Current Review of Genetically Modified Lactic Acid Bacteria for the Prevention and Treatment of Colitis Using Murine Models

Alejandra de Moreno de Leblanc, Silvina del Carmen, Jean-Marc J.-M. Chatel, Anderson Miyoshi, Vasco Azevedo, Philippe P. Langella, Luis L. Bermudez Humaran, Jean Guy Leblanc

Hindawi Publishing Corporation
Gastroenterology Research and Practice
Volume 2015, Article ID 146972, 8 pages
http://dx.doi.org/10.1155/2015/146972

Review Article

Current Review of Genetically Modified Lactic Acid Bacteria for the Prevention and Treatment of Colitis Using Murine Models

Alejandra de Moreno de LeBlanc,[1] Silvina del Carmen,[1] Jean-Marc Chatel,[2,3] Anderson Miyoshi,[4] Vasco Azevedo,[4] Philippe Langella,[2,3] Luis G. Bermúdez-Humarán,[2,3] and Jean Guy LeBlanc[1]

[1]*Centro de Referencia para Lactobacilos (CERELA-CONICET), T4000ILC San Miguel de Tucumán, Argentina*
[2]*INRA, Commensal and Probiotics-Host Interactions Laboratory, UMR1319 Micalis, 78350 Jouy-en-Josas, France*
[3]*AgroParisTech, UMR1319 Micalis, 78350 Jouy-en-Josas, France*
[4]*Federal University of Minas Gerais (UFMG), 31270-901 Belo Horizonte, MG, Brazil*

Correspondence should be addressed to Jean Guy LeBlanc; leblanc@cerela.org.ar

Received 23 November 2014; Revised 21 April 2015; Accepted 22 April 2015

Academic Editor: Mohamed Othman

Inflammatory Bowel Diseases (IBD) are disorders of the gastrointestinal tract characterized by recurrent inflammation that requires lifelong treatments. Probiotic microorganisms appear as an alternative for these patients; however, probiotic characteristics are strain dependent and each probiotic needs to be tested to understand the underlining mechanisms involved in their beneficial properties. Genetic modification of lactic acid bacteria (LAB) was also described as a tool for new IBD treatments. The first part of this review shows different genetically modified LAB (GM-LAB) described for IBD treatment since 2000. Then, the two principally studied strategies are discussed (i) GM-LAB producing antioxidant enzymes and (ii) GM-LAB producing the anti-inflammatory cytokine IL-10. Different delivery systems, including protein delivery and DNA delivery, will also be discussed. Studies show the efficacy of GM-LAB (using different expression systems) for the prevention and treatment of IBD, highlighting the importance of the bacterial strain selection (with anti-inflammatory innate properties) as a promising alternative. These microorganisms could be used in the near future for the development of therapeutic products with anti-inflammatory properties that can improve the quality of life of IBD patients.

1. Introduction

Inflammatory Bowel Diseases (IBD) describe a group of disorders of the gastrointestinal tract characterized by recurrent inflammation, with periods of relapse and remission, and epithelial injury. Ulcerative colitis (UC) and Crohn's disease (CD) are the two most frequent forms of IBD, clinically characterized by different intestinal location, nature, and the histological features of the inflammatory lesions as well as their association with specific deregulation of the host's immune response. The exact etiology of these pathologies is still unknown; however, it was described that in IBD patients there existed aberrant features of the interaction between intestinal microorganisms and gut immune and epithelial cells, which is manifested as chronic intestinal inflammation [1].

IBD require lifelong treatments, and although they are not generally associated with increased mortality, they can cause significant morbidity. Probiotic microorganisms have appeared as an alternative for IBD patients and their efficiency has been analyzed in experimental animal models and also in clinical trials [2, 3].

This paper will describe some of the mechanisms by which probiotic microorganisms can exert specific benefits against IBD, followed by a revision about the potential use of genetically modify lactic acid bacteria in the prevention and treatment of these recurrent diseases.

1.1. Mechanisms Involved in the Anti-Inflammatory Effects of Probiotic Lactic Acid Bacteria. Lactic acid bacteria are the most common microorganisms used as probiotics. Because of

specific documented beneficial effects, certain strains of LAB have been designated as probiotics, which have been defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [4]. Among these health benefits is the alleviation of IBD symptoms which was reported for some probiotic LAB strains [3].

In this sense, it has been shown that LAB and other probiotic microorganisms can counteract inflammatory processes in the gastrointestinal tract (GIT) through different mechanisms.

One of these is the modulation of the intestinal microbiota related to probiotic administration. It has been shown that *Lactobacillus* (*L.*) *reuteri* could be used to prevent colitis in IL-10$^{-/-}$ mice by increasing the number of lactobacilli in the GIT [5]. In a placebo-controlled trial, the oral administration of *L. salivarius* UCC118 reduced the prevalence of colon cancer and inflammatory activity in the mucosa of IL-10$^{-/-}$ mice by modifying the intestinal microbiota in these animals: *Clostridium* (*C.*) *perfringens*, coliforms, and enterococci counts also decreased significantly in the group treated with the probiotic [6]. Another mechanism by which probiotics can have a positive effect is inhibiting microorganisms through the production of antimicrobial substances such as bacteriocins. Many bacteriocins produced by various species of *Lactobacillus* have been reported [7]. The inhibitory activity of these bacteriocins varies from species to species. It was reported that Lacticin 3147, a broad spectrum bacteriocin produced by a *Lactococcus* (*Lc.*) *lactis*, inhibited a wide range of genetically distinct *C. difficile* strains isolated from healthy subjects and also from patients with IBD [8].

