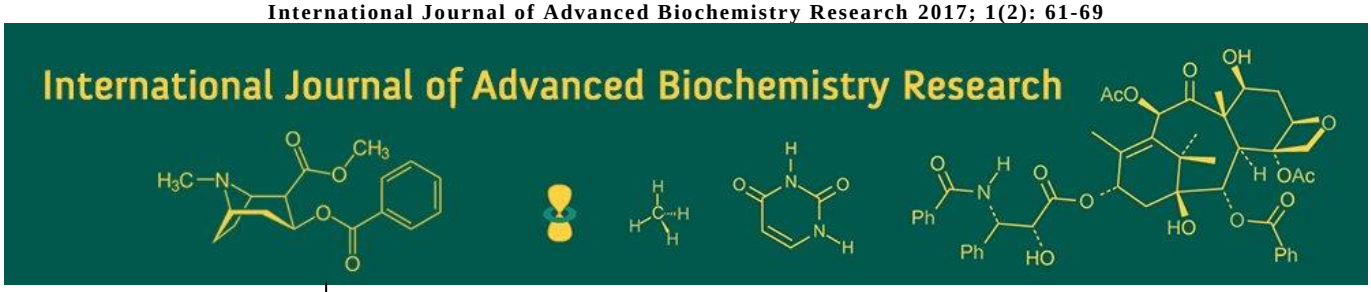

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Review of the possible mechanisms in the prevention of colorectal cancer by probiotic bacterial species

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Abstract
Several investigations, mainly using *in vitro* and animal models, have demonstrated a wide range of possible mechanisms, by which probiotics may play a role in colorectal cancer (CRC) prevention. In this context, the most well-studied probiotics are certain strains from the genera of *Lactobacillus* and bifidobacteria. In recent years, the consumption of over-the-counter probiotics to promote health has grown rapidly worldwide and become an independent industry. In medicine, various studies have demonstrated that probiotics can help improve the immune system and intestinal health. The gut microbiome can play an important role in maintaining homeostasis in the human body. An imbalance in the gut microbiome can lead to pro-inflammatory immune responses and the initiation of disease processes, including cancer. The research results prove some strains of probiotics by modulating intestinal microbiota and immune response can be used for cancer prevention or/and as adjuvant treatment during anticancer chemotherapy. An inverse relationship between the consumption of fermented dairy products, containing *Lactobacillus* or bifidobacteria (are the main probiotic groups) *Pediococcus*, *Lactococcus*, *Bacillus*, Shirota, Caseii, *Lactis*, *Rhamnosus*, Plantarum and yeasts and the incidence of colon, gastric cancer and breast cancer has also been reported in epidemiological and population-based case-control studies. A wealth of data implies that special receptors have essential roles in tumour development. A wealth of evidence emerging from laboratory studies indicates the anticancer activity of probiotics. This review presents the latest advances in research into the effectiveness of probiotics in the prevention and treatment support of cancer. The described issues concern the anticancer activity of probiotic microorganisms and their metabolites. In addition, we described the potential mechanisms of probiotic chemoprevention and the advisability of using probiotics.

Keywords: Probiotics, colorectal cancer prevention, *Lactobacillus*

1. Introduction
One hundred years ago, we ate beneficial bacteria all the time but pasteurization, sterilization and irradiation of food have ended much of that. The concept of probiotics evolved at the turn of the twentieth century from a hypothesis first proposed by Nobel Prize-winning Russian scientist Elie Metchnikoff, who propounded that the long and healthy life of Bulgarian people resulted from their consumption of fermented milk products [1]. Probiotic food can be defined as "food containing live microorganisms believed to actively enhance health by improving the balance of microflora in the gut". Several health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus, Bifidobacterium spp.,* and *Lactobacillus casei*. Because of the potential health benefits, these organisms are increasingly incorporated into dairy foods [2].

The human gastrointestinal tract is a reservoir of a complex and dynamic population of microorganisms (the gut microbiota) mainly containing bacteria (in number over 10^{14}), which exerts a significant influence on the host during homeostasis and disease [3]. The presence of such a large count of intestinal bacteria means that the human body has about 10 times more prokaryotic cells than eukaryotic cells [4]. In the human intestines are found bacterial phyla: Firmicutes, *Bacteroides*, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria and Spirochaetes [5].

Two bacterial phyla, gram-positive Firmicutes (*Bacillus* spp., *Lactobacillus* spp. And *Clostridium* spp.) and gram-negative Bacteroidetes, predominate in the human gut and represent about 90% of the bacterial population [6, 7].

