The purpose of this review is to discuss the potential mechanisms of probiotics action in colorectal cancer prevention. In this regard, the composition of the intestinal microbiota is considered as an important risk factor in the development of colorectal cancer, and probiotics are able to positively modulate the composition of this microbiota. Studies have shown that the regular consumption of probiotics could prevent the development of colorectal cancer. In this respect, in vitro and experimental studies suggest some potential mechanisms responsible for this anticarcinogenic action. The mechanisms include modification of the intestinal microbiota composition, changes in metabolic activity of the microbiota, binding and degradation of carcinogenic compounds present in the intestinal lumen, production of compounds with anticarcinogenic activity, immunomodulation, improvement of the intestinal barrier, changes in host physiology, inhibition of cell proliferation, and induction of apoptosis in cancer cells. In contrast, very few reports demonstrate adverse effects of probiotic oral supplementation. In light of the present evidence, more specific studies are needed on probiotic bacteria, especially regarding the identification of the bacterial strains with greater anticarcinogenic potential; the verification of the viability of these strains after passing through the gastrointestinal tract; the investigation of potential adverse effects in immunocompromised individuals; and finally establishing the dosage and frequency of use.

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Keywords:
Probiotics
Anticarcinogenic activity
Colorectal cancer
Intestinal microbiota
Health

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Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; B, Bifidobacterium; CLA, conjugated linoleic acid; CFU, colony-forming units; COX, cyclooxygenase; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; E, Escherichia; FR, free radical; GST, glutathione S-transferase; IgA, immunoglobulin A; IL, interleukin; L, Lactobacillus; S, Streptococcus; SCFA, short-chain fatty acid; TNF-α, tumor necrosis factor alpha.

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1. Introduction

Colorectal cancer (CRC) can affect the entire length of the large intestine and rectum. It is the third most prevalent cause of death among the different types of cancer worldwide, with the highest incidence being developed countries. It is estimated that by 2035, 24.4 million new cases of CRC will be diagnosed annually [1].

The etiological factors of CRC are multiple and involve modifiable and nonmodifiable risk factors. Therefore, the modifiable risk factors are identified as the main cause of sporadic CRC, which constitutes the majority of CRC cases (approximately 92%). Thus, it is assumed that most cases of CRC can be prevented [2].

In recent years, it has been observed that the intestinal microbiota composition is a risk factor for the development of CRC [3]. The intestinal microbiota can influence many aspects of the intestinal health, including its cellular features, physiology, metabolism, development, and immune homeostasis [4]. In addition, studies have shown that the composition of the intestinal microbiota in individuals with CRC differs from those who are healthy [5,6].

Therefore, modifying the intestinal microbiota composition by probiotics when ingested in adequate amounts may prevent the development of CRC because these microorganisms both influence the microbiota and potentially afford health benefits to the host [4,7]. Currently, in vitro, experimental, and clinical studies have shown promising results in regard to the anticarcinogenic properties of probiotics (Tables 1, 2, and 3). Many studies have proposed potential mechanisms whereby probiotics seem to inhibit the development of CRC. Thus, the present review aims to discuss the potential probiotics mechanisms of action in primary CRC prevention.

The studies included in this review were identified by a PubMed database search using the following descriptors in associations: colorectal cancer OR aberrant crypt foci OR colon cancer cells, AND probiotics OR potential probiotics OR probiotic bacteria OR probiotic yeasts OR lactic acid bacteria OR Lactobacillus OR Bifidobacterium, AND prevention OR anticarcinogenic activity. The search was filtered to limit only the past 10 years (2006-2016). Original studies that evaluated the preventive effect (anticarcinogenic) of a probiotic or potential probiotic microorganism on the risk of developing CRC in individuals of both sexes and all ages were considered. Classic articles on the topic and others resulting from reverse search were also selected. Review articles and those that used meta-analysis were excluded. In addition, original studies that investigated the preventive effect of the regular consumption of symbiotics or structural components of the microorganisms (such as exopolysaccharides), as well as the effect of probiotics in preventing adverse effects in CRC treatment, such as diarrhea, and the risk of infection in the preoperative and postoperative period, were excluded.

2. Potential mechanisms for probiotics action: anticarcinogenic activity

The following mechanisms are described in the scientific literature for being mainly responsible for the anticarcinogenic activity of probiotics: (1) modification of the intestinal microbiota composition; (2) changes in the metabolic activity of the intestinal microbiota; (3) binding and degradation of carcinogenic compounds present in the intestinal lumen; (4) production of compounds with anticarcinogenic activity, such as short-chain fatty acids (SCFAs) and conjugated linoleic acid (CLA); (5) immunomodulation; (6) improvement of the intestinal barrier; (7) changes in host physiology; and (8) inhibition of cell proliferation and induction apoptosis in cancer cells (Fig. 1). Each of these mechanisms is presented and discussed.

2.1. Modification of the intestinal microbiota composition

The exact intestinal microbiota composition and its relationship to the development of CRC remain unknown. However, a healthy intestinal microbiota must be composed in a way that the numbers of beneficial bacteria exceed the pathogenic bacteria (eubiose). Otherwise, it can trigger a chronic inflammation and raise the production of carcinogenic compounds (disbioses), which increases the risk of developing CRC [8].
Experimental studies suggest that regular consumption of probiotics can improve the quantitative and qualitative profile of the intestinal microbiota [9-11]. The same results were observed in clinical trials [12,13]. For example, the regular consumption of Lactobacillus plantarum CGMCC 1258, L. acidophilus LA-11, and Bifidobacterium longum BL-88 (2.6 × 10^14 colony-forming units (CFU)/d) for 16 days, was able to increase the diversity and microbial richness in individuals with CRC undergoing colorectomy. In this case, the intestinal microbiota composition of these patients resembled the healthy individuals [14].