Numerous studies have shown that certain strains of LAB may modulate the host's immune response by regulating the production of cytokines which are involved in regulation, activation, growth, and differentiation of immune cells. One of the ways by which probiotics may exert immunomodulatory activities is by stimulating the production of IL-10 (an anti-inflammatory cytokine). However, not all probiotic strains act in the same way. The anti-inflammatory effects such as the stimulation of IL-10 producing cells are strain-dependent characteristics, and their effectiveness is also dependent on the concentrations used and the method of administration. Thus, the anti-inflammatory effect associated with the administration of a potential probiotic yoghurt to mice was studied in models of acute and chronic intestinal inflammation induced by trinitrobenzene sulfonic acid (TNBS). Animals receiving yoghurt continuously improved immune response with increases in IL-10 and decreases in IL-17 (a proinflammatory cytokine) in the GIT of mice [9, 10]. In another study it was shown that the anti-inflammatory strain *L. salivarius* Ls 33 required the presence of NOD2 receptors to exert its protective effect, which was also related to the local production of IL-10 [11]. By studying the expression of different genes, it was observed that the probiotic strain *L. plantarum* Lp91 caused a significant decrease in the levels of TNFα (tumor necrosis factor α) and COX-2 (cyclooxygenase-2) in a mouse model of colitis, and this effect was related to significant increases of IL-10 expression [12]. It was also recently observed, through the use of both *in vitro* and *ex vivo* studies, that milk fermented with *L. paracasei* L74 CBA inhibited proinflammatory cytokines without affecting the levels of anti-inflammatory cytokines and that this anti-inflammatory activity depended on metabolic products released during the fermentation process [13]. A new study has also shown that a strain of *Streptococcus* (*S.*) *salivarius* inhibited the activation of the NF-κB *in vitro* by demonstrating anti-inflammatory properties *in vivo* [14].

The improvement of the intestinal barrier function is another mechanism by which probiotic bacteria can benefit the host. The exact mechanism by which probiotics enhance the barrier function and the intestinal mucus is unclear; however, it may be related to alterations in mucus secretion or changes in intercellular interactions of the mucosa and cell stability by modulating the phosphorylation of cytoskeletal proteins and tight junctions [15, 16]. Oral treatment with VSL # 3 (a mixture of eight probiotic bacteria including *Lactobacilli*, *Bifidobacterium*, and *Streptococcus* species) normalized the colonic physiological function and integrity of the mucosal barrier in IL-10$^{-/-}$ mice [15]. *L. plantarum* DSM 9843 and *L. reuteri* R2LC also improved barrier function in a model of enterocolitis induced by methotrexate in rats [17]. Some probiotic bacteria can modify the expression of *muc* genes and mucus secretion [18].

Probiotic can also act by reducing oxidative stress, which is characterized by an uncontrolled increase in the concentration of reactive oxidative species (ROS) in the GIT. Thus, another suggested mechanism to prevent inflammation by LAB administration is through the expression of enzymes that are be able to decrease the concentrations of ROS or affect their formation. The levels of these enzymes are often depleted in patients with IBD [19]. Probiotic LAB expressing high levels of antioxidant enzymes could increase these activities in specific locations of GIT and could then contribute to the prevention of oxidative damage, leading to potential applications for the treatment of IBD or posttherapy treatments for IBD or even cancer patients. In this sense, *L. rhamnosus* CNCM I-3690, selected for its antioxidant properties *in vitro*, showed anti-inflammatory activity in a model of colitis *in vivo* [20].

It is important to consider that probiotic characteristics are strain dependent and each probiotic should be tested to know if it has specific beneficial effects, and to describe the mechanism/s involved in their health-promoting properties. In addition, not many mechanisms are usually associated with one individual strain. So, genetic modification of LAB has been also described as a tool to development new treatments for IBD, using microorganisms with GRAS (General Recognized as Safe) status.

2. Genetically Modification of Lactic Acid Bacteria for Treatment of IBD

It is theoretically possible, using genetic engineering techniques, to obtain LAB strains that possess a variety of beneficial properties. *L. lactis* is a LAB used in various processes in the food industry and is has been characterized because it does not survive in the digestive tract of animals

and humans and thus has the potential to be used without the possibility of survival through the gastrointestinal tract [21]. *L. lactis* is normally used as a LAB model because (i) its genome has been completely sequenced, (ii) it is easy to manipulate genetically, and (iii) many genetic tools have already been developed for this species. Based on the identification and isolation of plasmids from native strains of *L. lactis* and other LAB, several cloning vectors have been developed. Using molecular biology techniques, these vectors have been engineered to become important tools for cloning genes of interest, and their products can be controlled with constitutive or inducible promoters.