The gut microbiota develops and matures during the first 3 years of human life [8]. Enterotype (the type and proportion of microorganisms found in the intestines) may indirectly affect the host's energy balance. The appropriate balance between bacterial populations ensures homeostasis of the gastrointestinal tract. However, the composition of the intestinal microbiome is susceptible to change. Thus, many factors such as improper diet, stress, gastrointestinal diseases, obesity or taking medications can lead to intestinal homeostasis disorders. As a result of an imbalance of the digestive system, proinflammatory immune responses initiate disease processes, including cancer. Intestinal dysbiosis may be the reason for the tumorigenesis of both local gastrointestinal cancers and tumours localized in distant sites of the body [9].

In recent years, studies on the use of probiotics for the prevention and treatment of human diseases have been performed globally [10]. At present, a variety of beneficial mechanisms have been identified, including regulating intestinal flora, enhancing intestinal barrier function, protecting intestinal epithelium from invasion by pathogens and strengthening immune function [11, 12].

Cancer patients have compromised immunity caused by primary diseases, chemotherapy and radiotherapy. The effects of probiotics in this population may differ from those of healthy people and raise several critical concerns [13]. Therefore, this article reviews whether cancer patients can take probiotics as well as their pros and cons (Figure 1).

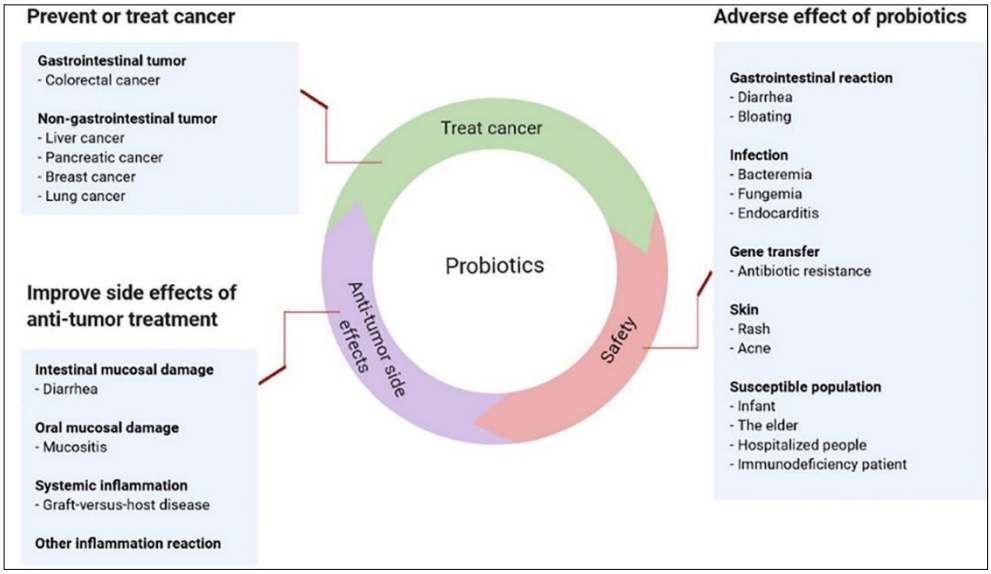

Fig 1: Pros and cons of probiotics in cancer

Studies have confirmed that probiotics can exert a variety of beneficial effects on the host. In addition, probiotic metabolites, such as short-chain fatty acids (SCFAs) and lactic acid, also play a significant role [14]. Using forward chemical genetic screening, a recent study found that multiple probiotic metabolites modulate host physiology by activating G protein-coupled receptors (GPCRs) [15]. Based on the contribution of probiotics to intestinal health, it is currently believed that the core benefit of probiotic management is to maintain healthy intestinal flora and support a healthy immune system through nonspecific and specific physiological effects, respectively [16]. (Figure 2).

Figure 2.

