Mechanisms Involved in the Anti-Inflammatory Properties of Native and Genetically Engineered Lactic Acid Bacteria

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Abstract: Lactic acid bacteria (LAB) represent a heterogeneous group of microorganisms that have been shown to possess therapeutic properties since they are able to prevent the development of some diseases, as shown mostly on animal models for cancer, infections and gastrointestinal disorders such as intestinal inflammation. LAB have been shown to regulate mucosal immune responses by modulating the production and liberation of regulatory agents such as cytokines by the host. Some of these cytokines, such as the anti-inflammatory interleukin-10 (IL-10), modulate the inflammatory immune response, thus immunomodulation is a mechanism by which LAB can prevent certain inflammatory bowel diseases (IBD). Since oxidative stress participates in the inflammatory processes and in the appearance of damages in pathologies of the gastrointestinal tract of humans such as IBD, LAB could also prevent inflammation by eliminating reactive oxygen species (ROS) through the activity of antioxidant enzymes. In order to obtain novel strains or enhance beneficial effects of LAB, genetic engineering has been used to produce either antioxidant enzymes (such as catalases and superoxide dismutases) or anti-inflammatory cytokines (such as IL-10) producing LAB. These novel strains have successfully been used to prevent inflammatory bowel diseases in animal models and could be evaluated in human clinical trials. Here, we present an overview of the current knowledge of the mechanisms by which LAB can be used to prevent undesired intestinal inflammatory responses and could be used as a therapeutic tool for IBD.

Keywords: Anti-inflammatory, antioxidant, catalase, immune regulation, interleukin-10, lactic acid bacteria, superoxide dismutase.

INTRODUCTION

Despite many years of study, the exact etiology and pathogenesis of inflammatory bowel diseases (IBD) remain unclear but great advances have been made using experimental animal models and have provided insights into the complex, multi-factorial processes and mechanisms that can result in chronic intestinal inflammation.

Lactic acid bacteria (LAB) represent a heterogeneous group of microorganisms that are naturally present in many foods and have long been consumed by humans without any obvious adverse effects [1]. Some selected strains, with well-defined characteristics, are frequently added as probiotics in order to confer specific benefits to consumers. Many studies have shown that LAB possess therapeutic properties since they can modulate the host immune system, act as a barrier or produce antimicrobial substances against certain pathogens, reduce cholesterol levels, decrease the frequency and duration of diarrhoea that are associated with antibiotic usage or rotavirus infections, and can prevent some diseases such as cancer, infections, and gastrointestinal disorders such as intestinal inflammation. Most of these investigations have been performed using animal models; however, clinical studies are now confirming that probiotic LAB are also effective in preventing and treating diseases in humans. Since probiotics are generally administered *via* the oral route, most of their beneficial effects take place at the intestinal level where many studies on the mechanisms involved in the prevention and treatment of intestinal disorders have been focused [2]. There is now mounting evidence suggesting that LAB have anti-inflammatory properties that prevent and alleviate certain intestinal disorders. The present review will give an overview of the current knowledge of the mechanisms of these anti-inflammatory properties.

IMMUNE SYSTEM AND LAB

The gut microbiota plays an important role in the control of certain human diseases. Moreover, an increasing number of clinical and experimental studies have demonstrated that the intestinal microbiota may modulate the inflammatory responses in allergic and inflammatory bowel diseases (IBD) [3]. Probiotic bacteria can counteract inflammatory processes by stabilizing the gut microbial environment and the intestine's permeability barrier, and by enhancing the degradation of enteral antigens and altering their immunogenicity (reviewed in [4]). Many beneficial effects of probiotics are related to their immunomodulatory effects: immune-enhancing

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as well as anti-inflammatory activities [5]. A healthy homeostasis in the gut may thus be achieved by optimizing the balance of pro- and anti-inflammatory cytokines and other mediators. Accumulating evidence indicates that the establishment and maintenance of intestinal and systemic tolerance are mainly dependent on suppressive cytokines, such as interleukin (IL)-10 and transforming growth factor-β (TGF-β), produced by regulatory T cells and T helper cells which are characteristic of the intestinal immune system [6-8]. The tolerogenic effects of the gut microbiota may partially be mediated by generation of these regulatory T cells. Indeed, certain LAB strains, normal inhabitants of the gut microbiota, have been shown to contribute to T helper cell populations which promote oral tolerance induction, preventing hypersensitivity and local inflammation [7, 9]. It was also shown that *Lactobacillus* (*L.*) *rhamnosus* GG conditioned media decreased tumor necrosis factor (TNF-α) production by macrophages *in vitro* by a contact independent mechanism [10]. Other studies have shown the specificity of bacterial strains in inducing anti- or proinflammatory cytokines. Indeed, some LAB, or their products, orally administered in mice (*L. reuteri* or *L. brevis*), had a stimulatory effect on the secretion of proinflammatory cytokines such as IL-1β and TNF-α [11]. The immunomodulatory properties of LAB, through the repression of proinflammatory cytokines, could be one of the mechanisms by which these probiotic microorganisms are able to prevent and treat certain inflammatory diseases in the gastrointestinal tract. However, it is important to note that the extrapolation from *in vitro* to *in vivo* results is not easy. In this sense, Mileti *et al*. [12] reported a work where three LABs were compared and showed that these probiotics inhibited direct or indirect DC activation by inflammatory bacteria, however some of these not only failed to protect against DSS-induced colitis in mice, they actually amplified the disease progression. Proper animal trials followed by human clinical trials are thus essential in confirming the probiotic anti-inflammatory effect of probiotic bacteria.

PROBIOTICS AS TREATMENTS FOR INFLAMMATORY BOWEL DISEASE

The effectiveness of probiotics in the alleviation of digestive diseases are first tested in animal models to elucidate their possible mechanisms of action. In this sense, several animal models have been used to test different probiotic bacteria and fermented products. There are also evidences that suggest that non-living probiotic bacteria can prevent the development of certain forms of intestinal inflammation. In this sense, Zakostelska *et al.* [13] reported that lysates of probiotic *L. casei* DN-114 001 reduced the severity of acute dextran sulfate sodium (DSS) colitis in BALB/c mice. This effect was mediated by beneficial changes on the intestinal microbiota and immune system (increases of IL-10 by Treg cells and decreases of pro-inflammatory cytokines). The anti-inflammatory effect of *L. salivarius* Ls33 and its peptidoglycan (PGN) was studied using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis model. This activity was IL-10-dependent, favoring the development of regulatory dendritic cells and CD4(+)Foxp3(+) regulatory T cells [14]. It is important to note that this protective capacity was not obtained with PGN purified from a non-anti-inflammatory strain. The structural analysis of PGNs from *L. salivarius* Ls33 showed the presence of an additional muropeptide, M-tri-Lys which also protected mice from colitis.

Another bacterial compound that could be implicated in the anti-inflammatory effect of LAB is the lipoteichoic acid (LTA), the major cell wall compound of lactobacilli. It is known that LTA molecules of certain bacteria can induce pro-inflammatory signaling in macrophages by interaction with TLR-2 [15], but it was also reported that changing the structure of LTA by removing D-alanine residues might affect its interactions with other surface molecules and therefore cause pleiotropic effects that can impact indirectly on the anti-inflammatory capacity of the lactobacilli [16]. This experiment was carried out with *L. rhamnosus* GG (LGG), comparing the wild-type and dltD mutant strains. Mice treated with the dltD mutant showed an improvement of some colitic parameters compared to LGG wild-type-treated mice, with a significant down-regulation of Toll-like receptor-2 expression and decrease of pro-inflammatory cytokines. These results with the LGG dltD mutant show the potential of modifying some structures of the probiotic cell surface to have an improvement in the treatment of IBD. Other modified LABs with potential in treatment of IBD will be described latter in this article.

