Chapter 57

Probiotics in Inflammatory Bowel Diseases and Cancer Prevention

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1 INTRODUCTION

Today’s consumers look for foods that, in addition to their intrinsic nutritional values, can offer some additional benefits to their health. For this reason, probiotics, which are live microorganisms that when administered in adequate amounts confer a health benefit on the host, are increasingly being consumed as diet supplements or pharmaceutical. Chronic pathological conditions such as intestinal inflammations and cancer usually require long treatments or are associated with alternate periods of remission and recurrence. Probiotics have been evaluated for some years now as an additional alternative for these patients to improve their quality of life with promising results.

Inflammatory bowel disease (IBD) describes a group of intestinal pathologies in which inflammation is a major feature. The two more common forms of IBD are ulcerative colitis (UC) (Head and Jurenka, 2003) and Crohn’s disease (CD) (Baumgart and Sandborn, 2007) which share etiological factors (Price, 1992); however, clinical manifestations (such as the location of the pathology) and the immunological response involved are distinctive between both. The exact etiology of these diseases remains unclear, but great advances have been made using experimental animal models and it had been demonstrated that multifactorial processes and mechanisms result in chronic intestinal inflammation (Elson and Weaver, 2003). The mechanisms by which probiotic microorganisms can exert beneficial effects against IBD were evaluated using animal models. In these models, the environmental conditions and genetic factors can be either controlled or defined and can then be evaluated in human clinical trials.

It is known that many factors are involved in cancer development, such as genetic, environmental, and dietary factors. Considering the promissory results demonstrated for different probiotics in IBD models, and the relation of chronic inflammation with increased probabilities of developing intestinal cancers, there is an increase in epidemiological reports and experimental studies that have shown the beneficial effect of certain probiotics against these cancers and even nonintestinal tumors.

This chapter summarizes some of the most relevant studies performed in the last 5 years regarding probiotics in the prevention and/or treatment of IBD and certain types of cancer, with emphasis being placed on the possible mechanisms involved.

2 PROBIOTICS AND INFLAMMATORY BOWEL DISEASES

Lactic acid bacteria (LAB) represent a heterogeneous group of microorganisms that are present in the normal diet of many people and also in the gastrointestinal and urogenital tract of animals, and some of these claimed to be probiotics. Many LAB and products containing these or other probiotic microorganisms were analyzed in different IBD models and different mechanisms of actions were implicated with their beneficial effects (del Carmen et al., 2011).

2.1 Mechanisms of Action of LAB Against IBD

2.1.1 Modulation of the Intestinal Microbiota

The intestinal microbiota composition and activity of patients suffering IBD are characterized by a decreased prevalence of dominant members of the human commensal microbiota such as Clostridium IXa and IV groups, Bacteroides,
Bifidobacterium and a concomitant increase of detrimental bacteria such as Escherichia coli and sulfate-reducing bacteria (Fava and Danese, 2011). In this sense, it has been shown that one of the mechanisms that probiotics can use to prevent IBD is by stabilizing the gastrointestinal microbiota (de Moreno de LeBlanc and LeBlanc, 2014).

It was reported that Lactobacillus (Lb.) reuteri prevented colitis in IL-10 knockout (KO) mice by increasing the number of lactobacilli in the gastrointestinal tract (GIT) (Madsen et al., 1999). A placebo-controlled trial showed that oral administration of Lb. salivarius UCC118 reduced mucosal inflammation in IL-10 KO mice and decreased the prevalence of colon cancer in those animals by modifying the gut microbiota with reduction of C. perfringens, coliforms, and enterococcus (O’Mahony et al., 2001). The administration of yogurt prepared with potential probiotic strains of Lb. delbrueckii subsp. bulgarius and Streptococcus (St) thermophilus, decreased the severity of the inflammation in a trinitrobenzene sulfonic acid-induced mouse model of IBD which was associated with beneficial modifications in the intestinal microbiota (Chaves et al., 2011a; de Moreno de LeBlanc et al., 2009).

There are also reports that showed the benefits associated with the use of probiotics in IBD patients; however, only a few on these have evaluated their effect on the fecal microbial composition (Table 57.1).

2.1.2 Enhancement of Intestinal Barrier Function

The exact mechanisms by which probiotic bacteria enhance gut mucosal barrier function are unclear, but may be related to alterations in mucus or changes in mucosal cell-cell interactions and cell stability through modulation of cytoskeletal and tight junction protein phosphorylation (Hilsden et al., 1996; Madsen et al., 2001; Meddings, 2008; Ng et al., 2009; Schmitz et al., 1999).

VSL#3 (a mix of eight probiotic strains) was orally administered IL-10 KO mice and it was reported that normalized colonic physiologic function and barrier integrity (Madsen et al., 2001). Barrier function was also enhanced by Lb. plantarum and Lb. reuteri administration in a methotrexate-induced enterocolitis model in rats (Mao et al., 1996); and the colonization of intestinal loops of healthy mice with Lb. brevis reduced intestinal permeability (Garcia-Lafuente et al., 2001). Lb. plantarum 299v induced intestinal mucin gene (muc2 and muc3) expression in vitro (Mack et al., 1999). VSL#3 also induced expression of mucus in vitro in intestinal epithelial cells, increased transepithelial resistance (TER), and prevented decrease of TER induced by pathogens, and stabilized tight junctions (Otte and Podolsky, 2004).

2.1.3 Reduction of the Oxidative Stress

The molecular mechanism of chronic inflammatory processes involves the participation of proinflammatory cytokines, chemokines, adhesion molecules, inflammatory mediators, and reactive oxygen species (ROS). ROS, such as superoxide anion (O\(_2^−\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radical (HO\(^•\)), are normal products of oxygen metabolism. Under normal conditions, ROS are in low concentrations in the GIT and can modulate the immune attack against some pathogens. However, in abnormal conditions, the overproduction of ROS leads to their accumulation and oxidative stress that can damage cellular structures and macromolecules such as DNA, RNA, proteins, and lipids. Oxidative stress is associated to recurrent and abnormal inflammation in both IBD patients and animal models of colitis (Damiani et al., 2007). When ROS concentration exceeds the capacity of cell defense systems, toxicity is triggered.

