Probiotics and Gastrointestinal Disorders

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The gastrointestinal (GI) tract is colonized by a vast community of symbionts and commensals that harbors a complex and diverse ecology of microorganisms comprised of 400-500 species with levels reaching 10^{11} cfu per gram of intestinal contents in the large intestine (1). These microbes have far-reaching implications for health in which they affect immunity and digestion of nutrients. Microbial interactions contribute to the homeostasis of the gut bacterial flora and destabilization of this microorganism ecosystem results in various GI disorders (2). It has been suggested that probiotics helps to maintain GI equilibrium of the indigenous microflora and benefit the host's health. They are thus defined as "live microorganisms, that when administered in an adequate amounts, confer a health benefit on the host" (3). Probiotic strains are considered as safe and non-pathogenic (4). The ability of probiotics to regulate gut microflora equilibrium is early recognized by Fuller in 1989 (5) who defined the term "probiotics" as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Havenaar et al. (6) further elaborated the term and redefined probiotics as "mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora".

In general, probiotics must meet the following criteria in order to exert their

beneficial effects in the GI tract: (1) to resist gastric acid and bile secretions (7), (2) to colonize and adhere to mucosa and be metabolically active in the GI tract (8-9), and (3) to produce antimicrobial substance with activity against potential pathogens such as *Clostridium* and *Salmonella* species (10-11). Currently, the best-studied probiotics include bacteria such as lactobacilli and bifidobacteria, as well as the yeast *Saccharomyces boulardii*. It has been proposed that probiotics maintain intestinal milieu and exert benefits to the gut by stimulating the immune system by prevention of adherence of antigens by competing adhesion sites, producing antimicrobial substances, inducing specific antibody secretions and mucus by epithelial cells (12), detoxifying colonic contents, promoting lactose tolerance, and producing metabolites that are essential to maintain intestinal health (13). Because of these potential beneficial effects, they have been used to treat various intestinal disorders such as inflammatory bowel disease, irritable bowel syndrome, acute gastroenteritis, lactose intolerance and colon cancer.

Gastrointestinal tract ulcer and lesions

Ulceration activates platelets and other inflammatory cells to produce various types of growth factors, cytokines and chemokines, which mediate re-epithelialization, restoration of muscular components, angiogenesis, tissue remodeling, and ultimately

ulcer healing (14-15). Halper et al. (16) discovered that metabolites of lactobacilli culture induced angiogenesis and proteoglycans deposition which is crucial for tissue remodeling. Indeed, polysaccharide fractions isolated from the cell wall of bifidobacteria, lactobacilli and streptococci were reported to have anti-ulcer effect and polysaccharide fractions from *Bifidobacterium bifidum YIT4007* were found to up-regulate epidermal growth factor and basic fibroblast growth factor (17). These findings provide evidence to support the notion that probiotics could indeed heal mucosa lesions and ulcer. In addition, Resta-Lenert and Barrett (18) demonstrated the effectiveness of both the live probiotics and its metabolites to increase trans-epithelial resistance, a parameter to measure intestinal epithelium layer integrity. However, it is evident that this effect is strain specific (19).

Indeed, yogurt containing *Lactobacillus gasseri* OLL2716 (LG21 yogurt) exhibited gastroprotective action and was found to protect against HCl-induced acute gastric lesions and antral ulcer in rats while non-fermented milk did not. This protective effect is suggested to be exerted through the increase in prostaglandin E_2 levels (20), which inhibit acute gastric lesions through the increase of gastric mucosal blood flow and bicarbonate secretion (21). In addition, culture supernatant of *L. acidophilus* and *B. adolescentis* repressed

5-bromo-2-(4-fluorophenyl)-3-(-4-methylsulfonylphenyl) thiophene (BFMeT)-induced ileum ulcer formation (22-23) probably by down-regulating the formation of thiobarbituric acid-reactive substances and resisting the colonizing of gram negative bacteria in the ileal mucosa (22).