Recently, several randomized controlled trials have now confirmed the beneficial effects of probiotics in humans with IBD including Crohn's disease, irritable bowel syndrome, pouchitis and ulcerative colitis. Some examples of these clinical trials with the most relevant beneficial effects of probiotics supplementation are shown in Table **1**.

Hedin *et al.*, [35] reported a case-control study about the use of probiotics and prebiotics in patients with IBD. They described that a high percentage of IBD patients used probiotics to manage their health compared to controls (without IBD). They noted that many patients rely on nonclinical sources of information and considered that not all probiotics can be useful for all the IBD, suggesting that healthcare providers should inquire about probiotic use in their patients and give evidence-based advice.

The results of the trials listed in Table **1** confirm the effectiveness of certain probiotic strains in the treatment and prevention of IBD. However, only a few of these reports describe mechanisms of these anti-inflammatory effects.

Gionchetti *et al*. [27] performed a double-blind randomized controlled trial (RCT) comparing the effects of VSL#3 probiotic mixture and a placebo to prevent the recurrence of chronic relapsing pouchitis, an IBD occurring after surgical resection of the colon. A relapse occurred in 15% of those in probiotics group versus 100% in the placebo group. This result was confirmed in a second multi-center double-blind RCT where the relapse at one year was 10% in the probiotics-treated patients versus 94% in the placebo-treated patients [26]. A link between VSL#3 anti-inflammatory capacities and Toll-like receptors (TLR) was established by Rachmilewitz *et al.* who showed that the probiotic treatment prevents inflammation *via* the recognition of bacterial DNA by TLR9 using a mouse colitis model [36]. Interestingly, the probiotic treatment increased the tissue levels of IL-10 in

Table 1. Examples of Human Clinical Trials (Randomized Controlled Trials) that have Demonstrated that Probiotics Improve Inflammatory Bowel Diseases Including Crohn's Disease (CD), Irritable Bowel Syndrome (IBS), Pouchitis (PCH) and Ulcerative Colitis (UC)

Disease	n=	Results	Probiotic *	Ref.
CD	32	Relapse in 6% of patients supplemented with probiotic strain vs. 38% with conventional treatment only.	*S. boulardii*	[17]
CD	6	Median pediatric CD activity index scores at 4 weeks were 73% lower than baseline and intestinal permeability improved in an almost parallel fashion.	*L. rhamnosus* GG	[18]
CD	21	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.	*L. rhamnosus* GG	[19]
IBS	77	Alleviation of IBS symptoms and normalization of the ratio of an anti-inflammatory to a proinflammatory cytokine in patients receiving probiotic strain vs. placebo group.	*B. infantis* 35624	[20]
IBS	48	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.	BIFICO (3 bifidobacteria species)	[21]
IBS	103	The total symptom score (abdominal pain + distension + flatulence + borborygmi) was reduced 42% in probiotic group compared with 6% in the placebo group.	*L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp *shermanii* JS, *B. breve* Bb99	[22]
IBS	25	The probiotic+prebiotic treatment showed short-term and long-term reductions in IBS symptoms.	Prescript-Assist (probiotic-prebiotic complex containing 29 soil-based, pH-resistant micro-flora)	[23]
IBS	25	The probiotic+prebiotic treatment was associated with significant reductions in 3 subsyndromic factors of IBS: general ill feelings/nausea, indigestion/flatulence, and colitis.	Prescript-Assist	[24]
IBS	67	The probiotic improved the quality of life in patient with IBS.	*S. boulardii*	[25]
PCH	36	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.	VSL#3 (probiotic preparation containing 3 *B.*, 4 *L.* and 1 *St.* strains)	[26]
PCH	40	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.	VSL#3	[27]
PCH	31	The probiotic mixture administration in patients with ileal pouch anal anastomosis modulated the disease activity and increased the number of mucosal regulatory T cells.	VSL#3	[28]
UC	18	Sigmoidoscopy scores were reduced in probiotic group compared with placebo. TNF-α and IL-1α were reduced after treatment with probiotic.	Symbiotic therapy (*B. longum* and Synergy1)	[29]
UC	327	The probiotic treatment was just as effective as conventional treatment (mesalazine) in maintaining remission.	*E. coli* Nissle 1917	[30]
UC	90	Probiotic supplementation improved remission compared to conventional treatment (balsalazide) alone.	VSL#3	[31]
UC	21	Probiotic preparation maintains remission (75%).	VSL#3	[32]
UC	120	62% improvement of symptoms and 0% relapse of intestinal disease while patients were on probiotics.	VSL#3	[33]
UC	29	Pediatric, randomized, placebo-controlled trial that suggests the efficacy and safety of this probiotic mixture in active UC and in maintenance of remission.	VSL#3	[34]

**microbial abbreviations: S. (Saccharomyces), B. (Bifidobacterium). L. (Lactobacillus), St. (Streptococcus), P. (Propionibacterium), E. (Escherichia).*

these patients [37]. IL-10 is an anti-inflammatory cytokine that is involved in the suppression of the release of pro-inflammatory cytokines such as TNF-α, interferon-γ (IFN-γ) and IL-1β [38]. Recently, the first pediatric, randomized, placebo-controlled trial using this probiotic mixture in patients with ulcerative colitis was published. Remission was achieved in 13 patients out of 29, treated with VSL#3 and IBD therapy and in 4 patients treated with placebo and IBD therapy ($P<0.001$). Three of the 14 patients treated with VSL#3 and IBD therapy and 11 of 15 patients treated with placebo and IBD therapy relapsed within 1 year of follow-up, the endoscopic and histological scores being significantly lower in the VSL#3 group than in the placebo group ($P<0.05$) [34].

A pilot study has suggested that *L. rhamnosus* GG may improve gut barrier function and clinical status in children suffering from mildly to moderately active, stable Crohn's disease (CD) [18]. In CD, abnormal activation of mucosal T lymphocytes against enteric bacteria is the key event triggering intestinal inflammation. Carol *et al.* [39] demonstrated that a probiotic strain of *L. casei* reduces the number of activated T lymphocytes in the lamina propria of CD mucosa, diminishing the release of IL-6 and TNF-α and lowering the expression of the anti-apoptotic protein Bcl-2. In addition, co-culture with *L. casei* significantly reduced the number of T cells displaying the IL-2 receptor in the lamina propria.

The results of these trials show that some probiotic strains can successfully modify the mucosal immune response to modulate the levels of specific activation molecules such as cytokines. By increasing IL-10 levels and, in consequence, decreasing inflammatory cytokines such as TNF-α and IFN-γ, some LAB can prevent the appearance of local inflammatory diseases and can successfully be used as an adjunct therapy with conventional treatments.