In order to compensate for the oxidative stress, the normal intestinal mucosa is equipped with a complex antioxidant defense system which includes enzymatic and nonenzymatic components having synergistic effects. The normal intestinal mucosa is equipped with a network of antioxidant enzymes such as catalase, glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-s-transferase (GST), and superoxide dismutase (SOD) that are able to neutralize most ROS. The activities of these enzymes are usually balanced to maintain a low and continual steady-state level of ROS; however, the levels of these enzymes are frequently depleted in IBD patients (Kruidenier et al., 2003). Probiotic microorganisms expressing high levels of antioxidant enzymes may increase the enzymatic activities at specific locations of the GIT and then contribute to the prevention of epithelial oxidative damage, leading to potential applications for the prevention or treatment of IBD. For example, the strain Lb. rhamnosus CNCM I-3690, selected for their antioxidant properties in vitro, showed anti-inflammatory activity in a model of colitis in vivo (Grompone et al., 2012).

SOD is considered as the first line of defense against ROS and is a member of the family of metalloenzymes that catalyze the oxido-reduction of superoxide anion to H\(_2\)O\(_2\). There are three different forms of this enzyme according to their metal center: manganese, copper-zinc, or iron. These enzymes are found in a broad range of organisms, which can use one, two, or all three enzymes. In most Streptococcus and Lactobacillus spp., Mn-SOD is the enzyme related to the elimination of ROS (Sanders et al., 1995). It has also been reported that two strains of Lb. fermentum (E-3 and E-18) and one strain of St. thermophilus exerted antioxidative activity through the production of Mn-SOD (Kullisaar et al., 2002). Furthermore, experimental data indicated that subcutaneous treatment with SOD reduced peroxidation reactions in the inflamed colon.
**TABLE 57.1** Examples of Human Clinical Trials (Randomized Controlled Trials) that Have Demonstrated that Probiotics Improve Inflammatory Bowel Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Probiotic$^b$</th>
<th>$n$</th>
<th>Mechanisms</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>Symbiotic therapy ($B. longum$ aNE Synergy 1)</td>
<td>18</td>
<td>Modulation of the immune response</td>
<td>Sigmoidoscopy scores were reduced in probiotic group compared with placebo. TNF-α and IL-1α were reduced after treatment with probiotic</td>
<td>Furrie et al. (2005)</td>
</tr>
<tr>
<td>UC</td>
<td>$E. coli$ Nissle 1917</td>
<td>327</td>
<td>NE</td>
<td>The probiotic treatment was just as effective as conventional treatment (mesalazine) in maintaining remission</td>
<td>Kruis et al. (2004)</td>
</tr>
<tr>
<td>UC</td>
<td>VSL-3</td>
<td>90</td>
<td>NE</td>
<td>Probiotic supplementation improved remission compared to conventional treatment (balsalazide) alone</td>
<td>Tursi et al. (2004)</td>
</tr>
<tr>
<td>UC</td>
<td>VSL-3</td>
<td>21</td>
<td>NE</td>
<td>Probiotic preparation maintains remission (75%)</td>
<td>Venturi et al. (1999)</td>
</tr>
<tr>
<td>UC</td>
<td>VSL-3</td>
<td>120</td>
<td>NE</td>
<td>62% improvement of symptoms and 0% relapse of intestinal disease while patients on probiotics</td>
<td>Karimi et al. (2005)</td>
</tr>
<tr>
<td>UC</td>
<td>VSL-3</td>
<td>29</td>
<td>NE</td>
<td>Pediatric, randomized, placebo-controlled trial that suggests the efficacy and safety of this probiotic mixture in active UC and in maintenance of remission</td>
<td>Miele et al. (2009)</td>
</tr>
<tr>
<td>CD</td>
<td>$S. boulardii$</td>
<td>32</td>
<td>NE</td>
<td>Relapse in 6% of patients supplemented with probiotic strain vs. 38% with conventional treatment only</td>
<td>Guslandi et al. (2000)</td>
</tr>
<tr>
<td>CD</td>
<td>$Lb. rhamnosus$ GG</td>
<td>6</td>
<td>NE</td>
<td>Median pediatric CD activity index scores at 4 weeks were 73% lower than baseline and intestinal permeability improved in an almost parallel fashion</td>
<td>Gupta et al. (2000)</td>
</tr>
<tr>
<td>CD</td>
<td>$Lb. rhamnosus$ GG</td>
<td>21</td>
<td>Modulation of the immune response</td>
<td>The number of specific IgA secreting cells in the class to β-lactoglobulin increased significantly from 0.2 to 1.4/106 cells and to casein from 0.3 to 1.0/106 cells</td>
<td>Malin et al. (1996)</td>
</tr>
<tr>
<td>IBS</td>
<td>VSL-3 (probiotic preparation containing 3 $B.$, 4 $Lb.$, and 1 $St.$ strains)</td>
<td>10</td>
<td>Detectable changes in the microbiota</td>
<td>Six patients showed improvement on global symptom assessment compared to baseline Significant reduction of the genus Bacteroides to levels similar to those obtained from healthy donors</td>
<td>Ng et al. (2013)</td>
</tr>
<tr>
<td>IBS</td>
<td>$Lb. acidophilus$, $Lb. plantarum$, $Lb. rhamnosus$, $B. breve$, $B. lactis$, $B. longum$, and $St. thermophilus$</td>
<td>50</td>
<td>Stabilization of intestinal microbiota</td>
<td>Adequate relief of overall IBS symptoms and improvement of stool consistency and quality of life</td>
<td>Ki Cha et al. (2012)</td>
</tr>
<tr>
<td>IBS</td>
<td>$B. infantis$ 35624</td>
<td>77</td>
<td>Modulation of the immune response</td>
<td>Alleviation of IBS symptoms and normalization of the ratio of an anti-inflammatory to a proinflammatory cytokines in patients receiving probiotic strain vs. placebo group</td>
<td>O’Mahony et al. (2005)</td>
</tr>
<tr>
<td>IBS</td>
<td>BIFICO (3 bifidobacteria species)</td>
<td>48</td>
<td>Modulation of the immune response</td>
<td>Relapse in 20% of patients in probiotic group vs. 93% in the placebo group. The probiotic impeded the activation of NF-κB, decreased the expressions of TNF-α and IL-1β and increased the expression of IL-10.</td>
<td>Cui et al. (2004)</td>
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<table>
<thead>
<tr>
<th>Disease</th>
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<th>n</th>
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</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td><em>Lb. rhamnosus</em> GG, <em>Lb. rhamnosus</em> Lc705, <em>P. freudenreichii</em> spp <em>shermanii</em> IS, <em>B. breve</em> Bb99</td>
<td>103</td>
<td>NE</td>
<td>The total symptom score (abdominal pain + distension + flatulence + borborygmi) was reduced 42% in probiotic group compared with 6% in the placebo group</td>
<td>Kajander and Korpela, (2006)</td>
</tr>
<tr>
<td>IBS</td>
<td>Prescript-Assist (probiotic-prebiotic complex containing 29 soil-based, pH-resistant micro-flora)</td>
<td>25</td>
<td>NE</td>
<td>The probiotic + prebiotic treatment showed short-term and long-term reductions in IBS symptoms</td>
<td>Bittner et al. (2007)</td>
</tr>
<tr>
<td>IBS</td>
<td><em>S. boulardii</em></td>
<td>67</td>
<td>NE</td>
<td>The probiotic improved the quality of life in patient with IBS</td>
<td>Choi et al. (2011)</td>
</tr>
<tr>
<td>PCH</td>
<td>VSL-3 (probiotic preparation containing 3 <em>Bifidobacterium</em>, 4 <em>Lactobacillus</em>, and 1 St. thermophilus strains)</td>
<td>36</td>
<td>NE</td>
<td>The probiotic mixture was effective in maintaining antibiotic introduced remission for at least a year in patients with recurrent or refractory pouchitis (85%) vs. 6% in the placebo group</td>
<td>Mimura et al. (2004)</td>
</tr>
<tr>
<td>PCH</td>
<td>Ecologic®825 (food supplement containing nine bacterial strains: one <em>Bifidobacterium</em> and six <em>Lactobacillus</em>)</td>
<td>16</td>
<td>Increase of bacterial diversity of mucosal pouch microbiota</td>
<td>Restores the mucosal barrier and increased microbial groups (Bacteroidetes and Clostridium clusters IX, XI, and XIVa) associated with healthy pouches</td>
<td>Persbom and Soderholm (2013)</td>
</tr>
<tr>
<td>PCH</td>
<td>VSL-3</td>
<td>40</td>
<td>NE</td>
<td>10% of patients treated with probiotics had an episode of acute pouchitis compared with 40% treated with placebo. Treatment with probiotic improved Inflammatory Bowel Disease Questionnaire score vs. placebo</td>
<td>Gionchetti et al. (2003)</td>
</tr>
<tr>
<td>PCH</td>
<td>VSL-3</td>
<td>31</td>
<td>Modulation of the immunoreponse</td>
<td>The probiotic mixture administration in patients with ileal pouch anal anastomosis modulated the disease activity and increased the number of mucosal regulatory T cells</td>
<td>Pronio et al. (2008)</td>
</tr>
</tbody>
</table>

