Oral administration of *Lactococcus lactis* expressing recombinant 15-lipoxygenase-1 (15 LOX-1) modulates chemically induced colitis in mice

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Abstract

Background: Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are characterized by extensive inflammation due to dysregulation of the innate and adaptive immune system whose exact etiology is not yet completely understood. Currently there is no cure for IBD, thus the search for new molecules capable of controlling IBD and their delivery to the site of inflammation are the goal of many researchers. The aim of this work was to evaluate the anti-inflammatory effect of the oral administration of a *Lactococcus* (*L.*) *lactis* strain producing 15-lipoxygenase-1 (15-LOX-1) using a dextran sodium sulfate (DSS)-induced IBD mouse model.

Methods: The anti-inflammatory strain *L. lactis* NCDO 2118 was modified to produce active 15-LOX-1 and tested in a DSS-induced IBD mouse model.

Results: The 15-LOX-1 produced by *L. lactis* was effective in the prevention of inflammatory bowel disease associated damage in the murine model and decreased pro-inflammatory cytokines such as IFN-γ and IL-4 while increasing the anti-inflammatory cytokine IL-10. *L. lactis* NCDO 2118 acts not just as a tool for delivery of 15-LOX-1 in the inflamed gut mucosa but still retains its anti-inflammatory effects.

Conclusions: This strain could be used in novel adjunct IBD treatment protocols.

Keywords colitis; inflammation; lactic acid bacteria; Lactococcus lactis; lipoxygenase; imunomodulation.

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1. Background

Inflammatory bowel diseases (IBD) are chronic, relapsing, immunologically mediated disorders confined to the gastrointestinal tract. The prevalence of IBD rapidly increased in Europe and North America in the second half of the Twentieth century and they are becoming more common worldwide [1]. The two main IBD are ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by inflammation that is limited to the colon while CD can affect any part of the gastrointestinal tract (GIT) [2, 3]. The etiology and pathogenesis of IBD are not fully understood, however, it is believed that IBD is caused by a dysfunctional interaction between the gut microbiota and the mucosal immune system, in genetically predisposed individuals [2].

Current IBD treatments, which include the use of anti-inflammatory drugs, are not curative and only partially induce and maintain remission. Their use is often accompanied by several side effects such as allergic reactions, pruritis, chills, fever, urticaria and liver problems [4, 5]. In this context, probiotics with anti-inflammatory properties have been proposed as an alternative in the prevention or treatment of IBD [6].

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [7] and, a growing number of studies have been demonstrated their positive effects in IBD mice models and also in human clinical trials [8]. The vast majority of probiotics currently used belong to lactic acid bacteria (LAB) group, mainly species of *Lactobacillus* and *Bifidobacterium* genus. LAB have been extensively explored for these purposes principally because they have been safely ingested for centuries by human beings in fermented foods, the reason for which they have been recognized as being generally regarded as safe (GRAS) by the U.S. Food and Drug Administration.

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Since several LAB strains are considered as probiotics, many of them have been genetically engineered in order to improve their intrinsic characteristics. *Lactococcus* (*L.*) *lactis*, the model LAB, appears as a good choice for the production of recombinant proteins with animal and human health importance due to the fact that it is a microorganism easy to handle and it has a large number of genetic tools available for cloning and expression of heterologous proteins [9, 10]. Moreover, *L. lactis* does not produce endotoxins or other toxic metabolic product [11]. That is why nowadays *L. lactis* is currently being used to express biotechnological molecules, especially bacterial and viral antigens [12].

The first work using genetically modified (GM) *L. lactis* aimed to treat IBD was published more than 10 years ago. It was showed that administration of *L. lactis* producing the anti-inflammatory cytokine IL-10 prevented the onset of colitis in IL-10 knockout mice and ameliorates the symptoms of colitis in a chemically induced IBD mice model [13]. Since then, others GM *L. lactis* strains producing active molecules with role in the pathogenesis of IBD have been constructed and tested. In this context, a *L. lactis* strain producing superoxide dismutase (SOD), an antioxidant enzyme able to neutralize the reactive oxygen species, showed anti-inflammatory effects in a mice model of IBD [14]. Motta et al. demonstrated that *L. lactis* producing Elafin (protease inhibitor possessing anti-inflammatory effects) decreased inflammation and restored gut homeostasis in different models of IBD, *in vivo* [15].

These works inspired us to construct a new *L. lactis* strain producing 15-lipoxygenase-1 (15-LOX-1). 15-LOX-1 plays a major role in the oxidative metabolism of polyunsaturated fatty acids (PUFAs) [16]. 15-LOX-1 oxidative metabolism of PUFAs catalyzes the formation of innumerous anti-

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inflammatory mediators, such as lipoxins, resolvins and protectins [17]. Several reports support an anti-inflammatory role for 15-LOX-1. Mangino et al. showed that mice treated with a selective 15-LOX-1 inhibitor (PD 146176) experienced significantly worse intestinal function during experimental colitis [18]. 15-LOX-1 downregulation is correlated with IL-1β upregulation, in human colorectal cancer [19]. It is known that the pro-inflammatory cytokine IL-1β plays a significant role in IBD pathogenesis [20]. 15-LOX-1 is also able to activate peroxisome proliferator receptor (PPAR)-γ [21]. PPAR-γ is a member of a nuclear receptor family and has been proposed as a therapeutic target in IBD due its ability to inhibit NF- κB pathway [22]. Moreover, Altmann et al. demonstrated that 13-Oxo-ODE, a metabolic mediator of 15-LOX-1, is an endogenous ligand for PPAR-γ in human colonic epithelial cells [23]. The aim of the present work was to evaluate the therapeutic role of 15-LOX-1-producing *L. lactis* in dextran sulfate sodium (DSS) induced colitis in mice.

