

Probiotics and their role in modulating CRC-associated intestinal microbiota and gut integrity

Unbalanced gut microbiota in CRC

The normal human GI tract usually maintains a delicate balance of the microbiota with about 10^{12} bacteria per gram of luminal content and over 1000 species (Qin *et al.*, 2010). The gut microbiota is responsible for metabolizing nutrients, producing vitamins, endogenous hormones and toxic products (e.g. carcinogens), especially in the large intestine (Guarner & Malagelada, 2003). The microbiota is responsible for degrading organic compounds including food additives, bile salts and cholesterol (Cummings, 1975; Pavlović *et al.*, 2012). In CRC, the gut microbiota has been

Table 1. Probiotic potential mode(s) of action in mitigating the factors responsible for CRC

Factors linked to CRC	Potential mode(s) of action of probiotics in mitigating factors of CRC
Unbalanced gut microbiota: ↑ *Bacteroides*, *Eubacterium*, *Fusobacterium*, *Proteobacteria*, *Salmonella* and *Prevotella*	**Modulation of gut microbiota:** ↑ *Bifidobacterium*, *Lactobacillus*, ↓ *Escherichia*, *Staphylococcus*
Disrupted colonic physico-chemical conditions: Alkalosis Water absorption in the colon Incomplete fermentation Genotoxic faecal water content	**Improvement of colonic physico-chemical conditions:** ↓ pH, Improve fermentation ↓ Putrefactive products: Putrescine, cadaverine and tryptamine
Damaged epithelial barrier: Normal epithelial cell death ↑ Permeability Tight junction protein rearrangement Pathogen translocation	**Reinforce gut epithelial barrier:** ↑ Defensins and mucus production by goblet cells ↑ Cytoprotective heat-shock proteins ↑ Normal epithelial cell survival
↑ **Harmful bacterial enzymes:** *β*-Glucuronidase, *β*-glucosidase, azoreductase, nitroreductase, alcohol dehydrogenase	↓ **Bacteria producing harmful enzymes:** *Bacteroides*, *Clostridium*, *Enterococcus*, *Salmonella*, *Enterobacter*, *Streptococcus*, *Citrobacter*, *Escherichia* and *Staphylococcus*
↑ **Carcinogenic products:** IQ, tryptophanase, urease, acetaldehyde, MNNG, AFLB1, TrpP-1, *N*-nitroso compounds, aromatic amines, sodium azide, benzo(α)pyrene, transformed secondary bile salts, aglycones hydrogen sulfide and indoles	**Binding, deactivation of carcinogens:** ↑ Detoxifying enzymatic antioxidants: GTS, glutathione, glutathione reductase, Glutathione peroxidase, superoxide dismutase and catalase
↑ **DNA damage:** ↑ Abnormal cell growth: Dysplasia, tumour formation	↑ **Anti-carcinogenic metabolites:** SCFAs, CLAs, phenols ↑ Apoptosis ↑ Differentiation in cancer cells
Intestinal inflammation: ↑ NF-κB, IL-8, IL6 ↓ Immune response against tumour cells	↓ **Intestinal inflammation:** ↓ TLR-4, ↑ IL-10, IL-8 secretion, NF-κB activation Immune response against tumour cells: ↑ TNF and NO production in epithelial cells ↑ Regulatory T-cell activity ↑ Bactericidal phagocytic activities of neutrophils ↑ IL-12, stimulation of DCs and NK cells

shown to be compromised and unbalanced (Frank *et al.*, 2007). Studies comparing human stool samples of healthy and CRC patients found a significant difference in bacterial genera (Sobhani *et al.*, 2011). Several *Lactobacillus* species from the intestinal flora were present in lower counts (Wong *et al.*, 2006), while *Fusobacterium* (Castellarin *et al.*, 2012; Kostic *et al.*, 2012), *Bacteroides*, *Eubacterium*, *Proteobacteria* and *Prevotella* (Shen *et al.*, 2010; Sobhani *et al.*, 2011), some *Salmonella* (Schiffman *et al.*, 1989) and *Clostridium* species (Scanlan *et al.*, 2008) were in higher counts in CRC patients.

Colon microbial carcinogenesis is a process that involves increased counts of CRC-causing bacteria such as enterotoxigenic *Bacteroides fragilis* that have been shown to induce colon tumour formation in multiple intestinal neoplasia (Min) mice (Sears & Pardoll, 2011). It has, therefore, been suggested that a colon microbial imbalance may increase the proliferation of carcinogenic bacteria that enhance the production of carcinogenic compounds, secondary bile acids and cholesterol metabolites, driving oncogenic transformations in the epithelium and CRC pathogenesis (Arthur & Jobin, 2011). However, further investigations are needed to establish this hypothesis.

Transient modulation of gut microbiota by probiotic bacteria

An unbalanced microbial composition can provide favourable conditions for colonic carcinogenesis (Rescigno, 2008). It has been reported that a daily consumption of specific probiotic strains can improve human health, restore the microbiota balance (Lee *et al.*, 2009) and inhibit intestinal colonization by pathogenic micro-organisms (Fig. 1). In a study using 1,2-dimethylhydrazine (DMH)-induced CRC rats, *Lactobacillus rhamnosus GG* administration reduced the number of coliforms and significantly elevated the count of lactobacilli (Bertkova *et al.*, 2010). According to a recent trial on goats, a mixture of *Lactobacillus reuteri* DDL 19, *Lactobacillus alimentarius* DDL 48, *Enterococcus faecium* DDE 39 and *Bifidobacterium bifidum* DDBA, significantly modified the faecal microbiota by reducing faecal enterobacteria and increasing bifidobacteria and LAB counts (Apás *et al.*, 2010).

In a clinical trial of CRC patients, the oral administration of probiotic treatment increased the counts of *Bifidobacterium*, *Lactobacillus and Enterococcus* and decreased the counts of *Escherichia coli* and *Staphylococcus aureus* (Zhang *et al.*, 2012; Zhu *et al.*, 2012). In addition, formulations of *Lactobacillus* and/or *Bifidobacterium* strains such as *Lactobacillus gasseri* OLL2716: LG21 (Ohara *et al.*, 2010) and *Bifidobacterium lactis* Bb12 (Worthley *et al.*, 2009) have increased *Bifidobacterium* and *Lactobacillus* in the faecal flora and decreased pathogen counts, including *Clostridium perfringens*.