The crucial role of IL-10 in the development of IBD has been demonstrated by experiments in IL-10–deficient mice. These animals develop a chronic bowel disease resembling CD in humans, which is in part caused by a loss of suppression of the mucosal immune response toward the normal intestinal microbiota [40]. Unfortunately, systemic IL-10 treatment of CD patients is not very effective in inducing clinical remission and is associated with considerable side effects, which are partly due to the fact that systemic IL-10 induces the pro-inflammatory cytokine IFN-γ [41]. However, studies in experimental models suggest that topical treatment with IL-10 is effective in preventing certain inflammatory diseases. LAB are potent candidates as delivery vehicles of beneficial compounds because of their GRAS status (generally recognized as safe) and expression of heterologous proteins and antigens, as well as various delivery systems are now available for these probiotic microorganisms [42]. Intragastric administration of a recombinant *Lactococcus* (*Lc.*) *lactis* strain secreting murine IL-10 prevented the onset of colitis in IL-10 knockout mice and caused a 50% reduction of the inflammation in dextran sulfate sodium-induced chronic colitis in these animals [43]. Braat *et al.* [44] have recently published the first report of a human clinical phase I trial with a genetically engineered therapeutic bacterium that secretes mature human IL-10. Ten CD patients were included in this phase I trial and received capsules, twice daily during 7 days, with the transgenic bacteria expressing IL-10. The lack of a control group, as is intrinsic to a phase I study, does not allow conclusions regarding the clinical efficacy of this specific bacterium, however, the lack of undesired side-effects shows that the use of genetically modified bacteria for mucosal delivery of proteins is a feasible strategy in human beings. This novel strategy avoids systemic side effects and can be biologically contained as described in this study; therefore, genetically modified LAB could be suitable for use as treatments for chronic intestinal diseases by delivering beneficial compounds (such as IL-10) to specific sites in the gastrointestinal tract where they are required.

PROBIOTICS AND INFLAMMATION BEYOND THE INTESTINAL TRACT

Probiotic microorganisms can also be efficient in the treatment of other inflammatory diseases that occur at other sites besides the gastrointestinal tract, such as in the case of allergic inflammation [45]. It has been reported that infants who were allergic to cow's milk and treated with hydrolyzed milk formula supplemented with *L. rhamnosus* GG, had a better clinical score and less TNF-α in their faeces than infants treated with the hydrolysed formula alone [46], showing a positive effect of this bacteria on food allergy induced inflammatory processes.

Perdigon *et al.* [5] have shown that fermented products and probiotics can activate immune cells at sites distant to the intestinal mucosa, such as bronchus and breast tissues. A fermented milk containing *L. helveticus* R389 was able to regulate the immune response in mammary glands in presence of a local inflammatory pathology (breast tumor) in mice [47]. The administration of milk fermented by this LAB strain delayed or stopped breast tumor development due to a modulation in the immune response which was manifested by an increase of the anti-inflammatory cytokine IL-10 [48]. A recent review by DeNardo and Coussens [49] showed that breast tumor promotion and rejection can be mediated by cytokines involved in chronic and acute inflammatory processes such as IL-10 which can be modulated by LAB.

REACTIVE OXYGEN SPECIES

Since oxidative stress and epithelial damages appear linked in pathologies of the gastrointestinal tract of humans such as IBD, another mechanism by which LAB could prevent inflammation is through the use of antioxidant enzymes that can degrade reactive oxygen species (ROS) or impair their formation.

ROS are small molecules (such as superoxide ions, free radicals and peroxides) that are formed as byproducts of the normal metabolism of oxygen. The biological sources of ROS are numerous: they can be generated in aerobiosis by flavoproteins [50] and by phagocytes during inflammatory reactions [51]. ROS, in low quantities, participate in cell signaling and regulatory pathways. When they are produced in large amounts, as is the case during inflammatory processes, they act to eliminate infectious agents by causing significant damages to cell structures and macromolecular constituents such as DNA, RNA, proteins and lipids [52]. Toxic-

ity occurs when the concentration of ROS exceeds the capacity of cell defence systems [53]. Large amounts of H_2O_2 are produced and excreted by human tumor cells [54] and might participate in tumor invasion and proliferation. In IBD patients, oxidative stress occurs as a result of recurrent and abnormal inflammation. A correlation between the increase in ROS production and disease activity in inflamed biopsies of IBD patients has been established in various studies [55-58]. Thus, oxidative stress plays an important role in pathologies of the gastrointestinal tract of humans such as IBD and certain types of cancers [59, 60].

The normal intestinal mucosa is equipped with a network of antioxidant enzymes that neutralize ROS in a two-step pathway. First, superoxide dismutases (SODs) convert the primary superoxide anion (O_2^-) into the more stable metabolite, hydrogen peroxide (H_2O_2). Second, H_2O_2 is converted to water by catalase (CAT) or glutathione peroxidase (GPO). The activities of these enzymes are usually balanced to maintain a low and continued steady-state level of ROS. However, the levels of these enzymes in inflammatory disease patients, such as those suffering from IBD, are frequently depleted [56, 59], highlighting the potential for increasing the local levels of these enzymes to function as a therapeutic. Probiotic LAB strains expressing high levels of SOD and CAT could increase these enzyme activities in specific locations of the gastrointestinal tract and could thus contribute to prevent oxidative epithelial damages, giving rise to potential applications for the treatment of inflammatory diseases or post-cancer drug treatments.

GENETICALLY ENGINEERED LAB-ANTIOXIDANT ENZYME AND IL-10 PRODUCTION

Catalases are widespread in aerobic (facultative or not) bacteria such as *Escherichia coli* and *Bacillus* (*B.*) *subtilis* [61]. Two classes of catalases are distinguished, according to their active-site composition: one is heme-dependent and the other, also named pseudocatalase, is manganese-dependent. Since the majority of LAB are not equipped with enzymes to detoxify oxygen-derived compounds, the insertion of genes coding for antioxidant enzymes (such as catalases or SOD) in probiotic bacteria could improve their anti-inflammatory properties beyond the modulation of the local immune-dependant inflammation response. Catalases of three lactobacilli have been successfully cloned and expressed in heterologous bacteria lacking catalase activity [62-65].

The food-grade *Lc. lactis* is a potential vector to be used as a live vehicle to deliver heterologous proteins for vaccine and pharmaceutical purposes. Since *Lc. lactis* has no catalase, Rochat *et al*. [61] introduced the *B. subtilis* heme catalase *KatE* gene into this industrially important microorganism giving rise to a strain capable of producing active catalase that can provide efficient antioxidant activity. A recent report has shown that this genetically engineered strain was able to prevent tumor appearance in an experimental DMH-induced colon cancer model [66]. The catalase producing *Lc. lactis* strain used in this study was able to slightly increase catalase activities in the intestines of mice treated with dimethylhydrazine (DMH), a colon cancer inducing drug. This increased antioxidant activity was sufficient to reduce H_2O_2 levels in the large intestines, a ROS involved in cancer promotion and progression, showing that this catalase-producing LAB could be used in novel therapeutic strategies for gastrointestinal pathologies.

Recently, the heterologous expression of a non-heme catalase in bacteria relevant to dairy industries has been reported [65]. A strain of *L. casei* was constructed to offer the advantage that no heme has to be added to the culture medium for catalase activity. Although this strain was able to reduce cecal and colonic inflammatory scores, no significant differences were observed compared to the use of the native non-catalase producing strain in a dextran sulfate sodium (DSS)-induced colitis mice model [67]. This is probably due to the insufficient production of catalase by this strain in the gastrointestinal tract. These authors suggest that in order to optimize their antioxidative strategy, evaluation of the effects of co-administration of *L. casei* strains producing high levels of catalase and SOD from *Lc. lactis* [68] will be relevant as some previous studies showed the positive impact of increased SOD activity in intestinal inflammation models [59, 69, 70].

In another study, using a different model of IBD, we have shown that a catalase producing strain of *L. casei* BL23 significantly decreased the physiological damages caused by the TNBS administration [71]. This result was related to the antioxidant capacity of the bacterial strain more than an immunomodulatory effect.

LAB have been used to locally deliver SOD directly to the intestines, an important breakthrough since oral administration of SOD is greatly limited by its short lifespan (5–10min) in the hostile conditions of the gastrointestinal tract.