2. Methods

2.1. Bacterial strains, plasmids and growth conditions

Bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* strains were grown in Luria-Bertani (LB) medium with shaking at 37°C during 18 hours. Where appropriate, the medium was supplemented with 100 μg/ml of ampicillin (Amp). *Lactococcus lactis* was grown in M17 medium supplemented with 0.5% glucose (GM17) or 1% xylose (XM17) at 30°C without agitation for a period of 20 hours. When necessary, the medium was supplemented with 10 μg/mL of chloramphenicol (Cm). Strains constructed in this work were frozen and placed in a - 80°C Ultra freezer.

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Table 1: Bacterial strains and plasmids used

Strain	Relevant characteristics	Source
Escherichia coli	TOP10 (*hsd*R; *mcr*A; *lac*ZΔM15; *end*A1; *rec*A1)	Invitrogen
L. lactis NCDO2118[a]	*L. lactis* subsp. l*actis*	LGCM
Escherichia coli 83 [b]	TG1 [*supE*, *hsd*, Δ5, *thi*, Δ*lac-proAB*), F' (*traD36* *proAB*-*lacZ*Δ *M*15)] Harboring p*XylT*:CYT:*nuc* plasmid	LGCM
Plasmids		
pLB202*15lox-1*	Plasmid containing the ORF from 15-lox-1	Lowrie et al., 1997
pCR®-Blunt II- TOPO ®	Cloning vector/Zeo^r-Km^r/pUC ORI	Invitrogen
pTP:c*15-lox-1*	pCR®-Blunt II-TOPO® vector containing the cytoplasmic form of 15-lox-1	This work
p*XylT*:CYT:*15lox-1*	pWV01/Cm^r; expression vector containing the fusion rbsUsp45::15-lox-1, under the control of PxylT	This work

[a]: *L. lactis* wild type strain from LGCM (Laboratory of Cellular and Molecular Genetics) strain collection.

[b]: *Escherichia coli* TG1 strain carrying pXylT:CYT:nuc plasmid from LGCM (Laboratory of Cellular and Molecular Genetics) strain collection.

2.2. Cloning and expression of recombinant 15 human LOX-1

The 15 human LOX-1 coding sequence containing 1989 bp was amplified by PCR from pLB202:15-LOX-1 plasmid. Primers containing one artificial restriction site at each end (*Nsi*I and *Eco*RI) were constructed based on the

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sequence of the Human 15-lipoxygenase mRNA (accession number "GenBank" M23892). Following PCR, amplicons corresponding to the cytoplasmic form of the 15-LOX-1 were purified and ligated into Zero Blunt®TOPO® vector generating the intermediate plasmid pTP:*c15lox-1*. Aliquots of the ligation reaction were electroporated into commercial *Escherichia coli* TOP10 and seven positive clones were selected out. In order to confirm the presence of c15-LOX-1 into pTP:*c15lox-1* vector, the colony was inoculated into 5 ml of LB medium containing Amp 100 µg/ml and culture was used for plasmid preparation using the Wizard ®PlusSV Minipreps DNA Purification System (Promega) kit. Insert was then amplified by PCR and digested with Nsi and EcoRI restriction endonucleases. The nucleotide sequence was determined using Big Dye Terminator Sequencing Ready Reaction kits (Applied Biosystems) and material was sequenced in ABI3130 (Applied Biosystems) sequencer. The sequence was then compared using CAP3 Sequence Assembly Program, and the final contig was analyzed using two BLAST (Basic Local Alignment Search Tool) programs, BLASTn and BLASTx. Digested insert, *c15lox-1* from pTP:*c15lox-1* vector, were purified and inserted between the *Nsi*I and *Eco*RI restriction sites into "p*Xyl*T:CYT" backbone. This backbone were previously purified from *Nsi*I/*Eco*RI cut of p*Xyl*T:CYT:*nuc* vector. The final plasmid, p*Xyl*T:CYT:*15lox-1*, was introduced in *Lactococcus lactis* NCDO2118 competent cells. The presence of this expression vector carrying *c15-LOX-1* was confirmed after plasmid extraction following manufacturer's specifications (Wizard ®PlusSV Minipreps DNA Purification System, Promega), PCR and *in silico* sequencing analysis.