The development of efficient systems to express genes and suitable protein secretion systems for use in LAB can permit these microorganisms to be used for the production and secretion of a number of heterologous proteins [22]. So, as was explained above, LAB are potential candidates for use as vehicles for the production and delivery of heterologous proteins of technological, medical, or prophylactic interest and several delivery systems are now available for those GRAS microorganisms [23]. The introduction of genes coding for antioxidant enzymes or cytokine production in LAB selected for their probiotic potential, such as the ability to modulate the immune response, may generate very useful strains that can be applied in the treatment of a variety of inflammatory diseases. However, before proposing the genetic modification of anti-inflammatory strains, innate mechanisms of potential vehicle strains should be demonstrated in appropriately designed clinical trials on a large scale. These tests are essential in future studies using genetically modified (GM) strains to demonstrate the differences between wild type and modified microorganisms.

The production of antioxidant enzymes and the production of the anti-inflammatory cytokine IL-10 are two of the most studied anti-inflammatory strategies using GM-LAB and both will be revised in the following sections along with a few examples of other anti-inflammatory compounds that have successfully been produced by GM-LAB.

2.1. Lactic Acid Bacteria That Are Genetically Modified to Produce Antioxidant Enzymes. As mentioned previously, in patients with IBD, oxidative stress occurs as the result of an abnormal and recurrent inflammation associated with increased concentrations of ROS. Because few microorganisms produce antioxidant enzymes in the concentrations required to exert biological effects, genetic engineering strategies have been used to obtain more efficient antioxidant producing LAB. Spyropoulos et al. have shown the potential uses of such strains in the treatment of IBD using a variety of animal models [24]. LAB have been used to locally deliver antioxidant enzymes such as superoxide dismutase (SOD) directly in the intestines. This was a major breakthrough because 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 shown that GM strains of *L. plantarum* and *L. lactis* that are able to produce and release SOD exhibited anti-inflammatory effects in a TNBS induced colitis model [25]. Another experimental study showed the anti-inflammatory activity *L. gasseri* strain producing SOD with an associated reduction of the severity of colitis in IL-10 deficient mice [26]. *L. casei* BL23 producing SOD was able to significantly reduce the damage induced by TNBS in mice as shown by increased survival, decreased weight loss, lower microbial translocation to liver, and more importantly a decrease in the damage of the large intestines [27]. These results agree with others presented previously in which the same strain was able to slightly reduce the histological damage degree in a dextran sulphate sodium (DSS) induced colitis model [28].

Because *L. lactis* lacks catalase (as is also the case for most LAB), the heme catalase gene *katE* from *B. subtilis* was added to this industrially important organism resulting in a GM strain able to produce catalase that provided active catalase activities [29]. It was demonstrated that this strain of *L. lactis* producing catalase was able to prevent the development of colon tumors in mice using a chemical induced model [30]. In another study, *L. casei* BL23 was modified to produce heme-independent catalase and this in turn decreased the intestinal inflammatory damage in a TNBS induced mouse model [27]. This result is similar to those obtained previously in which both the native strain *L. casei* BL23 and the derived GM strain producing catalase reduced inflammation degrees in the colon and cecum of mice, using a DSS induced model [31].

S. thermophilus CRL807 is a strain that was present in the starter mix, together with 12 other LAB, used to prepare yoghurt with anti-inflammatory and anticancer effects [9, 32]. The anti-inflammatory potential of this strain was demonstrated *in vitro* and *in vivo* [33]. So, the concept that LAB selected for their innate inflammatory potential can be genetically modified to produced antioxidant enzymes and obtain strains with more efficient anti-inflammatory effects was recently evaluated using *S. thermophilus* CRL 807 [33]. Unlike other studies using GM *L. lactis*, it was observed that the unmodified strain exerted anti-inflammatory effects in a TNBS-induced colitis model in mice. It was also observed that both genetically modified *S. thermophilus* CRL 807:CAT and *S. thermophilus* CRL807:SOD (used as suspension or in fermented milks) decreased the severity of inflammation, and these beneficial changes were increased compared to those observed in mice that received the wild type strain. The mixture of both GM-streptococci were also evaluated and shown to exert more anti-inflammatory properties than when each strain was given individually. These results prove that the use of LAB strains that are able to modulate the immune response (innate capacity of *S. thermophilus* CRL 807) and also express antioxidant enzymes show a combined effect and may be a useful strategy in the development of new therapeutics for patients suffering from IBD.

2.2. Genetically Modified LAB That Produce the Anti-Inflammatory Cytokine IL-10. Interleukin-10 (IL-10), as explained in the introduction, is one of the major anti-inflammatory cytokines involved in maintaining the homeostasis of the intestinal immune response. It is recognized for its ability to regulate inflammatory responses through the suppression of the proinflammatory cytokine cascades [34], and this is presented as a therapeutic candidate for the treatment of IBD [35]. Furthermore, oral administration of the IL-10 is not a viable option because of its extreme sensitivity to the

ambient of the GIT [36]. Although clinical trials conducted to date have shown relatively poor results, the use of new technologies for the delivery of IL-10 at the tissue level has recently been suggested that could become a viable treatment option for certain patients [37]. Thus, different strategies were designed to ensure that IL-10 reaches the GIT, including the use of microencapsulation techniques or viral vectors [38–40]. However, many of these methods are expensive, complicated, or risky methodologically. Therefore, the use of GM-LAB appeared as an attractive alternative for delivering this cytokine at mucosal surface level [22].