To determine whether a bacterial supply of SOD into the colon could improve an experimentally induced colitis, Han *et al*. [72] compared the effects of oral treatment with live recombinant *Lc. lactis* or *L. plantarum* producing different amounts of SOD with those of colonic infusion of commercial SOD. Macroscopic damages were reduced by the SOD producing strains in rats administered with trinitrobenzene sulfonate (TNBS) to induce colitis. Although not all of the anti-inflammatory effects could be attributed directly to SOD, the results of this study suggest that SOD-producing lactic acid bacteria could be used as a novel treatment of IBD.

Carroll *et al*. [73] have recently published a report where they investigated the ability of SOD from *Streptococcus thermophilus* to reduce colitis symptoms in IL-10 deficient mice using *L. gasseri* as a delivery vehicle. The *L. gasseri* producing SOD had significant anti-inflammatory activity which was associated with a reduction in the infiltration of neutrophils and macrophages that significantly reduced the severity of colitis in the IL-10-deficient mice.

Recent data has shown that SOD producing *L. casei* BL23 was able to significantly attenuate the TNBS-induced damages as shown by higher survival rates, decreased animal weight loss, lower bacterial translocation to the liver and the prevention of damage to the large intestines [71]. This is in agreement with previous results that have shown that the same SOD-expressing strain of *L. casei* was able to slightly attenuate the colonic histological damage score of a DSS-induced colitis model [74].

These results pave the way for the creation of novel genetically modified strains that could produce SOD and catalase concomitantly, giving rise to novel super-antioxidant strains that could be used for the treatment and/or prevention of inflammatory intestinal diseases caused by oxidative stress.

A novel regulated expression system with the ability of targeting heterologous proteins to the cytoplasm or to the extracellular medium has been described for *Lc. lactis* NCDO2118 strain. XIES is a food grade expression system that is tightly regulated by a sugar (xylose) that is rarely found in conventional foods and acts as inductor. When xylose is present in the media, IL-10 is expressed in high levels. On the other hand, the presence of glucose inhibits IL-10 expression. In this way, IL-10 expression can be up or down-regulated [75].

This expression system, using Lc. lactis that produce IL-10, showed that induction with xylose increased the cytokine levels (>500 pg/ml for the Cyt strain and >1000 pg/ml for the Sec strain). It has been demonstrated that *Lc. lactis* producing IL-10 in the cytoplasm showed a higher inmunomodulatory potential in a murine lung inflammation model; hypothesizing that the recombinant IL-10 produced in the cytoplasmic form and stored within the bacteria is probably kept under optimum conditions for a longer period of time and is slowly released in the tissue together with the bacterial host lysis [76].

This same *Lc. lactis* strain using the XIES expression system were employment to ferment milks as a new form of administration of IL-10 producing *Lc. lactis* and was effective in the prevention of IBD in a murine model. Mice that received milks fermented by *Lc. lactis* strains producing IL-10 in the cytoplasm (Cyt strain) or secreted to the product (Sec strain) showed lower macroscopic and microscopic damage scores in their large intestines, decreased IFN-γ levels in their intestinal fluids and lower microbial translocation to liver, compared to mice receiving milk fermented by the wild-type strain or those not receiving any treatment [77]. Furthermore, in healthy mice, without inflammatory (TNBS) stimulus, the number of cytokine positive immune cells and cytokine release in the large intestine tissue from the groups that received fermented milks compared to the control animals did not differ significantly, showing that changes in the cytokine profile observed in the TNBS model were induced by the inflammatory agent and not by the fermented product itself.

Some proven anti-inflammatory strains, both native and genetically modified and divided by their mechanism of action are described in Table **2**. The beneficial properties of these strains could be combined together with others to produce novel strains exerting a variety of beneficial effects. For example, the introduction of antioxidant enzyme genes (SOD and CAT) in current probiotic strains that have natural anti-inflammatory properties, such as the ability to modulate the immune-dependent anti-inflammatory processes or the mixture of probiotics with other genetically engineered LAB strains that produce anti-inflammatory cytokines could generate very potent products that could be used in the treatment of a variety of inflammatory diseases. These novel products with therapeutical purposes could be suitable for specific populations suffering from gastrointestinal or other inflammatory disorders, or prone to acquiring them.

Table 2. Examples of Bacterial Strains, Native and Genetically Modified (GM), or Probiotic Products with Proven Anti-inflammatory Properties Classified by their Mechanisms of Action: Immune Dependant Anti-inflammatory Properties (Immune) or Antioxidant Enzyme Producers such as Catalase (CAT) or Superoxide Dismutase (SOD)

Strain *	Type	Mechanism	Proven Effects	Ref.
B. longum	Native	Immune	Improvement of clinical appearance of chronic inflammation in patients. Decreases in TNF-α and IL-1α	[29]
BIFICO (3 bifidobacteria species)	Native	Immune	Prevention of flare-ups of chronic ulcerative colitis, inactivation of nuclear factor-kB (NF-kB), decreased the expressions of TNF-α and IL-1β and elevated the expression of IL-10.	[21]
L. salivarius ssp. *salivarius* CECT5713	Native	Immune	Recovery of inflamed tissue in TNBS model of rat colitis, increase in TNF-α and iNOS (inducible NO synthase) expression.	[78]
L. fermentum, L. reuteri	Native	Immune	Improvement of histology in a TNBS model of rat colitis, decreased levels of TNF-α and iNOS expression.	[79]
L. casei Shirota	Native	Immune	Improvement in murine chronic inflammatory bowel disease, down-regulation of pro-inflammatory cytokines such as IL-6 and IFN-γ.	[80]
L. casei DN-114 001	Native	Immune	Reduction in numbers of activated T lymphocytes in the lamina propria of Crohn's disease mucosa, decrease of IL-6 and TNF-α.	[39]
L. rhamnosus GG	Native	Immune	Alleviating intestinal inflammation, decrease TNF-α.	[46]
VSL#3	Native	Unknown	Delayed the relapse into pouchitis after surgical resection in human patients.	[27]

(Table 2) contd….