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2.3. Induction and protein expression of 15 human LOX-1 by *L. lactis* strains

Single colonies from *L. lactis* carrying p*Xyl*T:CYT:*15lox-1* plasmid and wild type strain were separately inoculated into 5 ml of GM17 medium supplemented or not with chloramphenicol. Cultures were diluted in M17 medium containing glucose or xylose; the last sugar required for 15-LOX-1 expression. Cultures were centrifuged (7 min. 4°C and 13,000 x g) at optical density (OD600nm) of 2.0, and both pellet and supernatant were collected for subsequent protein extraction. Supernatant and pellet were treated separately according to the protocol established by Le Loir and colleagues (Le Loir et al., 1998). Supernatant was filtered using a 0.22-µm filter (Corning), the filtrate was mixed with trichloroacetic acid (TCA, 100%), 10mM of dithiothreitol (DTT) (USB) and protease inhibitor cocktail (Sigma). Microtubes were incubated on ice for one hour, centrifuged for 20 minutes at 4°C/13,000 x g and pellet was resuspended in 50 mM NaOH (USB). Bacterial pellet was resuspended in 100 µL of TES-Lys buffer (25% sucrose, 1 mM EDTA, 50 mM Tris-HCl, pH 8.0, 10 mg/mL lysozyme) containing protease inhibitor cocktail (Sigma) and 10 mM DTT (USB). 20% of SDS was added to the mixture after 30 min. All materials were stored at -20°C for further analysis.

2.4. Western blot analysis

Proteins extracted from *L. lactis* were separated on denaturing polyacrylamide gels and analyzed by Western blotting according to Sambrook and Russell (2001). Proteins were transferred to PVDF membranes (Invitrogen) and then incubated with polyclonal mouse anti-lipoxygenase (1:100) (Novus Biologicals). Immunodetection blots were revealed using Western Breeze Chromogenic Western Blot Immunodetection®

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(Invitrogen) kit, following manufacturer's specification.

2.5. Animals

Female C57BL/6 mice were used for experimental colitis induced by dextran sodium sulfate (DSS). Animals were purchased from Federal University of Minas Gerais and kept under standard laboratory conditions, with free access to food and water. Experimental procedure was carried out according to the Ethical Principles in Animal Experimentation (CETEA/UFMG).

2.5.1. Induction of DSS colitis, oral treatment and measurement of disease activity

Experimental colitis was induced by adding to mice drinking water 1.5% of dextran sulfate sodium (DSS) (MP Biomedicals). In order to evaluate the therapeutic effect of *L. lactis* p*Xyl*T:CYT:*15lox-1* strain, C57BL/6 mice were divided into four groups containing five animals each. The control (CT) group (non-inflamed control) received water during 21 days; DSS group (inflamed control) was administered with DSS for 14 days, with an interval of four days receiving water; DSS+LL group was given DSS during seven days, after fresh GM17 medium with 5 x 10^9 *L. lactis* NCDO2118 wild type strain for 4 days and again DSS for seven days. The same DSS treatment described above was applied to DSS+LL-15LOX group, which was orally treated with the bacterial culture of *L. lactis* pXylT:CYT grown in XM17 (Figure 1A). It was measured the presence of occult blood in mice feces as well as the stool form and body weight loss of each mouse to determine the disease activity index (Table 2).

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Table 2: Disease activity index (DAI) of ulcerative colitis (UC) was analyzed with the weight loss and feces status. The weight loss of each mouse was calculated and scored.

Score	Body weight loss (%)	Diarrhea	Rectal Bleeding
0	None	Normal	None
1	1 to 5	__	__
2	5 - 10	Moderate	Occult
3	11 - 15	__	
4	> 15	Chronic	Visible

Colons, from the ileocecal junction to the anus, were removed, flushed with sterile PBS and one-third of each colon was carefully removed and fixed in formalin for histological analysis. Sections stained with hematoxylin and eosins were evaluated, based on the index previously described by Mccafferty and collaborators [32] (Table 3).

Table 3: Microscopic activity index for ulcerative colitis in mice

Activity	Classification
Crypt architectural distortion	0 quiescent, 1 mild, 2 moderate, 3 severe
Presence and degree of cellular infiltration	0 quiescent, 1 mild, 2 moderate, 3 severe
Extent of muscle thickening	0 quiescent, 1 mild, 2 moderate, 3 severe
Presence or absence of crypt abscesses	0 quiescent, 1 mild, 2 moderate, 3 severe
Depletion of goblet cells	0 quiescent, 1 mild, 2 moderate, 3 severe

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2.5.2. Cytokine and Secretory IgA measurement by ELISA

IL-6, IL-10, IFN-γ and IL-17A levels were determined in tissue extracts by ELISA [33] using cytokine detection Kit (BD Pharmingen, San Diego, CA, USA), according to the manufacturer's instructions. Secretory IgA titers were measured in mice fecal samples by ELISA as well using a commercial kit (Southern Biotechnology, Birmingman, AL). OD readings were determined at 405/492 nm (Bio-Rad Model 450 Microplate Reader).

2.6. Statistical analysis

Statistical analyses were performed using the GraphPad Prism 5.0 software (San Diego, CA, USA) and all results were expressed as mean ± standard deviation (SD). Significance of differences among groups was assessed by Student's t-test or analysis of variance (ANOVA) followed by a Tukey comparison post-hoc test. Means were considered statistically different when $p < 0.05$.

