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Evaluation of the effect of soymilk fermented by a riboflavin-producing *Lactobacillus plantarum* strain in a murine model of colitis

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RESEARCH ARTICLE

Abstract

Inflammatory bowel diseases (IBD) are idiopathic diseases of the gastrointestinal tract characterised by recurrent inflammation that require lifelong treatments. It has been shown that certain strains of lactic acid bacteria (LAB) can produce specific health-promoting compounds in foods or in the gastrointestinal tract that can in turn prevent and/or treat IBD. This study was designed to evaluate the possible therapeutic potential of soymilk fermented by the riboflavin-producing strain *Lactobacillus plantarum* CRL 2130 in a trinitrobenzene sulfonic induced colitis mouse model. Mice that received soymilk fermented by *L. plantarum* CRL 2130 showed a decrease in weight loss, lower damage scores in their large intestines, lower microbial translocation to liver and decreased cytokines levels in their intestinal fluids compared to animals that received unfermented soymilk or soymilk fermented by a non-riboflavin-producing *L. plantarum* strain. This is the first report that demonstrates that a riboflavin-producing LAB was able to prevent experimental colitis in a murine model.

Keywords: vitamin production; lactic acid bacteria; inflammatory bowel disease

1. Introduction

Inflammatory bowel diseases (IBD) are chronic, progressive, and even destructive because they can cause irreversible structural damages resulting in the loss of intestinal function. Although the exact aetiology of IBD remains unclear, it is multifactorial. Environmental factors (i.e. lifestyle, diet), as well as infectious, immunological, and psychological causes, together with genetic susceptibility could be some of the major causes. The dysbiosis of the intestinal microbiota has also been shown to be a key factor for IBD development (De Moreno de LeBlanc and LeBlanc, 2014). Although the prevalence and incidence of IBD is continuously increasing especially in developed countries (affecting 150-250/100,000 population), they are rarely fatal. However, they negatively influence the quality of life of the patients (Moura *et al.*, 2015). Conventional IBD therapies can prevent and reduce mucosal inflammation and are based on the use of aminosalicylates, corticosteroids, immunosuppressive compounds, and antibiotics. These drugs must be taken throughout life of IBD patients and can cause adverse side effects, such as gastrointestinal problems, mucositis, anaemia, carcinogenesis, hepatotoxicity, nephrotoxicity and hypersensitivity reactions (Meyer *et al.*, 2015). In addition, some patients do not respond to current treatment protocols. Therefore, novel alternative therapeutic options for treating IBD must be developed.

Riboflavin (vitamin B_2) plays an essential role in cellular metabolism, being the precursor of the coenzymes flavin mononucleotide and flavin adenine dinucleotide, and it is therefore required by all flavoproteins, such as glutathione reductase which protects cells from harmful effect of reactive oxygen species (ROS). In addition, riboflavin on its own can work as an antioxidant by scavenging ROS. Oral administration of riboflavin prevented carbon tetrachloride induced liver damage in rats causing the normalisation of serum hepatic enzymes (aspartate transaminase, alanine

transaminase and alkaline phosphatase) and oxidant parameters in liver (glutathione and malondialdehyde). This vitamin intake also inhibited the release of the pro-inflammatory cytokine tumour necrosis factor (TNF)-α from rat leukocytes (Al-Harbi *et al.*, 2014).

Lactic acid bacteria (LAB) are a heterogeneous group of Gram-positive microorganisms that are technologically important, both for food production and for its beneficial properties. Certain strains of LAB are able to produce, release and/or increase specific beneficial compounds in foods. These ingredients can be macronutrients, micronutrients or non-nutritive compounds and can be naturally present in certain foods or added during processing (Arena *et al.*, 2016; LeBlanc *et al.*, 2011a; Russo *et al.*, 2016). In recent years, the use of LAB for vitamin synthesis was proposed to obtain fermented bio-enriched foods. In this context, some LAB strains have been described to be able to synthesise B-group vitamins, particularly riboflavin and folates (LeBlanc *et al.*, 2011a; Russo *et al.*, 2014; Thakur *et al.*, 2015). It was previously shown by our group that *Lactobacillus plantarum* CRL 2130 was able to significantly increase the initial concentration of riboflavin in aqueous soy extract, commonly referred to as soymilk (Juarez del Valle *et al.*, 2014). This fermented soymilk was shown to be able to revert and prevent arriboflavinosis in a mouse model (unpublished data). The use of riboflavin producing LAB is considered a valid alternative and a more natural approach to the obligatory fortification of foods with chemically purified forms of the vitamin.

Therefore the aim of this study was to evaluate the anti-inflammatory/anti-oxidant effect of a soymilk fermented by the riboflavin producing strain *L. plantarum* CRL 2130 in the prevention/treatment of a trinitrobenzene sulfonic (TNBS)-induced colitis murine model.