For a better understanding of the action of probiotics on oncogenic/pathogenic bacteria further investigations were required. It was found that probiotic bacteria, delivered to the gut, rely on their antimicrobial, competitive, adhesive and anti-invasive properties to act on other micro-organisms and regulate gut microbial activity (Prakash *et al.*, 2011; Jones *et al.*, 2013). In addition, probiotics can provide intestinal and epithelial haemostasis, specifically improving epithelial barrier integrity (Rafter, 2003). Probiotics were found to produce antimicrobial substances, such as bacteriocins, lactic acid, reuterin, hydrogen peroxide and deconjugated bile acids, providing mechanisms for inhibition of pathogenic and carcinogenic microbes (Šušković *et al.*, 2010). Some probiotic bacteria can bind or compete with pathogens for nutrients/molecules, adhere to epithelial cells and block the adherence of pathogens (competitive exclusion) (Prince *et al.*, 2012; Ranadheera *et al.*, 2012), and outcompete pathogens by forming biofilms (Hancock *et al.*, 2010).

Effects on the gut epithelial barrier

In the intestinal epithelium, the cells form an impermeable barrier (Amasheh *et al.*, 2009) and are covered with a mucus layer (Mennigen *et al.*, 2009). This barrier protects the intestinal wall from physical and chemical damage, as well as from pathogens (Watson *et al.*, 2005). If pathogenic bacteria penetrate the intestinal epithelium, an inflammatory response is initiated at the site and in the adjacent intestinal mucosa causing damage to this epithelial barrier, increasing CRC risk (Karin *et al.*, 2006). It has been found that probiotic consumption can reinforce the epithelial barrier by preventing tight junction protein rearrangement (Worthley *et al.*, 2009) and by increasing the production of defensins and mucus by goblet cells (Kleessen & Blaut, 2005), as well as reducing the leakage of harmful solutes, micro-organisms and antigens (Fig. 1) (Watson *et al.*, 2005; Mennigen *et al.*, 2009). A recent study indicated that components of *E. coli* strain *Nissle* 1917 decreased the permeability of ^{14}C-mannitol by restoring a disrupted epithelial barrier (Stetinova *et al.*, 2010). Preparations of *L. rhamnosus* GG and *B. lactis* Bb12, tested on CRC patients, also significantly improved epithelial integrity (Ko *et al.*, 2007; Karczewski *et al.*, 2010). Probiotics prevented epithelial barrier damage by inducing the production of cytoprotective heat-shock-proteins in stressed epithelial cells to maintain haemostasis (Yan & Polk, 2012) and promote cell survival (Mennigen *et al.*, 2009; Khailova *et al.*, 2010). Interestingly, the epithelial cell signalling implicated is not only stimulated by bacterial metabolites but also by whole bacteria formulations (Madsen, 2012).

Effects on the gut physico-chemical conditions

Physico-chemical properties of digesta in the colon such as bulking, water retention, pH, viscosity and levels of bile acids were disrupted in CRC subjects (Ohara *et al.*, 2009; Roessler *et al.*, 2011; Clark *et al.*, 2012). This environment can be altered by probiotics to increase the resistance to carcinogenesis. As demonstrated by Lan *et al.*, upon exposure to probiotic propionibacteria short chain fatty acids (SCFAs, propionate and acetate), an acidic

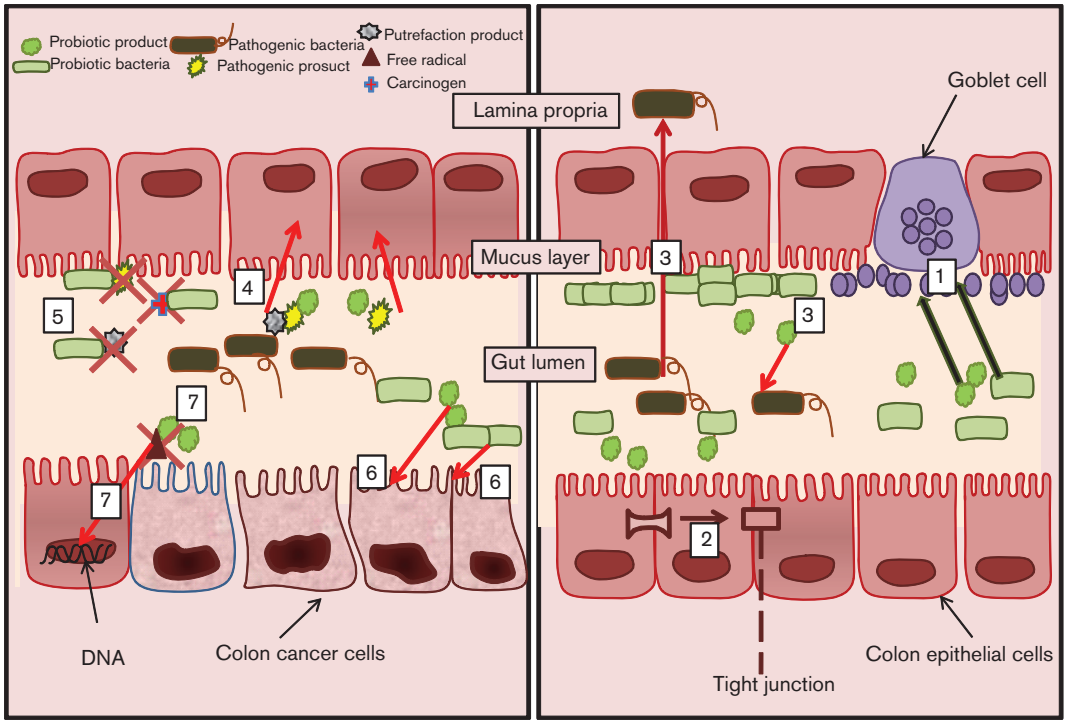

Fig. 1. Mechanisms of action of probiotic bacteria in the induction of the immune and the anti-inflammatory response in the gut: involvement of macrophages and DCs. (1) Preventing the inflammatory response against pathogens: probiotic bacteria may prevent the immune response against pathogens by inhibiting NF-κB in macrophages thus decreasing IL-8 (which prevents recruitment of neutrophils). (2) Enhancing the inflammatory response through macrophages: probiotic bacteria activate the immune response by inducing epithelial cells to produce TNF, activation of NF-κB in macrophages and thus IL-8 production. (3) Damping the inflammatory response through DCs: probiotic bacteria reduce the communication between DCs and pathogenic bacteria and prevent activation of CD4+ T-cells and TNF production as an inflammatory response to pathogens. (4) Inducing the anti-inflammatory response through DCs: probiotic bacteria can stimulate the innate immune system by signalling DCs which activates T-regulatory cells and induces the production of anti-inflammatory cytokines, mainly IL-10 and TGF-β.