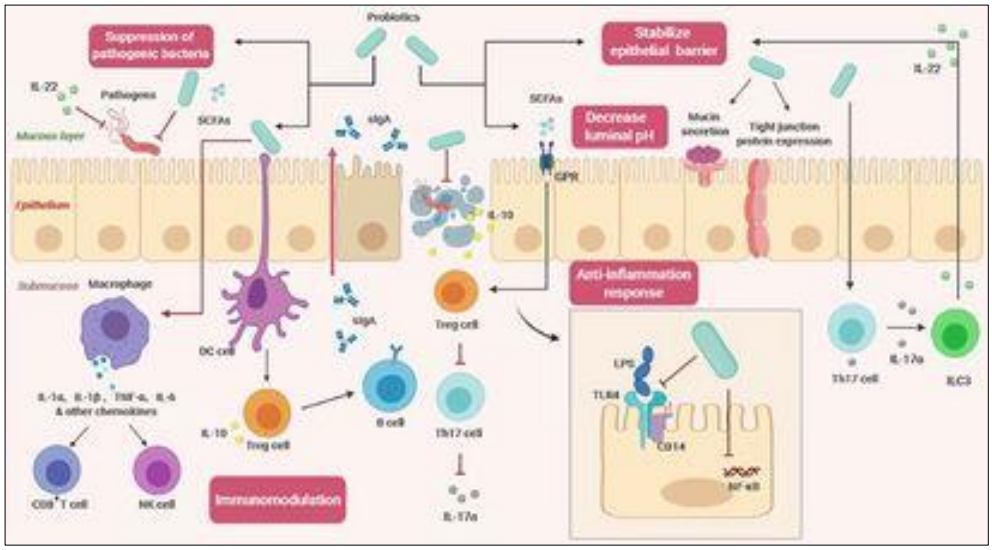

Fig 2: The effects of probiotics on the host (SCFA)

Increasingly, the microbiological scientific community is relying on molecular biology to define the complexity of the gut flora and to distinguish one organism from the next. Current techniques, including genetic fingerprinting, gene sequencing, oligonucleotide probes and specific primer selection, discriminate closely related bacteria with varying degrees of success. Additional molecular methods being employed to determine the constituents of complex microbiota in this area of research are community analysis, denaturing gradient gel electrophoresis (DGGE)/temperature gradient gel electrophoresis (TGGE), fluorescent in situ hybridisation (FISH) and probe grids [17].

2. Mechanism of Probiotics Action in Cancer Prevention and Therapy

The anticarcinogenic activity of probiotics is based on: (1) modification of the intestinal microbiota composition, (2) metabolic activity of the intestinal microbiota, (3) inhibition of cell proliferation and induction apoptosis in cancer cells, (4) binding and degradation of carcinogenic compounds present in the intestinal lumen, and (5) improvement of the intestinal barrier. Figure 3 shows potential mechanisms of action of probiotics in the prevention of colorectal cancer development.

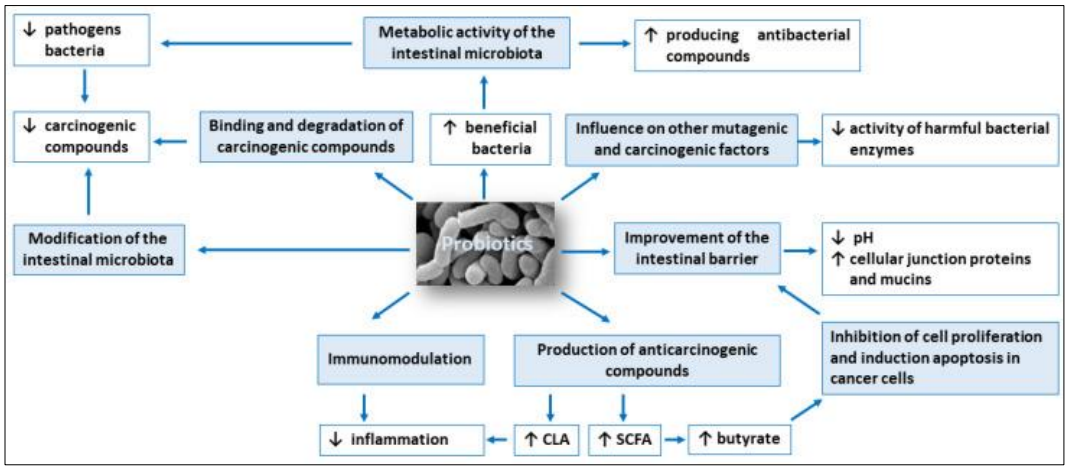

Fig 3: Potential mechanisms of probiotics action in the prevention of colorectal cancer development. Symbols: ↓ decrease, ↑ increase

2.1 Modification of the Intestinal Microbiota Composition

The healthy intestinal microbiota must be properly balanced and diversified to ensure homeostasis (eubiosis). Disturbance of the intestinal microbiota balance may result in a shortage of beneficial bacteria and an excess of pathogens (dysbiosis). Moreover, dysbiosis can cause a chronic inflammation and raise the production of carcinogenic compounds which increases the risk of developing colorectal cancer [18, 19].