2. Materials and methods

Preparation of soymilk

Aqueous soy extract was obtained as described previously (Juarez del Valle *et al.*, 2014). Briefly, whole soy beans obtained from local market were soaked overnight in distilled water. The outer seed coat was removed and blended in a kitchen blender (Home Electric TS-696, China) with 1 volume of distilled water to get a thick paste. The slurry was then cooked with 3 volumes of water at 80 °C for 15 min, after which 2 additional volumes of water were added. Then, soymilk was filtered using a double layered cheese-cloth and autoclaved (10 min at 121°C).

Bacterial strain and fermentation of soymilk

The strain of *L. plantarum* used in this study belongs to the Culture Collection of CERELA (Centro de Referencia para Lactobacilos, CONICET, San Miguel de Tucumán, Argentina). *L. plantarum* CRL 2130, previously known as *L. plantarum* CRL 725 roseoflavin resistant variant (G), is a high riboflavin producing strain able to produce 1.6 mg/ml of this vitamin in soymilk (Juarez del Valle *et al.*, 2014). *L. plantarum* CRL 691 is a strain that does not produce riboflavin and was used as a negative control. Before use, the bacteria were grown for 16 h at 30 °C without agitation in 5 ml of riboflavin-free culture medium (Riboflavin Assay Medium; Difco, Sparks, MD, USA) for *L. plantarum* CRL 2130 or of De Man-Rogosa-Sharp (MRS; Britania, Buenos Aires, Argentina) for *L. plantarum* CRL 691 because this latter strain requires riboflavin for its growth. Then, 5 ml of the freshly grown culture were harvested by centrifugation (5,000×*g*, 8 min, at room temperature), washed twice with saline solution (0.85% w/v NaCl) to remove all extracellular contaminants (such as vitamins including riboflavin) and resuspended in this solution at the original volume in saline solution. Cell suspensions were inoculated into sterile soymilk at a calculated initial optical density (OD_{600}) of 0.2 (equivalent to 2×10^7 cfu/ml), and incubated statically at 30 °C during 12 h. Unfermented soymilk was also used for the animal assays. This milk was acidified to pH 4.5 (the same obtained after bacterial fermentation) by adding lactic acid.

Animal model of colitis

Conventional female BALB/c mice weighing 20-25 g (six week old) were used. The animals were obtained from the inbred closed colony maintained at CERELA. They were housed under normalised conditions (12 h light/ 12 h darkness cycle and temperature 18-20 °C). Mice received commercial riboflavin-free diet (Dyets, Bethlehem, PA, USA) (in order to avoid interference by exogenous riboflavin) and different soymilk (fermented or unfermented) *ad libitum* throughout the experimental period. Animal protocols were approved by the Animal Protection Committee of CERELA (CRL-BIOT-LT-2010/1A) and all experiments comply with the current laws of Argentina and all international organisations for the use of experimental animals.

Colitis was induced with TNBS as previously described (De Moreno de LeBlanc *et al.*, 2009). Briefly, mice were fully anesthetised with an intraperitoneal injection using a mix of ketamine hydrochloride (100 mg/kg body weight; Holliday-Scott S.A., Buenos Aires, Argentina) and xylazine hydrochloride (5 mg/kg body weight; Rompun, Bayer, Division Sanidad Animal, Buenos Aires, Argentina). Intestinal inflammation was then induced by intrarrectal instillation using a 4 cm length catheter. Each mouse received 100 ml of TNBS (Sigma, St. Louis, MO, USA) solution (2 mg/mouse) dissolved with 1:1 absolute ethanol and phosphate-buffered saline solution (PBS 0.01 M, pH 7.4). Mice from the control group (mock group) did not receive TNBS and they but were inoculated with the ethanol-PBS mixture.

The mock group (5 mice) received unfermented soymilk. Mice inoculated with TNBS were sub-divided into four experimental groups with ten animals in each group. TNBS+soy group received unfermented soymilk; TNBS+soy+CRL 2130 group received soymilk fermented by the riboflavin producing strain *L. plantarum* CRL 2130; TNBS+soy+CRL 691 group received soymilk fermented by the riboflavin auxotroph strain *L. plantarum* CRL 691; and TNBS+soy+B_2 group received unfermented soymilk supplemented with commercial riboflavin (1.6 mg/ml) in a concentration similar to the one obtained previously in the soymilk fermented by *L. plantarum* CRL 2130 (Juarez del Valle *et al.*, 2014).