NE, not specified.

*aUC, Ulcerative colitis; CD, Crohn’s disease; IBS, irritable bowel syndrome; PCH, pouchitis.*

*bMicrobial abbreviations: S, Saccharomyces; B, Bifidobacterium; bL, Lactobacillus; St, Streptococcus; P, Propionibacterium; E, Escherichia.*
and prevented colonic inflammatory damages in a TNBS-induced colitis model in rats (Segui et al., 2004). In addition, in this study, treatment with SOD decreased oxidative stress and adhesion molecule upregulation in response to abdominal irradiation in mice.

Catalase is another major antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. Catalases are widespread in aerobic (facultative or not) bacteria such as *E. coli* and *Bacillus (B.) subtilis* (Rochat et al., 2005). There are two different classes of catalases according to their active-site composition: one is heme-dependent and the other, also named pseudocatalase, is manganese-dependent.

### 2.1.3.1 Genetic Modification as an Alternative for Antioxidant Producing Lactic Acid Bacteria

By definition, LAB are catalase negative microorganisms, and because few microorganisms produce antioxidant enzymes in sufficient concentrations to exert biological effects, genetic engineering strategies have been performed to obtain more efficient antioxidant producing LAB (Spyropoulos et al., 2010). LAB have been used to locally deliver antioxidant enzymes such as SOD or catalase directly in the intestines. This is an important strategy if considered that oral administration of SOD is largely limited by its short half-life (5-10 min) in the hostile conditions of the GIT. It has been reported that genetically modified (GM) strains of *Lb. plantarum* and *Lc. lactis* that are able to produce and release SOD exhibited anti-inflammatory effects in a TNBS induced colitis model (Han et al., 2006). *Lb. gasseri* strain producing SOD also showed anti-inflammatory activity associated to reduction of the severity of colitis in IL-10 deficient mice (Carroll et al., 2007). *Lb. casei* BL23 producing SOD was able to significantly reduce the intestinal damage score induced by TNBS in mice (LeBlanc et al., 2011) and previously this same strain was able to slightly reduce the histological damage degree in a dextran sulfate sodium (DSS) induced colitis model (Watterlot et al., 2010).

Since *Lc. lactis* does not produce catalase, the heme catalase gene *katE* from *B. subtilis* was added to this industrially important organism (Rochat et al., 2005). It has been shown that this catalase producing *Lc. lactis* strain administered to mice prevented the development of chemical-induced colon tumors in these animals (de Moreno de LeBlanc et al., 2008b). *Lb. casei* BL23 was also modified to produce heme-independent catalase and its oral administration decreased the intestinal inflammatory damages in a TNBS-induced mouse model (LeBlanc et al., 2011). This result agreed to those obtained previously in which both the native strain *Lb. casei* BL23 and the derived GM catalase-producing strain reduced histological inflammation score in the colon and cecum of mice, induced by DSS (Rochat et al., 2007).