The first evidence of GM-LAB as therapeutic vehicle for IL-10 was published in 2000, when it was shown that a strain of *L. lactis* secreting IL-10 prevented the onset of colitis in IL-10$^{-/-}$ mice [41] and reduced inflammation in a model of DSS induced colitis [42]. An important step for the safe use of this GM-LAB for therapeutic purposes in humans was the construction of a biological containment system in a GM strain of *L. lactis* for intestinal delivery of human IL-10 cytokine [43]. In that study, the thymidylate synthase gene of *L. lactis* was replaced by the human IL-10 gene, making this strain unable to grow in the absence of thymidine or thymine. This strain did not contain any antibiotic resistance marker and since thymidine is auxotrophic, the strain could not be spread to the environment making it one of the safest built GM-LAB so far. This containment system was evaluated in patients with CD and was shown not to produce any adverse side effects, and these GM-LAB could only be recovered in feces with the addition of thymidine [44]. However, the clinical outcomes in these patients revealed no statistically significances between those individuals who received the GM-LAB compared with the placebo group. These results showed the need to evaluate new methods of administration to achieve a more effective delivery of IL-10 in the intestinal mucosa using therapeutic LAB [45–49].

L. lactis subsp. *lactis* NCDO2118 pXYL:IL-10 is a LAB that produced IL-10 using an expression system that has a food-grade inducer in the xylose-inducible expression system (XIES) [48]. Anti-inflammatory properties were also described for the wild type strain (*L. lactis* subsp. *lactis* NCDO2118) in a DSS induced colitis model in mice [50]. It was demonstrated that milk fermented by *L. lactis* NCDO2118 pXYLCYT:IL-10, strain capable to produce and maintain IL-10 in the bacterial cytoplasm, exerted an anti-inflammatory effect in an acute TNBS induced model of IBD in mice, which was more pronounced than the ones observed with milk fermented by the wild type strain [51]. This effect was related to decreased levels of proinflammatory cytokines in the GIT of mice. The results showed the use of fermented milks as a new form of administration of IL-10 producing *L. lactis*. In this system milk acts as a matrix to protect 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), appropriate for a specific population that suffers gastrointestinal disorders.

L. lactis subsp. *cremoris* MG1363 pGroESL:IL-10 produces IL-10 using the protein delivery expression system SICE (Stress-Inducible Controlled Expression) and is based on a stress inducible promoter (pGroESL) that allows the production of the heterologous protein *in situ* (e.g., colon) [45]. *L. lactis* capable of delivering the IL-10 protein using the SICE system has the advantage that it does not require an inductor because the adverse conditions of the GIT can by themselves induce this system and all the IL-10 can be locally produced by this LAB in the intestine. Recent studies showed that this strain exerted a protective effect against inflammation in a model of low-grade colon inflammation [52].

Even though protein delivery by GM-LAB showed promising results, DNA delivery by GM-LAB was also evaluated for the production of IL-10 locally by the host's intestinal cells. *L. lactis* subsp. *cremoris* MG1363 pValac:IL-10 is a GM-LAB used for the delivery of IL-10 cDNA. This system is based on a new vector for DNA delivery using lactococci called pValac (vaccination using lactic acid bacteria) that delivers the DNA to the intestinal cells and gives these cells the capacity to produce IL-10 directly at the site of inflammation [46]. It was reported that *L. lactis* MG1363 engineered to express fibronectin binding protein A (FnBPA) was used as a vehicle to deliver the cDNA for IL-10 using the plasmid pValac::*il-10*. *L. lactis* MG1363 FnBPA + pValac:IL-10 exerted a significant anti-inflammatory effect in a TNBS induced acute model of colitis in mice maintaining high ratios of anti-/proinflammatory cytokines in the intestinal fluids and tissues [53]. The importance of the presence of FnBPA in the GM-LAB was demonstrated using a recombinant strain of *L. lactis* that expresses FnBPA under the control of the nisin inducible expression system [54]; however, the use of the noninvasive strain *L. lactis* subsp. *cremoris* MG1363 pValac:IL-10 was also able to provide the IL-10 cDNA to the host's cells and exerted an in-inflammatory effect in a DSS induced colitis model in mice [55] showing that this DNA delivery system could be used in noninvasive strains.

The effectiveness of both protein and DNA delivery systems was also recently compared using a TNBS induced chronic inflammation model. It was demonstrated that *L. lactis* pValac:IL-10 (noninvasive strain) exerted similar anti-inflammatory effects than *L. lactis* pGroESL:IL-10, when they were administered to the mice during the remission period [56]. Even though the animals that received the strain delivering IL-10 cDNA had higher levels of IL-10 in the intestinal tissues, both systems were effective in maintaining the remission of inflammation which is one of the major problems in current IBD treatments.

2.3. Genetically Modified LAB That Produce Other Anti-Inflammatory Compounds. Since TNF is one of the most important proinflammatory cytokines in immune regulated inflammatory processes, the objective of certain groups has been not only to reduce the expression of this cytokine but also to prevent it from being active. *L. lactis* was engineered to secrete monovalent and bivalent murine (m)TNF-neutralizing nanobodies as therapeutic proteins. It was shown that the oral administration of nanobody-secreting *L. lactis* resulted in local delivery of anti-mTNF nanobodies at the colon that significantly reduced inflammation in DSS-induce chronic colitis in mice [57].