Ulcers sites are rapidly colonized by various bacteria such as *Escherichia coli*, streptococci and enterococci and Gram-negative bacteria and their lipopolysaccharides may also play a role in ulcer formation (24-25). It was reported that Gram-positive probiotic strains such as bifidobacteria and lactobacilli (26-29) antagonized the adhesion and colonization of pathogenic bacteria to the intestinal mucosa and induction of lactobacillus colonization accelerated gastric ulcer healing in rats (24). These abilities are probably mediated through competition with enteropathogens for the same carbohydrate receptors in the gut (30) and by passive forces such as exerting steric hindrance at enterocytic pathogens receptors (31). Lipoteichoic acids (LTA), cell wall components isolated from many Gram-positive bacteria (32) and adhesion-promoting protein (33) of the probiotic strains may also play a role in resisting the colonization of pathogenic bacteria. The inhibition of adherence of pathogenic bacteria to intestinal epithelial cells by probiotics bacteria may also exert through the stimulation of intestinal mucins production. The mucus

layer plays an important role in resistance to damage to the epithelial cells by gastric acid produced in the stomach and by foreign substances such as chemicals. Reports showing various probiotic strains can induce both MUC2 and MUC3 mRNA and protein expression (19, 34-36). This further supports the protective role of probiotics in ulcer formation. Furthermore, it was observed that probiotic bacteria stimulated gut epithelial cell proliferation in rats (37). Since reduced epithelial cell proliferation and mucosal atrophy of the gut increase the susceptibility of gut lumen to pathogenic invasion, the ability of probiotics to stimulate cell proliferation is a preventative measure against pathogenic damage to gastric cells. The potential actions of probiotics on ulcer healing are summarized in Table 1.

Table 1. Probiotics may affect the following biological events during ulcer healing in the gastrointestinal tract.

Actions:	Possible mechanisms of action
(1)↑Re-epithelialization	Induction of growth factors
(2)↑Angiogenesis	Induction of growth factors
(3)↑Proteoglycan deposition	Induction of growth factors
(4)↑Trans-epithelial resistance	Production of prostaglandins
(5)↑ Mucus & bicarbonate secretions & mucosal blood flow	Production of prostaglandins

Growth factors include epidermal growth factor and basic fibroblast growth factor.

***Helicobacter pylori* infections**

H. pylori infection is associated with the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma and gastric mucosal-associated lymphoid tissue (MALT) lymphoma (38-39). Some strains of lactic acid-producing bacteria (LAB) were found to inhibit the growth of *H. pylori*. Culture supernatant of *L. acidophilus La 1* inactivated *H. pylori* adhesion to HT-29 cells and inhibited their growth both in vitro and in vivo (40). The authors suggested that the inhibitory effect was partly contributed by lactic acid produced by the bacteria. Aiba et al. (41) also emphasized on the inhibitory role of lactic acid on *H. pylori* growth. They confirmed the suppression of *H. pylori* growth by *L. salivarius* in vivo using *H. pylori*-infected gnotobiotic murine model. *L. casei strain Shirota* isolated from fermented milk product Yakult also resulted in a significant reduction in *H. pylori* colonization in the antrum and body mucosa in *H. pylori*-infected mice (42).

The anti-infection effects of probiotics are suggested to be mediated through the production of antimicrobial substances, competition for adhesion of pathogens, enhancement of mucus barrier, alteration of intestinal permeability and motility, stimulation of specific and non-specific immune response (43). *Bacillus subtilis 3* was demonstrated to suppress the growth of *H. pylori* (44) and its heat stable, protease

insensitive, pH and organic acid concentration independent cell-free supernatant was suggested to be responsible for the anti- *H. pylori* effect. Amicoumacin A was one of the identified substances. Another probiotic strain, *Weissella confusa strain PL9001*, reduced infectivity and persistence of *H. pylori* by rupturing its cell wall and inhibiting its binding (45).