3. Results

3.1. *L. lactis* is able to express the cytoplasmic form of human *15lox-1*

The first step of this work was to amplify by PCR the open reading frame (ORF) of *15lox1* from pcDNA3:*15lox1* plasmid. The amplicon of approximately 1978 base pairs corresponding to the sequence encoding the cytoplasmic form of *15lox1* was cloned into pCR®-Blunt II-TOPO® plasmid, generating the intermediate vector pTP:c*15lox1*. This vector was then successfully transformed in *E. coli* TOP10 electrocompetent cells. Five positive colonies, which were confirmed by PCR analysis as harboring the insert, had their DNA extracted in order to purify pTP:*c15lox1* plasmid. *C15lox-1* was correctly excised from pTP:*c15lox1* using restriction enzymes (*Nsi*I e *Eco*RI), purified and ligated into "p*Xyl*T:CYT" purified backbone. The final vector, p*Xyl*T:CYT:*15lox1* (Figure 1A), was properly inserted in both *E. coli* TOP10 and *L. lactis* NCDO2118

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electrocompetent cells. Clones containing the recombinant vector were confirmed by restriction enzymes digestion, PCR analysis and *in silico* DNA sequencing (data not shown).

Expression of recombinant 15LOX-1 (r15LOX-1) by *L. lactis* recombinant strain was then evaluated. In order to do that bacterial culture was grown in the presence of xylose, to induce the expression of recombinant r15LOX-1. As negative control, culture was also grown in the presence of glucose, a sugar that represses p*XylT* promoter. Total protein was extracted to assess the capacity of *L. lactis* recombinant strain to correctly address r15LOX-1 using XIES expression system. The identification of r15LOX-1 was accomplished by either SDS-PAGE or immunoblotting experiments (Fig. 1B and C respectively). A protein fragment of 74.6 kDa was detected, which is the expected molecular weight for 15LOX-1, for proteins extracted from the induced *L. lactis* recombinant culture. No signals were visualized for proteins extracted from the non-induced culture. These results demonstrate that *L. lactis* is able to efficiently produce recombinant human 15-LOX-1 in its cytoplasmic compartment (Fig. 1B, C).

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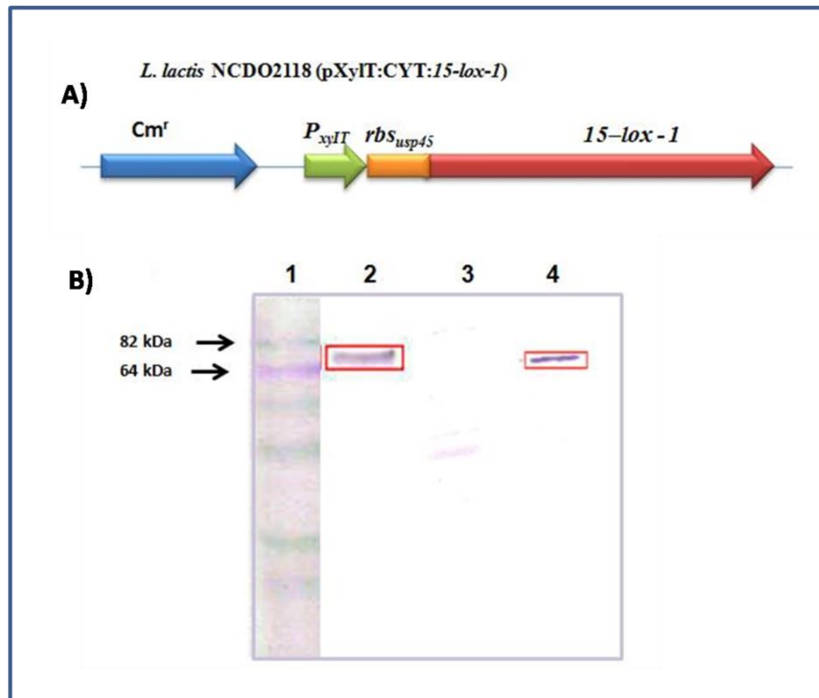

Figure 1: (A) Schematic representation of XIES expression vector used for heterologous expression of r15LOX-1 in *L. lactis*. P*xylT*: Promoter induced by xylose. rbsUsp45 and spUsp45: Ribosome binding site and the signal peptide of lactococcal protein Usp45 respectively. 15LOX-1: ORF from human 15LOX-1. Cmr: Gene which confers resistance to chloramphenicol**. (B)** Immunodetection of r15LOX-1 from induced and non-induced *L. lactis* pCYT:*15lox-1* culture. Lane 1: Pre-Stained Protein Ladder (Invitrogen); Lane 2: Commercial 15LOX-1 protein; Lane 3: Cytoplasmic protein fraction from non-induced culture; Lane 4: Cytoplasmic protein fraction from induced culture. Red rectangle highlights the protein fragment referring to human 15LOX-1. Blot membrane was revealed using monoclonal mouse anti-human 15LOX-1 (1:100).