Interleukin-27 (IL-27) plays a role in the regulation of T helper (Th) cell differentiation inducing Th1 differentiation and suppressing immune responses. For this reason, it has been proposed that IL-27 could be useful in therapy of diseases mediated by inflammatory cytokines [58]. The 2 genes encoding mouse IL-27 were introduced in *L. lactis* together with a signal sequence which allowed for this cytokine to be secreted. This IL-27 secreting strain was able to reduce colitis, induced via transfer of CD4(+)CD45RB(hi) T cells into Rag(−/−) mice, by increasing the production of IL-10 [59].

Transforming Growth Factor-β1 (TGF-β) is an inhibitory cytokine recognized as a key regulator of immunological homeostasis and inflammatory responses and was successfully expressed by *L. lactis* and when administered to DSS-induced mice, this GM-LAB decreased colon damage scores [60].

Besides immune regulators, other compounds have also been shown to possess anti-inflammatory potential. The Trefoil Factor (TFF) family of peptides, TFF1 (formerly pS2), TFF2 (formerly spasmolytic peptide, SP), and TFF3 (formerly Intestinal Trefoil Factor, ITF), is involved in the protection of the gastrointestinal tract since they play an essential role in epithelial restitution and repair of mucosal damage [61]. Intragastric administration of TFF-secreting *L. lactis* led to active delivery of TFF at the mucosa of the colon and, in contrast to administration of purified TFF, proved to be very effective in prevention and healing of acute DSS-induced colitis [62]. These same authors then produced a mouth rinse formulation of *L. lactis* secreting human TFF1 which provided a safe and efficient therapeutic tool for treating oral mucositis [61].

It was previously shown that colonic tissues of patients with IBD have increased proteolytic activity and that the use of protease inhibitor Elafin was able to prevent intestinal inflammation in mouse models of colitis [63]. For this reason, serine protease inhibitors such as Elafin and Secretory Leukocyte Protease Inhibitor were expressed in recombinant *L. lactis* and were shown to be very effective anti-inflammatory molecules in a DSS-induced mouse model of colitis [60].

The enzyme 15-lipoxygenase-1 (15-LOX-1) is another molecule that has been proposed as a potential candidate for the resolution of IBD because of its potent anti-inflammatory action. It was shown that the administration of milk fermented by a *L. lactis* strain 15-LOX-1 was effective in the prevention of the intestinal damage associated with inflammatory bowel disease in a TNBS murine model [64].

3. Risk Assessment of Genetically Modified Lactic Acid Bacteria with Anti-Inflammatory Properties

Although there is no scientific evidence to support the notion that GM organisms are dangerous for human consumption, it is necessary to show that it is safe to use genetically modified probiotics designed to extend the range of applications covered by natural probiotics.

Consumption of GM microorganisms by human is still a highly controversial issue due to the public perception that genetic manipulation is not "natural." Scientists need through well-designed studies to report the results for the general population to inform consumers of the benefits that these techniques can confer with minimal risk to the health and the environment, such was the case of the IL-10 producing LAB that was shown to be safe in human clinical trials [44].

4. Conclusions

The revision presented here not only showed the potential associated with probiotic microorganisms to be used in patients suffering diseases associated with gastrointestinal inflammation, but also showed the potential use of GM-LAB as new therapies for these patients. It is important to restate the genetic modification of lactic acid bacteria with innate anti-inflammatory properties to produce anti-inflammatory compounds (such as antioxidant enzymes or anti-inflammatory cytokines). It is a promissory tool to obtain new more effective strains with potential applications for IBD patients. These powerful strains should be given as an adjunct treatment to current protocols for IBD patients, and because of the beneficial properties, these could actually improve the quality of life of these patients and aid in preventing the unbalance of beneficial/pathogenic microbiota present in the GIT.

Conflict of Interests

There is no conflict of interests to disclose for all authors.

Authors' Contribution

Alejandra de Moreno de LeBlanc and Silvina del Carmen contributed equally to this work.

Acknowledgments

The authors would like to thank (i) Consejlho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), (ii) Centro Brasileiro-Argentino de Biotecnologia (CBAB), (iii) Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES), (iv) Consejo Nacional de Investigaciones Cientıficas y Tecnicas (CONICET), (v) Agencia Nacional de Promocion Cientıfica y Tecnologica (ANPCyT), and (vi) ECOS-SUD-MinCyT for their financial support.

References

[1] P. J. Basso, M. T. Fonseca, G. Bonfá et al., "Association among genetic predisposition, gut microbiota, and host immune response in the etiopathogenesis of inflammatory bowel disease," *Brazilian Journal of Medical and Biological Research*, vol. 47, no. 9, pp. 727–737, 2014.

[2] E. De Greef, Y. Vandenplas, B. Hauser, T. Devreker, and G. Veereman, "The use of probiotics in IBD and IBS," *Minerva Pediatrica*, vol. 66, no. 5, pp. 491–500, 2014.

[3] S. del Carmen, J. G. LeBlanc, and A. de Moreno de LeBlanc, "Use of probiotics in the treatment of Crohn's disease," in *Crohn's Disease: Etiology, Diagnosis and Treatment Options*, J. G.

LeBlanc and A. de Moreno de LeBlanc, Eds., pp. 287–306, Nova Science, Hauppauge, NY, USA, 2013.