*St. thermophilus* CRL807 is a strain that was present in the starter mix, together with 12 other LAB, used to prepare a yogurt with anti-inflammatory properties that will be discussed in the next sections (Chaves et al., 2011b; de Moreno de LeBlanc et al., 2009). The anti-inflammatory potential of this strain was demonstrated in vitro and in vivo (del Carmen et al., 2014a). In this article, the concept that LAB selected for their innate inflammatory potential can be GM to produce antioxidant enzymes and thus produce more efficient anti-inflammatory effects. It was observed that the wild type strain (*St. thermophilus* CRL807) exerted anti-inflammatory effects in a TNBS-induced colitis model in mice; however, genetically modification of *St. thermophilus* CRL807 to produce catalase or SOD (used as suspension or in fermented milks) increased its innate anti-inflammatory potential. These results suggest that the use of LAB strains that are able to modulate the immune response and also express antioxidant enzymes may be a useful strategy in the development of new therapies for patients suffering from IBD.

### 2.1.4 Improvement of the Host’s Immune Response

Many beneficial effects of probiotics are associated to their immunomodulatory potential as well as anti-inflammatory activities. Considering that most of probiotics enter the host orally, these can modulate the Gut Associated Lymphoid Tissue, which is a well-organized immune network that protects the host from pathogens and prevents the hyperstimulation of the immune response by ingested proteins through the oral tolerance (Weiner et al., 1997).

Nuclear factor κB (NF-κB) is a molecule that was analyzed to understand the anti-inflammatory mechanism of action of certain probiotics. It is one of the central transcription factors mediating inflammatory responses and is required for the transcriptional activation of a number of inflammatory effectors, including IL-8, TNF-α, IL-6, Cox2, iNOS. Its deregulation has been demonstrated in many inflammatory conditions. It was shown that a number of LABs can suppress inflammatory signals mediated by NF-κB by blocking IκB-α (inhibitor κB/nuclear factor κB pathway) degradation (Neish, 2004; Thomas and Versalovic, 2010). To evaluate this mechanism, colonic biopsies from UC patients were cocultured with *Bifidobacterium (B.) longum* and it was showed that this probiotic decreased NF-κB activation and downregulated inflammatory cytokine secretion (Bai et al., 2006).

The modulation of cytokine (mediators produced by immune cells) production is another characteristic evaluated for probiotics because these molecules are implicated in the regulation, activation, growth, and differentiation of immune cells.
In this sense, interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines involved in the homeostasis of the gut immune system (de Moreno de Leblanc et al., 2011). IL-10 has the capacity of downregulating inflammatory cascades through the suppression of proinflammatory cytokines (Mocellin et al., 2004) and its role as an essential immune-regulator in the intestinal tract has been demonstrated using IL-10-deficient mice (Kuhn et al., 1993). These animals develop a chronic bowel inflammation, resembling CD in humans and even colon cancer. The importance of IL-10 modulation by probiotics in gut inflammatory diseases was recently reviewed (de Moreno de Leblanc et al., 2011). This regulatory capacity is related with the potential of probiotic microorganisms to prevent and treat certain inflammatory diseases in the GIT through the repression of proinflammatory cytokines. However, it is important to clarify that not all probiotic strains act in the same manner and anti-inflammatory effects, such as stimulation of IL-10 producing cells, are strain-dependent traits.

Modifications in the cytokine profiles were evaluated using yogurt prepared with potential probiotic strains using a TNBS-induced acute intestinal inflammation model in mice where it was demonstrated that this product prevented intestinal damages and improved gut anti-inflammatory responses (de Moreno de Leblanc et al., 2009). This effect was related with increases of IL-10 and decreases of IL-17 in the IBD-induced mice given yogurt and in the remission period in a mouse model of recurrent inflammation (Chaves et al., 2011).

Recently, it was reported that Lb. plantarum CECT 7315/7316 administration modulated the acute and chronic inflammatory response in rat models. Probiotic supplementation decreased IL-1β, IL-6 and increased IL-10 systemic levels compared to the placebo group (Vilahur et al., 2014). E. coli Nissle 1917 (EcN) is another probiotic strain for which anti-inflammatory potential was demonstrated using different models. Recently, it was shown that oral EcN administration to rats after TNBS induction of colitis decreased the levels of TNF-α and tended to increased IL-10 in a dose dependently manner (Sha et al., 2014). Oral administration of Lc. lactis NCDO 2118 to mice during the remission period of colitis induced by DSS resulted in a milder form of recurrent colitis due to an early increase in IL-6 production and sustained IL-10 production in colonic tissue and an increase in the numbers of regulatory CD4(+) T cells (Tregs) in the mesenteric lymph nodes and spleen (Luerce et al., 2014).

Modulation of the host immune response was also reported in many human clinical trials in which different probiotic strains exerted beneficial effect with alleviation of IBD symptoms. Table 57.1 summarizes some of these results obtained with specific probiotic strains.

Probiotic yogurt containing Bifidobacterium and Lactobacillus ssp. was administered to patients in remission phase of IBD. It was observed that those that received the probiotic yogurt decreased serum levels of IL-1β, TNF-α, and CRP compared to the patients that received conventional yogurt and the baseline values. These results were accompanied by increase of IL-6 and IL-10 showing a positive modulation of the immune response by this probiotic product (Shadnoush et al., 2013).

2.1.4.1 Genetically Modified Lactic Acid Bacteria that Produce the Anti-inflammatory Cytokine IL-10

Interleukin-10 (IL-10), as explained above, is one of the major anti-inflammatory cytokines involved in maintaining the homeostasis of the gut immune response and is thus a therapeutic candidate for the treatment of IBD (de Moreno de Leblanc et al., 2011). However, oral administration of IL-10 is not an option because of its sensitivity to the hostile conditions of the GIT (Egilmez and Sikora, 2005). This aroused the interest to develop new technologies for the delivery of IL-10 at the intestinal level (Marlow et al., 2013). Therefore, the use of GM LAB appeared as an attractive alternative for delivering this cytokine at mucosal surface level (LeBlanc et al., 2013).