Moreover, the probiotic microorganisms are capable of reducing the population of pathogenic bacteria in different ways, including competition for nutrients, growth factors, and adhesion receptors. Some probiotics can produce antibacterial substances such as bacteriocins, reuterin, hydrogen peroxide, and lactic acid, which inhibit the growth or eliminate pathogenic bacteria from the intestinal lumen (Fig. 2) [4]. The beneficial modification in the composition of the intestinal microbiota is directly related to the decreased risk of developing CRC.

2.2. Changes in 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 β-glucosidase, β-glucuronidase, nitrate reductase, azoreductase, and 7-α-dehydroxylase, all of which are capable of converting polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, and primary bile acids into active carcinogens and synthesize aglycones, phenols, cresols, ammonia, and N-nitroso compounds. These metabolites have cytotoxic and genotoxic activities, which can lead to abnormal cell growth and activation of antipapoptotic pathways in the colonocytes, thereby contributing to the development of CRC [15]. Changing the microbial metabolism by modulating the activity of these enzymes is one of the mechanisms proposed by which the consumption of probiotics can reduce the risk of developing CRC. Some in vitro [16], in vivo [9,10,17,18], and clinical [12] studies have demonstrated that the consumption of certain strains of probiotic bacteria can reduce the activity of these enzymes. Moreover, this effect was observed mainly for β-glucuronidase and nitrate reductase.

Some species of pathogenic bacteria, such as Clostridium, Bacteroides, Eubacterium, and Escherichia coli, exhibit higher activity of these enzymes responsible for the synthesis of carcinogenic compounds [19]. Thus, as presented in Section 2.1, the regular consumption of probiotic microorganisms can reduce the populations of pathogenic bacteria in the intestinal microbiota and consequently reduce the intestinal production of carcinogenic compounds [9,12,17].

2.3. Binding and degradation of carcinogenic compounds present in the intestinal lumen

In vitro studies showed that carcinogenic compounds present in the medium may bind to the cell wall of some probiotic bacteria [20,21]. This ability seems 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 [21]. Some strains of probiotics are able to metabolize and inactivate these compounds, especially N-nitroso compounds and heterocyclic aromatic amines (Fig. 2) [15].

The binding capacity and degradation appear to be highly dependent on the strain used; the viability of the microorganism; the carcinogenic compound; probiotic dose; and environmental conditions, such as pH, the presence of bile salts, and gastrointestinal enzymes. The actual occurrence of this mechanism in vivo is questioned because the conditions found in the human gastrointestinal tract may reverse this process.

More experimental and clinical studies are needed to clarify how this mechanism occurs in the human intestinal tract. Future studies should address how to enhance the effects of bacteria on inactivation of carcinogenic compounds and determine the precise mechanisms involved in the prevention of CRC.

2.4. Production of compounds with anticarcinogenic activity

Probiotic microorganisms are able to produce compounds with anticarcinogenic activity, such as the SCFAs and CLA (Fig. 3). Because they are different in nature, each of these compounds exerts specific anticarcinogenic activities, as discussed below.

2.4.1. Short-chain fatty acids

The SCFAs are the end products of bacterial fermentation of nondigestible carbohydrates from the diet and from endogenous origins, such as mucus. It is estimated that 100 to 450 mmol of SCFA is produced daily in the human intestine, and the approximate molar ratios for acetate (C2), propionate (C3), and butyrate (C4) are 60:25:15, respectively [22].

Butyrate is the most studied SCFA when it comes to CRC, as it helps to regulate the balance between proliferation, differentiation, and apoptosis of colonocytes [4]. Butyrate can be found in higher quantities in the feces of healthy individuals compared with individuals with CRC; in addition, it is estimated that reducing 1 μg/L of butyric acid concentration in feces increases the risk of developing CRC by 84.2% [23,24].

It is estimated that 200 mmol of butyrate is produced in the human colon every day. However, its concentration progressively diminishes as the chyme moves toward the cecum region of the descending colon, where, once along this path, it is rapidly absorbed by the colonocytes. The production of butyrate in the descending colon is small because of the low availability of substrate from the food that is consumed [25]. Butyrate-producing bacteria belong to the clusters of Clostridium IV and XIVa, and the main producing species include Faecalibacterium prausnitzii, Eubacterium rectale, and Roseburia [22]. The lactic acid bacteria do not produce butyrate, but some species of bacteria such as E. hallii and Anaerostipes caccae are capable of converting acetate and lactate into butyrate [23].

Consequently, the production of butyrate is dependent on the composition of the intestinal microbiota, the diet, the chemical composition of the carbohydrates ingested, and the
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<td>↓ Cell proliferation</td>
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<td>SCD</td>
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<td>cells at 37°C for 72 h.</td>
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<td>Human colonic cancer cells lines Caco-2, HT-29, and SW480</td>
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<td>LPS</td>
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<td>?</td>
<td>Kim et al [70]</td>
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<td><strong>L. rhamnosus GG and B latis Bb12</strong></td>
<td>Human colonic cancer cell line Caco-2</td>
<td>10⁶ CFU/mL at 37°C for 6 or 12 h.</td>
<td>–</td>
<td>↑ Apoptosis</td>
<td>Activation of the apoptosis through the mitochondrial pathway: ↑ BAX translocation, cytochrome c release, and caspase-9 and -3 cleavage</td>
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<td>↓ Cell proliferation and colony formation ↑ Apoptosis</td>
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<td>–</td>
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<td>Inhibited the ErbBs 2 and 3 receptors’ expression and their downstream molecules including the cyclin D1 and its transcriptional regulator E2F-1</td>
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<td><strong>Bacillus polyfermenticus</strong></td>
<td>Human colonic epithelial cell line NCM460</td>
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<td>AOM</td>
<td>Did not affect cell colony formation normal colonocytes ↓ Cell colony formation in cancer cells</td>
<td>Inhibited the ErbBs 2 and 3 receptors’ expression and their downstream molecules including the cyclin D1 and its transcriptional regulator E2F-1 Antioxidant and SCFA activities</td>
<td>Ma et al [74]</td>
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<td>Grishima et al [75]</td>
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</tbody>
</table>
### Skimmed milk kefir and ayran
Human colonic cancer cells lines HT-29 and Caco-2