[4] C. Hill, F. Guarner, G. Reid et al., "Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic," *Nature Reviews Gastroenterology & Hepatology*, vol. 11, no. 8, pp. 506–514, 2014.

[5] K. L. Madsen, J. S. Doyle, L. D. Jewell, M. M. Tavernini, and R. N. Fedorak, "Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice," *Gastroenterology*, vol. 116, no. 5, pp. 1107–1114, 1999.

[6] L. O'Mahony, M. Feeney, S. O'Halloran et al., "Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice," *Alimentary Pharmacology and Therapeutics*, vol. 15, no. 8, pp. 1219–1225, 2001.

[7] T. R. Klaenhammer, "Bacteriocins of lactic acid bacteria," *Biochimie*, vol. 70, no. 3, pp. 337–349, 1988.

[8] M. C. Rea, E. Clayton, P. M. O'Connor et al., "Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains," *Journal of Medical Microbiology*, vol. 56, no. 7, pp. 940–946, 2007.

[9] A. de Moreno de LeBlanc, S. Chaves, and G. Perdigón, "Effect of yoghhurt on the cytokine profile using a murine model of intestinal inflammation," *European Journal of Inflammation*, vol. 7, no. 2, pp. 97–109, 2009.

[10] S. Chaves, G. Perdigon, and A. de Moreno de LeBlanc, "Yoghurt consumption regulates the immune cells implicated in acute intestinal inflammation and prevents the recurrence of the inflammatory process in a mouse model," *Journal of Food Protection*, vol. 74, no. 5, pp. 801–811, 2011.

[11] E. M. Fernandez, V. Valenti, C. Rockel et al., "Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide," *Gut*, vol. 60, no. 8, pp. 1050–1059, 2011.

[12] R. K. Duary, M. A. Bhausaheb, V. K. Batish, and S. Grover, "Anti-inflammatory and immunomodulatory efficacy of indig enous probiotic *Lactobacillus plantarum* Lp91 in colitis mouse model," *Molecular Biology Reports*, vol. 39, no. 4, pp. 4765–4775, 2012.

[13] E. Zagato, E. Mileti, L. Massimiliano et al., "*Lactobacillus paracasei* CBA L74 metabolic products and fermented milk for infant formula have anti-inflammatory activity on dendritic cells *in vitro* and protective effects against colitis and an enteric pathogen *in vivo*," *PLoS ONE*, vol. 9, no. 2, Article ID e87615, 2014.

[14] G. Kaci, D. Goudercourt, V. Dennin et al., "Anti-inflammatory properties of *Streptococcus salivarius*, a commensal bacterium of the oral cavity and digestive tract," *Applied and Environmental Microbiology*, vol. 80, no. 3, pp. 928–934, 2014.

[15] K. Madsen, A. Cornish, P. Soper et al., "Probiotic bacteria enhance murine and human intestinal epithelial barrier function," *Gastroenterology*, vol. 121, no. 3, pp. 580–591, 2001.

[16] S. C. Ng, A. L. Hart, M. A. Kamm, A. J. Stagg, and S. C. Knight, "Mechanisms of action of probiotics: recent advances," *Inflammatory Bowel Diseases*, vol. 15, no. 2, pp. 300–310, 2009.

[17] Y. Mao, S. Nobaek, B. Kasravi et al., "The effects of *Lactobacillus* strains and oat fiber on methotrexate-induced enterocolitis in rats," *Gastroenterology*, vol. 111, no. 2, pp. 334–344, 1996.

[18] C. Caballero-Franco, K. Keller, C. De Simone, and K. Chadee, "The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 292, no. 1, pp. G315–G322, 2007.

[19] L. Kruidenier and H. W. Verspaget, "Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease—radicals or ridiculous?" *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 12, pp. 1997–2015, 2002.

[20] G. Grompone, P. Martorell, S. Llopis et al., "Anti-inflammatory *Lactobacillus rhamnosus* CNCM I-3690 strain protects against oxidative stress and increases lifespan in *Caenorhabditis elegans*," *PLoS ONE*, vol. 7, no. 12, Article ID e52493, 2012.

[21] S. Drouault, G. Corthier, S. D. Ehrlich, and P. Renault, "Survival, physiology, and lysis of *Lactococcus lactis* in the digestive tract," *Applied and Environmental Microbiology*, vol. 65, no. 11, pp. 4881–4886, 1999.

[22] J. G. LeBlanc, C. Aubry, N. G. Cortes-Perez et al., "Mucosal targeting of therapeutic molecules using genetically modified lactic acid bacteria: an update," *FEMS Microbiology Letters*, vol. 344, no. 1, pp. 1–9, 2013.

[23] A. Miyoshi, L. G. Bermúdez-Humarán, M. S. P. D. Azevedo, P. Langella, and V. Azevedo, "Lactic acid bacteria as live vectors: heterologous protein production and delivery systems," in *Biotechnology of Lactic Acid Bacteria Novel Applications*, F. Mozzi, R. R. Raya, and G. M. Vignolo, Eds., pp. 161–176, Wiley-Blackwell, Ames, Iowa, USA, 2010.

[24] B. G. Spyropoulos, E. P. Misiakos, C. Fotiadis, and C. N. Stoidis, "Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis," *Digestive Diseases and Sciences*, vol. 56, no. 2, pp. 285–294, 2011.