GM LAB have been reported for the first time as therapeutic vehicle for IL-10 in 2000, when it was shown that a IL-10 producing Lc. lactis strain prevented the onset of colitis in IL-10−/− mice (Schotte et al., 2000), and reduced inflammation in DSS induced colitis model (Steidler et al., 2000). An important phase for the safety use of this GM LAB for therapeutic purposes in humans was the construction of a biological containment system in the GM strain for intestinal delivery of human IL-10 cytokine (Steidler et al., 2003). For this purpose, the thymidylate synthase gene of Lc. lactis was replaced by the human IL-10 gene, making this strain unable to grow in the absence of thymidine or thymine. This containment system was evaluated in patients with CD and was reported that it did not produce any adverse side effects (Braat et al., 2006). However, the clinical outcomes in these patients showed no statistical significances between those patients who received the GM LAB compared to the placebo group. These results showed the need to evaluate new methods of IL-10 administration to achieve a more effective delivery of this cytokine in the intestinal mucosa using therapeutic LAB (Benbouziane et al., 2013; Guimaraes et al., 2009; Innocentin et al., 2009; Miyoshi et al., 2004).

Lc. lactis subsp. lactis NCDO2118 pXYL:IL-10 is a LAB that produced IL-10 using the xylose-inducible expression system (XIES) that has a food-grade inducer (Miyoshi et al., 2004). Milk fermented by Lc. lactis NCDO2118 pXYLCYT:IL-10, a strain capable to produce and maintain IL-10, in the bacterial cytoplasm reduced the levels of
proinflammatory cytokines at the intestinal level in TNBS-induced mice (del Carmen et al., 2012). This study also showed the use of fermented milks as a new form of administration of IL-10 producing LAB since milk acts as a protector matrix for the bacteria and the cytokine during the passage through the GIT. This new approach could lead to the development of new therapeutic fermented products (functional foods) focused for a specific population that suffers gastrointestinal disorders.

The protein delivery expression system Stress-Inducible Controlled Expression (SICE) was also used to produce and secrete IL-10 by *Lc. lactis* subsp. *cremoris* MG1363 (Benbouziane et al., 2013). *Lc. lactis* pGroE:IL-10 capable of delivering the IL-10 protein using the SICE system that has the advantage that it does not require an inductor because the hostile conditions of the GIT can themselves induce gene expression and the IL-10 can be locally produced by this LAB in the intestine. In this sense, it was recently demonstrated that this strain protected against inflammation in a model of low-grade colon inflammation (Martin et al., 2014).

DNA delivery by GM LAB was also evaluated for the production of IL-10 by the host’s intestinal cells. *Lc. lactis* subsp. *cremoris* MG1363 pValac:IL-10 is a GM LAB used for the delivery of IL-10 cDNA to the intestinal cells and gives these host cells the capacity to produce IL-10 directly at the site of inflammation (Guimaraes et al., 2009). It was reported that *Lc. lactis* MG1363 engineered to express fibronectin binding protein A (FnBPA) was used as a vehicle to deliver the IL-10 cDNA using the plasmid pValac:il-10. *Lc. lactis* MG1363 FnBPA+pValac: IL-10 and decreased the inflammatory damages in the intestine of mice in a TNBS induced acute model of colitis by maintaining high ratios of anti-/proinflammatory cytokines in the intestine (del Carmen et al., 2013). It was also recently demonstrated that the use of the noninvasive strain *Lc. lactis* subsp. *cremoris* MG1363 pValac:IL-10 (without FnBPA) was also able to deliver the IL-10 cDNA to the host’s cells and exerted an inflammatory effect in a DSS induced colitis model in mice (Zurita-Turk et al., 2014).

Finally, the effectiveness of both protein and DNA delivery systems was also recently compared using a TNBS induced chronic inflammation model. It was demonstrated that *Lc. lactis* pValac:IL-10 (noninvasive strain) exerted similar anti-inflammatory effects than *Lc. lactis* pGroE:IL-10, when they were administered to the mice during the remission period (del Carmen et al., 2014b).

### 3 PROBIOTICS IN CANCER PREVENTION

The use of probiotics is constantly growing due to increasing numbers of studies proving that certain strains present health promoting properties; among these is the prevention or treatment in the early stages of some types of cancers (de Moreno de Leblanc and Perdigon; 2010; Kahouli et al., 2013).

The value of experimental animal models is the insight they can provide into the complex, multifaceted processes and mechanisms that can result in cancer development. It is also important to state that *in vitro* assays allow understanding the mechanisms of action involved in the probiotic effects. However, the application of probiotics as a biotherapeutic against cancer needs to be ultimately tested in human trials.

#### 3.1 Probiotics and Fermented Products in Colon Cancer Prevention and Treatment

Colorectal cancer (CRC) is one of the most common cancers worldwide; its incidence rates being higher in the Western world. This is the type of cancer in which the effects of probiotics and fermented products have been the most extensively studied considering that these microorganisms enter the organism orally and can positively modulate the intestinal microbiota and modulate the gut immune response. The benefits of probiotics on the gut immune system in the prevention of cancer has also been previously described (de Moreno de LeBlanc et al., 2007b; Frenkel et al., 2013; Kato et al., 1984).

It is important to consider the association of chronic inflammation with several malignant diseases (Prescott and Fitzpatrick, 2000). This relationship would be mediated by cytokines (Feghali and Wright, 1997) or by ROS generated by inflammatory phagocytes that can cause injury to target cells, thus contributing to cancer development (Weitzman and Gordon, 1990). At the intestinal level, examples include intestinal cancer after chronic intestinal inflammation (Collins et al., 1987; Korelitz, 1983). It has been suggested that inflammatory mediators can be useful as important biomarkers for colon cancer progression (McClellan et al., 2012). In this sense, the anti-inflammatory effect reported for certain probiotics at the intestinal level indirectly can turn in an antitumor effect by preventing the development of cancer cells.