Sobhani *et al.* [20] compared samples of feces from healthy people and colorectal cancer patients. Their research shows that the number of *Bacteroides* and *Prevotella* genus was significantly higher in the colorectal cancer group. In the intestinal ecosystem, several species of the *Lactobacillus* type were present in lower amounts than bacteria of the genera *Bacteroides*, *Eubacterium*, *Fusobacterium*, *Prevotella* and Proteobacteria. It was also found that several species of the genus *Salmonella* and *Clostridium* were present in greater numbers in patients with colorectal cancer [21]. Some strains of *Bacteroides spp.* and *Clostridium spp.* are classified as bacteria which are involved in the pathogenesis of colorectal cancer. *Bacteroides fragilis* produces enterotoxigenic toxin (fragilysin), which affects the induction of inflammatory mediators, which leads to the progression of cancer [22]. The pathogenic strain of *Escherichia coli* can synthesize several toxins, for example, cytotoxic necrotizing agent (CNF), cytolethal distending toxins (CDT), and other various virulence factors. *Streptococcus gallolyticus* and *Lactobacillus gasseri* can also be connected to colorectal cancer [23, 24].

The results of few clinical trial studies showed the beneficial effect of probiotics on the composition of gut microbiota and, thus on the host by improving intestinal barrier integrity, inhibiting the growth of pathogens, and reducing the metabolism of pro-carcinogenic substances. Ohara *et al.* investigated the differences between the intestinal flora of colorectal cancer patients and healthy subjects and assessed the possibility of using probiotics to prevent colorectal carcinogenesis [25]. After ingestion of the probiotic (*Lactobacillus gasseri* OLL2716: LG21), the *Lactobacillus* detection rate increased, and a decrease in the total amount of *Clostridium perfringens* was found. Moreover, faecal pH indicated acidosis and synthesis of faecal putrefaction products were inhibited, while the increase in the short-chain fatty acids and isobutyric acid concentration was observed.

A deterioration of the intestinal environment was observed in the colorectal cancer patients in comparison to the healthy controls, and the intestinal environment improved when probiotics was taken, suggest the possibility of preventing colorectal carcinoma with probiotics. Kotzampassi *et al.* demonstrated beneficial effects of probiotics (*Lactobacillus: acidophilus, plantarum; Bifidobacterium Lactis* and *Lactobacillus acidophilus*) in patients undergoing colorectal surgery for cancer [26]. The probiotics formulation significantly decreased the risk of postoperative complications, namely mechanical ventilation, infections and anastomotic leakage.

2.2 Metabolic Activity of the Intestinal Microbiota

Some bacteria present in the human intestines are capable of producing carcinogenic compounds from the diet, as well as from the bile salts endogenously produced. This ability is due to the presence and activity of some enzymes, such as azoreductase, β-glucuronidase, β-glucosidase, nitrate reductase, all of which are capable of converting heterocyclic aromatic amines, polycyclic aromatic

hydrocarbons, and primary bile acids into active carcinogens and synthetize aglycones, ammonia, cresols, phenols and N-nitroso compounds [27]. These metabolites have genotoxic and cytotoxic activities, which can lead to abnormal cell growth and activation of anti-apoptotic pathways in the colonocytes, thereby contributing to the development of colorectal cancer [28]. Changing the microbial metabolism by modulating the activity of these enzymes may be one of the mechanisms by which the probiotics can reduce the risk of developing colorectal cancer.

Metabolites of lactic acid bacteria (LAB) play an important role in controlling the intestinal microbiota. Among compounds that inhibit the development of pathogenic microorganisms, organic acids are considered to be the most important, in particular lactic and acetic acid as well as hydrogen peroxide and bacteriocins [29].

Furthermore, probiotics produce bacteriocins, which are deadly or bacteriostatic on sensitive microorganisms, affecting the cell membranes of bacteria having receptors capable of attaching them. Bacteriocins can cause: A portion of the bacterial cytoplasmic membrane that leads to the dissipation of the transmembrane potential and induces the leakage of K + ions, ATP and amino acids from the cytoplasm of attacked cells; cell lysis and interfering or inhibiting the synthesis of DNA, RNA and proteins (act as DNAase or RNAase) [30, 31]. The bacteriocins produced by probiotics e.g., are bifidocin B produced by *Bifidobacterium bifidum*, nisin from *Lactococcus Lactis* and lactacin B from *Lactobacills acidophilus*. Bifidocin B, which is produced by *Bifidobacterium bifidum* NCFB 1454, exerts a strong inhibitory activity against several pathogenic bacteria, including *Salmonella Typhimurium* SL1344 and *Escherichia coli* C1845 [32, 33]. On the other hand, Drissi *et al*. explored the role of bacteriocins may have in the GIT. In a genome mining research, 641 genomes (307 whole genomes and 334 draft genomes) from microorganisms in the human gut were received. The genomes represented 199 bacterial genera, including *Lactobacillus*, *Streptococcus*, *Clostridium*, and *Bacillus*. Of the 317 bacteriocins, 175 were from Firmicutes (which includes LAB), 79 from Proteobacteria, 34 from Bacteroidetes, and 25 from Actinobacteria. The authors suggested that bacteriocins in the GIT may have low levels of antimicrobial activity and may thus not have such a drastic effect on microbial populations. However, it was also suggested if bacteriocins play a lesser role in population dynamics, they may have a greater role to play in quorum sensing, or possibly in host immune modulation [34].