3.2. Oral administration of *L. lactis* expressing 15-LOX-1 alleviates colitis symptoms

As described in the method section, *L. lactis* expressing 15-LOX-1 (LL-15LOX) was continuously fed to C57BL/6 mice for four consecutive days, during the remission period of colitis. This experimental protocol mimics the remission and active periods of IBD (Fig. 2a). Fig. 1b shows that body weight of mice significantly decreased during the second cycle of DSS treatment compared to the body weight of the other groups

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(control, DSS-LL and DSS-LL-15LOX groups). According to this, a reduction in colon length was also observed at day 21 in the DSS group but not in *L. lactis* (LL) or *LL* 15-LOX treated groups (Fig. 1c). Thus, oral treatment of mice with *L. lactis* or *L. lactis* expressing 15-LOX recovered their body weight and ameliorated the colon shortening. Despite that *L. lactis* decreased the clinical symptoms (macroscopic inflammatory score) of colitis, the recombinant *L. lactis*- 15LOX dramatically reduced the severity of the disease (Fig. 2d).

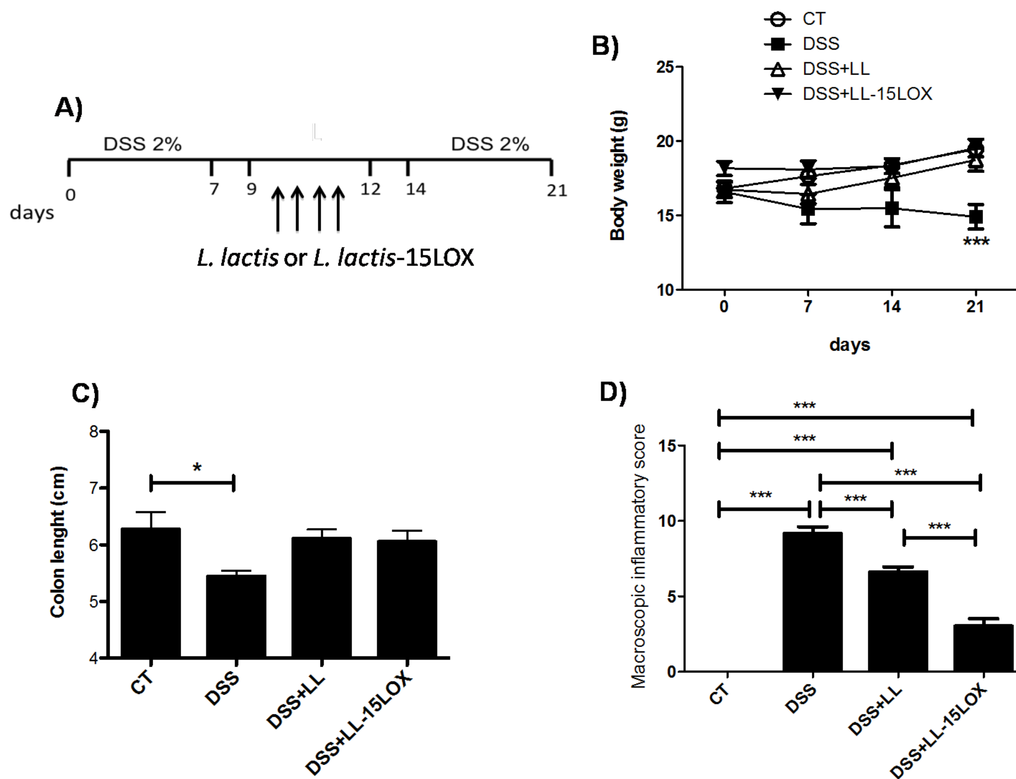

Figure 2: Oral administration of *L. lactis* expressing 15-LOX improved macroscopic score of colitis. **(A)** Experimental protocol. C57BL/6 mice received 1.5% DSS for 7 days. Native *L. lactis* (LL) *or L. lactis* expressing 15 LOX (LL-15LOX) was continually administered for 4 consecutive days during the remission period (arrows) between the first and second course of colitis. The control group received medium. Mice were sacrificed at day 21. **(B)** Body weight from day 0 to day 21. **(C)** Colon length measured in cm. **(D)** Macroscopic score of colitis, including scores related to body weight, diarrhea and rectal bleeding. Bars are the mean of 6 mice/group, and the data are representative of two independent experiments; ANOVA, Tukey post-test. *, $p<0.05$, ***, $p<0.001$.

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3.3. *L. lactis*-15 LOX prevents intestinal inflammation

In order to determine if native and engineered LAB could improve the intestinal inflammation, histological scores were devised to allow quantification of histological changes (Fig. 3a). Colon sections from mice of the DSS group showed severe inflammatory lesions throughout the mucosa and submucosa with areas of intense ulceration (arrow). *L. lactis* expressing 15-LOX presented a significant decrease of the damage score when compared to the DSS group.