Strain *	Type	Mechanism	Proven Effects	Ref.
L. rhamnosus GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS and *B. breve* Bb99.	Native	Unknown	Alleviating irritable bowel syndrome symptoms.	[22]
*B.*and *L.plantarum*	Native	Unknown	Improvement of the disease activity index in an induced rat colitis model.	[81]
L. rhamnosus GG	Native	Unknown	Improvement in the clinical status in children with mildly to moderately active stable Crohn's disease.	[18]
L. casei Shirota	Native	Unknown	Improvement in the clinical condition of murine model of ulcerative colitis.	[82]
E. coli Nissle 1917	Native	Immune	Ameliorated colitis and decreased pro-inflammatory cytokine secretion via TLR-2- and TLR-4-dependent pathways in DSS-induced model of IBD.	[83]
L. rhamnosus Lr32 or *L. salivarius* Ls33	Native	Immune	Stimulation of DC regulatory functions by targeting specific pattern-recognition receptors and pathways.	[84]
L. plantarum HY115 and *L. brevis* HY7401	Native	Immune	Inhibition of NF-kB pathway, with decrease of pro-inflammatory cytokines. Reduction of degradation activities of chondroitin sulfate and hyaluronic acid of intestinal bacteria, induced by DSS.	[85]
Lc. lactis subsp. cremoris FC	Native	Unknown	inhibition of inflammatory cell infiltration	[86]
B. longum HY8004 and L. plantarum AK8-4	Native	Immune	Inhibition of NF-kB pathway and TLR4 expression, with decrease of pro-inflammatory cytokines. Reduction of intestinal bacterial glycosaminoglycan (GAG) degradation.	[87]
VSL#3-derived L. casei	Native	Immune	Induction of posttranslational degradation of T-cell chemokine interferon-inducible protein (IP)-10 in intestinal epithelial cells contributes to the anti-inflammatory effects of VSL#3.	[88]
B. bifidum 17	Native	Immune	Inhibition of Th1-driven intestinal inflammation with reduction of pro-inflammatory cytokines (IL-1β, IL-6), keratinocyte derived chemokine (KC), cyclooxygenase 2 (Cox-2) and myeloperoxidase (MPO) using a TNBS– induced model.	[89]
L. plantarum Lp91	Native	Immune	Down regulation of TNF-a and COX2 and up-regulation of IL-10 gene expression.	[90]
Probiotic yoghurt	Native	Immune	Diminution of the acute episode and prevention of recurrence in a TNBS induced mouse model. Decrease of pro-inflammatory cytokines (IFNγ, IL-12 and IL-17) and TLR4 expression. Increase of IL-10 and TLR-9. Changes in the intestinal microbiota.	[91, 92]
L. plantarum NCIMB8826 *ΔDlt*	GM	Immune	Reduction of secretion of proinflammatory cytokines by peripheral blood mononuclear cells and monocytes and increase in IL-10 production in a murine colitis model.	[93]
Lc. lactis IL-10	GM	Immune	Reduction in colitis in mice treated with DSS.	[43]
L. casei BL23 MnKat	GM	CAT	Reduction of cecal and colonic inflammatory scores.	[67]
Lc. lactis + KatE	GM	CAT	Slight increase in catalase activities in the intestines and prevention of colon cancer of mice administered the cancer inducing drug DMH.	[66]
Lc.lactis NZ9800 and *L.plantarum* NCIMB8826 + pNZ804 sodA	GM	SOD	Reduction in macroscopic damages in rats administered with TNBS to induce colitis	[72]

(Table 2) contd….

Strain *	Type	Mechanism	Proven Effects	Ref.
L. gasseri NC1501	GM	SOD	Reduction in inflammation in IL-10-deficient mice.	[73]
L. casei BL23 MnSOD	GM	SOD	Reduction of oxidative stress and intestinal inflammation scores in the DSS model, using Lb. casei MnSOD alone or in combination with Lb. casei MnKat.	[74]
L. casei BL23 MnKat and MnSOD	GM	CAT or SOD	Faster recovery of initial weight loss, increased enzymatic activities in the gut, reduction of inflammatory scores and microbial traslocation to liver using a TNBS-induced model in mice.	[71]
Lc. lactis IL-10	GM	Immune	Modulation of acute allergic airway inflammation in mice.	[76]
Lc. lactis IL-10	GM	Immune	Reduction of damage scores in the large intestines, IFN-γ levels in their intestinal fluids and microbial translocation to liver using a TNBS-induced model in mice.	[77]

**microbial abbreviations: B. (Bifidobacterium). L. (Lactobacillus), St. (Streptococcus), P. (Propionibacterium), Lc. (Lactococcus).*

These preliminary studies would also need to be performed in larger mammals before implying to safe use in the design of phase I human clinical trials and the removal of antibiotic resistance markers in the producing strains is also necessary before their employment in the design of novel therapeutical products that could be used in human studies.

The consumption of engineered strains by humans is still highly controversial due to public perception that genetic manipulation is not "natural". Scientist must perform well-designed studies where the results are divulged to the general populations in order to inform consumers of the obvious beneficial effects these novel techniques can confer with the minimum of risk to their health and to the environment. Throughout the course of history most novel treatments have been met with resistance from potential benefactors, it is thus important to show that the potential benefits are highly superior to the risks for novel treatments to be completely accepted by the population as a whole.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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DISCLOSURE

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REFERENCES

[1] Fuller, R.History and development of probiotics. In *Probiotics - The Scientific Basis*, R. Fuller, ed.; Chapman and Hall: New York, **1992**, pp. 1-8.

[2] Heyman, M.; Menard, S. Probiotic microorganisms: how they affect intestinal pathophysiology. *Cell Mol. Life Sci.* **2002**, *59*, 1151-1165.

[3] Guarner, F.; Malagelada, J. R. Role of bacteria in experimental colitis. *Best Pract Res Clin Gastroenterol.* **2003**, *17*, 793-804.

[4] Isolauri, E.; Salminen, S.; Ouwehand, A. C. Microbial-gut interactions in health and disease. Probiotics. *Best Pract Res Clin Gastroenterol.* **2004**, *18*, 299-313.

[5] Perdigon, G.; Vintini, E.; Alvarez, S.; Medina, M.; Medici, M. Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. *J. Dairy Sci.*, **1999**, *82*, 1108-1114.

[6] de Moreno de LeBlanc, A.; Perdigon, G. Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. *Med Sci. Monit.*, **2004**, *10*, BR96-104.

[7] Pessi, T.; Sutas, Y.; Hurme, M.; Isolauri, E. Interleukin-10 generation in atopic children following oral Lactobacillus rhamnosus GG. *Clin. Exp. Allergy,* **2000**, *30*, 1804-1808.

[8] Rautava, S.; Kalliomaki, M.; Isolauri, E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J. Allergy Clin. Immunol.*, **2002**, *109*, 119-121.

[9] Perdigon, G.; Medina, M.; Vintini, E.; Valdez, J. C. Intestinal pathway of internalisation of lactic acid bacteria and gut mucosal immunostimulation. *Int. J. Immunopathol. Pharmacol.*, **2000**, *13*, 141-150.

[10] Pena, J. A.; Versalovic, J. Lactobacillus rhamnosus GG decreases TNF-alpha production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. *Cell Microbiol.*, **2003**, *5*, 277-285.

[11] Maassen, C. B.; van Holten-Neelen, C.; Balk, F.; den Bak-Glashouwer, M. J.; Leer, R. J.; Laman, J. D.; Boersma, W. J.; Claassen, E. Strain-dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains. *Vaccine,* **2000**, *18*, 2613-2623.

[12] Mileti, E.; Matteoli, G.; Iliev, I. D.; Rescigno, M. Comparison of the immunomodulatory properties of three probiotic strains of Lactobacilli using complex culture systems: prediction for *in vivo* efficacy. *PLoS One,* **2009**, *4*, e7056.

[13] Zakostelska, Z.; Kverka, M.; Klimesova, K.; Rossmann, P.; Mrazek, J.; Kopecny, J.; Hornova, M.; Srutkova, D.; Hudcovic, T.; Ridl, J.; Tlaskalova-Hogenova, H. Lysate of Probiotic Lactobacillus casei DN-114 001 Ameliorates Colitis by Strengthening the Gut Barrier Function and Changing the Gut Microenvironment. *PLoS One*, *6*, e27961.

[14] Macho Fernandez, E.; Valenti, V.; Rockel, C.; Hermann, C.; Pot, B.; Boneca, I. G.; Grangette, C. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut*, *60*, 1050-1059.

[15] Matsuguchi, T.; Takagi, A.; Matsuzaki, T.; Nagaoka, M.; Ishikawa, K.; Yokokura, T.; Yoshikai, Y. Lipoteichoic acids from Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. *Clin Diagn Lab Immunol*. **2003**, *10*, 259-266.

[16] Claes, I. J.; Lebeer, S.; Shen, C.; Verhoeven, T. L.; Dilissen, E.; De Hertogh, G.; Bullens, D. M.; Ceuppens, J. L.; Van Assche, G.; Vermeire, S.; Rutgeerts, P.; Vanderleyden, J.; De Keersmaecker, S. C. Impact of lipoteichoic acid modification on the performance of the probiotic Lactobacillus rhamnosus GG in experimental colitis. *Clin. Exp. Immunol.*, **2010**, *162*, 306-314.