2.3 Inhibition of Cell Proliferation and Induction Apoptosis in Cancer Cells

Proliferation and apoptosis of cancer cells determine the rate of cancer development. During the cancer development process, these cells proliferate more than undergo apoptosis. Probiotics that can modulate cellular proliferation and apoptosis are of great interest because cancer cells would be eliminated less aggressively, and apoptosis brings no damage to the neighbour cells and does not cause inflammation [35]. The apoptosis signalling pathways can be activated by probiotic bacteria (e.g., lactic acid bacteria; LAB) through a mitochondria-dependent (intrinsic) and a death receptor-dependent, mitochondria-independent (extrinsic) pathway (Figure 4) [36].

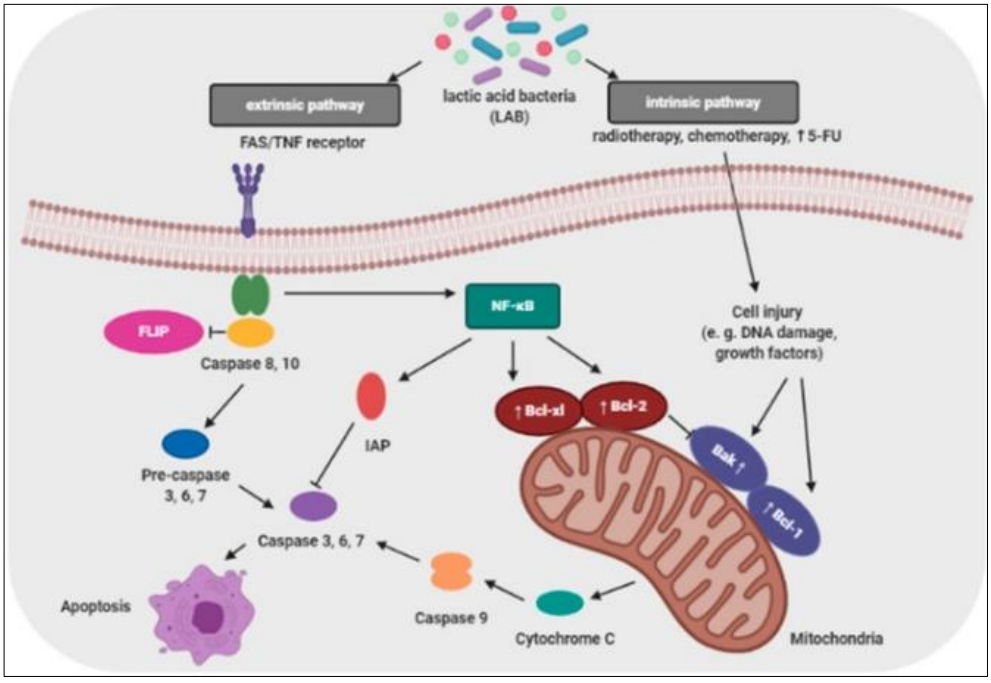

Fig 4: Potential mechanisms of action of lactic acid bacteria in pathways of apoptosis

Baldwin *et al*. evaluated the antiproliferative activity of *Lactobacillus acidophilus* and *Lactobacillus casei* against LS513 gastric cell lines through cellular apoptosis [37]. Hwang *et al*. demonstrated that probiotic induced apoptosis in gastric cancer cells (KATO3) by inhibiting NF-κB and mTOR-mediated signaling [38]. Cousin *et al*. demonstrated that probiotic bacteria induce chromatin condensation, apoptotic bodies, DNA fragmentation, caspase activation, inactivation of mitochondrial trans-membrane potential and cell cycle arrest [39]. Probiotic strains such as *Lactobacillus reuteri* have been reported to influence hematological cancers, which enhanced TNF-induced apoptosis in human chronic myeloid leukemia derived cells [40].