- 20, 50, 100, and 200 μL/mL of the fermented milk supernatant at 37°C for 30 min
- Fecal water from individual genotoxic activity positive
- ↓ Genotoxicity of fecal water added to the medium
- Did not affect intestinal tight junctions

### Heat-killed L. paracasei IMPC2.1 and L. rhamnosus GG
Human colonic cancer cell line DLD-1

- 10⁸ CFU/mL at 37°C for 24 or 48 h
- ↓ Cell proliferation
- Activation of the apoptosis
- Orlando et al [76]

### Pediococcus pentosaccharus FP3, L. salivarius FP25 and FP35, and E. faecium FP51
Human colonic cancer cell line Caco-2

- Different concentrations of the probiotic live whole cells and the cultured medium were added to cells at 37°C for 24 h.
- ↓ Cell proliferation
- Adhesion of probiotic bacteria to colon cancer cells
- ↓ Bioproduction of SCFA
- Thirabunyanon et al [77]

### Lactobacillus spp isolated from Philippine commercial dairy products
Human colonic cancer cells line HT-29

- Different concentrations of the probiotic cell-free supernatant were added to cells at 37°C for 72 h.
- LPS
- ↓ Cell proliferation
- ↓ Expression of the early apoptotic gene (cfos and cjun)
- ↓ Expression of the proinflammatory cytokine genes (TNF-α and IL1-β)
- Shyu et al [78]

### Clostridium butyricum ATCC 23857 and Bacillus subtilis ATCC 19398
Human colonic cancer cells lines HCT116, SW1116, and Caco-2

- Different concentrations of the conditioned medium were added to cells for 24, 48, or 72 h at 37°C.
- 1%, 2%, 5%, 10%, and 20% of probiotics supernatants or lysates at 37°C
- ↓ Cell proliferation and expression of inflammatory genes
- The presence of bacitracin or butyrate in the conditioned medium induced cell cycle arrest and apoptosis activation.
- Chen et al [79]

### L acidophilus ATCC 4356 and L. casei ATCC 39392
Human colonic cancer cells lines Caco-2

- Different concentrations of the conditioned medium were added to cells for 24, 48, or 72 h at 37°C.
- 1%, 2%, 5%, 10%, and 20% of probiotics supernatants or lysates at 37°C
- ↓ Cell proliferation
- ↓ Cell apoptosis through caspase-3 activation; ↓ capacity to degrade collagen matrix. Through direct effect, not via secreted substances
- Dallal et al [80]

### L. crispatus SJ-3C-US and L. rhamnosus GG
Human colonic cancer cell HT-29

- 5%, 10%, 15%, 20%, or 25% of probiotics culture supernatants for 24 h at 37°C
- ↓ Cell proliferation
- ↓ The expression of MMP2, MMP9, and CASP3 genes
- ↑ The expression of TIMP1 and TIMP2
- Nouri et al [81]

