# Anti-inflammatory Properties of Lactic Acid Bacteria: Current Knowledge, Applications and Prospects

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**Abstract:** Lactic acid bacteria (LAB) represent a heterogeneous group of microorganisms that are naturally present in many foods. Some selected strains are frequently added as probiotics in order to confer specific benefits to consumers.

Many studies have shown that LAB 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.

They 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 to the inflammatory processes and to 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.

Engineering LAB to produce either antioxidant enzymes (such as catalases and superoxide dismutases) or anti-inflammatory cytokines (such as IL-10) is a strategy currently exploited by several groups. 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.

## INTRODUCTION

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 researches 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 most 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 to 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 and 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 in-

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1871-5214/08 $55.00+.00 

flammatory process 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 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 is 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]. Recently, it was also shown that *Lactobacillus* (*L.*) *rhamnosus* GG conditioned media decreased tumour 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 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.

## PROBIOTICS AND INTESTINAL INFLAMMATION

The effectiveness of probiotics in the alleviation of digestive diseases are tested in animal models. 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**.

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*. [19] 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-centre double-blind RCT where the relapse at one year was 10% in the probiotics-treated patients versus 94% in the placebo-treated patients [18]. A link between VSL#3 anti-inflammatory capacities and Toll-like receptors (TLR) was recently 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 [25]. Interestingly, the probiotic treatment increased the tissue levels of IL-10 in these patients [26]. 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 [27].

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) [28]. In CD, abnormal activation of mucosal T lymphocytes against enteric bacteria is the key event triggering intestinal inflammation. Carol *et al.* [29] 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 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 [30]. 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 proinflammatory cytokine IFN-γ [31]. However, studies in experimental models suggest that topical treatment with IL-10 is effective to prevent 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 [32]. Intragastric administration of a recombinant *Lactococcus* (*Lc.*) *lactis* strain secreting murine IL-10 prevented 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 [33]. Braat *et al*. [34] 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

**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* | [12] |
| IBS | 77 | 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 | *B. infantis* 35624 | [13] |
| 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) | [14] |
| 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 | [15] |
| 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) | [16] |
| 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 | [17] |
| 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) | [18] |
| 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 | [19] |
| 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) | [20] |
| UC | 327 | The probiotic treatment was just as effective as conventional treatment (mesalazine) in maintaining remission | *E. coli* Nissle 1917 | [21] |
| UC | 90 | Probiotic supplementation improved remission compared to conventional treatment (balsalazide) alone | VSL#3 | [22] |
| UC | 21 | Probiotic preparation maintains remission (75%) | VSL#3 | [23] |
| UC | 120 | 62% improvement of symptoms and 0% relapse of intestinal disease while patients on probiotics | VSL#3 | [24] |

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

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 NON-INTESTINAL INFLAMMATION

Probiotic microorganisms can also be efficient in the treatment of other inflammatory disease besides intestinal disorders, such is the case of allergic inflammation [35]. It has been reported that that cow's milk allergic infants, treated with extensively hydrolysed 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 [36].

Perdigon *et al.* [5] have shown that orally administered fermented products and probiotics can modify the immune cell activation in distant mucosal sites 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 tumour) in mice [37]. The administration of milk fermented by this LAB strain delayed or stopped breast tumour development due to a modulation in the immune response which was manifested by an increase of the anti-inflammatory cytokine IL-10 [38]. A recent review by DeNardo and Coussens [39] has shown 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 [40] and by phagocytes during inflammatory reactions [41]. 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 [42]. Toxicity occurs when the concentration of ROS exceeds the capacity of cell defence systems [43]. Large amounts of $H_2O_2$ are produced and excreted by human tumor cells [44] 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 have been established in various studies [45-48]. Thus, oxidative stress plays an important role in pathologies of the gastrointestinal tract of humans such as IBD and certain types of cancers [49, 50].

The normal intestinal mucosa is equipped with a network of antioxidant enzymes that neutralize ROS in a two-steps 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 continual steady-state level of ROS. However, the levels of these enzymes in inflammatory disease patients, such as those suffering from IBD, are frequently depleted [46, 49], 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 treatment of inflammatory diseases or post-cancer drug treatments.

## GENETICALLY ENGINEERED LAB – ANTIOXIDANT ENZYME PRODUCTION

Catalases are widespread in aerobic (facultative or not) bacteria such as *Escherichia coli* and *Bacillus* (*B.*) *subtilis* [51]. 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 [52-55].

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*. [51] 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 [56]. 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 [55]. 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 caecal 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 [57]. 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* [58] will be relevant as some previous studies showed the positive impact of increased SOD activity in intestinal inflammation models [49, 59, 60].

To determine whether a bacterial supply of SOD into the colon could improve an experimentally induced colitis, Han *et al*. [61] 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 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*. [62] 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

**Table 2. Examples of Bacterial Strains, Native and Genetically Modified (GM), 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 | [20] |
| 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 | [14] |
| *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 | [63] |
| *L. fermentum, L. reuteri* | native | immune | improvement of histology in a TNBS model of rat colitis, decreased levels of TNF-α and iNOS expression | [64] |
| *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 | [65] |
| *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 | [29] |
| *L. rhamnosus* GG | native | immune | alleviating intestinal inflammation, decrease TNF-α | [36] |
| VSL#3 | native | unknown | delayed the relapse into pouchitis after surgical resection in human patients | [19] |
| *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS and *B. breve* Bb99. | native | unknow | alleviating irritable bowel syndrome symptoms. | [15] |
| *B.*and *L.plantarum* | native | unknown | improvement of the disease activity index in an induced rat colitis model. | [66] |
| *L. rhamnosus* GG | native | unknown | improvement in the clinical status in children with mildly to moderately active stable Crohn's disease. | [28] |
| *L. casei* Shirota | native | unknown | Improvement in the clinical condition of murin model of ulcerative colitis | [67] |
| *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 | [68] |
| *Lc. lactis* IL-10 | GM | immune | reduction in colitis in mice treated with DSS | [33] |
| *L. casei* BL23 MnKat | GM | CAT | reduction of cecal and colonic inflammatory scores. | [57] |
| *Lc. lactis* + KatE | GM | CAT | slight increase catalase activities in the intestines and prevention of colon cancer of mice administered the cancer inducing drug DMH. | [56] |
| *Lc.lactis* NZ9800 and *L.plantarum* NCIMB8826 + pNZ804 sodA | GM | SOD | reduction in macroscopic damages in rats administered TNBS to induce colitis | [61] |
| *L. gasseri* NC1501 | GM | SOD | reduction in inflammation in IL-10-deficient mice | [62] |

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

neutrophils and macrophages that significantly reduced the severity of colitis in the IL-10-deficient mice.

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.

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-dependant anti-inflammatory processes, could gen-

erate very potent strains that could be used in the treatment of a variety of inflammatory diseases. These strains could also be included in certain foods for the elaboration of products that could be consumed as part of a normal diet that in addition to their intrinsic nutritional values could be used in a general program to prevent the development of inflammatory diseases in certain population groups with increased risks for these diseases.

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.

## ACKNOWLEDGEMENTS

The authors would like to thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and the Centro Argentino Brasileño de Biotecnología (CABBIO) for their financial support.

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**Received: November 06, 2007 Revised: January 04, 2008 Accepted: ????????????????**