The increased incidence of apoptosis of cancer cells induced by probiotics has been attributed to SCFAs, particularly butyrate, which can induce epigenetic changes, paralyze the

cell cycle, and stimulate the expression of proapoptotic genes. A relationship exist between the amount of SCFAs in the faeces and cell proliferation in the colonic crypts [41]. Probiotic *Lactobacillus spp*. induced selective genotoxic, cytotoxic, pro-apoptotic effects on leukaemia and colon cancer cell lines, and as anti-inflammatory effects on macrophage cells at the molecular level.

In vitro, experimental evidence suggests that probiotics used for their anti-cancer activity operate via a process of genotoxicity and cytotoxicity against tumour cells. Liu *et al*. explored the effects of *Lactobacillus casei* 01 on 4-nitroquinoline Noxide (4-NQO) induced genotoxicity and colon cancer cell line (HT29) [42]. Nami *et al*. reported that the metabolites from *Lactobacillus acidophilus* 36 YL exhibited the most potent cytotoxic effect against human cervical cancer cell lines (HeLa) and colorectal cancer cell lines (HT-29) [43].

Since *in vitro* studies using cell lines indicated that probiotics had proapoptotic effects on carcinoma cells probiotics-based regimens might be used as an adjuvant treatment during anticancer chemotherapy [44, 45, 46, 47].

2.4 Binding and Degradation of Carcinogenic Compounds Present in the Intestinal Lumen

Carcinogenic compounds may bind to the cell wall of some probiotic bacteria. This to be associated with the occurrence of cationic exchange between the carcinogenic compounds and the peptidoglycan present in the cell walls of some probiotic microorganisms. Thus, carcinogenic compounds would be eliminated together with the bacteria through the feces [48].

Studies have shown, 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), 3-amino-1-methyl 5 h pyrido[4, 3-b] indole acetate (TrpP2) [49].

Rowland and Grasso studied *in vitro* the effect of intestinal microorganisms (of the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus*) to dimethyl-nitrosamine and showed that bacteria of the genus *Lactobacillus* most actively degraded these substances [50]. The amine was transformed into its precursor, dimethylamine, as well as to other volatile metabolites. However, Morotomi and Mutai showed a high ability of *Lactobacillus casei* to detoxify mutagenic heterocyclic amines [51]. They studied effects of live and heat-inactivated bacteria, and only live showed such ability. This may indicate that live bacteria produce metabolites or catalyze reactions that lead to amine detoxification.

The literature also shows that probiotics may have the ability to detoxify mycotoxins that may have carcinogenic properties [52]. El-Nezami *et al*. demonstrated that 5-week supplementation of probiotics reduced the urinary excretion of aflatoxin B (1)-N (7)-guanine, a marker for hepatocyte carcinogenesis, and synbiotic consumption for 12-week significantly reduced colorectal cancer risk [53].

2.5. Improvement of the Intestinal Barrier

The microorganisms of the intestinal microbiota may change the intestinal barrier and permeability. Some probiotics can reduce intestinal permeability because they can modify components of the intestinal barrier, such as intracolonic pH, the production of mucins and the cellular junction proteins [54].

Lower intracolonic pH values (more acidic) inhibit the proliferation of pathogenic and putrefactive bacteria, as well as the activity of bacterial enzymes responsible for the production of carcinogenic compounds [55]. In several *in vitro* studies has been confirmed that probiotic bacteria inhibit the growth of gram-negative pathogenic microorganisms. This growth-inhibiting activity has generally been attributed to the fact that probiotic strains lower the pH and/or produce lactic acid. For example, strains of *L. acidophilus*, *L. casei* subsp. *Rhamnosus*, and *Lactobacillus bulgaricus* inhibited the growth of clinical isolates of *Helicobacter pylori* [56, 53] and *L. casei subsp. Rhamnosus* strain Lcr35 reduced the growth of enteropathogenic and enterotoxigenic *Escherichia coli*, and *Klebsiella pneumoniae* [51]. It was shown that inhibition occurred when the pH of the incubation medium was acid and that no growth inhibition occurred when the pH of the incubation medium was neutral.