However, the probiotic characteristics should be evaluated in appropriated models for specific pathologies. It is also important to note that a strain or product with benefits against a type of cancer will not necessarily exert a beneficial effect against other types of tumors. As was described for IBD, different mechanisms can be involved in the beneficial effects of probiotics against CRC, among them, the modulation of the intestinal microbiota, the inactivation of carcinogenic compounds, antioxidant effects, and the modulations of the host’s immune response.
3.1.1 Modifications in the Gut Microbiota

It has been reported that the phylogenetic core of human microbiota was significantly different in the stools of CRC patients compared to those from individuals with normal colonoscopy (Marchesi et al., 2011). Changes in the composition of the microbiota of CRC patients could impact on their mucosal immune response (Sobhani et al., 2012).

Probiotics can beneficially modify the intestinal microbiota and this can be related with reduction of the level of certain enzymes such as β-glucuronidase, nitroreductase, produced by certain microorganisms, which convert procarcinogens to carcinogens in the intestine (Fernandes and Shahan, 1990; Goldin and Gorbach, 1984). Goldin and Gorbach evaluated the effect of administering two *Lb. acidophilus* strains on the activity of these bacterial enzymes in 21 healthy volunteers. It was also shown using a DMH-induced colon cancer model in mice that animals fed cyclically with yogurt (the yogurt with anti-inflammatory properties reported in IBD models and explained above) presented lower β-glucuronidase and nitroreductase activity levels in the intestinal content than the tumor control group, which increased the activity of these microbial enzymes contributing in this way to the cancer development (de Moreno de LeBlanc and Perdigon, 2005). *Lb. rhamnosus* GG and *Lb. acidophilus* suppressed DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats (Verma and Shukla, 2013).

Modifications in the gut microbiota can also be associated to changes in the presence of short-chain fat acids (SCFA) produced by bacterial fermentation of undigested carbohydrates. It was demonstrated that microbe-derived SCFAs (such as butyrate) regulate host gene expression involved in intestinal homeostasis as well as carcinogenesis through modulation of microRNAs (Hu et al., 2011). Butyrate was also associated with induction of differentiation, suppression of proliferation, and enhanced apoptosis *in vitro* (Heerdt et al., 1997). The probiotic mixture VSL#3 was able to convert linoleic acid into conjugated linoleic acid inducing the upregulation of PPARγ, a reduction in colonic tumor cells viability, and the induction of apoptosis (Ewaschuk et al., 2006; Le Leu et al., 2010). The symbiotic combination of resistant starch (RS) and *B. lactis* protected against CRC development in a rat model, and this was in part related to an increase in the SCFA by RS intake (Le Leu et al., 2005).

Recently, it was reported that *Lb. salivarius* Ren modulated colonic microbiota structures and the luminal metabolisms (increased SCFA levels), and prevented the early colorectal carcinogenesis in DMH-induced rat model (Zhu et al., 2014).

3.1.2 Inactivation of Carcinogenic Compounds

There is an increase in the relative risk of developing CRC in people that consume large amounts of red meat (Larsson and Wolk, 2006). This relationship can be explained by the presence of heterocyclic aromatic amines (HCA) that result from cooking meat at high temperatures (Felton et al., 2007; Turesky, 2007). The detoxification of cooked food mutagenic compounds, especially from the Western meat-rich diets, may be a mechanism by which probiotics prevent CRC.

LAB and other commensal bacteria can bind or metabolize several carcinogens, including HCA and N-nitrous compounds, which were correlated with the reduction in mutagenicity observed after exposure of HCA to the bacterial strains (Orrhage et al., 1994). It was demonstrated that strains of *Lb. gasseri* and *B. longum* strongly bind 3-amino-1,4-dimethyl-5H-pyrido-[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) (Sreekumar and Hosono, 1998a,b). *Lb. acidophilus* NCFB1748 and *B. longum* BB536 decreased the bioavailability of Trp-P-2 in their GITs and other tissues of mice (Orrhage et al., 2002). It was also reported that cell fractions of *Lb. acidophilus* and *Bifidobacterium* spp. bond Trp-P-1 and decreased its genotoxicity (Zhang and Ohta, 1993).

Recently, the effect of four probiotic *Lactobacillus* strains to detoxify N-nitrosodimethylamine was evaluated and it was shown that all the LAB tested have a beneficial effect, *Lb. brevis* 0945 being the one that lowered the concentration and genotoxicity of N-nitrosodimethylamine most effectively (Nowak et al., 2014).

3.1.3 Antioxidant Effects

Oxidative stress and epithelial damage are normally linked to pathologies of the GIT such as CRC, so another mechanism by which probiotics could prevent this cancer is through the production of antioxidant enzymes that can degrade ROS or impair their formation.

Since the majority of LAB do not produce enzymes that can degrade reactive oxygen species, genes coding for antioxidant enzymes (such as catalases or SOD) were inserted in LAB as an antioxidant and anti-inflammatory strategies. GM *Lc. lactis* to produce catalase exerted efficient antioxidant activity (Rochat et al., 2005) and prevented tumor appearance in a DMH-induced CRC model in mice (de Moreno de LeBlanc et al., 2008a). The catalase producing *Lc. lactis* strain was able to slightly increase catalase activity in the intestines of mice treated with DMH; however, this was sufficient to reduce H$_2$O$_2$ levels in the large intestines.
Other LAB strain GM to produce antioxidant enzymes that showed their anti-inflammatory properties using IBD models in mice should be evaluated in appropriate models to assess their antitumor potential.

Recently it was reported that the administration of a symbiotic (\(Lb.\) rhamnosus + \(Lb.\) acidophilus + inulin) before DMH-induced CRC in rats decreased tumor incidence compared with the DMH control group and this effect was related with a decreased level of malondialdehyde, a measure of lipid peroxidation, and increased levels of GR, SOD, and glutathione peroxidase in the animals given the symbiotic (Verma and Shukla, 2014).

### 3.1.4 Improvement of the Host’s Immune Response

Several studies using animal models suggest that probiotics have potential to prevent CRC by modulation of the host’s immune system. \(Lb.\) casei strain Shirot (LeS) has been demonstrated to exert beneficial effects in carcinogenesis animal models by modulating the host immune response (Matsuzaki et al., 2004). The administration of \(Lb.\) acidophilus SNUL, \(Lb.\) casei YIT9029, and \(B.\) longum HY8001 increased the survival rate of mice injected with tumor cells, which was correlated with increased cellular immunity (total T cells, natural killer (NK) cells and Major histocompatibility complex (MHC) class II+ cells, and CD4+CD8+ T cells) (Lee et al., 2004).