extracellular pH shifts cancer cell death from apoptosis to necrosis (Lan *et al.*, 2007). Moreover, a slight change in pH conditions (a lower pH in the faeces) can block harmful enzymatic activity of the commensal bacteria and its binding to the surrounding epithelial cell wall and molecules (Wollowski *et al.*, 2001). The toxicity of faecal water content (Prescott, 1912; Grishina *et al.*, 2011) and the degree of water absorption by the colon are one the first signs of irritation of the colonic mucosa (Jensen *et al.*, 1976). Rats consuming *Bifidobacterium adolescentis* SPM1207 had less faecal water content than did control rats, decreasing colon toxicity, due to reduced exposure to soluble toxic compounds (Wollowski *et al.*, 2001; Lee *et al.*, 2009). A clinical trial on the daily consumption of *L. gasseri* OLL2716: LG21 for 12 weeks in CRC patients demonstrated a decrease in alkalosis in stool and faecal product synthesis (oxidized products from incomplete fermentation) such as putrescine, a cancer marker (Apás *et al.*, 2010; Ohara *et al.*, 2010). Thus, mounting evidence suggests that the improvement of colonic environment by probiotic bacteria is strongly linked to a decrease in colonic irritation and lesions that cause inflammation and abnormal cell growth.

Effect of probiotics on metabolic and carcinogenic compounds

Activity of bacterial enzymes in CRC

An unbalanced gut microbiota may favour the secretion of bacterial enzymes such as β-glucuronidase, β-glucosidase, azoreductase (Gorbach & Goldin, 1990) and nitroreductase, which produce carcinogens (Kim *et al.*, 1994; Hambly *et al.*, 1997; Ohno *et al.*, 2001). These harmful enzymes generate toxic metabolites such as aromatic amines (Gorbach & Goldin, 1990; Roldán *et al.*, 2008), transformed secondary bile salts (McGarr *et al.*, 2005), hydrogen sulfide (Ramasamy *et al.*, 2006), aglycones (McBain & Macfarlane, 1998), acetaldehydes (Seitz & Becker, 2007) and reactive oxygen species (ROS) (Kumar *et al.*, 2007). β-Glucosidase, for example, can hydrolyse the detoxifying compound glucuronide, and produce other carcinogens. Bacterial β-glucuronidase produced by *Clostridium perfringens* (Fujisawa & Mori, 1996) increases the genotoxicity of food mutagens, such as 2-amino-3-methylimidazo [4,5-f] quinolin (IQ) in the colon (Abdelali *et al.*, 1995). The bacterial enzymes azoreductase and nitroreductase, produced by

bacteria such as *Bacteroides*, *Clostridium*, *Enterococcus*, *Salmonella* and *Staphylococcus* (Chung *et al.*, 1992), metabolize colourants, drugs and aromatic nitro compounds to generate toxic aromatic amines (Gorbach & Goldin, 1990). *Enterobacter*, *Enterococcus*, *Streptococcus*, *Citrobacter* and *Escherichia* increase alcohol dehydrogenase activity and the production of acetaldehyde, a carcinogen (Azcárate-Peril *et al.*, 2011).

Inhibition of harmful enzymatic activity

In CRC patients, bile acids and cholesterol are converted to microbial products faster in the colon, leading to a disrupted enzymatic activity of the faecal flora and the generation of harmful enzymes (Mal *et al.*, 2012). These are reduced by the administration of probiotic formulations. Interestingly, several studies showed that *Bifidobacterium* or *Lactobacillus* consumption may limit the formation of toxic metabolites by decreasing the dehydroxylation of primary bile acids and reducing faecal deoxycholic acid concentrations (De Preter *et al.*, 2011). *L. rhamnosus* GG significantly decreased the activity of β-glucuronidase (Bertkova *et al.*, 2010). Indeed, the activity of harmful bacterial enzymes can be reduced by certain LAB, as observed with *Butyrivibrio fibrisolvens* supplementation in a mouse CRC model (Ohkawara *et al.*, 2005) and with *Lactobacillus plantarum* given to rats with DMH-induced CRC (Bertkova *et al.*, 2010). Furthermore, *B. adolescentis* SPM1207 (Lee *et al.*, 2009) and *B. adolescentis* SPM0212 (Kim *et al.*, 2008) reduced intestinal β-glucosidase, and β-glucuronidase (Kekkonen *et al.*, 2011), as well as tryptophanase and urease, producers of putrefactive products linked to higher incidence of CRC, such as indoles and ammonia (An *et al.*, 2010, 2011).

Removal of carcinogenic products by probiotics

Carcinogenic compounds in the gut. In CRC cases, high oxidative and genotoxic levels in the gut have been observed (Mai *et al.*, 2009; Boleij & Tjalsma, 2012). In fact, high levels of bile acids in the aqueous phase of faeces were detected. Bile acids can exert cytotoxic effects on the colonic epithelium and increase malignant cell proliferation (Fotiadis *et al.*, 2008). Bile acids (e.g. deoxycholic acid and lithocholic acid) are potentially carcinogenic and are negatively correlated with the levels of antineoplastic products in the colon, such as SCFAs (Ou *et al.*, 2012). The colonic mucosa is exposed to cancer-causing compounds (Nancey *et al.*, 2001; Nau, 2011) that are mutagens and pro-mutagens such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), IQ, benzo(α)pyrene and sodium azide (Cheah, 1990; Schiffman *et al.*, 1990; Pearson *et al.*, 2009). Also, a high level of food-borne generated compounds (Cross & Sinha, 2004), such as aflatoxin B1 (AFLB1) and 3-amino-I,4-dimethy-5H-pyrido (4,3-b) indole (TrpP-1), a fungal dietary contaminant, can increase gut genotoxicity (Nancey *et al.*, 2001; Nau 2011). Carcinogens such as *N*-nitroso compounds and indoles, generated from the intestinal metabolism of proteins, may increase faecal mutagenicity and increase CRC risk (Kelloff *et al.*, 1996; Davis & Milner, 2009).