The inflammatory and carcinogenic processes increase intestinal permeability, mainly because they change the structure and expression of the cellular junction proteins, which makes colonocytes adhere to each other. These proteins are found mostly in the apical region between the colonocytes and are formed by a complex of transmembrane proteins that bind to the colonocyte cytoskeleton through the junction of transmembrane proteins, forming the tight junctions [17]. Probiotics can reduce intestinal permeability because they can change the distribution of cell junction proteins and improve the distribution of these proteins throughout the colonic epithelium, making it more continuous. In the small intestine of healthy subjects, administration of *Lactobacillus plantarum* WCFS1 induced changes in the epithelial tight junctions, resulting in increased staining of the scaffold protein zonula occludens-1 and the transmembrane protein occluding. *Lactobacillus plantarum* induced translocation of zonula occludens-1 to the tight-junction region was also seen in an *in vitro* model of the human epithelium, and this significantly protected against chemically induced disruption of the tight junction and the associated increase in epithelial permeability. The mechanism was shown to be dependent on Toll-like receptor 2 signalling and highlights the homeostatic role of innate signalling pathways in maintaining human intestinal epithelial barrier functions [49].

3. Probiotics enhance innate immune functions

Mammalian defensins, which are part of the innate immune system in the GI, have pharmaceutical potential given their antimicrobial, antiviral and immunomodulatory activities. About cancer, defensins have been implicated in exerting cytotoxic activity on tumour cells involving membrane lysis and DNA damage, as well as anti-angiogenic effects [57]. Defensins have the potential to overcome the evasion of tumour cells on immunosurveillance. For instance, murine β-defensin 2 has been shown to promote dendritic cell maturation, which in turn triggers type 1 polarized immune responses, such as the production of proinflammatory cytokines IL-12, IL-1α, IL-1β and IL-6 [58]. Beta-defensins are expressed in the mucosa and epithelial cells [57, 59]. Treatment of human colon cancer cells Caco-2 with

probiotics L. plantarum significantly upregulates human β-defensin 2 (HDB-2) mRNA expression and HBD-2 secretion in a dose-dependent manner, through the induction of TLR2 [59]. A probiotic mixture Symbioflor 2, containing several E. coli genotypes has been shown to increase HDB-2 synthesis in humans, and a dose-dependent induction of HDB-2 in Caco-2 cells [60]. Similar *in vitro* findings have been reported for the probiotic mixture VSL#3 and *Lactobacillus* [61].

Immunoglobulin A (IgA) at mucosal surfaces is a major component of specific immunity against the invasion of pathogenic microorganisms [62]. Antibody production, particularly IgA, is markedly reduced in the intestines of germ-free mice [63]. Indeed, TLR ligation by microbiota on intestinal epithelial cells was shown to promote the production of IgA by B cells into the lumen [64]. Animal studies have revealed certain probiotic strains such as *Lactobacillus* elicit antigen-specific IgA responses at the mucosal surfaces. Probiotics administration in humans was shown to increase both serum and faecal IgA levels [62]. IgA has been implicated to have anti-inflammatory function and direct cytotoxic effect on tumour cells [65]. A study showed administration of yoghurt containing probiotics was effective in suppressing the development of carcinoma in the large intestine of mice treated with carcinogen 1, 2-dimethylhydrazine [66]. The anticarcinogenic action of probiotics observed in this study was associated with upregulation of IgA, T-lymphocyte and colonic macrophage activities. Furthermore, both animal and human studies have demonstrated increases in natural killer (NK) cells and its activity in response to probiotics administration [67]. For instance, a clinical trial revealed three-week intake of *L. casei* strain Shirota enhanced the cytotoxicity activity of natural killer (NK) cells [68]. Dietary supplementation of *Bifidobacterium Lactis* led to increases in T-lymphocyte and NK cells in the systemic circulation, as well as upregulation of NK cell cytotoxicity activity [69]. Consumption of a probiotic drink, containing *L. casei* Shirota, was also effective in enhancing NK cell activity in healthy elderly subjects [70].

Furthermore, *L. casei* Shirota was shown to stimulate the production of inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), which in turn suppressed the development of a tumour and prolonged the survival in mice treated with a carcinogen [71]. IFN-γ is involved in the activation of NK cells and macrophages; therefore it plays a critical role in cancer prevention [72]. Humans and animals constantly produce IFN-γ as a defence against neoplasms [73]. *Lactobacillus Rhamnosus* E509 was shown to be effective in stimulating IFN-γ in human peripheral blood mononuclear cells [74]. In a study involving 24 human subjects, a daily intake of 450 g of yoghurt for 4 months led to an increased IFN-γ production in T-lymphocytes [75]. It has been suggested that ageing can result in a decline in physiological interferon response, which increases the tendency of tumour development during the ageing process [73]. In this regard, a study showed that *Lactobacillus* were able to restore the IFN-γ status in aged mice [76]. The excessive inflammatory response is not desirable because of its association with CRC development [77, 78, 79]. Interestingly, probiotics can induce and control Tregs activity as discussed above, which may promote a balance of cytokine production (i.e. a balancing act between proinflammatory and anti-inflammatory immune response).