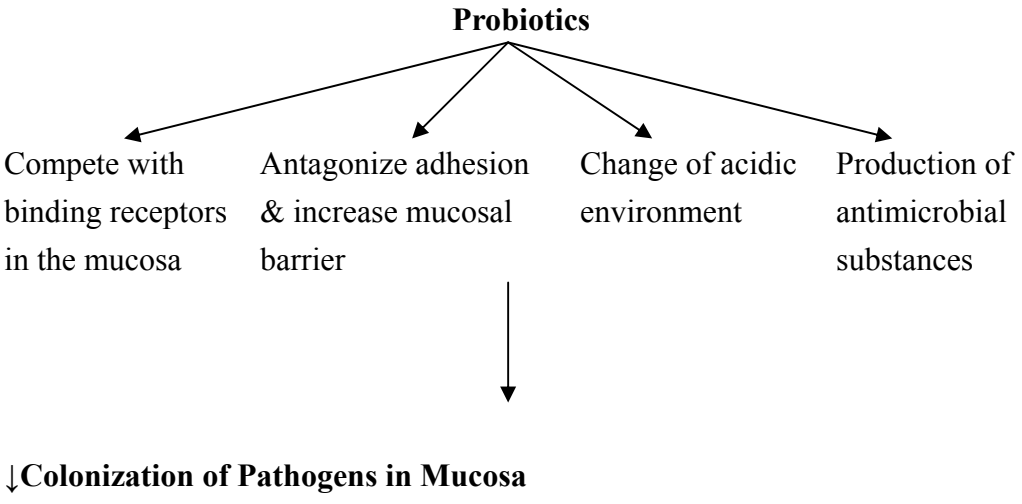
The role of probiotics in *H. pylori* eradications was also investigated. Lyophilized and inactivated *L. acidophilus* increased eradication rates of *H. pylori* (46). *L. acidophilus CRL 639* showed a decrease of viable *H. pylori* cell count and this effect was related to the intracellular proteinaceous compound (47). *Bifidobacterium* in yogurt was found to improve eradication rates of *H. pylori* in an intention-to-treat analysis (48). However, others showed the ineffectiveness of a probiotic supplemented yogurt (containing *L. acidophilus*, *L. casei*, *L. bulgaricus* and *Streptococcus thermophilus*) (49) and *Bacillus clausii* (50) in eradicating *H. pylori* infection. The overall mechanisms in the modification of pathogen infection in the intestinal mucosa are described in Figure 1.

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a chronic relapsing idiopathic

inflammation of the GI tract which embraces Crohn's disease (CD) and ulcerative colitis (UC). The prevalence of IBD ranges from 20 to 200 per 100,000 in North American and European populations (51). However, the etiology and pathogenesis of IBD remains undefined. No single agent or distinct mechanism has been implicated as a causative agent for IBD. Suspected factors include genetic disorders (52-53); microbial and viral infections (54-55); inappropriate immunological responses (53) such as defective immunoregulation of gut-associated lymphoid tissue (GALT) (56), activation of macrophages (57), intestinal permeability defects (58) and hypersensitivity reactions to antigens in the intestinal lumen or mucosa (59), thrombosis (60), neutrophil infiltration and free radical production (61) and other environmental factors such as nutrition and smoking (62).

Figure 1. Modification of pathogen infection by probiotics.



In the past decades, the importance of probiotics on relieving and treating IBD symptoms has raised much attention. It was reported that experimental colitis did not develop when mice were kept in a germ-free environment (63). This study shows that normal mucosal microflora is required to initiate or maintain the inflammatory process and exaggerated mucosal immune response to normal constituents of the mucosal microflora could lead to IBD. Both *L. plantarum* (64) and the multi-strain probiotics mixture VSL#3 (containing *S. salivarius subsp. thermophilus, L. casei, L. plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus, B. longum, B. infantis* and *B. breve*) decreased colitis score in IL-10 deficient mice which spontaneously develop colitis (65). Clinical studies showed that probiotic combination therapies using VSL#3 (66) and *S. boulardii* (67) benefit patients with UC and CD, respectively. Others indicated a difference in efficacy of different strains of probiotics in treating UC and CD. VSL#3 has been proven to prevent the onset and relapses of pouchitis (68), postoperative recurrences of CD (18, 69) and UC (70) whereas a placebo-controlled study showed that *L. rhamnosus GG* (LGG) was ineffective to prevent endoscopic recurrence and severity of recurrent lesions in patients with CD after surgery (71).

It has been proposed that these anti-inflammatory abilities are mediated through: 1. suppression of pathogenic bacteria growth by decreasing luminal pH via production of short chain fatty acids and secretion of bactericidal proteins. For instance, *B. infantis* suppressed the growth of IBD and colitis associated bacteria *Bacteroides vulgantus* (72-74); 2. prevention of epithelial binding and invasion of pathogenic bacteria by competing with pathogenic receptors on epithelium and prevent adherence; 3. modulation of immune response of GALT and epithelial cells; 4. enhancement of mucosa barrier function or immunoregulatory activities and 5. induction of T cell apoptosis in the mucosal immune compartment (11, 75). The ability of some probiotic strains to up-regulate immunologlobulin IgA (76-78), down-regulate inflammatory cytokines and enhance gut immunological barrier functions may also account for these anti-inflammatory effects. Several studies showed that several probiotic strains could induce protective cytokines, including IL-10 and TGFβ (79-80). *Lactobacillus* species, *B. longum* and VSL#3 inhibited the secretion of inflammatory cytokine interleukin-8 (IL-8), IL-4, IL-5, TNF-α and interferon-γ (16, 81-83). Immune and inflammatory responses in the GI tract often involve the transcription factor NF-κB which has been proven to be a key factor in regulating inflammatory response, apoptosis or tumorigenesis. Recently, the inhibitory effect of probiotics on IκB/NF-κB pathway and MAPK pathways is suggested for its immunosuppressive ability. Both