Recent studies have demonstrated that probiotic bacteria can reduce carcinogen levels by deactivation or mechanical sequestration, reducing the impact on epithelial cells (Fig. 1) (Bomba *et al.*, 2012).

Binding of carcinogens. *L. rhamnosus* GG and *L. rhamnosus* LC-705 were shown to bind carcinogens such as indole and AFLB1 and excrete them in the faecal matter (Eaton & Gallagher, 1994; El-Nezami *et al.*, 1998). It was also demonstrated that *Bifidobacterium longum*, *Lactobacillus acidophilus* and *Streptococcus salivarius* strains could bind and cause the release in faeces of heterocyclic amines and mutagens such as 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (MeIQ), 2-amino-3-methyl-3H-imidazo [4,5-f] quinoline (MHIQ), and 5-phenyl-2-amino-l-methylimidazo [4,5-f] pyridine (PhMIP) (Bolognani *et al.*, 1997). The administration of *L. reuteri* DDL 19, *L. alimentarius* DDL 48, *Enterococcus faecium* DDE 39 and *B. bifidum* DDBA, to animals, and *L. gasseri*, to CRC patients, decreased mutagen faecal concentrations such as putrescine (Apás *et al.*, 2010), cadaverine and tryptamine (toxic amines) (Ohara *et al.*, 2010). Better methodology for the investigation of binding capacity of probiotic bacteria as well as their effects on mutagens is still required.

Inactivation of carcinogens. LAB can decrease the activity of carcinogens such as MNNG and DMH by scavenging reactive intermediates and producing carcinogen-deactivating and antioxidative enzymes such as glutathione-S-transferase (GST), glutathione, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase (Liong, 2008). Remarkably, the treatment of colon cells with a supernatant from bacterial fermentation increased GST activity, an enzyme considered as having chemopreventive potential (Scharlau *et al.*, 2009). The probiotic suppression of DMH-induced rat CRC can be related to the detoxifying effect of antioxidant enzymes (Kumar *et al.*, 2012).

Anti-carcinogenic and antioxidant metabolites produced by probiotics prevent CRC

Probiotics enhance the fermentation of dietary fibres (Borowicki *et al.*, 2011) and increase the levels of antitumour compounds such as SCFAs, conjugated linoleic acids (CLAs) or phenols, with potential therapeutic effects against CRC (Wollowski *et al.*, 2001; Le Leu *et al.*, 2010). SCFAs are an energy source for colonocytes (Floch, 2010) and promote acidosis and apoptosis of CRC cells (Pufulete, 2008). *B. lactis* increased the production of SCFAs promoting an acidic environment that is problematic to the formation of high levels of secondary bile acids (Zampa *et al.*, 2004) and lowering the incidence and multiplicity of colonic neoplasms (Le Leu *et al.*, 2010). A number of probiotic bacteria produce, from lactic fermentation, phenols with antioxidant capacity (Lai *et al.*, 2013) and bioactive fatty acids such as CLAs

(Mladenova *et al.*, 2011), a group of isomers of linoleic acid, that possess anti-inflammatory and anti-carcinogenic properties (Bertkova *et al.*, 2010). During growth, *Pediococcus pentosaceus* 16:1, *L. plantarum* 2592 and *Lactobacillus paracasei* F19 produce antioxidants corresponding to almost 100 mg of vitamin C (Kruszewska *et al.*, 2002). This antioxidant capacity may inhibit peroxidation and scavenge free radicals, preventing tumour formation (Kumar *et al.*, 2012). On the other hand, Watson has stated in his recent review that the antioxidant nutritional supplements may cause more cancers than they prevent (Watson, 2013). It is clear that more research is needed in this field.

Several anti-carcinogenic and antioxidant probiotic products potentially repress and prevent colon neoplastic growth (Pufulete, 2008) by the acceleration of apoptosis (Borowicki *et al.*, 2011) and the inhibition of cancer cell proliferation. In addition, probiotic bacteria and their metabolites were found to promote cell differentiation (Linsalata & Russo, 2008; Linsalata *et al.*, 2010) and reduce DNA damage in the colonic epithelium (Table 2) (Gozuacik & Kimchi, 2004; Kim *et al.*, 2010).

Probiotics favourably modulate the host immune response to reduce CRC risk

Probiotics can both suppress and enhance the intestinal and systemic immune response, offering therapeutic and preventive options against inflammatory diseases and CRC (Takagi *et al.*, 2008; Elmadfa *et al.*, 2010). Probiotics affect immunological and cellular responses by enhancing the epithelial barrier and stimulating the production of anti-inflammatory, antioxidant and anti-carcinogenic compounds. Increasing evidence suggests that probiotics, interacting via Toll-like receptors (TLRs), induce anti-inflammatory cytokine production, initiate TNF production in epithelial cells, inhibit NF-κB in macrophages and influence the production of IL-8 needed for the recruitment of neutrophils (Fig. 2) (Gareau *et al.*, 2010). Some strains of lactobacilli can also promote regulatory T-cell activity, stimulate bactericidal phagocytic activities of neutrophils in peripheral blood and natural killer (NK) cell activity involved in the suppression of tumorigenesis (Fig. 2) (Ohara *et al.*, 2009).

Lactobacillus and *Bifidobacterium* have been shown to decrease the expression of TLR-4, IL-8 secretion and NF-κB activation (Grimoud *et al.*, 2010), potentially caused by the release of bacterial products such as proteins, flagellin and LPS, and to decrease the expression of peroxisome proliferator-activated receptors (PPAR) γ, a ligand for CLAs (Bassaganya-Riera *et al.*, 2002; Ewaschuk *et al.*, 2006; Bassaganya-Riera & Hontecillas, 2010). SCFAs have immunomodulatory functions that affect the inflammatory response, in some cases through interactions with G-protein-coupled receptors in the gut (Kimura *et al.*, 2011). Recent animal and human studies have discussed the cellular and immunological effects of bacterial cells and products of recent probiotic formulations.