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**Abbreviations and symbols:** AOM, azoxymethane; LPS, lipopolysaccharide; ↓, decrease; ↑, increase.
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<td>Bacillus polyfementicus SCD</td>
<td>Male F344 rats (5 wk old) DMH-induced CRC model. Diet rich in fat (12% of lard) and low in fiber (2%)</td>
<td>The probiotic was mixed in the diet (3 × 10^6 CFU), and the animals were fed diet 1 wk before the injection of AOM and continued until the end of the study. 10 wk</td>
<td>↓ ACF incidence</td>
<td>?</td>
<td>Lee et al [69]</td>
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<tr>
<td>Bacillus polyfermenticus</td>
<td>Male F344 rats (5 wk old) DMH-induced CRC model. Diet rich in fat (12% of lard) and low in fiber (2%)</td>
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<td>Inactivation of the EGFR-Mek-Erk pathway signaling. ↑ Apoptosis</td>
<td>Chen et al [73]</td>
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<td>Fermented milk produced by EPS-producing S. thermophilus 5581 or 4239 or PH or Lactococcus lactis ssp. cremoris JFR or L. delbrueckii ssp. bulgaricus 3984</td>
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<td>The probiotic was mixed in the diet (5 × 10^6 CFU/g). 19 wk</td>
<td>↓ Tumor incidence and size.</td>
<td>Anti-inflammatory activity: ↓ infiltration of inflammatory cells, 1αB degradation, and COX-2 expression</td>
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<td>Kumar et al [86]</td>
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<td>Male Sprague–Dawley rats (5 wk old) AOM-induced CRC model. AIN-76a modified</td>
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<td>Did not alter the neoplasm incidence, the SCFA incidence, cell proliferation, and spontaneous apoptosis</td>
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<td>Bacillus polyfermenticus</td>
<td>Female CD-1 nude mice (8 wk old) injected subcutaneously with DLD-1 colon cancer cells</td>
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<td>↓ Tumor incidence and size. Did not induce tumor necrosis and leukocyte infiltration</td>
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<td>Yogurt fermented by L delbrueckii subsp bulgaricus 2038 and S salivarius subsp thermophilus 1131</td>
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<td>↓ Tumor incidence ?</td>
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<td>Yogurt fermented by L delbrueckii subsp Bulgaricus 2038 and S salivarius subsp thermophilus 1131</td>
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<td>The yogurt was mixed in the diet (10% w/w), and the animals were fed that diet 3 wk before the injection of DMH and continued until the end of the study. 4 wk</td>
<td>↓ Tumor incidence ?</td>
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<td>The probiotic was administrated in drinking water (5 × 10^10 CFU/100 g of body weight) from 1 wk before colitis-associated CRC induction until the end of the study. 10 wk</td>
<td>None of the treated animals developed CRC. ↑ Microbial richness and angiostatin levels</td>
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<td>↓ β-Glucuronidase and β-glucosidase activity; fecal pH; the intestinal population of aerobic bacteria and <em>E. coli</em></td>
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<td>↑ Apoptosis through ↑ caspase-9 and caspase-3 and ↓ Bcl-2 expression</td>
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<td>↓ Intestinal inflammation through ↑ CD8 cells</td>
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<td>↓ β-Glucuronidase activity and the hepatic lipid peroxidation ↑ GST activity ↑ Nitroreductase, β-glucuronidase, and β-glucosidase activity</td>
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<td><em>L. rhamnosus</em> GG MTCC #1408, L. casei MTCC #1423, <em>L. plantarum</em> MTCC #1407, <em>L. acidophilus</em> NCDC #15, and <em>B. bifidum</em> NCDC #234</td>
<td>Sprague-Dawley rats DMH-induced CRC model. Standard diet</td>
<td>The probiotic was administrated orally daily (1 × 10⁸ lactobacilli/0.1 mL), and the animals started the treatment 1 wk before the injection of DMH and continued until the end of the study. 7 wk</td>
<td>↓ ACF incidence</td>
<td>↑ GSH, SOD, and GPx activity</td>
<td>Verma and Shukla [18]</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG MTCC #1408 or <em>L. acidophilus</em> NCDC #15</td>
<td>Male Sprague-Dawley rats DMH-induced CRC model. Standard diet</td>
<td>The probiotic was administrated orally daily (1 × 10⁸ lactobacilli/0.1 mL), and the animals started the treatment 1 wk before the injection of DMH and continued until the end of the study. 7 wk</td>
<td>↓ Tumor incidence, burden and multiplicity; lipid peroxidation</td>
<td>↑ GSH, SOD, and GPx activity</td>
<td>Verma and Shukla [93]</td>
</tr>
<tr>
<td>Probiotic</td>
<td>Species</td>
<td>Age</td>
<td>Model</td>
<td>Diet</td>
<td>Dose</td>
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<tr>
<td>L. salivarius Ren</td>
<td>Male rats F344 (5 wk old) DMH-induced CRC model. Purified basal rodent diets</td>
<td>19 wk</td>
<td>The probiotic was administrated orally daily as a low dose (5 × 10^8 CFU/kg) or as a high dose (1 × 10^10 CFU/kg). The treatment started 2 wk before the injection of DMH and continued until the end of the study.</td>
<td>↓ ACF incidence</td>
<td>↓ Azoreductase activity and the intestinal population of one Bacilllus-related strain and Ruminococcaceae strain</td>
</tr>
<tr>
<td>Clostridium butyricum ATCC 23857 and Bacillus subtilis ATCC 19398</td>
<td>Male C57BL/6 mice (8 wk old) in SPF conditions DMH-induced CRC model</td>
<td>28 wk</td>
<td>The probiotic was administrated orally (2.5 × 10^8 CFU/0.3 mL) 3×/wk.</td>
<td>↓ Tumor incidence and size.</td>
<td>↓ Th2 and Th17 lymphocytes spleen population and the expression of proinflammatory genes</td>
</tr>
<tr>
<td>Dead nanosized L. plantarum</td>
<td>Male Balb/c (6 wk old) DSS and AOM-induced CRC model.</td>
<td>8 wk</td>
<td>The probiotic was administrated orally daily as a low dose (4 × 10^8 CFU/kg) or as a high dose (4 × 10^11 CFU/kg). Two weeks after the AOM injection, the probiotic treatment started.</td>
<td>↓ Tumor incidence; areas of dysplasia, adenocarcinoma, and structural disruption</td>
<td>↓ Overexpression of proinflammatory cytokines and inflammatory genes</td>
</tr>
<tr>
<td>L. plantarum AdF10 and L. rhamnosus GG</td>
<td>Female Sprague-Dawley rats DMH-induced CRC model</td>
<td>8 wk</td>
<td>The probiotic was administrated orally daily (2 × 10^10 cells/d) 16 wk</td>
<td>↓ Tumor incidence, multiplicity, and size</td>
<td>↓ COX-2 protein expression</td>
</tr>
<tr>
<td>L. salivarius Ren</td>
<td>Male F344 rats (5 wk old) DMH-induced CRC model</td>
<td>19 wk</td>
<td>The probiotic was administrated</td>
<td>↓ Tumor incidence</td>
<td>↓ Intestinal population of Ruminococcus sp and</td>
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presence of other metabolites [26]. These factors help to explain the conflicting results that can be found in literature.