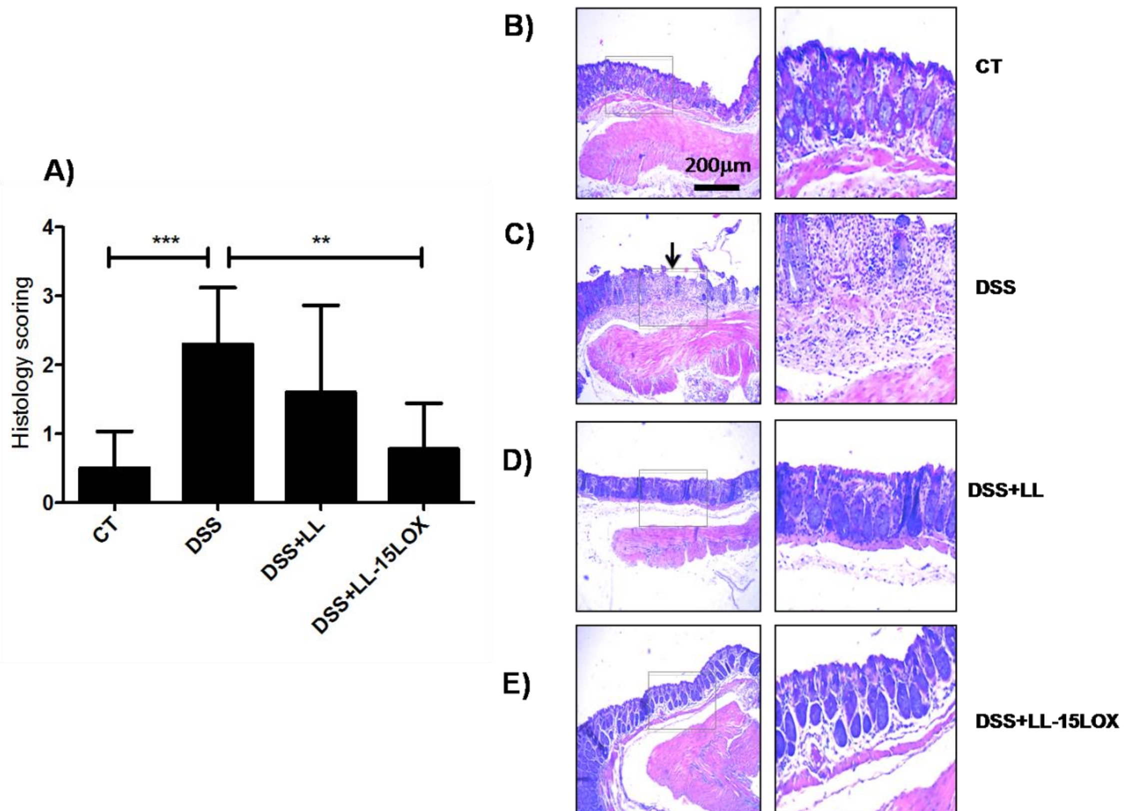

Figure 3. Oral administration of *L. lactis* expressing 15-LOX prevented histological damage induced by colitis. (A) Histological scores of colon sections of DSS-colitis mice with or without oral administration of *L. lactis* or *L. lactis*-15LOX. Values represent the means ± MSE (n=10). **, p<0.01,***, p<0.001. Photograph (X100) of H&E-stained paraffin sections of a representative colon from control **(B)**, DSS **(C)** DSS + LL **(D)** and DSS+ LL-15LOX groups at day 21**(E)**.

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3.4. Oral administration of *L. lactis* expressing 15-LOX is associated with decreased levels of pro-inflammatory cytokines

To address the mechanisms involved in colitis prevention by oral administration of *L. lactis* expressing 15-LOX, cytokine profiles in intestines were evaluated. As shown in Fig. 4, DSS significantly increased the levels of TNF-α, IL-6, IL-12, IFN-γ and IL-4 while reduced the levels of IL-17 and IL-10. Native *L. lactis* was able to maintain the levels of TNF-α, IL-12 and IL-10 at a level similar to that of the control group. IL-6 and IL-4 were not affected by *L. lactis* (Fig. 4b,4f). However, the oral treatment with *L. lactis* expressing 15-LOX-1 significantly decreased levels of INF-γ and IL-4, compared to both DSS and DSS-LL groups (Fig. 4d,f). *L. lactis* expressing 15-LOX-1 administration increased IL-6 and decreased IL-17 significantly compared to the control and the other test groups. Lastly, the sIgA level was increased in DSS-treated mice while the sIgA level in the native *L. lactis*-treated mice was maintained at a level similar to that of the control group. Oral administration of *L. lactis* expressing 15-LOX did not alter sIgA production (Fig. 4h), compared to the group that received the wild type strain.