Animal studies

Lactobacillus fermentum FERM P-13857 and *Lactobacillus casei shirota* elicited IL-12 production in bone marrow cell-derived dendritic cells (DCs) in mice (Takagi *et al.*, 2008), which stimulates DCs and activates NK cells, involved in tumour-immune surveillance (Takagi *et al.*, 2008). Also, *L. rhamnosus* GG and *B. adolescentis* bacterial extracts, given to rats, induced macrophage activation and significantly increased the production of TNF-α (Bertkova *et al.*, 2010) and nitric oxide (NO) by macrophages (Lee *et al.*, 2008), which can be cytotoxic or cytostatic to tumour cells (Switzer *et al.*, 2011). Potential immunomodulatory and anti-tumorigenic properties of microencapsulated *L. acidophilus* (Urbanska *et al.*, 2009) and *Saccharomyces boulardii* (Chen *et al.*, 2009) in a yogurt formulation administered to *Apc* (Min/+) mice was demonstrated. There was a correlation between the reduction of intestinal tumour growth, dysplasia and inflammation with the oral administration of probiotics (Urbanska *et al.*, 2009). The mechanisms involved were related to the downregulation of extracellular-signal-regulated kinases (Erk)1/2 activities through the inactivation of growth receptors such as EGFR (epidermal growth factor receptor) and EGFR-Erk pathways (Chen *et al.*, 2009).

Human studies

In a recent animal study, *L. gasseri* OLL2716: LG21 increased IL-1β, a cytokine that plays a central role in the regulation of immune responses, and enhanced NK cell activity in the blood (Ohara *et al.*, 2010). The daily ingestion of fermented milk containing *L. casei shirota* for 3 weeks restored NK cell activity in healthy subjects. Peripheral blood mononuclear cells (PBMCs) from healthy humans were cultured in the presence of heat-killed *L. casei shirota*, which increased the activity of NK cells (Nanno *et al.*, 2011), which play a role in tumour-immune surveillance (Uccello *et al.*, 2012). *L. rhamnosus* GG, *B. lactis* Bb12 and/or inulin enriched with oligofructose demonstrated immune stimulatory effects by inducing the maturation of DC (Roller *et al.*, 2007), reinforcing the immune response against tumour cells (Elmadfa *et al.*, 2010). This formulation has shown anti-inflammatory effects by the activation of IL-10-secreting cells linked to the induction of apoptosis in colon cancer and suppressing pro-carcinogenic factors (Ewaschuk *et al.*, 2006; Zhu *et al.*, 2011).

Application of probiotics as a supplement to advanced-CRC treatments

Based on their anticancer properties, probiotics can be used in combination with conventional CRC therapies (such as surgery and chemotherapy) (Baldwin *et al.*, 2010). Data obtained, although based on a limited number of patients and samples, suggest an effective approach for achieving clinical benefits in immune-compromised hosts by improving their intestinal environments (Wada *et al.*,

Table 2. Inhibition of cancer cell proliferation and prevention of malignant transformation: effects and mechanisms of probiotics

Probiotics	CRC model and treatment	Effects	Potential mechanisms	References
Enterococcus faecium RM11 and *L. fermentum* RM2	Caco-2 cells; live probiotic cells and supernatant	↓ Cell viability	↑ Adherence	Thirabunyanon *et al.* (2009)
			↑ Apoptosis	
			↑ Pan-caspases	
Saccharomyces boulardii	HT-29, SW-480 or HCT-116; probiotic cells	↓ Colony formation and induction of apoptosis	↓ EGFR-Erk and EGFR-Akt pathways	Chen *et al.* (2009)
	Apc (Min/+) mice; oral administration of probiotic cells	↓ Intestinal tumour growth and dysplasia	↓ EGFR and receptor tyrosine kinase signalling	
			↓ HER-2, HER-3, and insulin-like growth factor-1 receptor	
L. delbrueckii CU/22	HT-29 cells; probiotic supernatant	↑ Apoptosis and necrosis	↑ Bacterial hydrogen peroxide and superoxide radicals	Strus *et al.* (2009)
L. acidophilus 606 EPS	HT-29 cells; isolated cell-bound exopolysaccharides (cb-EPS)	↑ Tumour cell death via autophagy	↑ Beclin-1 and GRP78	Kim *et al.* (2010)
		Alteration of cell morphology	↓ Bcl-2 and Bak regulation	
L. rhamnosus GG and *B. lactis* Bb12 (aleurone (+))	HT29 and LT97 cells; fermentation supernatant	↓ Cell growth	↑ Cell cycle arrest in G(0)/G(1) and alkaline phosphatase activity	Borowicki *et al.* (2011)
			↑ Apoptosis and p21 and WNT2B	
B. lactis and *L. rhamnosus*	Caco-2 cancer cell line; live probiotic bacteria	↑ Apoptosis	BAX translocation, cytochrome *c* release, and caspase-9 and -3 cleavage	Altonsy *et al.* (2010)
Bacillus polyfermenticus	Colon, breast, cervical and lung cancers and azoxymethane-treated NCM-460 colonocytes; bacterial cell-free supernatant	↓ Colony formation on soft agar	↓ ErbB receptor-dependent pathway	Ma *et al.* (2010)
	Tumours implanted in the skin of nude mice; Injection of bacterial cell-free supernatant	↓ Carcinogen-induced colony formation by normal colonocytes	↓ ErbB2 and ErbB3 protein and mRNA expression	
		↓ Tumour growth	↓ E2F-1-dependent transcriptional regulation of cyclin D1	
L. paracasei subsp. *paracasei* M5, *L. paracasei* subsp. *paracasei* X12, *L. fermentum* K11, *L. fermentum* K14 and *L. casei* X11	HT-29 cells; cell walls and cytoplasm extracts	↓ Cell proliferation	↑ Apoptosis	Wang *et al.* (2012)
			S-phase accumulation	
S. thermophilus 14085 and *Bifidobacterium infantis* 14603	HT-29 and Caco-2 cells; extracts from fermented soymilk with organic solvents	↓ Cell proliferation	Antitumour bioactive compounds from bacterial fermentation	Lai *et al.* (2013)
L. plantarum AS1	CRC induced by DMH in rats; pre- and post-treatment with 1 ml containing 10^9 c.f.u. of *L. plantarum* AS1 in saline day^{-1}	↓ Mean tumour volume diameter and total number of tumours	Altering lipid peroxidation and antioxidant enzyme activities in the colon and in the plasma	Kumar *et al.* (2012)
Propionibacterium freudenreichii	HGT-1 cells; fermented milk supernatant	↑ Apoptosis	↑ Chromatin condensation and formation of apoptotic bodies	Kumar *et al.* (2013)
		↑ Cytotoxicity of camptothecin, a drug used in chemotherapy	↑ DNA laddering and cell cycle arrest	
			↑ ROS	
			↑ Caspase activation and cytochrome c release	