Moreover, butyrate can contribute in the prevention of CRC because it is capable of improving the intestinal barrier through the increase in mucus production, and in the proliferation of healthy cells because this SCFA is the major energy substrate for colonocytes. Butyrate also stimulates the production of growth factors and anti-inflammatory cytokines, such as interleukin (IL)-10. In addition, this SCFA has the ability to decrease the production of inflammatory cytokines by inhibiting the activation of nuclear transcription factor kappa B; increasing the immunogenicity of tumor cells; regulating the activity of proteins involved in apoptosis, such as Bcl-2, Bak, and caspases 3 and 7; increasing the activity of the antioxidant enzyme glutathione S-transferase (GST); suppressing cyclooxygenase (COX)-2 activity; stimulating the production of antimicrobial peptides; and inhibiting the deacetylation of histones. These effect can result in silencing or up-regulation of genes involved in the control of cell cycle proliferation, differentiation, and apoptosis [4,22,25].

However, work in animals show that the beneficial effects of butyrate vary according to the experimental model used, the degree of inflammation, the dose, the stage of the carcinogenic process, and even the genetics of the individual [26]. In this manner, the probiotic treatment (Lactobacillus salivarius Ren, low dose: 5 × 10⁸ CFU/kg, high dose: 1 × 10¹⁰ CFU/kg, for 15 weeks) in 1,2-dimethylhydrazine (DMH) induced CRC rat model was able to increase the amount of total SCFA and butyrate in the feces. In addition, the incidence of aberrant crypt foci (ACF) in the colon was decreased significantly in these animals [10]. In contrast, rats treated with Lactobacillus delbrueckii subsp. rhamnosus and B lactis Bb12 (daily dose of 5 × 10⁸ CFU of each strain/g of diet, for 31 weeks) were not able to decrease the number of adenomas in the colon, and did not change the amount of total SCFA in the feces [27]. Similar results were observed in a clinical trial in which the participants were treated with 5 g of B. lactis (10⁹ CFU/g) for 4 weeks [28].

Furthermore, the acetic and propionic acids also exhibit anti-inflammatory activity because they are able to suppress the activation of nuclear transcription factor kappa B, and regulate the gene expression of proinflammatory cytokines [29]. In addition, propionic acid is able to stimulate apoptosis of tumor cells and exhibits an antiproliferative activity. After butyrate, propionic acid is the second SCFA preferably used as an energy source for colonocytes [30].

SCFAs are naturally produced by the bacteria that compose the intestinal microbiota. However, the amount produced may not be sufficient for inhibiting the development of CRC. Thus, consumption of probiotics may contribute to the increase of the daily production of SCFA. Probiotics may also be offered together with prebiotics, the latter of which can increase SCFA production by acting as a substrate for the intestinal microbiota.

2.4.2. Conjugated linoleic acid

Some species of probiotic bacteria, such as L acidophilus, L casei, L plantarum, Propionibacterium freudenreichii, and all the strains present in the probiotic VSL#3 (L casei, L plantarum, L acidophilus, L delbrueckii subsp bulgaricus, B infantis, B breve, B longum, Streptococcus salivarius subsp thermophilus), are capable
<table>
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<th>Probiotics</th>
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<th>Treatments</th>
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<tr>
<td><em>L. rhamnosus</em> LC705 and <em>P. freudenreichii</em> ssp <em>hermanii</em> JS</td>
<td>38 men aged 24 to 55 y</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>The subjects consumed daily 2 capsules containing viable microorganisms (2 × 10^{10} CFU/d of each strain). 4 wk</td>
<td>↓ β-Glucosidase and urease activities</td>
<td>Hatakka et al [12]</td>
</tr>
<tr>
<td><em>B. lactis</em> LAFTI B94</td>
<td>17 healthy subjects aged 45 to 75 y</td>
<td>Randomized, double-blind, placebo-controlled, crossover</td>
<td>One capsule containing 5 g (10^9 CFU/g) 4 wk</td>
<td>↑ <em>B. lactis</em> intestinal population</td>
<td>Worthley et al [28]</td>
</tr>
<tr>
<td><em>L. gasseri</em> OLL271 6: LG21</td>
<td>10 colorectal cancer patients and 20 healthy subjects</td>
<td>Randomized, double-blind, placebo-controlled</td>
<td>↑ Lactobacillus intestinal population, fecal SCFA isobutyric acid, and NK cell activity</td>
<td>↓ Clostridium perfringens intestinal population, fecal pH, and the synthesis of fecal putrefaction products</td>
<td>Ohara et al [13]</td>
</tr>
<tr>
<td>Yogurt produced by <em>S. thermophilus</em> and <em>L. delbrueckii</em> subsp <em>bulgaricus</em></td>
<td>45 241 adults</td>
<td>Prospective study with 12 y of follow-up</td>
<td></td>
<td>↓ Risk of developing CRC</td>
<td>Pala et al [95]</td>
</tr>
</tbody>
</table>

Abbreviations: NK, natural killer; hs-CRP, high-sensitivity C-reactive protein.
of producing CLA from linoleic acid. This fatty acid is produced in the distal ileum by bacteria and can be absorbed by or interact with the colonocytes in the intestinal lumen, thus exerting its beneficial effects locally [31].

The antiproliferative and proapoptotic activities of CLA result from its ability to increase the expression of the peroxisome proliferator-activated gamma receptor, which is involved in the modulation of lipid metabolism, apoptosis, and immune system function. In this manner, mice induced to colitis-associated CRC and treated with VSL#3 (1.2 × 10^{12} bacteria/d for 68 days) expressed higher amounts of peroxisome proliferator-activated gamma receptor in the colonic tissues and lower amounts of adenomas and adenocarcinomas compared with the control group [32].

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**Fig. 1** – Potential mechanisms of action of probiotics in the prevention of colorectal cancer development. Symbols: ↓, decrease; ↑, increase.