probiotics conditioned medium (84) and DNA (82) were reported to block NF-κB activation through inhibition of IκB ubiquitination which was probably mediated through the inhibition of proteasome activity. The blockade of IκB degradation reduced the translocation of NF-κB into the nucleus leading to the down-regulation of transcription and translation of many inflammatory chemokines. Besides, probiotic bacteria (85) and probiotic DNA (82) reduced phosphorylation of p38 MAPK indicating the involvement of the MAPK pathway in regulating chemokine release and hence intestinal inflammation and lesions.

Aggressive Th1-like cytokine expression is a strong initiator of IBD (86). Bacteria DNAs have a 20-fold greater frequency of unmethylated CpG dinucleotides than vertebrate DNA (87), which can stimulate immune cells to secrete IFN-γ (88), Th1-like cytokines (89) and B cell proliferation (90) through the toll-like receptor 9 (TLR9) recognition (91-92). Recently, VSL#3 bacteria DNA were found to suppress the proinflammatory effects of other bacteria DNAs such as *E. coli*, *Salmonella* Dublin and *Salmonella* Typhimurium (82) suggesting a novel mechanism on its anti-inflammatory actions.

Heat shock proteins (HSPs) (also known as stress proteins) are induced by a

variety of physiological stressors including proinflammatory cytokines, prostaglandins and reactive oxygen metabolites (ROS). ROS and proinflammatory cytokines are known strong factors that contribute to the pathogenesis of IBD. Our previous studies showed that heat shock protein 32 (HSP32) acted as an inflammatory defensive factor and played a protective role in the colonic damage in TNBS-induced colitis in rats (93) while HSP47 as a collagen-specific molecular chaperon contributing significantly to gastric ulcer healing (94). Interestingly, it was recently demonstrated that VSL#3 induced HSP25 and HSP72 expression in young adult mouse colon cells (84). Whether the beneficial effects of probiotics on IBD are mediated through the up-regulation of HSPs could be another focus for future studies.

Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is a multi-factorial GI disorder characterized by symptoms of abdominal pain, excessive flatus, variable bowel habit, abdominal bloating and diarrhea. The etiology of IBS remains unknown although experimental evidence indicates the involvement of antibiotics administration (95), abnormal colonic fermentation (96) and colonic microflora imbalance (97). Fecal microflora in IBS has been shown to have lower number of lactobacilli and bifidobacteria but higher numbers of facultative bacteria than healthy subjects and have an abnormal

fermentation of food residues (98).

Based on the critical role of microflora on IBS development, probiotics have been used to treat IBS in many clinical trials with encouraging results and effects with the use of *L. plantarum* strain and the multiple strains VSL#3. The administration of a rose-hip drink containing *L. plantarum* decreased pain and flatulence in patients with IBS (99). Niedzielin et al. (100) also showed the effectiveness of *L. plantarum* in liquid suspension on abdominal pain resolution and stool frequency normalization in constipated patients. Recently, the short-term effects of *L. plantarum* and *B. breve* on relieving pain in IBS patients fulfilled Rome II criteria were also demonstrated (101). However, *L. plantarum* strain did not improve colonic fermentation in patients with IBS as reflected by no change in total hydrogen production (102). Another *Lactobacillus* strain LGG in the capsulated formed did not significantly reduce IBS regarding pain, urgency and bloating but improved diarrhea and stool consistency (103) in patients fulfilled the Rome Criteria. Interestingly, it was reported that even heat-inactivated *L. acidophilus* significantly demonstrated therapeutic benefit in 50% of patients in a double-blind, placebo-controlled, cross-over trial considering abdominal pain, bloating or gas, daily number of stools, consistency, mucus content and general physical state (104). In addition, Kim et al. (105) found that VSL#3 was

effective to relief abdominal bloating but not abdominal pain, gas and urgency in IBS patients predominant with diarrhea.