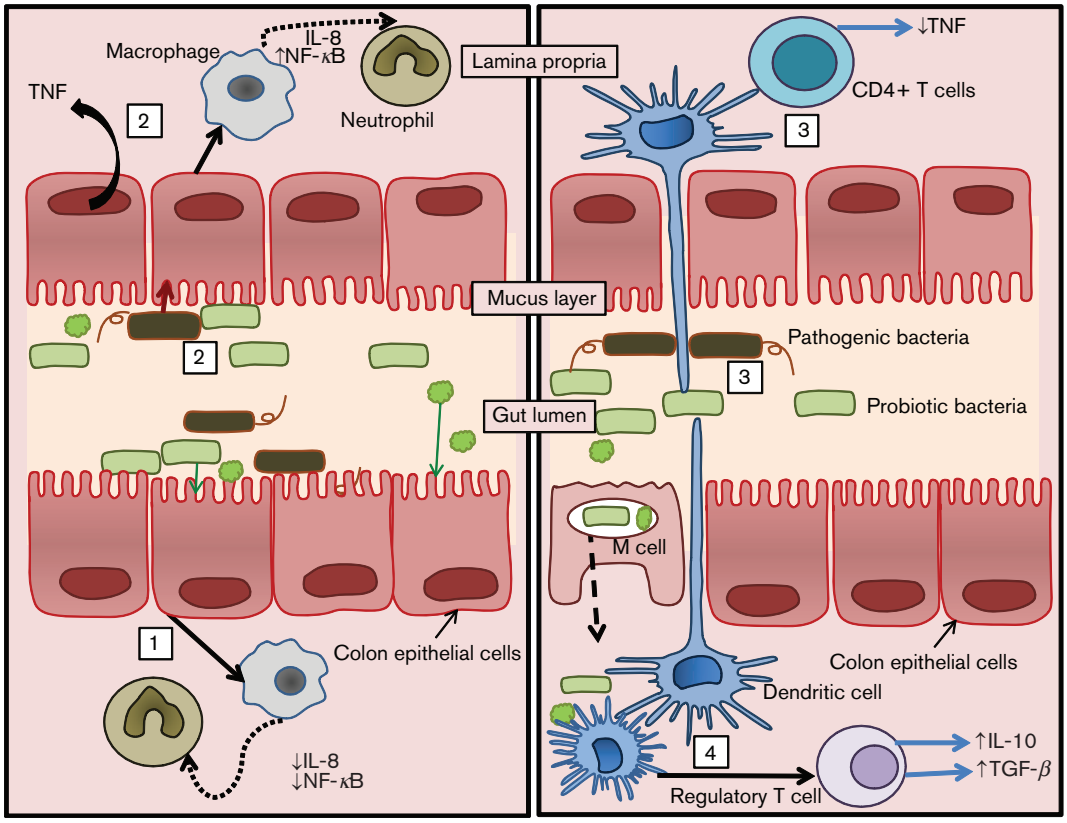

Fig. 2. Potential mechanisms of action of probiotic bacteria in the improvement of the physico-chemical conditions and the microbiota balance in the colon while producing beneficial metabolites and reducing toxic compounds. (1) Enhancing mucus production from goblet cell. (2) Reinforcing intercellular integrity by increasing the intercellular integrity of apical tight junctions and producing beneficial metabolites that improve the growth of epithelial cells. (3) Antimicrobial activity by blocking pathogen entry into the epithelial cells and also by producing antimicrobial compounds. (4) Reducing carcinogens production by inhibiting the activity of harmful enzymes that generate potential carcinogens from bile salts, food and other products. (5) Detoxification of toxic compounds by decreasing faecal putrefaction, degrading and binding certain molecules. (6) Inhibiting cancer cell proliferation by producing anti-carcinogenic metabolites that suppress malignant growth and induce apoptosis in cancer cells. (7) Decreasing oxidative stress and genotoxicity by producing antioxidants that scavenge free radicals, such as reactive oxygen species, and reduce DNA damage in colon cells.

2010). The administration of probiotics along with CRC treatment may alleviate the secondary effects related to chemotherapy (Osterlund *et al.*, 2007). Moreover, clinical reports show that probiotics can improve the integrity of the gut mucosal barrier and decrease infectious complications in surgical CRC patients (Liu *et al.*, 2011). Some of the recent applications of probiotic strains in CRC are summarized in Table 3.

With chemotherapy

Recent studies showed the ability of LAB to enhance the apoptosis-induction capacity of 5-fluorouracil (5-FU), a chemotherapeutic agent (Baldwin *et al.*, 2010). According to Osterlund *et al.*, *L. rhamnosus* GG supplementation reduced several undesirable effects of 5-FU-based chemotherapies such as the frequency of severe diarrhoea and abdominal discomfort (Osterlund *et al.*, 2007). Patients receiving *L. rhamnosus* GG along with 5-FU-based regimens needed less hospital care, had less bowel toxicity, received fewer chemotherapy doses and suffered less from abdominal pain and diarrhoea than patients with no probiotic administration (Osterlund *et al.*, 2007). Nagata *et al*, concluded from their study that the enteral administration of *Bifidobacterium breve Yakult* to cancer patients on chemotherapy was shown to prevent infections and particularly improve the faecal microbiota; the frequency of fever and the use of intravenous antibiotics were also reduced (Wada *et al.*, 2010).