Yogurt feeding inhibited tumor growth in a DMH-induced CRC model in mice. Yogurt administration to mice resulted in increased number of IgA secreting cells and CD4+ T lymphocytes in the lamina propria of the large intestine (de Moreno de LeBlanc et al., 2004b; Perdigon et al., 2002). The analysis of cytokines in this model suggested that yogurt feeding modulated the immune response by stimulating cytokine production when this was required or inducing downregulation of the immune cells to avoid an exacerbated immune response. This effect was associated with the regulatory cytokine IL-10, which was increased in the tissue of the mice given yogurt during all the assayed periods. A comparative study between yogurt consumption and indomethacin treatment was carried out in a DMH-induced CRC model in mice. Mice treated with indomethacin showed fewer immune cells infiltrating into the large intestine than the animals given yogurt in which a great increase in the number of immune cells infiltrating the lamina propria was observed (de Moreno de LeBlanc et al., 2004). It was also observed that indomethacin treatment maintained the effects during the administration; however, when the treatment was stopped due to cachexia produced in the animals, the tumor developed with the same characteristics as in the control without treatment. When yogurt feeding was stopped at the end of the experiment (6 months), the animals were observed over a 9-month period and did not develop tumors. Another study with the same model of DMH-induced CRC showed that a preventive yogurt feeding was not enough to inhibit the tumor in the initiation stage and only delayed tumor appearance (de Moreno de LeBlanc and Perdigon, 2004). Unlike this, mice that did not receive yogurt previous to DMH injection but were fed cyclically with yogurt after tumor induction did not develop tumors. These observations suggested that yogurt exerted its antitumor activity by the inhibition of tumor progression and that this effect was achieved by long-term cyclical yogurt consumption.

Probiotic Dahi containing \(Lb.\) acidophilus LaVK2 and \(B.\) bifidum BbVK3 alone or in combination with piroxicam modulated the development of early biomarkers of CRC in male DMH injected rats (Mohania et al., 2014).

The antitumor potential of the probiotic mix VSL#3 was evaluated as a pretreatment in patients with long-standing colitis and it was reported that attenuated inflammatory-associated parameters, delaying transition to dysplasia and cancer, offering a potential therapeutic use for these patients (Appleyard et al., 2011).

### 3.2 Effects in the Prevention and/or Treatment of Nonintestinal Tumors

The oral administration of probiotic microorganisms and fermented products can influence mucosal sites different to the intestines due to the existence of the common mucosal immune system. In this sense, after intestinal stimulation, both B and T cells can migrate from Peyer’s patches to mucosal membranes of the respiratory, gastrointestinal, and genito-urinary tract, as well as to exocrine glands such as the lacrimal, salivary, mammary, and prostatic glands (Brandtzæg and Pabst, 2004).

Probiotic \(Lb.\) casei CRL431 orally administered to mice induced an immune stimulation not only at the intestinal level but also in bronchus and mammary glands (de Moreno de LeBlanc et al., 2005a). It was shown that the administration of this probiotic strain inhibited the growth of a fibrosarcoma induced by methylcholanthrene in mice in a dose-dependent form (Perdigón et al., 1993). Probiotic administration stimulated the immune system with high levels of macrophage activation (the main infiltrative cells in the tumor) and high levels of TNFα.

In addition to containing LAB, fermented milk can possess nonbacterial components produced during milk fermentation that may contribute to their antitumor activities, as was reported for a peptide fraction obtained from milk fermented by \(Lb.\) helveticus R389 (LeBlanc et al., 2002).

\(Lb.\) plantarum 5BL was evaluated in vitro and showed remarkable anticancer activity against different human cancer cell lines, but no cytotoxic effects were exerted on HUVEC normal cells (Nami et al., 2014a). Similarly, \(Lb.\) acidophilus
36YL, isolated from the vagina of healthy and fertile Iranian women, showed anticancer effects on the four tested cancer cell lines, without cytotoxicity on normal cell line. This effect was associated to apoptosis induction (Nami et al., 2014b). Table 57.2 summarizes the effect of LAB or fermented products containing these microorganisms in nonintestinal tumors reported during the last 3 years (2011-2014). Results were obtained searching the words “probiotic and cancer” in PubMed database; however results about breast cancer were not included because they will be discussed in the following section.

### 3.2.1 Probiotics in Breast Cancer Prevention

There are many studies that reported the beneficial effect of probiotics against breast cancer. Soy isoflavone ingestion was evaluated accompanied with the co-administration of probiotic bacteria, and it was observed that high concentrations of probiotics may alter the metabolism of isoflavones (Cohen et al., 2007). Recently, it was stated that the regular consumption of beverages containing Lb. casei Shirota and soy isoflavones was inversely associated with the incidence of breast cancer in Japanese women (Toi et al., 2013). The cooperative mechanism of prevention exerted by soymilk and Lb. casei Shirota