Acute Gastroenteritis

There have been a number of clinical trials to attest the efficacy of probiotics in the prevention and treatment of diarrheal conditions. The ability of probiotics to reduce the severity and shorten the duration and frequency of symptoms has been well established. LGG is probably the most well studied strain with proven efficacy in acute gastroenteritis.

Diarrhea in children

Rotavirus is one of the leading etiologic agents of nosocomial diarrhea, which is a major problem in pediatric hospitals. Extensive clinical trials have been focused on the efficacy of probiotic strains to treat and prevent diarrhea in infant and children, particularly in rotavirus gastroenteritis. *B. bifidium* and *S. thermophilus* supplemented infant formula reduced the incidence of diarrhea and rotavirus shedding in hospitalized infants (106). LGG is the most potent strain to reduce the duration of diarrhea in children with rotavirus gastroenteritis, compared with *L. casei* or a combination of *S. thermophilus* and *L. delbruckii subsp. bulgaricus* (107). This is

accompanied by increased IgA specific antibody-secreting cells to rotavirus and serum IgA antibody level at convalescent stage in the LGG-treated group. LGG was also found to promote recovery from non-bloody acute diarrhea in children (108). Szajewska & Mrukowicz (109) reviewed a number of published, randomized, double-blind, placebo-controlled trials on probiotics in the treatment and prevention of acute diarrhea in infants and children. The authors concluded that probiotics significantly reduced the duration of diarrhea when compared with placebo but only LGG showed consistent effects although the efficacy of other strains has been reported (110-111). The enhancement of intestinal immune response to rotavirus may explain the consistency of LGG on the treatment and prevention of rotavirus gastroenteritis.

In a double-blind, placebo-controlled clinical study, live preparation of LGG plus rehydration solution shortened the duration of diarrhea, lessened the chance of a protracted course and fastened discharge from hospital in children with acute-onset diarrhea (112). In addition, early supplementation of LGG at the start of oral rehydration demonstrated shortest duration of diarrhea, best weight gain and fastest correction of acidosis in young children (113). Instead of using viable probiotic supplement, Simakachorn et al. (114) demonstrated similar results using lyophilized

heat-killed *L. acidophilus LB* in rotavirus-positive children with acute diarrhea in only 24 hours of treatment.

The preventive effect of probiotics on diarrhea has also been investigated. The incidence of diarrhea was significantly reduced in healthy children supplemented with *L. casei* fermented milk when compared with yogurt (115) and in undernourished infants given LGG in flavored gelatin (116). Likewise, LGG was found to reduce the risk of nosocomial diarrhea and rotavirus gastroenteritis in a double-blind trial in children who were hospitalized for reasons other than diarrhea (117). Another probiotic strain, *B. lactis* supplemented in milk formula also reduced the risk of getting diarrheal episodes and delayed the first onset of diarrhea as compared with the placebo (118). However, others (119) reported that LGG was ineffective in preventing nosocomial rotavirus infections.

Antibiotic-associated diarrhea (AAD)

The use of antibiotics, particularly broad-spectrum antibiotic regimen, disturbs intestinal flora balance and results in changes in colonic carbohydrate digestion, decreases short-chain fatty acid absorption and finally leads to an osmotic diarrhea. It was found that 5-25% of patients receiving antimicrobial agents developed AAD

(120). Probiotic strains including lactobacilli, enterococci, bifidobacteria and *S. boulardii* have been studied in the prevention of AAD. Co-administration of LGG with oral antibiotic significantly reduced stool frequency and increased stool consistency during the antibiotic therapy in children with acute infectious disorders (121). The same strain was also found to prevent intestinal side-effects and diarrhea in children receiving antibiotic treatment for respiratory infections (122). Healthy subjects treated with erythromycin plus LGG yoghurt decreased AAD and other side effects of erythromycin (123). Another controlled-trial showed that LGG significantly reduced diarrhea, nausea and taste disturbance in *H. pylori* positive subjects who received rabeprazole, clarithromycin and tinidazole (124). However, another randomized, double-blind, placebo-controlled trial involved 267 adult patients taking antibiotics showed no reduction in AAD with LGG supplementation (125). Recently, D'Souza et al. (126) reviewed 9 randomized, double blind, placebo controlled studies and suggested that *S. boulardii* and lactobacilli can be used to prevent AAD. In another meta-analysis (127), 22 placebo-controlled studies were evaluated. The authors concluded a strong benefit of probiotic administration on AAD. Both studies agree that further data are needed to prove the efficacy. Besides *Lactobacillus* species, *Enterococcus SF 68* was shown to reduce the incidence of AAD (8.7% compared with 27.2% in placebo) (128). Preventative effect of non-pathogenic yeast *S. boulardii* on