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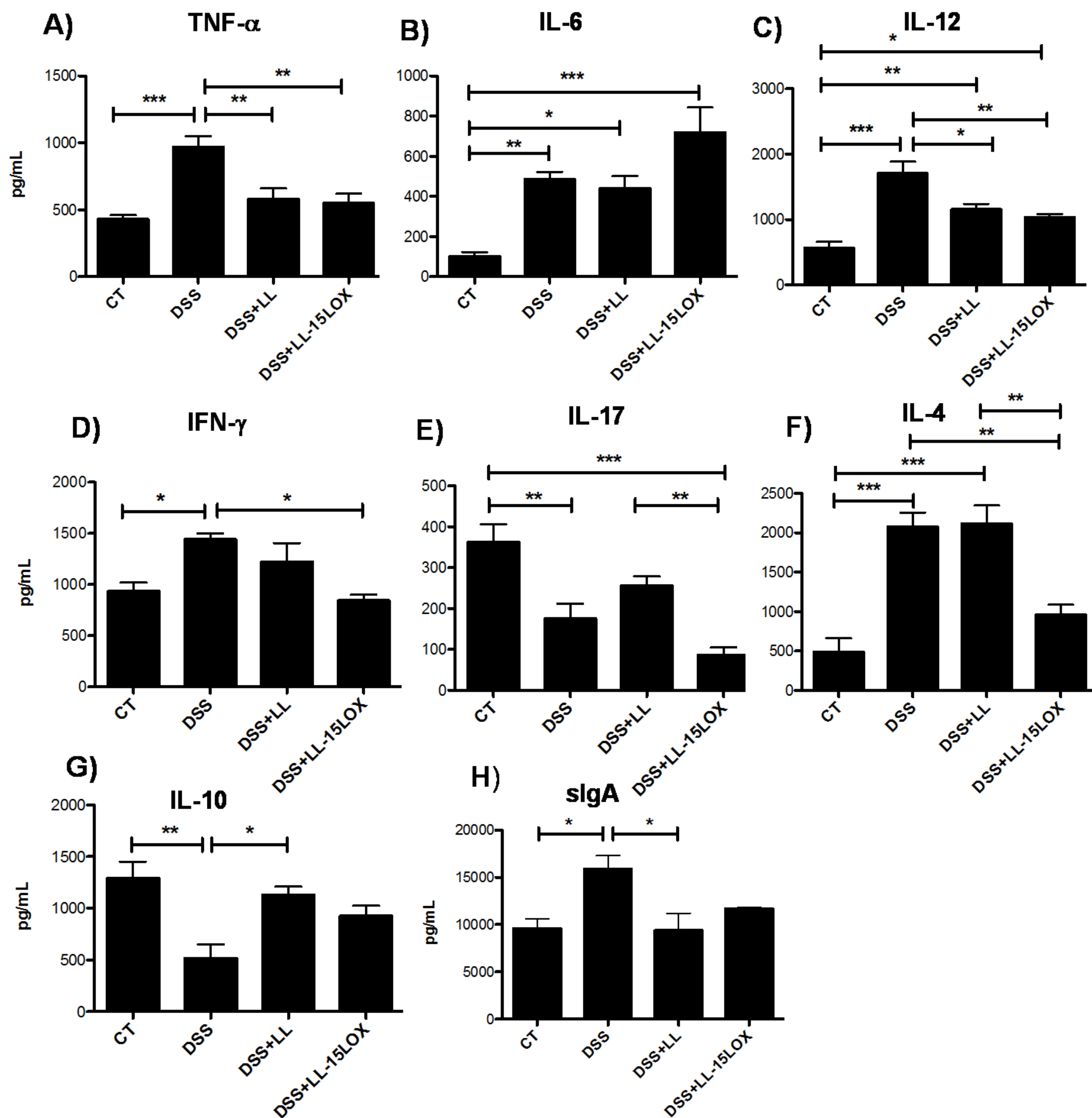

Figure 4. Effects of *L. lactis* expressing 15 LOX on cytokine production by colonic cells and in secretory IgA levels. Colonic TNF-α (**A**), IL-6 (**B**), IL-12 (**C**), INF-γ (**D**), IL-17 (**E**), IL-4 (**F**) and IL-10 (**G**) were measured by ELISA in mice from control, DSS, DSS+LL and DSS+LL-15LOX groups. Intestinal feces were collected and total sIgA was measured by ELISA (**H**). One representative result from two independent repetitions is shown. Bars represent the mean ± MSE of 5 mice per group. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

4. Discussion

15-Lipoxygenase is an inducible and highly regulated lipid-peroxidative enzyme whose expression and arachidonic acid metabolites are implicated in several important inflammatory conditions

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including airway, glomerular inflammation, atherosclerosis and colitis [18, 24, 25]. Studies demonstrated that 15-LOX-1 significantly contributes to resolution of inflammation as well as in the maintenance of self-tolerance [26, 27].

Here, the lactic acid bacteria *Lactococcus lactis* was genetically modified to express 15-LOX-1. Although the exact mechanism by which 15-LOX-1 gain the gut mucosa is unknown, it is possible that the enzyme is slowly released in the inflamed gut mucosa after its bacterial host lysis. Therefore, 15-LOX-1 could increase the local enzymatic activity in the large intestine to produce the anti-inflammatory mediators.