Soymilk (fermented or unfermented) was changed daily in order to guarantee their qualities. The animals received fermented or unfermented soymilk three days before inflammation induction until the end of the experiment (four days post-TNBS) as a replacement of their drinking water in addition to their diet. Soymilk were given *ad libitum* in feeding dishes which were hung on top of the cages to prevent the animals from bathing and contaminating these supplements. The average daily intake was calculated and was each mouse consumed approximately 3 ml per day. Body weight and animal mortality rates were controlled daily. Five animals of each group were sacrificed at the end of the study and samples were collected. The experiment was repeated twice and the results from both individual trials (5 mice were sacrificed in each trial) were analysed together (n=10 for each group, considering that except for the live body weight measurement, 5 mice were sacrificed in each trial).

Assessment of colonic inflammation

To evaluate the extent of colonic damage and inflammation, large intestines were dissected, macroscopically examined and then fixed in formaldehyde solution (10% v/v in PBS). Macroscopic lesions were assessed using a previously described scoring system. Each parameter (erythema, haemorrhage, oedema, stricture formation, ulceration, faecal blood, presence of mucus, diarrhoea and adhesions) consisted of 1 point that were summed after visual tissue examination. For histologic analysis, tissues were embedding in paraffin and serial sections of 4 µm were made and stained with haematoxylin and eosin an observed by light microscopy. The degree of microscopic damage was assessed using a histopathological score described previously (Del Carmen *et al.*, 2013). Animals were then placed in different groups where animals were: (1) identical to normal mice (grade 0); (2) showed mild mucosal and / or submucosal inflammatory infiltrate (admixture of neutrophils) and oedema, punctate mucosal erosions often associated with capillary proliferation, muscularis mucosa intact (grade 1); (3) grade 1 changes involving 50% of the specimen (grade 2); (4) prominent inflammatory infiltrate and oedema (neutrophils usually predominating) frequently with deeper areas of ulceration extending through the muscularis mucosae into the sub mucosa; rare inflammatory cells invading the muscularis propria but without muscle necrosis (grade 3); (5) grade 3 changes involving 50% of the specimen (grade 4); (6) extensive ulceration with coagulative necrosis bordered inferiorly by numerous neutrophils and lesser numbers of mononuclear cells; necrosis extends deeply into the muscularis propria (grade 5); (7) grade 5 changes involving 50% of the specimen (grade 6).

To evaluate microbial translocation toward extra-gut organs, the liver was aseptically removed, weighed, and homogenised in 5.0 ml of sterile 0.1% (w/v) peptone solution. Serial dilutions of the homogenates were plated in triplicate in the following media MRS, MacConkey (Britania) and LAPTg (1% glucose, 1.5% peptone, 1% tryptone, 1% yeast extract and 0.1% Tween 80). Bacterial growth was evaluated after incubation at 37 °C for 48 to 72 h.

Determination of cytokines in the intestinal contents

Large intestines were removed and their contents collected with 500 µl of PBS containing Complete Mini EDTA-free Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Mannheim, Germany), centrifuged (3,000×*g*, 10 min, 4 °C), and the supernatants were stored at -80 °C until cytokines determinations. Cytometric bead array (CBA) Mouse Inflammation Kit (BD Bioscience, San Diego, CA, USA) was used to measure interleukin-6 (IL-6), IL-10, monocyte chemoattractant protein-1 (MCP-1), interferon-γ (IFN-γ), TNF-α and IL-12p70 levels, following the manufacturer's instructions. The concentration of each cytokine in the intestinal fluid of each mouse was obtained and the results were expressed as pg/ml.

Statistical analysis

Results were expressed as the mean values of n=10 ± the standard deviation (DS). Statistical analyses were performed using MINITAB 15 software (Minitab, State College, PA, USA). Comparisons were performed by an ANOVA general linear model followed by Tukey's post hoc test and $P<0.05$ was considered significant.

3. Results

Changes in the animal live weights

TNBS inoculation was associated to loss of body weight in all the groups when compared to the mock group; however, significant differences at the end of the experimental protocol were only observed in mice that received unfermented soymilk or soymilk fermented by the non-riboflavin-producing LAB strain CRL 691 (Figure 1). Mice that received soymilk fermented by the riboflavin-

producing strain (TNBS+soy+CRL 2130 group) showed lower weight loss than the animals from TNBS+soy group, and these differences were significant at the end of the experiment (day 4 post-TNBS). Mice from TNBS+soy+CRL 2130 group maintained percentages of initial body weight similar to the group given soymilk fortified with commercial B_2 (Figure 1). There was no significant difference in soy intake between the different experimental groups (data not shown).