[17] Guslandi, M.; Mezzi, G.; Sorghi, M.; Testoni, P. A. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig. Dis. Sci*., **2000**, *45*, 1462-1464.

[18] Gupta, P.; Andrew, H.; Kirschner, B. S.; Guandalini, S. Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. *J. Pediatr. Gastroenterol. Nutr*., **2000**, *31*, 453-457.

[19] Malin, M.; Suomalainen, H.; Saxelin, M.; Isolauri, E. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG. *Ann. Nutr. Metab*., **1996**, *40*, 137-145.

[20] O'Mahony, L.; McCarthy, J.; Kelly, P.; Hurley, G.; Luo, F.; Chen, K.; O'Sullivan, G. C.; Kiely, B.; Collins, J. K.; Shanahan, F.; Quigley, E. M. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology,* **2005**, *128*, 541-551.

[21] Cui, H. H.; Chen, C. L.; Wang, J. D.; Yang, Y. J.; Cun, Y.; Wu, J. B.; Liu, Y. H.; Dan, H. L.; Jian, Y. T.; Chen, X. Q. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J. Gastroenterol*., **2004**, *10*, 1521-1525.

[22] Kajander, K.; Korpela, R. Clinical studies on alleviating the symptoms of irritable bowel syndrome. *Asia Pac. J. Clin. Nutr*., **2006**, *15*, 576-580.

[23] Bittner, A. C.; Croffut, R. M.; Stranahan, M. C.; Yokelson, T. N. Prescript-assist probiotic-prebiotic treatment for irritable bowel syndrome: an open-label, partially controlled, 1-year extension of a previously published controlled clinical trial. *Clin. Ther*., **2007**, *29*, 1153-1160.

[24] Bittner, A. C.; Croffut, R. M.; Stranahan, M. C. Prescript-Assist probiotic-prebiotic treatment for irritable bowel syndrome: a methodologically oriented, 2-week, randomized, placebo-controlled, double-blind clinical study. *Clin. Ther*., **2005**, *27*, 755-761.

[25] Choi, C. H.; Jo, S. Y.; Park, H. J.; Chang, S. K.; Byeon, J. S.; Myung, S. J. A randomized, double-blind, placebo-controlled multicenter trial of saccharomyces boulardii in irritable bowel syndrome: effect on quality of life. *J. Clin. Gastroenterol*., **2011**, *45*, 679-683.

[26] Mimura, T.; Rizzello, F.; Helwig, U.; Poggioli, G.; Schreiber, S.; Talbot, I. C.; Nicholls, R. J.; Gionchetti, P.; Campieri, M.; Kamm, M. A. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut,* **2004**, *53*, 108-114.

[27] Gionchetti, P.; Rizzello, F.; Helwig, U.; Venturi, A.; Lammers, K. M.; Brigidi, P.; Vitali, B.; Poggioli, G.; Miglioli, M.; Campieri, M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology,* **2003**, *124*, 1202-1209.

[28] Pronio, A.; Montesani, C.; Butteroni, C.; Vecchione, S.; Mumolo, G.; Vestri, A.; Vitolo, D.; Boirivant, M. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. *Inflamm. Bowel. Dis*., **2008**, *14*, 662-668.

[29] Furrie, E.; Macfarlane, S.; Kennedy, A.; Cummings, J. H.; Walsh, S. V.; O'Neil D, A.; Macfarlane, G. T. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut,* **2005**, *54*, 242-249.

[30] Kruis, W.; Fric, P.; Pokrotnieks, J.; Lukas, M.; Fixa, B.; Kascak, M.; Kamm, M. A.; Weismueller, J.; Beglinger, C.; Stolte, M.; Wolff, C.; Schulze, J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut,* **2004**, *53*, 1617-1623.

[31] Tursi, A.; Brandimarte, G.; Giorgetti, G. M.; Forti, G.; Modeo, M. E.; Gigliobianco, A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med. Sci. Monit*., **2004**, *10*, PI126-131.

[32] Venturi, A.; Gionchetti, P.; Rizzello, F.; Johansson, R.; Zucconi, E.; Brigidi, P.; Matteuzzi, D.; Campieri, M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther*., **1999**, *13*, 1103-1108.

[33] Karimi, O.; Pena, A. S.; van Bodegraven, A. A. Probiotics (VSL#3) in arthralgia in patients with ulcerative colitis and Crohn's disease: a pilot study. *Drugs Today (Barc),* **2005**, *41*, 453-459.

[34] Miele, E.; Pascarella, F.; Giannetti, E.; Quaglietta, L.; Baldassano, R. N.; Staiano, A. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am. J. Gastroenterol*., **2009**, *104*, 437-443.

[35] Hedin, C. R.; Mullard, M.; Sharratt, E.; Jansen, C.; Sanderson, J. D.; Shirlaw, P.; Howe, L. C.; Djemal, S.; Stagg, A. J.; Lindsay, J. O.; Whelan, K. Probiotic and prebiotic use in patients with inflammatory bowel disease: a case-control study. *Inflamm. Bowel. Dis.*, **2010**, *16*, 2099-2108.

[36] Rachmilewitz, D.; Katakura, K.; Karmeli, F.; Hayashi, T.; Reinus, C.; Rudensky, B.; Akira, S.; Takeda, K.; Lee, J.; Takabayashi, K.; Raz, E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology,* **2004**, *126*, 520-528.

[37] Ulisse, S.; Gionchetti, P.; D'Alo, S.; Russo, F. P.; Pesce, I.; Ricci, G.; Rizzello, F.; Helwig, U.; Cifone, M. G.; Campieri, M.; De Simone, C. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am. J. Gastroenterol,* **2001**, *96*, 2691-2699.

[38] de Moreno de LeBlanc, A.; del Carmen, S.; Zurita-Turk, M.; Santos Rochat, C.; van de Guchte, M.; Azevedo, V.; Miyoshi, A.; LeBlanc, J.G. Importance of IL-10 modulation by probiotic microorganisms in gastrointestinal inflammatory diseases. *ISRN Gastroenterology,* **2011**, doi:10.5402/2011/892971.

[39] Carol, M.; Borruel, N.; Antolin, M.; Llopis, M.; Casellas, F.; Guarner, F.; Malagelada, J. R. Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. *J. Leukoc Biol*., **2006**, *79*, 917-922.

[40] Kuhn, R.; Lohler, J.; Rennick, D.; Rajewsky, K.; Muller, W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell,* **1993**, *75*, 263-274.

[41] Tilg, H.; van Montfrans, C.; van den Ende, A.; Kaser, A.; van Deventer, S. J.; Schreiber, S.; Gregor, M.; Ludwiczek, O.; Rutgeerts, P.; Gasche, C.; Koningsberger, J. C.; Abreu, L.; Kuhn, I.; Cohard, M.; LeBeaut, A.; Grint, P.; Weiss, G. Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma. *Gut,* **2002**, *50*, 191-195.

[42] Agresti, P. M.; Gala-García, A.; LeBlanc, J. G.; de Moreno de LeBlanc, A.; Azevedo, V.; Miyoshi, A. Uso potencial de bacterias lácticas como vehículos vacúnales, *Vacunas,* **2012**, *13*, 15-20.

[43] Steidler, L.; Hans, W.; Schotte, L.; Neirynck, S.; Obermeier, F.; Falk, W.; Fiers, W.; Remaut, E. Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. *Science,* **2000**, *289*, 1352-1355.