AAD was also documented. *S. boulardii* resulted in significant reduction of AAD in 193 patients received beta-lactam antibiotics (129) and in 180 patients received multiple antibiotics (130). However, the therapeutic effect of *S. boulardii* was not observed in elderly patients (131).

Traveler's diarrhea

Traveler's diarrhea is defined as the passage of three or more unformed stools in 24 hours during or shortly after travel, or any number of loose stools if accompanied by fever, cramping, abdominal pain or vomiting (132-133). Approximately 80% of episodes of traveler's diarrhea are due to intestinal infections (134). Infections are mostly caused by enterotoxigenic *E. coli*, *Shigella* species, *Campylobacter jejuni*, protozoa, viruses and helminthes. Several studies have investigated the efficacy of probiotics in prevention of traveler's diarrhea. A placebo-controlled double-blind study (135) involved 756 subjects traveled to two destinations to Southern Turkey showed that LGG effectively reduced the occurrence of traveler's diarrhea by overall 11.8% in one destination but not the other destination. The non-pathogenic yeast *S. boulardii* also showed some preventive effect on traveler's diarrhea by reducing the incidence of diarrhea (136) in 3,000 Austrian travelers traveled to distant regions. However, in another randomized double blind clinical trial (137), prophylactic

ingestion of Lactinex® (a commercial preparation contained *L. acidophilus* and *L. bulggaricus*) did not reduce the incidence or duration of traveler's diarrhea in volunteers traveled from US to Mexico. The ability of probiotics to resist pathogenic bacteria adhesion to the GI mucosa and their antimicrobial effects could contribute to the beneficial effects on traveler's diarrhea.

Maldigestion and lactose intolerance

Lactose maldigestion is a common genetic trait in up to 70% of the world's population (138). Primary lactose intolerance is due to a reduction of lactase activity in the intestinal brush border after weaning while secondary forms of lactose malabsorption may be due to inflammation or functional loss of the small intestinal mucosa and by protein-energy malnutrition (139). Maldigestion of lactose leads to signs of lactose intolerance with increased abnormal gas bloating, flatus, abdominal pain and diarrhea (140). *Lactobacillus* and *S. thermophilus* exert their lactase activity in vivo in the gut lumen and alleviate lactose intolerance (141-142) by hydrolysis of lactose in the small intestine. However, the consumption of VSL#3 did not improve parameters of lactose maldigestion (138). It is also proposed that some strains of probiotic bacteria can enhance lactose digestion if the β-galactosidase of the bacteria is released. de Vrese et al. (139) reported that human lactose malabsorbers consumed

diet containing active microbial β-galactosidase but killed lactobacilli with partly broken cell walls reduced symptoms of lactose intolerance but the effect was not observed for those consumed sterilized lactobacilli.

Colon cancer

Mortality from colorectal cancer (CRC) is the third only to that of lung cancer and prostate cancer in men and lung cancer and breast cancer in women (143). Diet makes an important contribution (approximately 70%) to the risk of CRC (144).

In animal studies, probiotics were found to suppress tumor growth and tumorigenesis, chemical carcinogen-induced DNA damage and aberrant crypt foci (ACF) formation. ACF are putative preneoplastic lesions that may develop into adenomas and carcinomas. Oral administration of LGG before and during 1,2-dimethylhydrazine (DMH) treatment interfered with initiation or early promotional stages of DMH-induced tumorigenesis and significantly decreased the incidence of colon tumor (145). *L. casei YIT 9018* inhibited the growth of tumor cells injected into mice (146) and whole peptidoglycan extracted from *B. infantis strain ATCC15697* also suppressed tumor growth (147). Moreover, synbiotics (combination of probiotics and prebiotics) supplementation suppressed colon carcinogenesis by

modulating gut-associated lymphoid tissue in carcinogen-treated rats (148). However, others reported the inability of LAB to inhibit the progression of colon tumor in DMH-treated animals (149).