[25] W. Han, A. Mercenier, A. Ait-Belgnaoui et al., "Improvement of an experimental colitis in rats by lactic acid bacteria producing superoxide dismutase," *Inflammatory Bowel Diseases*, vol. 12, no. 11, pp. 1044–1052, 2006.

[26] I. M. Carroll, J. M. Andrus, J. M. Bruno-Bárcena, T. R. Klaenhammer, H. M. Hassan, and D. S. Threadgill, "Anti-inflammatory properties of *Lactobacillus gasseri* expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 293, no. 4, pp. G729–G738, 2007.

[27] J. G. LeBlanc, S. del Carmen, A. Miyoshi et al., "Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice," *Journal of Biotechnology*, vol. 151, no. 3, pp. 287–293, 2011.

[28] L. Watterlot, T. Rochat, H. Sokol et al., "Intragastric administration of a superoxide dismutase-producing recombinant *Lactobacillus casei* BL23 strain attenuates DSS colitis in mice," *International Journal of Food Microbiology*, vol. 144, no. 1, pp. 35–41, 2010.

[29] T. Rochat, A. Miyoshi, J. J. Gratadoux et al., "High-level resistance to oxidative stress in *Lactococcus lactis* conferred by *Bacillus subtilis* catalase KatE," *Microbiology*, vol. 151, no. 9, pp. 3011–3018, 2005.

[30] A. de Moreno de LeBlanc, S. Chaves, G. Perdigón et al., "Oral administration of a catalase-producing *Lactococcus lactis* can prevent a chemically induced colon cancer in mice," *Journal of Medical Microbiology*, vol. 57, no. 1, pp. 100–105, 2008.

[31] T. Rochat, L. G. Bermúdez-Humarán, J.-J. Gratadoux et al., "Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice," *Microbial Cell Factories*, vol. 6, article 22, 2007.

[32] A. de Moreno de LeBlanc and G. Perdigón, "Yogurt feeding inhibits promotion and progression of experimental colorectal cancer," *Medical Science Monitor*, vol. 10, no. 4, pp. BR96–BR104, 2004.

[33] S. del Carmen, A. de Moreno de LeBlanc, R. Martin et al., "Genetically engineered immunomodulatory *Streptococcus thermophilus* strains producing antioxidant enzymes exhibit enhanced anti-inflammatory activities," *Applied and Environmental Microbiology*, vol. 80, no. 3, pp. 869–877, 2014.

[34] S. Mocellin, F. Marincola, C. R. Rossi, D. Nitti, and M. Lise, "The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle," *Cytokine & Growth Factor Reviews*, vol. 15, no. 1, pp. 61–76, 2004.

[35] A. de Moreno de LeBlanc, S. del Carmen, M. Zurita-Turk et al., "Importance of IL-10 modulation by probiotic microorganisms in gastrointestinal inflammatory diseases," *ISRN Gastroenterology*, vol. 2011, Article ID 892971, 11 pages, 2011.

[36] N. Egilmez and K. Sikora, "Method for treating inflammatory bowel disease by oral administration of IL-10," Google Patents, 2005.

[37] G. J. Marlow, D. van Gent, and L. R. Ferguson, "Why interleukin-10 supplementation does not work in Crohn's disease patients," *World Journal of Gastroenterology*, vol. 19, no. 25, pp. 3931–3941, 2013.

[38] N. Huyghebaert, A. Vermeire, S. Neirynck, L. Steidler, E. Remaut, and J. P. Remon, "Development of an enteric-coated formulation containing freeze-dried, viable recombinant *Lactococcus lactis* for the ileal mucosal delivery of human interleukin-10," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 60, no. 3, pp. 349–359, 2005.

[39] J. O. Lindsay, A. Sandison, P. Cohen, F. M. Brennan, and H. J. F. Hodgson, "IL-10 gene therapy is therapeutic for dextran sodium sulfate-induced murine colitis," *Digestive Diseases and Sciences*, vol. 49, no. 7-8, pp. 1327–1334, 2004.

[40] H. Nakase, K. Okazaki, Y. Tabata et al., "New cytokine delivery system using gelatin microspheres containing interleukin-10 for experimental inflammatory bowel disease," *Journal of Pharmacology and Experimental Therapeutics*, vol. 301, no. 1, pp. 59–65, 2002.

[41] L. Schotte, L. Steidler, J. Vandekerckhove, and E. Remaut, "Secretion of biologically active murine interleukin-10 by *Lactococcus lactis*," *Enzyme and Microbial Technology*, vol. 27, no. 10, pp. 761–765, 2000.

[42] L. Steidler, W. Hans, L. Schotte et al., "Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10," *Science*, vol. 289, no. 5483, pp. 1352–1355, 2000.

[43] L. Steidler, S. Neirynck, N. Huyghebaert et al., "Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10," *Nature Biotechnology*, vol. 21, no. 7, pp. 785–789, 2003.

[44] H. Braat, P. Rottiers, D. W. Hommes et al., "A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 6, pp. 754–759, 2006.

[45] B. Benbouziane, P. Ribelles, C. Aubry et al., "Development of a Stress-Inducible Controlled Expression (SICE) system in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces," *Journal of Biotechnology*, vol. 168, no. 2, pp. 120–129, 2013.