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**Fig. 2** – The regular consumption of probiotics can modulate the composition of the intestinal microbiota through (1) the competition for nutrients, (2) adhesion receptors, as well as (3) the production of bacteriocin, which can eliminate potential pathogenic bacteria or carcinogenic producing bacteria. In addition, some probiotics are able (4) to bind and degrade carcinogenic compounds present in the intestinal lumen.
CLA is also reported to influence the expression of genes involved in the apoptosis process (caspase 3, caspase 9, and Bcl-2) and the cellular response to cell growth factors, such as insulin-like growth factor [32]. Ewaschuk et al [31] observed that CLA produced by the strains of VSL#3 were capable of inducing apoptosis and reducing the viability of colon cancer cells (HT-29 and Caco-2). In addition, CLA is able to suppress the production of eicosanoids in colonocytes in 2 ways. The first consists of the replacement of arachidonic acid in the cell membranes by CLA, and the second is the result of the interference of CLA in the activity of the cyclooxygenase and lipoxygenase enzymes, which are responsible for the synthesis of eicosanoids [32].

The anticarcinogenic activity of CLA is dose dependent. Thus, the consumption of probiotics that can enhance the production of this fatty acid may increase the amount of CLA sufficient to promote an anticarcinogenic effect [31].

### 2.5. Immunomodulation

The occurrence of chronic inflammation in the intestine increases the risk for CRC development. Therefore, individuals with inflammatory bowel disease are 5 times more likely to develop CRC compared with healthy individuals [19]. The chronic inflammation can affect the composition of the intestinal microbiota and increase its genotoxic potential, which indicates the existence of a strong relationship between intestinal microbiota, immune system, and CRC risk [26,33].

The intestinal microbiota is essential for maturation of the immune system and developing immunological tolerance, a mechanism by which the immune system is modulated to protect the host organism against pathogens. Providing the proper amount of probiotics and a favorable microbiota for the immune system is one approach to immunomodulation to benefit the host organism [8].

The use of probiotics for immunomodulation is a common and growing practice [8]. It occurs through the interaction between the immune cells present in the gastrointestinal tract and the probiotic microorganisms or their metabolites. The metabolites are recognized by receptors of the immune and epithelial cells, such as Toll-like and NOD-like receptors [34,35]. After being recognized, the immune and epithelial cells begin to secrete cytokines that can help to regulate the innate and adaptive immune response [35].

In the case of CRC, the proinflammatory cytokines IL-1β, IL-6, IL-8, IL-17, IL-12, and tumor necrosis factor-α (TNF-α) can be associated with the development of cancer. In contrast, the anti-inflammatory cytokines IL-10 and transforming growth factor beta exhibit inhibitory effects [15]. Because probiotics are able to increase the production of anti-inflammatory cytokines and decrease the production of proinflammatory cytokines (Fig. 3), the development of the colon cancer cells can be delayed. In addition, probiotics may decrease the expression of COX-2, an enzyme that catalyzes the production of prostaglandins from arachidonic acid, which has been linked to an
increased risk of developing CRC because it stimulates cell proliferation and the proinflammatory process [36].

Another important immunomodulatory pathway consists of the increased production of immunoglobulin A (IgA). Because of its resistance to proteolysis, this immunoglobulin acts on the intestinal barrier, limiting the contact of potentially carcinogenic compounds present in the intestinal lumen with colonocytes [34]. Furthermore, IgA creates an anti-inflammatory environment because it is unable to activate the complementary system and the proinflammatory response [37]. However, the results of studies that evaluated the effect of probiotic treatment on the production of IgA remain controversial [38-41].

Some probiotics can also affect the immune response through the activation of phagocytes and contribute to the maintenance of the state of vigilance, which can eliminate cancer cells in their early stages of development [35]. For example, Vinderola et al [42] concluded that despite being stimulated, the phagocytes present in the Feyer patches and in the peritoneum of the animals treated with the liquid and the solid fractions of kefir did not cause tissue damage. So, the consumption of kefir probably keeps the immune system in a state of vigilance.

It is important to highlight that the immunomodulatory activity of probiotics is dependent on their survival and persistence in the gastrointestinal tract, as well as the strain, dosage, and frequency used. Besides, their type of interaction with the host immune system can affect their immunomodulatory activity [35]. Therefore, not all probiotics are able to modulate the immune system and prevent the occurrence of CRC [34]. According to Galdeano et al [43], it would require a dose of 10⁹ to 10⁹ CFU/d of a strain with immunomodulatory effect and a permanence time in the intestine between 48 and 72 hours to induce immunostimulation on the host.

2.6. Improved intestinal barrier

The main function of the intestinal barrier is to protect our body from physical and chemical damage, as well as from invasion of microorganisms present in the intestinal lumen. This barrier consists of a layer of epithelial cells (colonocytes), some immune cells, goblet and Paneth cells, cell junction proteins, mucus layers, IgA, pH, antimicrobial peptides, and microorganisms that comprise the intestinal microbiota [44].

The symbiotic relationship between the intestinal microbiota and our body is dependent on the existence of this anatomical separation. Any disturbance in this barrier enhances the interaction of the host with the intestinal microbiota, which may lead to chronic inflammation and, consequently, the development of CRC [44].

The microorganisms of the intestinal microbiota may change the intestinal barrier and make it more or less permeable (Fig. 3). Besides, some species of probiotics are able to reduce intestinal permeability [45] because they can modify 3 important components of the intestinal barrier, such as the intracolonic pH, the cellular junction proteins, and the production of mucins.

2.6.1. Intracolonic pH

Individuals with CRC may exhibit higher intracolonic pH values when compared with healthy individuals. Thus, decreased intracolonic pH has been associated with lower incidence of this type of cancer. Higher pH values can be attributed to the low amounts of organic acids and SCFA present in the stools of these individuals [23,46]. These acids can be produced from the metabolic activity of probiotic microorganisms as previously described. Therefore, the fecal pH has been used as an indirect marker of the presence and activity of probiotic microorganisms [47].