[44] Braat, H.; Rottiers, P.; Hommes, D. W.; Huyghebaert, N.; Remaut, E.; Remon, J. P.; van Deventer, S. J.; Neirynck, S.; Peppelenbosch, M. P.; Steidler, L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin. Gastroenterol Hepatol*., **2006**, *4*, 754-759.

[45] Menard, S.; Candalh, C.; Bambou, J. C.; Terpend, K.; Cerf-Bensussan, N.; Heyman, M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut,* **2004**, *53*, 821-828.

[46] Majamaa, H.; Isolauri, E. Probiotics: a novel approach in the management of food allergy. *J. Allergy Clin. Immunol*., **1997**, *99*, 179-185.

[47] de Moreno de LeBlanc, A.; Matar, C.; Theriault, C.; Perdigon, G. Effects of milk fermented by Lactobacillus helveticus R389 on immune cells associated to mammary glands in normal and a breast cancer model. *Immunobiology,* **2005**, *210*, 349-358.

[48] de Moreno de LeBlanc, A.; Matar, C.; LeBlanc, N.; Perdigon, G. Effects of milk fermented by Lactobacillus helveticus R389 on a murine breast cancer model. *Breast Cancer Res*., **2005**, *7*, R477-486.

[49] Denardo, D. G.; Coussens, L. M. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Res*., **2007**, *9*, 212.

[50] Condon, S. Responses of lactic acid bacteria to oxygen. *FEMS Microbiol. Lett.,* **1987**, *46*, 269-280.

[51] Ross, S. The involvement of oxygen radicals in microbicidal mechanism of leukocytes and macrophages. *Klin Wochenschr*. **1991**, *69*, 975-980.

[52] Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem*., **1997**, *272*, 20313-20316.

[53] Farr, S. B.; Kogoma, T. Oxidative stress responses in Escherichia coli and Salmonella typhimurium. *Microbiol. Rev*., **1991**, *55*, 561-585.

[54] Szatrowski, T. P.; Nathan, C. F. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res*., **1991**, *51*, 794-798.

[55] Keshavarzian, A.; Banan, A.; Farhadi, A.; Komanduri, S.; Mutlu, E.; Zhang, Y.; Fields, J. Z. Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut*, **2003**, *52*, 720-728.

[56] Lih-Brody, L.; Powell, S. R.; Collier, K. P.; Reddy, G. M.; Cerchia, R.; Kahn, E.; Weissman, G. S.; Katz, S.; Floyd, R. A.; McKinley, M. J.; Fisher, S. E.; Mullin, G. E. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig. Dis. Sci*., **1996**, *41*, 2078-2086.

[57] Sedghi, S.; Fields, J. Z.; Klamut, M.; Urban, G.; Durkin, M.; Winship, D.; Fretland, D.; Olyaee, M.; Keshavarzian, A. Increased production of luminol enhanced chemiluminescence by the inflamed colonic mucosa in patients with ulcerative colitis. *Gut,* **1993**, *34*, 1191-1197.

[58] Simmonds, N. J.; Allen, R. E.; Stevens, T. R.; Van Someren, R. N.; Blake, D. R.; Rampton, D. S. Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. *Gastroenterology,* **1992**, *103*, 186-196.

[59] Kruidenier, L.; van Meeteren, M. E.; Kuiper, I.; Jaarsma, D.; Lamers, C. B.; Zijlstra, F. J.; Verspaget, H. W. Attenuated mild colonic inflammation and improved survival from severe DSS-colitis of transgenic Cu/Zn-SOD mice. *Free Radic. Biol. Med*., **2003**, *34*, 753-765.

[60] Kruidenier, L.; Verspaget, H. W. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease--radicals or ridiculous? *Aliment Pharmacol. Ther*., **2002**, *16*, 1997-2015.

[61] Rochat, T.; Miyoshi, A.; Gratadoux, J. J.; Duwat, P.; Sourice, S.; Azevedo, V.; Langella, P. High-level resistance to oxidative stress in Lactococcus lactis conferred by Bacillus subtilis catalase KatE. *Microbiology,* **2005**, *151*, 3011-3018.

[62] Abriouel, H.; Herrmann, A.; Starke, J.; Yousif, N. M.; Wijaya, A.; Tauscher, B.; Holzapfel, W.; Franz, C. M. Cloning and heterologous expression of hematin-dependent catalase produced by Lactobacillus plantarum CNRZ 1228. *Appl. Environ Microbiol.,* **2004**, *70*, 603-606.

[63] Knauf, H. J.; Vogel, R. F.; Hammes, W. P. Cloning, sequence, and phenotypic expression of katA, which encodes the catalase of Lactobacillus sake LTH677. *Appl. Environ Microbiol*., **1992**, *58*, 832-839.

[64] Noonpakdee, W.; Sitthimonchai, S.; Panyim, S.; Lertsiri, S. Expression of the catalase gene katA in starter culture Lactobacillus plantarum TISTR850 tolerates oxidative stress and reduces lipid oxidation in fermented meat product. *Int. J. Food Microbiol*., **2004**, *95*, 127-135.

[65] Rochat, T.; Gratadoux, J. J.; Gruss, A.; Corthier, G.; Maguin, E.; Langella, P.; van de Guchte, M. Production of a heterologous nonheme catalase by Lactobacillus casei: an efficient tool for removal of H2O2 and protection of Lactobacillus bulgaricus from oxidative stress in milk. *Appl. Environ Microbiol*., **2006**, *72*, 5143-5149.

[66] de Moreno de LeBlanc, A.; LeBlanc, J. G.; Perdigón, G.; Miyoshi, A.; Langella, P.; Azevedo, V.; Sesma, F. Oral administration of a catalase-producing Lactococcus lactis can prevent a chemically induced colon cancer in mice. *J. Med. Microbiol*., **2008**, *58*.

[67] Rochat, T.; Bermudez-Humaran, L.; Gratadoux, J. J.; Fourage, C.; Hoebler, C.; Corthier, G.; Langella, P. Anti-inflammatory effects of Lactobacillus casei BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice. *Microb. Cell Fact*., **2007**, *6*, 1-10.

[68] Sanders, J. W.; Leenhouts, K. J.; Haandrikman, A. J.; Venema, G.; Kok, J. Stress response in Lactococcus lactis: cloning, expression analysis, and mutation of the lactococcal superoxide dismutase gene. *J. Bacteriol*., **1995**, *177*, 5254-5260.

[69] Ogawa, Y.; Kanatsu, K.; Iino, T.; Kato, S.; Jeong, Y. I.; Shibata, N.; Takada, K.; Takeuchi, K. Protection against dextran sulfate sodium-induced colitis by microspheres of ellagic acid in rats. *Life Sci*., **2002**, *71*, 827-839.

[70] Segui, J.; Gironella, M.; Sans, M.; Granell, S.; Gil, F.; Gimeno, M.; Coronel, P.; Pique, J. M.; Panes, J. Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine. *J. Leukoc Biol*., **2004**, *76*, 537-544.

[71] LeBlanc, J. G.; del Carmen, S.; Miyoshi, A.; Azevedo, V.; Sesma, F.; Langella, P.; Bermudez-Humaran, L. G.; Watterlot, L.; Perdigon, G.; de Moreno de LeBlanc, A. Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice. *J. Biotechnol*., **2011**, *151*, 287-293.

[72] Han, W.; Mercenier, A.; Ait-Belgnaoui, A.; Pavan, S.; Lamine, F.; van, S., II; Kleerebezem, M.; Salvador-Cartier, C.; Hisbergues, M.; Bueno, L.; Theodorou, V.; Fioramonti, J. Improvement of an experimental colitis in rats by lactic acid bacteria producing superoxide dismutase. *Inflamm Bowel Dis*., **2006**, *12*, 1044-1052.