[46] V. Guimarães, S. Innocentin, J.-M. Chatel et al., "A new plasmid vector for DNA delivery using lactococci," *Genetic Vaccines and Therapy*, vol. 7, article 4, 2009.

[47] S. Innocentin, V. Guimarães, A. Miyoshi et al., "*Lactococcus lactis* expressing either *Staphylococcus aureus* fibronectin-binding protein A or *Listeria monocytogenes* internalin A can efficiently internalize and deliver DNA in human epithelial cells," *Applied and Environmental Microbiology*, vol. 75, no. 14, pp. 4870–4878, 2009.

[48] A. Miyoshi, E. Jamet, J. Commissaire, P. Renault, P. Langella, and V. Azevedo, "A xylose-inducible expression system for *Lactococcus lactis*," *FEMS Microbiology Letters*, vol. 239, no. 2, pp. 205–212, 2004.

[49] S. Termont, K. Vandenbroucke, D. Iserentant et al., "Intracellular accumulation of trehalose protects *Lactococcus lactis* from freeze-drying damage and bile toxicity and increases gastric acid resistance," *Applied and Environmental Microbiology*, vol. 72, no. 12, pp. 7694–7700, 2006.

[50] T. D. Luerce, A. C. Gomes-Santos, C. S. Rocha et al., "Anti-inflammatory effects of *Lactococcus lactis* NCDO 2118 during the remission period of chemically induced colitis," *Gut Pathogens*, vol. 6, no. 1, article 33, 2014.

[51] S. del Carmen, A. de Moreno de Leblanc, G. Perdigón et al., "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," *Journal of Molecular Microbiology and Biotechnology*, vol. 21, no. 3-4, pp. 138–146, 2012.

[52] R. Martín, F. Chain, S. Miquel et al., "Effects in the use of a genetically engineered strain of *Lactococcus lactis* delivering in situ IL-10 as a therapy to treat low-grade colon inflammation," *Human Vaccines & Immunotherapeutics*, vol. 10, no. 6, pp. 1611–1621, 2014.

[53] S. del Carmen, M. Zurita-Turk, F. Alvarenga Lima et al., "A novel interleukin-10 DNA mucosal delivery system attenuates intestinal inflammation in a mouse model," *European Journal of Inflammation*, vol. 11, no. 3, pp. 641–654, 2013.

[54] J. F. Almeida, D. Mariat, V. Azevedo et al., "Correlation between fibronectin binding protein A expression level at the surface of recombinant lactococcus lactis and plasmid transfer in vitro and in vivo," *BMC Microbiology*, vol. 14, article 248, 2014.

[55] M. Zurita-Turk, S. del Carmen, A. C. Santos et al., "*Lactococcus lactis* carrying the pValac DNA expression vector coding for IL-10 reduces inflammation in a murine model of experimental colitis," *BMC Biotechnology*, vol. 14, no. 1, p. 73, 2014.

[56] S. del Carmen, R. Martín Rosique, T. Saraiva et al., "Protective effects of *Lactococci* strains delivering either IL-10 protein or cDNA in a TNBS-induced chronic colitis model," *Journal of Clinical Gastroenterology*, vol. 48, supplement 1, pp. S12–S17, 2014.

[57] K. Vandenbroucke, H. de Haard, E. Beirnaert et al., "Orally administered *L. lactis* secreting an anti-TNF Nanobody demonstrate efficacy in chronic colitis," *Mucosal Immunology*, vol. 3, no. 1, pp. 49–56, 2010.

[58] H. Yoshida and M. Yoshiyuki, "Regulation of immune responses by interleukin-27," *Immunological Reviews*, vol. 226, no. 1, pp. 234–247, 2008.

[59] M. L. Hanson, J. A. Hixon, W. Li et al., "Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice," *Gastroenterology*, vol. 146, no. 1, pp. 210.e13–221.e13, 2014.

[60] L. G. Bermúdez-Humarán, J. P. Motta, C. Aubry et al., "Serine protease inhibitors protect better than IL-10 and TGF-β anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci," *Microbial Cell Factories*, vol. 14, no. 1, article 26, 2015.

[61] S. Caluwaerts, K. Vandenbroucke, L. Steidler et al., "AG013, a mouth rinse formulation of *Lactococcus lactis* secreting human Trefoil Factor 1, provides a safe and efficacious therapeutic tool

for treating oral mucositis," *Oral Oncology*, vol. 46, no. 7, pp. 564–570, 2010.

[62] K. Vandenbroucke, W. Hans, J. Van Huysse et al., "Active delivery of trefoil factors by genetically modified *Lactococcus lactis* prevents and heals acute colitis in mice," *Gastroenterology*, vol. 127, no. 2, pp. 502–513, 2004.

[63] J. P. Motta, L. Magne, D. Descamps et al., "Modifying the protease, antiprotease pattern by elafin overexpression protects mice from colitis," *Gastroenterology*, vol. 140, no. 4, pp. 1272–1282, 2011.

[64] T. Saraiva, K. Morais, V. Pereira et al., "Milk fermented with a 15-lipoxygenase-1-producing *Lactococcus* lactis alleviates symptoms of colitis in a murine model," *Current Pharmaceutical Biotechnology*, vol. 16, no. 5, pp. 424–429, 2015.