Lower intracolonic pH values inhibit the proliferation of putrefactive and pathogenic bacteria, as well as the activity of bacterial enzymes responsible for the production of carcinogenic compounds [46]. Thus, Chang et al [9] attributed the reductions in the intestinal populations of Escherichia coli and aerobic bacteria to the low intracolonic pH exhibited by the animals treated with L acidophilus KFRI342 (2 × 10⁹ CFU/mL for 10 weeks) in comparison to the control group.

Besides lowering the intracolonic pH, lactic and acetic acids increase peristalsis, hindering the adhesion of pathogenic bacteria to colonocytes and consequently reducing the time that carcinogenic compounds could be in contact with the intestinal mucosa [47].

2.6.2. Cellular junction proteins

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 (occludins and claudins) that bind to the colonocyte cytoskeleton through the junction transmembrane proteins, forming the tight junctions [48,49].

The regular consumption of probiotics can reduce intestinal permeability because they can change the distribution of cell junction proteins (Fig. 3) [45,50]. It decreases the amount of potential carcinogenic and inflammatory compounds absorbed and prevents the occurrence of damage to the colonocytes and, consequently, the development of CRC.

Treatment with a mixture of probiotics (L plantarum CGMCC 1258, L acidophilus LA-11, and B longum BL-88 at a dose of 2.6 × 10¹⁴ CFU/d for 16 days) in individuals with CRC was able to reduce intestinal permeability. In addition, the probiotic treatment increased the amount of cell junction proteins, such as claudin, occludin, and JAM-1, and improved the distribution of these proteins throughout the colon epithelium, making it more continuous [14].

2.6.3. Mucins production

The carcinogenic process decreases the production of mucins and makes their composition less glycosylated [48]. This increases the chance of contact between the carcinogenic compounds, intestinal microbiota, and colonocytes, which may contribute to the development of inflammation and, consequently, CRC.

The barrier formed by the mucus is dynamic, and the composition and quantity of mucus produced are influenced by the composition of the intestinal microbiota [51]. Thus, some probiotics are able to increase the production of mucins by goblet cells (Fig. 3) through the upregulation of the MUC genes, mainly MUC 2. In this manner, the treatment with
2.7. Changes in host physiology

Probiotics may change the physiology of the host and thus contribute to the prevention of CRC [26]. For example, some probiotic microorganisms are able to change the activity of some enzymes involved in the cellular detoxification process, such as catalase, superoxide dismutase, and GST, thus preventing the activity of free radicals (FRs) and carcinogenic substances (Fig. 3).

The FR is naturally produced by the intestinal microbiota during cellular respiration and inflammatory processes. The production of FR by the intestinal microbiota can be significant and may exert carcinogenic activity if not controlled. In this manner, the colon is susceptible to the deleterious effects of these compounds and should have an active and efficient detoxification system [15,26].

Kumar et al [54] concluded that the anticarcinogenic effect of _L. plantarum_ AS1 is a consequence of its antioxidant properties. These authors observed that the activity of catalase, superoxide dismutase, and GST increased in animals treated with probiotics. Similarly, the fermented milk Dahi supplemented with _L. acidophilus_ LaVK2 and _L. plantarum_ Lp9 was able to increase the activity of GST in the liver and in the colon tissue of the treated animals (2 × 10⁹ CFU/g of each strain during 32 weeks). These animals also showed a reduced amount of lipid peroxidation products in these tissues and lower incidence of colon tumors in comparison with the control group [17].

GST is an antioxidant enzyme with detoxifying activity that belongs to the group of enzymes of phase II biotransformation process; it inactivates the carcinogens compounds that have been absorbed by the body. It is believed that probiotics are able to increase the activity of this enzyme through the action of butyric acid. This SCFA could change the status of histone acetylation, thus increasing the expression of GST [55].

Another method by which probiotics can modify the physiology of the host is related to polyamines, which are positively charged molecules capable of binding to proteins, phospholipids, DNA, and RNA present in the cells. Consequently, polyamines can regulate gene expression, cell proliferation, and differentiation [56].

Because of their physiological functions, the biosynthesis, catabolism, absorption, and cellular efflux of polyamines are strictly controlled. However, with the development of CRC, this control is lost, which increases the intracellular concentrations of these molecules. Thus, polyamines may be used as biomarkers of the proliferation of CRC cells [56].

In this manner, Singh et al [57] observed that F344 rats induced to CRC with azoxymethane and fed a diet containing 2% of _B. longum_ (4 × 10⁹ cells/g diet for 40 weeks) showed decreased ornithine decarboxylase enzyme activity in the intestinal mucosal cells compared with the control. Associated with this result, the probiotic-treated animals exhibited lower incidence and multiplicity of the colon tumor. Ornithine decarboxylase is a limiting enzyme in the synthesis of polyamines and is more active in tumors than in healthy cells based on the hyperproliferative state [56].

Overall, few experimental and clinical studies have investigated the ability of probiotics to alter the physiology of the host with CRC (Tables 2 and 3). Thus, this mechanism needs to be studied for a better understanding of its impact in the host and for CRC risk.

2.8. Inhibition of proliferation and induction of apoptosis of cancer cells

The occurrence of proliferation and apoptosis of tumor cells is what defines the speed of cancer development. Because of the changes that occur during the cancer development process, these cells proliferate more than undergo apoptosis [58]. Thus, probiotics that are able to modulate the cellular proliferation and apoptosis are of great interest because cancer cells would be eliminated less aggressively and because apoptosis brings no damage to the neighbor cells and does not cause inflammation, unlike the chemotherapy and radiotherapy treatments (Fig. 3) [10].