In our study, we choose administrated *L. lactis* expressing 15-LOX-1 after the onset of colitis due to two reasons. First of all, because this experimental protocol resembles the clinical scenario, as it is not possible to predict when the disease will start or when it will become active. Second, because the enzyme 15-LOX-1 catalyzes lipid peroxidation of arachidonic acid and an inflammatory stimulus is necessary to activate the cascade.

Recently, we identified *Lactococcus lactis* NCDO2118 as a new probiotic strain with a potential role in the treatment of IBD [28]. Thus, our intention was to associate the probiotic capacity of *L. lactis* NCDO2118 with the ability of the enzyme 15-LOX-1 to generate anti-inflammatory mediators such as lipoxins and resolvins. At the macroscopic level, administration of native *L. lactis* improved body weight loss, colon shortening and the clinical score as previously published by our group [28], without significant differences with the genetically modified strain. However, at the histological level, mice treated orally with *L. lactis*-15LOX-1 significantly reduce the tissue damage induced by DSS, demonstrating its enhanced anti-inflammatory potential.

The most abundant immunoglobulin produced at the mucosal sites is IgA in its

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secretory form. Gut secretory IgA (sIgA) is bound to a secretory component that protects the molecule from digestive enzymes and maintains its activity in the mucus layer that recovers luminal epithelial cells. In the luminal mucous layer, sIgA protects the intestinal epithelium against colonization and/or invasion of pathogens or commensals. Our results showed that administration of DSS significantly increased the s-IgA levels, similarly to results obtained by other authors where the induction of colitis with 2% DSS was associated to increases in the number of IgA-containing cells in the colons of the mice, at differences to the use of 5% DSS that was associated to decreases of these cells, suggesting that the model using the first doses reflects the behavior of B cells in human quiescent colitis [29]. In the present work, we observed that both *L. lactis* strains were able to maintain the sIgA levels as found in the control group; however, the lack of significant differences between the groups that received the wild type strain and the GM strain discards the possibility that IgA modulation might be a regulatory mechanism mediated by the production of 15LOX by the *L. lactis*.

Since cytokines are major mediators of inflammation and regulatory activity in the gut mucosa, we analyzed the ability of *L. lactis*-15LOX-1 to modulate the production of cytokines in colonic tissues. TNF-α, IL-6, IL-12, INF-γ, IL-4 were the cytokines that we have found to be significantly increased in the colonic tissue after DSS administration when compared with non-inflamed control mice. It is well accepted that TNF-α, IL-6, and IL-12 are major cytokines involved in the acute inflammation induced by DSS, and this profile switch to a Th2-mediated inflammatory response (with increase in IL-4 and IL-10) when the disease is in a chronic state [30]. Despite the role of lymphocytes are less documented in this colitis experimental model, Dieleman et al. (1998) showed that chronic DSS-

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induced colitis is characterized by focal epithelial regeneration and a Th1 and Th2 cytokine profile [31]. It is possible that chronic immune activation mediated by both populations of Th cells can interfere with colonic healing and can play a role in the pathogenesis of chronic colitis. The results obtained in this study showed that the administration of the wild type *L. lactis* induced an anti-inflammatory response by decreasing mainly TNF-α and IL-12 production; cytokines that can be related to macrophage infiltration in the colonic tissues [29]. Interestingly, oral administration of *L. lactis* expressing 15-LOX-1 not only maintained this anti-inflammatory effect but also decreased other cytokines that were incremented in the inflamed control, which are mostly related to T cells, such as IFN-γ and IL-4. Both are cytokines typically produced by polarized lymphocytes Th1 and Th2, respectively. We speculate that the enzyme 15-LOX-1 delivered by *L. lactis* could interfere in the production of those inflammatory cytokines due to the production of lipoxins and protectins. Actually, LXA_4 and PD1 inhibited inflammatory cytokine secretion from T lymphocytes. Moreover, PD1 are produced during epithelial injury and mediate wound repair in addition to counteracting inflammation.

Another immune modulation associated to *L. lactis* administration (independently of the use of wild type or GM strain) was the increases of the anti-inflammatory IL-10 to values similar to the ones observed in the non-inflamed control.

5. Conclusions

The lactic acid bacteria *Lactococcus lactis* was genetically modified to express 15-LOX-1. Mice treated orally with *L. lactis*-15LOX-1 significantly reduce the tissue damage induced by DSS, demonstrating its enhanced anti-inflammatory potential and also decreased other cytokines that were incremented in

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the inflamed control, which are mostly related to T cells, such as IFN-γ and IL-4.

Thus, *L. lactis* act not just as a tool to delivery 15-LOX-1 in the inflamed gut mucosa but still had anti-inflammatory effects. The nature of this *L. lactis* NCDO 2118 (host strain) components that are responsible for its anti-inflammatory effects is under investigation.

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Authors' contributions:

RDOC, KM, VBP, ACG, TDL, MA, CSR, CCP and DCC performed the experiments consisting of the construction of the strain, the evaluation of 15-lox production and evaluation of the 15-lox-1 producing strain in a rodent model. AMCF, LBH, HB, PL, AdMdL, JGL, AM and VA participated in the elaboration of the project, obtaining the funding and all authors participated in the evaluation of the experimental data and writing of the manuscript.

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