Effects on complications related to surgery

In patients with CRC, supplementation with viable probiotics, before surgery, can improve bacterial dysbiosis (Zhang *et al.*, 2010). *L. casei shirota* was given to patients whose colonic polyps were surgically removed in order to

suppress the recurrence of CRC (Nanno *et al.*, 2011). Infection following abdominal operation, considered as a factor affecting the morbidity of patients, was reduced using preoperative administration of probiotics. Patients who received daily encapsulated treatment containing *B. longum* BL-88, *L. acidophilus* La-11 and *L. plantarum* CGMCC No. 1258, before and after their operation, had better recovery of peristalsis, lower incidence of diarrhoea (Liu *et al.*, 2011) and reduced infection-related complications (Liu *et al.*, 2011). Likewise, Zhang and colleagues found that the preoperative use of viable *Bifidobacterium* stabilized the immune status and prognosis of patients undergoing CRC resection and diminished postoperative septic complications (Zhang *et al.*, 2010). Probiotic mixtures supported the intestinal barrier function following CRC surgery, which may have prevented cancer recurrence (Xia *et al.*, 2010). Polypectomized patients and CRC patients who have undergone curative resection while receiving *B. lactis* and *L. rhamnosus* had greater PBMCs producing IFN-γ and IL-2, both cytotoxic to cancer cells (Roller *et al.*, 2007).

Effects on inflammation

Lactobacillus johnsonii La1, given orally pre- and post-operatively, adhered to the colonic mucosa, reducing the counts of potentially pathogenic bacteria in the stool (enterobacteria and enterococci). Gianotti and colleagues used *L. johnsonii* La1 in a formulation with *B. longum* BB536 and demonstrated the increased expression of naive and memory lymphocyte subsets while reducing dendritic phenotypes, dampening an overinflammatory response at the intestinal and distant sites in case of surgery (Gianotti *et al.*, 2010). In addition to alleviating several undesirable complications associated with CRC treatments, the administration of probiotics to patients may prevent cancer recurrence and improve their quality of life (Xia *et al.*, 2010). On the other hand, a mixture of probiotic bacteria: *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *L. paracasei* subsp. *paracasei* and *L. plantarum*, with bioactive plant fibres β-glucans, inulin, pectin, resistant starch, postoperatively elevated the levels of the anti-inflammatory cytokine IL-6 and prevented mild wound infection with faecal secretion. In this case, the synbiotic formulation did not have an anti-inflammatory effect, probably due to absence of bowel cleaning (Horvat *et al.*, 2010). As described, specific probiotic strains administered in different ways (mixture, period, dose) were effective to a certain extent in bringing clinical benefits to CRC patients. However, more investigations are needed to improve probiotic formulations for better efficacy.

Significance and future directions of probiotic formulations in CRC

Very few reports demonstrate any limitations and negative aspects of probiotic oral supplementation. Some studies suggest that an increased bacterial translocation was related to mortality after supplementation with *Lactobacillus delbrueckii* UFV-H2b20 and *B. lactis* Bb12 in mice with DMH-induced injuries. These findings alert us to the potentially severe side-effects associated with the use of probiotics under stressful situations, such as change in environmental and experimental conditions (Liboredo *et al.*, 2010). The variability observed in the documented benefits of probiotics in humans was shown to be dependent on the concomitant therapies and the health baseline status of the patient, the dosing and the addition of prebiotics or many strains into the formulation. Many reports brought to attention another important player minimizing the efficacy of orally administrated probiotics which is the loss in the viability of probiotics reaching the large intestine (Tomaro-Duchesneau *et al.*, 2012b). Subsequently, microencapsulation, defined as the entrapment of viable cells in a polymer matrix, has been suggested to improve cell viability during GI transit (McConnell *et al.*, 2008; Del Piano *et al.*, 2010; Prakash *et al.*, 2011). Microencapsulation of probiotics can confer a significant resistance to gastric juice, thus protecting the bacterial cells during gastric and duodenal transit (Kailasapathy, 2002; Del Piano *et al.*, 2011). Indeed, the use of artificial cell microcapsules allows for a 'pH controlled delivery' of the probiotic bacteria through the gut. Concurrently, it allows the diffusion of oxygen, nutrients and metabolites while preventing white lymphocytes, antibodies and cytokines from accessing the microcapsule (Sultana *et al.*, 2000; Kailasapathy, 2002; Tomaro-Duchesneau *et al.*, 2012a). As supported by previous research, this technology may assume a lot of importance in the near future for the development of active probiotic bacterial preparations in treating many diseases, including CRC.

Concurrently, recent research continues to support the idea that probiotic consumption may reduce tumour growth, modulate the host immune response and re-establish healthy gut conditions in CRC subjects. Recent studies continue to provide evidence that probiotic formulations have the potential to protect the gut and colon epithelial cells against toxic substances digested or produced within the intestine, reactive metabolites and from compromising activity of pathogens or endogenous commensal bacteria (Iacono *et al.*, 2011; Circu & Aw, 2012). Several studies have shown the immunomodulatory impact of probiotics on the inhibition of tumour growth by the modulation of cytokines production and signalling pathways related to carcinogenesis initiation and epithelial cell growth (Azcárate-Peril *et al.*, 2011; Ullman & Itzkowitz, 2011; Zhu *et al.*, 2011). Research in this field still has to progress towards a solid understanding of the molecular interactions of the micro-organisms with both healthy and compromised hosts (Kleerebezem & Vaughan, 2009). The current treatments of CRC include invasive procedures and toxic drugs that not only attack cancer cells but also affect normal cells (Siegel *et al.*, 2012). As a current view, it seems challenging to portray probiotics as a therapy that can replace these treatments, but, the emerging outcomes of