L. acidophilus, *L. gasseri*, *W. confusa*, *S. thermophilus*, *B. breve* and *B. longum* were antigenotoxic against DMH and N'-nitro-N-nitrosoguanidine (MNNG) (150) and prevented DNA damage induced by MNNG and DMH. Burns & Rowland (151) also reported a significant decrease in DNA damage by several LAB strains. In addition, treatment with *L. plantarum* together with fructooligosacccharide was very effective in increasing resistance to fecal water genotoxicity in HT-29 cells. DMH- (152), azoxymethane (AOM)- (153), and 2-amino-3-methyl-3H-imidazo(4,5-f)quinoline (IQ)- (154) induced ACF formation was also suppressed by bifidobacteria (152-153) , *L. acidophilus* (155), *B. breve* (156) and *B. longum* (157). The consumption of probiotic mixture of *E. faecalis*, *C. butyricum* and *B. mesentericus* significantly decreased DNA adduct (a cancer susceptibility biomarker) formation in the colonic epithelium induced by 2-amino-alpha-carboline (AAC) in animals (158).

Human studies concerning the efficacy of probiotics in preventing colon cancer

is limited and controversial. Yet, Shahani & Ayebo (159) suggested that consumption of yoghurt and fermented milk supplemented with lactobacillus or bifidobacterium might reduce the incidence of colon while others argued that the intake of dairy products was not associated with lower risk of colon cancer (160). In another study, feces collected from nine healthy subjects consumed probiotic yogurt or standard yoghurt were incubated with human tumor cells HT-29 clone19A (161). It was shown that probiotic yogurt intervention significantly lowered fecal water genotoxicity compared with standard yogurt.

The mechanism by which probiotics exert their anti-tumorigenesis is unclear. However, indirect effects of probiotics on tumorigenesis have been suggested. It has been shown that LAB may bind to mutagenic amines formed by cooking protein-rich food (162) and mutagenic pyrolyzates (163) and degrade nitrosamines (164). Bacterial enzymes such asβ-glucuronidase hydrolyzed many glucuronides and produced the carcinogen aglycones. *L. casei* (165) and *L. acidophilus* (166) were shown to significantly inhibit bacterial enzymes-β-glucuronidase, nitroreductase and azoreductase and the level of these enzymes increased again when lactobacillus supplementation is terminated. This indicated the importance of continuous supplementation. LAB or their secreted soluble compounds may also interact with and

inhibit the growth of tumor cells (154, 167). In addition, the tumor promoters- and putative precarcinogens- producing putrefactive bacteria in the human colon was significantly reduced with the consumption of *L. acidophilus* supplemented fermented milk (168). This could be resulted from the reduction of intestinal pH (169). Indeed, *L. acidophilus* and *B. bifidum* consumption has been shown to reduce fecal pH significantly (170). Another mechanism by which probiotics inhibit tumor growth is the stimulation of the immune response. Matsuzaki (171) demonstrated the anti-tumor and anti-metastatic effects of *L. casei strain Shirota* on transplantable tumor cells. The author reported that intrapleural administration of the strain also effectively inhibited tumor growth in rodents by inducing the production of several cytokines, such as IFN-gamma, IL-1beta and TNF-alpha.

Conclusion

The GI tract harbors a complex ecology of microorganisms. A good balance in microbiota promotes good gut health while disturbance in the micro-ecosystem results in various gastrointestinal disorders. Probiotics support the intestinal flora balance and their beneficial effects on various GI disorders (ulcer and lesions, *H. pylori* infections, inflammatory bowel disease, irritable bowel syndrome, acute gastroenteritis, maldigestion and lactose intolerance and colon cancer) are fully discussed. The

underlying mechanisms proposed for these effects are also evaluated. However, the functional role of probiotics on certain GI diseases remained unclear and controversial. In addition, it is important to emphasize that the actions of probiotics are strain-specific and it is inappropriate to extrapolate the properties of one strain to another. Moreover, conflicting results have been observed that even the same strain produces dissimilar actions. Further studies including basic experiments and clinical trials are required to confirm all these actions and verify their mechanistic signaling pathway before the establishment of their therapeutic values for different GI diseases. Currently, there are increasing interests in research to elucidate the actions of synbiotics for the well-being of mankind. This is a new direction of approach in the development of therapeutic agents for GI disorders.

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