### TABLE 57.2 Examples of Non-Intestinal Cancer That Have Demonstrated Beneficial Effects of LAB

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>LAB</th>
<th>Host</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Lb. salivarius REN</td>
<td>Rats</td>
<td>Probiotic administration suppressed 4NQO-induced oral carcinogenesis in rats. Probiotic administration decreased the expression of proliferating cell nuclear antigen and induced apoptosis in a dose-dependent manner</td>
<td>Zhang et al. (2013)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>NE</td>
<td>Human</td>
<td>Nutritional supplement containing probiotics improved body weight as well as serum albumin and prealbumin levels in patients with head and neck cancer cachexia</td>
<td>Yeh et al. (2013)</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>Lb. rhamnosus GG &amp; Lb. casei strain Shirota</td>
<td>Rats</td>
<td>Probiotic fermented milk and chlorophyllin administration reduced precarcinogenic events in AFB1 induced liver carcinogenesis in rats. This effect was associated with increased antioxidant status and decreased expression of oncogenes</td>
<td>Kumar et al. (2011, 2012)</td>
</tr>
<tr>
<td>Cervical</td>
<td>NE</td>
<td>Human</td>
<td>Probiotic promotes the clearance of HPV-related cytological abnormalities in women</td>
<td>Verhoeven et al. (2013)</td>
</tr>
<tr>
<td>Cervical</td>
<td>Vaginal lactobacilli</td>
<td>In vitro</td>
<td>Cytotoxic effects on cervical tumor cells was exerted by common vaginal lactobacilli. The cytotoxicity was independent of pH and lactate</td>
<td>Motevaseli et al., (2013)</td>
</tr>
<tr>
<td>Cervical</td>
<td>Lc. lactis and Lb. casei displaying E7 antigen</td>
<td>Mice</td>
<td>Non-genetically modified lactic acid bacteria displaying E7 antigen at their surface protected mice against HPV type 16-induced tumors</td>
<td>Ribelles et al. (2013)</td>
</tr>
<tr>
<td>Skin</td>
<td>Lb. acidophilus KCTC 3155</td>
<td>Mouse</td>
<td>The administration of maeisol (a member of the genus Rosaceae) fermented with probiotics reduced the development of DMBA-TPA induced skin carcinogenesis in mouse. This effect was related to the enhancing of total antioxidant capacity and phase II detoxifying enzymes</td>
<td>Lee et al. (2013)</td>
</tr>
</tbody>
</table>

Microbial abbreviations: Lb, Lactobacillus; Lc, Lactococcus; NE, not specified.
was evaluated in a rat carcinogenic model and it was demonstrated that soymilk prevented the development of mammary tumors and that *Lb. casei* Shirota suppressed tumor growth (Kaga et al., 2013).

In Western diets, fermented milks are more common as dairy foods than soy-based products. Milks fermented by different LAB and bifidobacteria strains (*B. infantis, B. bifidum, B. animalis, L. acidophilus, and L. paracasei*) were evaluated *in vitro*, and they inhibited the growth of a breast cancer cell line (Biffi et al., 1997). Other studies performed in humans showed negative association between yogurt consumption and breast cancer development (Le et al., 1986). van’t Veer et al. (1989) showed similar results in The Netherlands, and suggested that these effects would be related to changes in the intestinal microbiota (which could alter the metabolism of estrogen) and to the modulation on the immune system.

Milk fermented by *Lb. helveticus* R389 (a strain with high proteolytic activity) was studied comparatively with the milk fermented by a proteolytic deficient mutant, and both were able to delay tumor growth in a breast cancer model in mice using the injection of 4T1 cells (de Moreno de LeBlanc et al., 2005b,c). This effect was related to the immunoregulatory capacity of the fermented milks, which was more evident in the mice fed with milk fermented by *Lb. helveticus* R389, demonstrating the importance of the components released into the milk after the fermentation with the proteolytic strain.

Kefir was another fermented product evaluated in a breast cancer model in mice. Kefir and its cell-free fraction (KF) possess several substances that can exert beneficial effects on the immune system and prevent certain types of cancer (Vinderola et al., 2005). It was reported that mice given 2 days cyclical feeding with whole kefir diminished tumor growth, and the same cyclical feeding with KF showed the most significant delay of the tumor growth (de Moreno de LeBlanc et al., 2006). The results also demonstrated that the most important effect in this tumor model was due to substances released during milk fermentation (and not the microorganisms themselves) (de Moreno de Leblanc et al., 2007a).

It was reported that *Lb. acidophilus* isolated from traditional homemade yogurt and also from neonatal stool induced a significant decrease in breast tumor growth pattern using a mouse model induced by 4T1 cell injection by modulating the host’s immune response (Maroof et al., 2012). *Lb. casei* spp. *casei* ATCC 39392 was administered to mice bearing invasive ductal carcinoma and it was shown that decreased tumor growth rate and prolongation of mice survival by an immunomodulating mechanism (Soltan Dallal et al., 2012). The administration of selenium nanoparticle-enriched *Lb. plantarum* induced a reduction of tumor volume and increase in rate of survival in 4T1 breast cancer-bearing mice compared to mice that received probiotic alone or control mice (Yazdi et al., 2012). This effect was associated with an efficient immune response caused by the elevation of the proinflammatory cytokines IFN-γ, TNF-α, and IL-2 levels and increased NK cell activity.

The importance of the stimulation of host immune cells by LAB and their beneficial effect against mammary carcinoma was analyzed using two mice models (Lakritz et al., 2014). In one model, mice were fed a Westernized chow increasing risk for development of mammary tumors. The other model consisted of FVB strain erbB2 (HER2) mutant mice, genetically susceptible to mammary tumors. Animals received *Lb. reuteri* ATCC-PTA-6475 in drinking water. It was observed that oral supplementation of the probiotic strain inhibited features of mammary neoplasia in both models. The protective mechanism was associated to CD4+CD25+ lymphocytes because when they were isolated and transplanted into other subjects conferred anticancer protection in the cell recipient animals.

It was also demonstrated that long-term administration of *Lb plantarum* LS/07 with and without inulin (as a probiotic or as probiotic/prebiotic mix) was effective against a chemically induced breast cancer by modulating the immune response against the tumor (Kassayova et al., 2014).

Recently, our research group evaluated the effect of milk fermented by the probiotic bacterium *Lb. casei* CRL 431 on a murine breast cancer model. It was observed that the administration of this probiotic fermented milk stimulated the immune response against this breast tumor, and avoided or delayed its growth. This effect was observed when the probiotic product was administered preventively and also when its administration was after tumor induction (Aragon et al., 2014).

### 4 CONCLUSIONS

There are many reports about the anti-inflammatory and antitumor effect of probiotic strains and fermented products that contain these microorganisms against intestinal and nonintestinal pathologies. Different mechanisms of action can be involved in these beneficial effects, being the modulation of the host’s immunity one of the most important mechanism reported using *in vitro* assays and also in animal models. However, there are not enough human trials where the application of probiotics as biotherapeutics against chronic inflammation and mainly cancer was tested. It is also important to evaluate the safety of these products in hosts that can be immunosuppressed for the received treatments. These assays are very important before the medical community can accept the addition of probiotic as complementary therapies for IBD and cancer patients.
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