Table 3. Clinical applications of probiotic formulations in CRC patients

Probiotics	Treatment	Trial design and CRC conditions	Clinical study outcomes	References
L. rhamnosus GG LGG and *B. lactis* Bb12	10^{10} c.f.u. of *L. rhamnosus* GG LGG and *B. lactis* Bb12 +10 g of oligofructose-enriched inulin	Randomized, double-blinded, placebo-controlled trial	↑ Faecal *Bifidobacterium* and *Lactobacillus*	Rafter *et al.* (2007)
	In a capsugel	For 12 weeks	↓ *Clostridium perfringens*	
	Orally; daily for 12 weeks	37 CRC and 43 polypectomized patients	↓ CRC proliferation ↓ Faecal water-induced necrosis in cancer cells ↓ Exposure to genotoxins ↓ Secretion of IL- 2 ↑ Production of IFN-γ	
L. rhamnosus GG and *B. lactis* Bb12	10^{10} c.f.u. of *L. rhamnosus* GG and 10^{10} c.f.u. of *B. lactis* Bb12 +10 g of inulin enriched with oligofructose	Randomized double-blinded, placebo-controlled trial	↑ IL-2 secretion by activated PBMCs	Roller *et al.* (2007)
	Encapsulated		↑ Capacity of PBMC to produce IFN-γ	
	Orally; daily for 12 weeks	34 CRC patients with curative resection and 40 polypectomized patients	Minor stimulatory effects on the systemic immune system	
B. longum BB536 and *L. johnsonii* La1	10^{7} or 10^{9} c.f.u. of a mixture of *B. longum* BB536 and *L. johnsonii* La1	Randomized, double-blinded	Probiotic adherence to the colonic mucosa	Gianotti *et al.* (2010)
	Orally; two daily doses for 3 days preoperatively and 5 days postoperatively	31 subjects with elective resection for CRC	↓ Pathogens	
			↓ Dendritic phenotypes CD83-123, CD83-HLADR; CD83-11c	
Pediococcus pentosaceus, *Leuconostoc mesenteroides*, *L. paracasei* 19 and *L. plantarum* 2362	10^{10} c.f.u. of each probiotic + 10 g fibre Orally; every 8 h, 2 days preoperatively and at day 2 postoperatively till day 4	Prospective double-blinded randomized placebo-controlled trial	↑ IL-6 after 72 h	Horvat *et al.* (2010)
		68 patients having mechanical bowel cleaning preoperatively	↓ Mild wound infection with faecal secretion	
L. plantarum CGMCC No. 1258, *L. acidophilus* La-11 and *B. longum* BL-88	2×10^{11}c.f.u.	100 patients with CRC	↓ Bacterial translocation	Liu *et al.* (2011)
	L. plantarum CGMCC No. 1258, 1×10^{10} c.f.u. of *L. acidophilus* La-11 and 5×10^{10} c.f.u. of *B. longum* BL-88		↑ Transepithelial resistance ↓ Transmucosal permeation of horseradish peroxidase and lactulose/mannitol ratio	
	Daily		↓ Ileal-bile acid binding protein Positive rate of blood bacterial DNA	
	Encapsulated formulation 6 days preoperatively and 10 days postoperatively		↑ Mucosal tight junction protein expression ↓ Blood enteropathogenic bacteria Post-operative recovery of peristalsis Improved infectious-related complications	

Table 3. cont.

Probiotics	Treatment	Trial design and CRC conditions	Clinical study outcomes	References
			↓ Incidence of diarrhoea	
L. rhamnosus LGG	2×10^{10} c.f.u. of *L. rhamnosus* LGG	150 patients having 5-FU-based regimens	↓ Frequency of severe diarrhoea and abdominal discomfort	Osterlund *et al.* (2007)
	Daily for 24 weeks on cycle days 7–14, for 8 days/month		↓ Chemotherapy dose	
			↓ Abdominal discomfort and diarrhoea	
B. breve Yakult	Enteral	42 CRC patients on chemotherapy	↓ Risk infection	Wada *et al.* (2010)
			Improved faecal micro flora and intestinal environments	
			↓ Frequency of fever	
			↓ Intravenous antibiotics use	
L. casei Shirota	After surgery	Patients with surgically removed colonic polyps	↓ Recurrence of CRC with moderate/severe atypia	Nanno *et al.* (2011)
L. johnsonii La1 and *B. longum* BB536	2×10^{7} *L. johnsonii* La1 and 2×10^{9} c.f.u. day^{-1}*B. longum* BB536, Orally for 3 days pre- and 6 days postoperatively	21 CRC patients	↓ Pathogens	Gianotti *et al.* (2010)
			↑ Expression of naive and memory lymphocyte subsets	
			↓ Expression of dendritic phenotypes	
Bifidobacterium	Administration of viable bacteria with routine enteral nutrition	60 patients undergoing CRC resection	↓ Postoperative *Bifidobacterium*/*E. coli* (B/E) ratio as compared to preoperative	Zhang *et al.* (2010)
			↑ Both preoperative and postoperative B/E ratios	
			↑ Stool SIgA, while ↓ serum IgG, IgM, IgA, IL-6, CRP	
			↓ Postoperative septic complications	
n/a	1 day bowel preparation with probiotics for 3 days	60 patients with colonic surgery	Maintain the intestinal barrier function after surgery CRC	Xia *et al.* (2010)
Enterococcus. faecium M-74	For 3 months	60 CRC patients with colonic adenoma	↑ Biopsies with intracellular bacteria in adenoma and carcinoma group	Mego *et al.* (2005)
			↑ Intraepithelial bacteria in patients with large bowel adenoma and carcinoma	

probiotic applications in CRC or other diseases (e.g. IBS, diabetes, allergies) (Collado *et al.*, 2009) suggest the consideration of probiotics for therapeutic and prophylactic purposes. Probiotics have shown clinical latency as a supplement for CRC patients especially when administrated prior/post surgery or during prolonged hospitalization to manage symptoms related to the severity of the disease or the side-effects and other complications related to the treatments. Still, further human studies are needed to guide the decision of their establishment as complementary treatment in CRC.

Acknowledgements

This work was supported by funding from the Canadian Institutes of Health Research (CIHR) MOP 64308 to S. P. The authors wish to acknowledge a McGill Internal scholarship from the George G. Harris Fellowship Program to I. K. and a Doctoral Alexander Graham Bell Canada Scholarship from NSERC to C. T.-D.

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