In an in vitro study, _Enterococcus faecium_ RM11 and _L. fermentum_ RM28, strains of bacteria that can be found in fermented milk, were able to inhibit Caco-2 cell proliferation by 21% and 23%, respectively [59]. Similarly, Sadeghi-Alibadi et al [60] observed an antiproliferative activity of _L. plantarum_ A7 and _L. rhamnosus_ GG inactivated by the heat and the cell-free supernatant produce by them. This suggests that the effects of probiotics on cancer cells may not depend on the microorganism viability.

A study with rats induced to CRC with DMH and treated with different doses of _B. longum_ BCRC 910051 for 15 weeks showed decreased mitotic index of colonocytes and cell proliferation in the colonic crypts compared with the untreated group. This effect may have led to a decrease of 25% to 30% in the amount of ACF present in the probiotic-treated animals [40]. Zhu et al [10] observed that the treatment with _L. salivarius_ Ren at different doses for 15 weeks reduced cell proliferation in the colonic crypts of F344 rats induced to CRC with DMH. Consequently, the probiotic treatment was able to reduce the incidence of ACF by 40%.

The increased incidence of apoptosis of cancer cells induced by the consumption of probiotics has been attributed to the SCFA, particularly butyrate. This SCFA is able to induce epigenetic changes, paralyze the cell cycle, and stimulate the expression of proapoptotic genes [25]. Therefore, it was observed that an inverse relationship existed between the amount of SCFA in the feces and cell proliferation in the colonic crypts [28].
Immunomodulation is another possible pathway that contributes to the proapoptotic activity induced by the consumption of probiotics, especially increased TNF-α production [61]. In addition, Wan et al [62] concluded that the ability to induce apoptosis of the tumor cells SW620 by the probiotic L. delbrueckii was a consequence of the increased expression of caspase-3 and reduced expression of Bcl-2. Thus, through immunomodulation, increased production of SCFA, and increased expression of genes and proteins involved in the regulation of the apoptotic process, probiotics can inhibit tumor development (Table 1). However, this effect of probiotic consumption on cancer cells should not extend to healthy colonocytes because this would lead to dysfunction in the intestinal barrier, which is directly related to the development of CRC.

3. Future research

Dietary interventions for preventing CRC, such as probiotics, have emerged as viable alternatives to manage CRC; in addition, very few reports demonstrate any adverse effects of probiotic oral supplementation. However, this is an aspect that needs to be further investigated, especially regarding the consumption of probiotics by immunocompromised individuals as well as individuals with altered intestinal permeability, as in the case of some individuals with CRC.

Some contradictory results are found in the scientific literature regarding the anticarcinogenic activity of probiotics (Tables 1, 2, and 3). These discrepancies can, at least partly, be explained by the fact that the protective mechanisms are strain specific. Therefore, further studies should be conducted to identify the strains involved in the prevention of CRC. Besides, the studies used varying doses, treatment times, and frequency use, as well as delivery of probiotic product (lyophilized, microencapsulated, or ready for consumption, such as yogurt). These aspects can lead to different results and make it challenging to compare the results from such studies.

Another important aspect that could minimize the efficacy of orally administrated probiotics is the loss of viability of probiotics reaching the colon. Thus, developing and applying techniques to ensure the viability of the probiotics strains, such as microencapsulation, could enhance the preventive effect of such strains on the CRC development. In addition, it is necessary to establish the dosage and consumption frequency recommended to obtain consistent protective effects against CRC.

4. Unknown aspects and missing knowledge

In 2011 Sears and Pardoll [63] proposed that certain microbiome members possessing unique virulence traits, the so-called alpha-bugs, were not only directly prounocenogen but capable of remodeling the colonic bacterial community to one that enhances and further promotes the induction of mucosal immune responses and epithelial changes resulting in CRC. Subsequently, based on the alpha-bugs hypothesis, Tjalsma et al [19] proposed the bacterial driver-passenger model for CRC. According to this model, the CRC would be initiated by the “driver” bacteria (alpha-bugs), which would eventually be replaced by “passenger” bacteria that could either promote or stall the carcinogenic process.

Based on this hypothesis, studies have been conducted to identify which bacterium is capable of triggering the development of CRC. Thus, the presence or absence of certain bacteria or groups of bacteria has been associated with the increased risk for developing CRC [3,64–67]; however, so far, triggering the process of carcinogenesis cannot be attributed to a single bacterium or a group. Thus, it is crucial that the bacteria drivers are identified so that the use of probiotics can be directed to fight the establishment and proliferation of these specific bacteria in the intestinal lumen. However, the choice of probiotic to be used is not a simple task, and a recent study found that the choice of the probiotic depends on individualized features of the host’s resident microbiome. Furthermore, the modulatory capacity and persistence of a probiotic in the intestine would be influenced by the specific core members of the gut microbiome and functional genes associated with them [68]. Based on these criterion, specific host microbiome analysis could assist in a probiotic personalized prescription. In this way, it is believed that the regular consumption may help prevent the development of CRC in a more effective way.

5. Conclusions

Evidence suggests that the consumption of probiotics can contribute to the prevention of CRC. Presently, the scientific evidence suggests that probiotics exert anticarcinogenic activity by potential physiological mechanisms usually co-dependents and strain-specific. Although more studies are needed to elucidate what mechanisms are effective in humans, the results with probiotics and the host are promising. Furthermore, considering the lack of evidence of adverse effects associated with it, the regular consumption of probiotics should not be an impediment for general health.

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