[73] Carroll, I. M.; Andrus, J. M.; Bruno-Barcena, J. M.; Klaenhammer, T. R.; Hassan, H. M.; Threadgill, D. S. Anti-inflammatory properties of Lactobacillus gasseri expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. *Am J Physiol Gastrointest Liver Physiol*., **2007**, *293*, G729-738.

[74] Watterlot, L.; Rochat, T.; Sokol, H.; Cherbuy, C.; Bouloufa, I.; Lefevre, F.; Gratadoux, J. J.; Honvo-Hueto, E.; Chilmonczyk, S.; Blugeon, S.; Corthier, G.; Langella, P.; Bermudez-Humaran, L. G. Intragastric administration of a superoxide dismutase-producing recombinant Lactobacillus casei BL23 strain attenuates DSS colitis in mice. *Int. J. Food Microbiol*., **2010**, *144*, 35-41.

[75] Miyoshi, A.; Jamet, E.; Commissaire, J.; Renault, P.; Langella, P.; Azevedo, V. A xylose-inducible expression system for Lactococcus lactis. *FEMS Microbiol Lett*., **2004**, *239*, 205-212.

[76] Marinho, F. A.; Pacifico, L. G.; Miyoshi, A.; Azevedo, V.; Le Loir, Y.; Guimaraes, V. D.; Langella, P.; Cassali, G. D.; Fonseca, C. T.; Oliveira, S. C. An intranasal administration of Lactococcus lactis strains expressing recombinant interleukin-10 modulates acute allergic airway inflammation in a murine model. *Clin. Exp. Allergy,* **2010**, *40*, 1541-1551.

[77] del Carmen, S.; de Moreno de LeBlanc, A.; Perdigon, G.; Bastos Pereira, V.; Miyoshi, A.; Azevedo, V.; LeBlanc, J. G. Evaluation of the anti-inflammatory effect of milk fermented by a strain of IL-10 producing Lactococcus lactis using a murine model of Crohn´s disease. *J. Mol. Microbiol. Biotechnol.*, **2012**, *21*, 138-146.

[78] Peran, L.; Camuesco, D.; Comalada, M.; Nieto, A.; Concha, A.; Diaz-Ropero, M. P.; Olivares, M.; Xaus, J.; Zarzuelo, A.; Galvez, J. Preventative effects of a probiotic, Lactobacillus salivarius ssp. salivarius, in the TNBS model of rat colitis. *World J Gastroenterol*. **2005**, *11*, 5185-5192.

[79] Peran, L.; Sierra, S.; Comalada, M.; Lara-Villoslada, F.; Bailon, E.; Nieto, A.; Concha, A.; Olivares, M.; Zarzuelo, A.; Xaus, J.; Galvez, J. A comparative study of the preventative effects exerted by two probiotics, Lactobacillus reuteri and Lactobacillus fermentum, in the trinitrobenzenesulfonic acid model of rat colitis. *Br. J. Nutr*., **2007**, *97*, 96-103.

[80] Matsumoto, S.; Hara, T.; Hori, T.; Mitsuyama, K.; Nagaoka, M.; Tomiyasu, N.; Suzuki, A.; Sata, M. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. *Clin. Exp. Immunol*., **2005**, *140*, 417-426.

[81] Osman, N.; Adawi, D.; Ahrne, S.; Jeppsson, B.; Molin, G. Modulation of the effect of dextran sulfate sodium-induced acute colitis by the administration of different probiotic strains of Lactobacillus and Bifidobacterium. *Dig. Dis. Sci*., **2004**, *49*, 320-327.

[82] Herias, M. V.; Koninkx, J. F.; Vos, J. G.; Huis in't Veld, J. H.; van Dijk, J. E. Probiotic effects of Lactobacillus casei on DSS-induced ulcerative colitis in mice. *Int. J. Food Microbiol*., **2005**, *103*, 143-155.

[83] Grabig, A.; Paclik, D.; Guzy, C.; Dankof, A.; Baumgart, D. C.; Erckenbrecht, J.; Raupach, B.; Sonnenborn, U.; Eckert, J.; Schumann, R. R.; Wiedenmann, B.; Dignass, A. U.; Sturm, A. Escherichia coli strain Nissle 1917 ameliorates experimental colitis via toll-like receptor 2- and toll-like receptor 4-dependent pathways. *Infect Immun*., **2006**, *74*, 4075-4082.

[84] Foligne, B.; Zoumpopoulou, G.; Dewulf, J.; Ben Younes, A.; Chareyre, F.; Sirard, J. C.; Pot, B.; Grangette, C. A key role of dendritic cells in probiotic functionality. *PLoS One,* **2007**, *2*, e313.

[85] Lee, H. S.; Han, S. Y.; Bae, E. A.; Huh, C. S.; Ahn, Y. T.; Lee, J. H.; Kim, D. H. Lactic acid bacteria inhibit proinflammatory cytokine expression and bacterial glycosaminoglycan degradation activity in dextran sulfate sodium-induced colitic mice. *Int. Immunopharmacol*., **2008**, *8*, 574-580.

[86] Nishitani, Y.; Tanoue, T.; Yamada, K.; Ishida, T.; Yoshida, M.; Azuma, T.; Mizuno, M. Lactococcus lactis subsp. cremoris FC alleviates symptoms of colitis induced by dextran sulfate sodium in mice. *Int. Immunopharmacol*., **2009**, *9*, 1444-1451.

[87] Lee, B.; Lee, J. H.; Lee, H. S.; Bae, E. A.; Huh, C. S.; Ahn, Y. T.; Kim, D. H. Glycosaminoglycan degradation-inhibitory lactic acid bacteria ameliorate 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice. *J. Microbiol. Biotechnol*., **2009**, *19*, 616-621.

[88] Hormannsperger, G.; Clavel, T.; Hoffmann, M.; Reiff, C.; Kelly, D.; Loh, G.; Blaut, M.; Holzlwimmer, G.; Haller, D. Posttranslational inhibition of proinflammatory chemokine secretion in intestinal epithelial cells: implications for specific IBD indications. *J Clin. Gastroenterol*., **2010**, *44 Suppl 1*, S10-15.

[89] Philippe, D.; Heupel, E.; Blum-Sperisen, S.; Riedel, C. U. Treatment with Bifidobacterium bifidum 17 partially protects mice from Th1-driven inflammation in a chemically induced model of colitis. *Int. J. Food Microbiol*., **2011**, *149*, 45-49.

[90] Duary, R. K.; Bhausaheb, M. A.; Batish, V. K.; Grover, S. Anti-inflammatory and immunomodulatory efficacy of indigenous probiotic Lactobacillus plantarum Lp91 in colitis mouse model. *Mol. Biol. Rep*.

[91] de Moreno de LeBlanc, A.; Chaves, S.; Perdigon, G. Effect of yoghhurt on the cytokine profile using a murine model of intestinal inflammation. *Eur. J. Inflam.,* **2009**, *7* 97-109.

[92] Chaves, S.; Perdigon, G.; de Moreno de LeBlanc, A. Yoghurt consumption regulates the immune cells implicated in acute intestinal inflammation and prevents the recurrence of the inflammatory process in a mouse model. *J. Food Prot*., **2011**, *74*, 801-811.

[93] Grangette, C.; Nutten, S.; Palumbo, E.; Morath, S.; Hermann, C.; Dewulf, J.; Pot, B.; Hartung, T.; Hols, P.; Mercenier, A. Enhanced anti-inflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids. *Proc. Natl. Acad. Sci. U.S.A*., **2005**, *102*, 10321-10326.

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