In summary, there is a strong body of evidence which supports the modulation of immune responses as one of the key anti-CRC mechanisms of probiotics. In particular, probiotics have been well demonstrated to regulate inflammatory processes and enhance intestinal barrier functions and innate immune responses, all of which are critical to prevent colorectal tumorigenesis.

4. Safety of probiotics use

The long history of use of probiotic bacteria, especially *Lactobacillus* and bifidobacteria in foods (e.g. fermented dairy products) and their ubiquitous presence in the human GI tract, provides reasonable evidence regarding the safety of dietary LAB [80, 81]. Nevertheless, given the mechanisms of action of probiotics as discussed above, there are potential theoretical risks of probiotics use. These risks could include excessive immune stimulation, adverse metabolic activities, infection (endocarditis and bacteremia), transmigration of pathogenic bacteria, and transfer of antibiotic-resistance from probiotics to pathogenic bacteria (e.g. Tetracycline resistance in *Lactobacillus plantarum*; vancomycin resistance in many *Lactobacillus* strains) [81].

One of the key safety concerns is that LAB has been associated with cases of infection, i.e. infective endocarditis and bacteremia [82]. Nonetheless, two Finnish studies demonstrated that there was no significant increase in *Lactobacillus* or probiotics-related infection cases between 1990 and 2000, even though there had been a significant increase in *Lactobacillus* GG consumption over that decade [80, 83]. The LAB, *Lactobacillus* and bifidobacteria which are the most commonly used probiotics have been issued the 'generally regarded as safe' (GRAS) status [81]. A review reported that dietary intake of LAB was well-tolerated and demonstrated to be safe in 143 human clinical studies conducted between 1961 and 1998, involving a total of 7526 subjects [84]. Concerning the safety of probiotics use in patients receiving nutritional support, Whelan and Myers (2010) conducted a systematic review of case reports, randomized controlled trials, and nonrandomized trials, covering a total of 53 trials and 4131 patients received probiotics [85]. This extensive review found that probiotics had either no or positive effects on safety outcomes (e.g. mortality, infection) [85]. Consistently, other reviews also concluded that clinical evidence available to date supports the safety of consumption of LAB probiotics [81, 86, 87, 88]. Nevertheless, it is noteworthy that not all probiotics have GRAS status. Some members of enterococci, streptococci and bacilli, which are also used as probiotic organisms, are not generally regarded as safe since they contain opportunistic pathogens, especially enterococci [80, 81, 89].

In summary, there is a reasonable body of evidence to support the use of probiotics. However, individuals with immune deficiency should use probiotics with caution because of the risk of infection such as endocarditis, pneumonia and sepsis [89, 90, 91, 92, 93]. Microorganisms with known virulence genes should not be developed for probiotic use [92]. Readers are referred to recommendations of the EU-PROJECT workshop for more details on the biosafety assessment of microorganisms for human consumption, including taxonomy and typing, *in vitro* assessment of virulence and *in vivo* investigations [92].

5. Conclusion

As a dietary supplement, probiotics lack strict standards for efficacy and safety certification. Although the efficacy of several strains has been experimentally supported, the health-promoting effects of most probiotics have not been proven. Relevant publicity of probiotic products rarely mentions the potential risks. In conclusion, there is a convincing body of evidence suggesting various potential mechanisms of action of probiotics in CRC prevention. The chemopreventive effects of probiotics are dependent on the strain of the microorganism. Emerging data suggest viability may not be a prerequisite for probiotics to exert their anti-CRC activity. Synbiotics are likely to be more effective than either prebiotics or probiotics alone, as indicated by the growing body of data. More *in vivo*, especially human studies are warranted to further elucidate and confirm the potential chemopreventive role of probiotics (viable and non-viable), prebiotics and synbiotics in CRC. However, the presented research results confirm the effectiveness of probiotics only for potential prevention of cancer or as adjuvant treatment during anticancer chemotherapy. Clinical trials are still not enough to unambiguously confirm the potential of probiotic microorganisms in this regard. Therefore, it is very important and desirable to continue research on the anti-cancerogenic properties of specific probiotic strains and their mechanisms of action (especially during treatment). In addition, a randomized, double-blind, placebo-controlled clinical trial should be conducted to obtain approval from the medical community and validate the potential of probiotics as an alternative cancer therapy.

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