Macroscopic and histologic analysis of large intestine

The severity of the colitis induced by TNBS was further evaluated by macroscopic and histologic examinations. Macroscopic observations of the large intestines showed a significant decrease (P<0.05) in the inflammation score of mice from TNBS+soy+CRL 2130 and TNBS+soy+B_2 groups compared to mice from the TNBS+soy and TNBS+soy+CRL 691 groups (Figure 2). These results were confirmed by histological evaluation. Mice from TNBS+soy+CRL 2130 group as well as those receiving commercial riboflavin (TNBS+soy+B_2 group) showed significantly lower intestinal damage scores compared to the TNBS+soy and TNBS+soy+CRL 691 groups (Figure 2).The intestines of the mice from TNBS+soy group showed prominent inflammatory infiltrates, altered architecture of the crypts, and blood vessel proliferation. Similar observations were obtained in the samples of mice from TNBS+soy+CR 691 group (Figure 2). Mice from TNBS+soy+CRL 2130 group showed no thickening of the mucosa muscular layer and higher presence of goblet cells which were depleted in animals from TNBS+soy group.

Microbial translocation to liver

The observed intestinal damage was associated with the results obtained from the microbial counts in the liver. Mice that received soymilk fermented by the riboflavin-producing strain (TNBS+soy+CRL 2130 group) showed a significant decrease (P<0.05) for the liver microbial growth in all evaluated media compared to microbial counts obtained from mice that received unfermented soymilk (TNBS+soy group). Mice that received the non-riboflavin-producing strain did not shown significantly different bacterial counts in MacConkey and LAPTg media, compared to the TNBS+soy group. No significant differences between TNBS+soy+CRL 2130 and TNBS+soy+B_2 groups were found (Figure 3).

Intestinal cytokine profiles

The assessment of the cytokines concentrations in the intestinal fluids were performed in the groups that received the riboflavin-producing strain in comparison with the mock group, and the mice from TNBS+soy and TNBS+soy+B_2 groups. The results showed that TNBS+soy group increased significantly (P<0.05) the levels of all the assayed cytokines compared to the mock group (Figure 4). Both the administration of soymilk fermented by the overproducing riboflavin strain (TNBS+soy+CRL 2130 group) and the supplementation with commercial riboflavin (TNBS+soy+B_2 group) significantly decreased the levels of cytokines, compared to mice from TNBS+soy group (Figure 4). Some cytokines that were analysed with the CBA kit

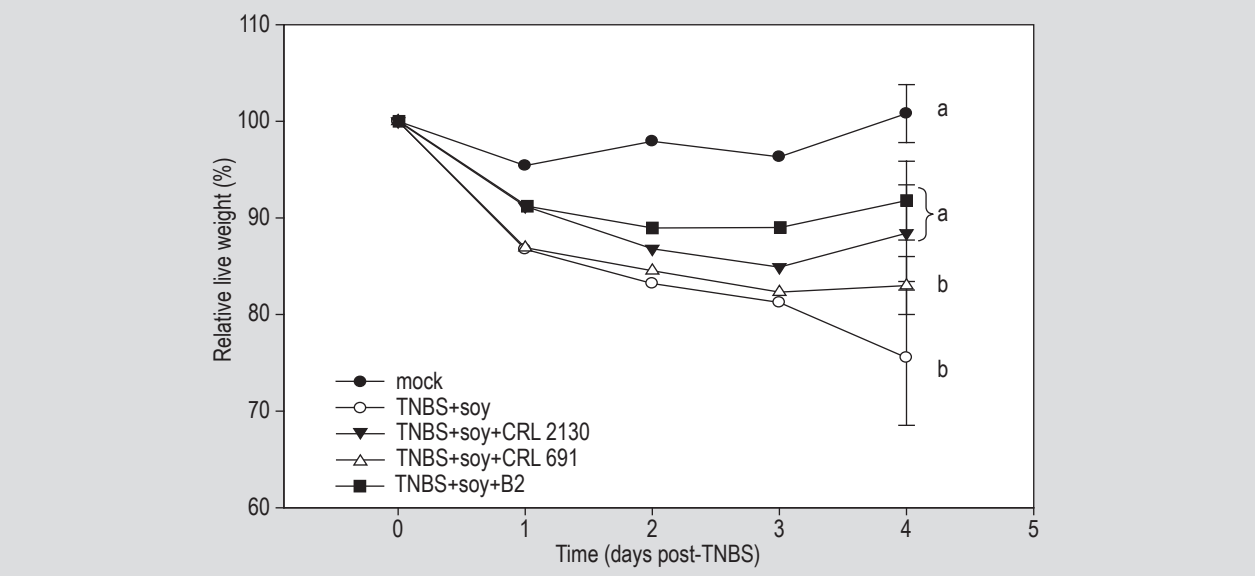

Figure 1. Soymilk fermented by the riboflavin producing lactic acid bacteria reduced the body weight loss in the trinitrobenzene sulfonic (TNBS) colitis model. Body weight is shown as the percentage of the initial mice body weight. Each value represents the mean of n=10 from 2 individual trials. Standard deviation is only shown at day 4 where significant differences between the groups were observed. [a,b,c] Means for each value without a common letter differ significantly (P<0.05).

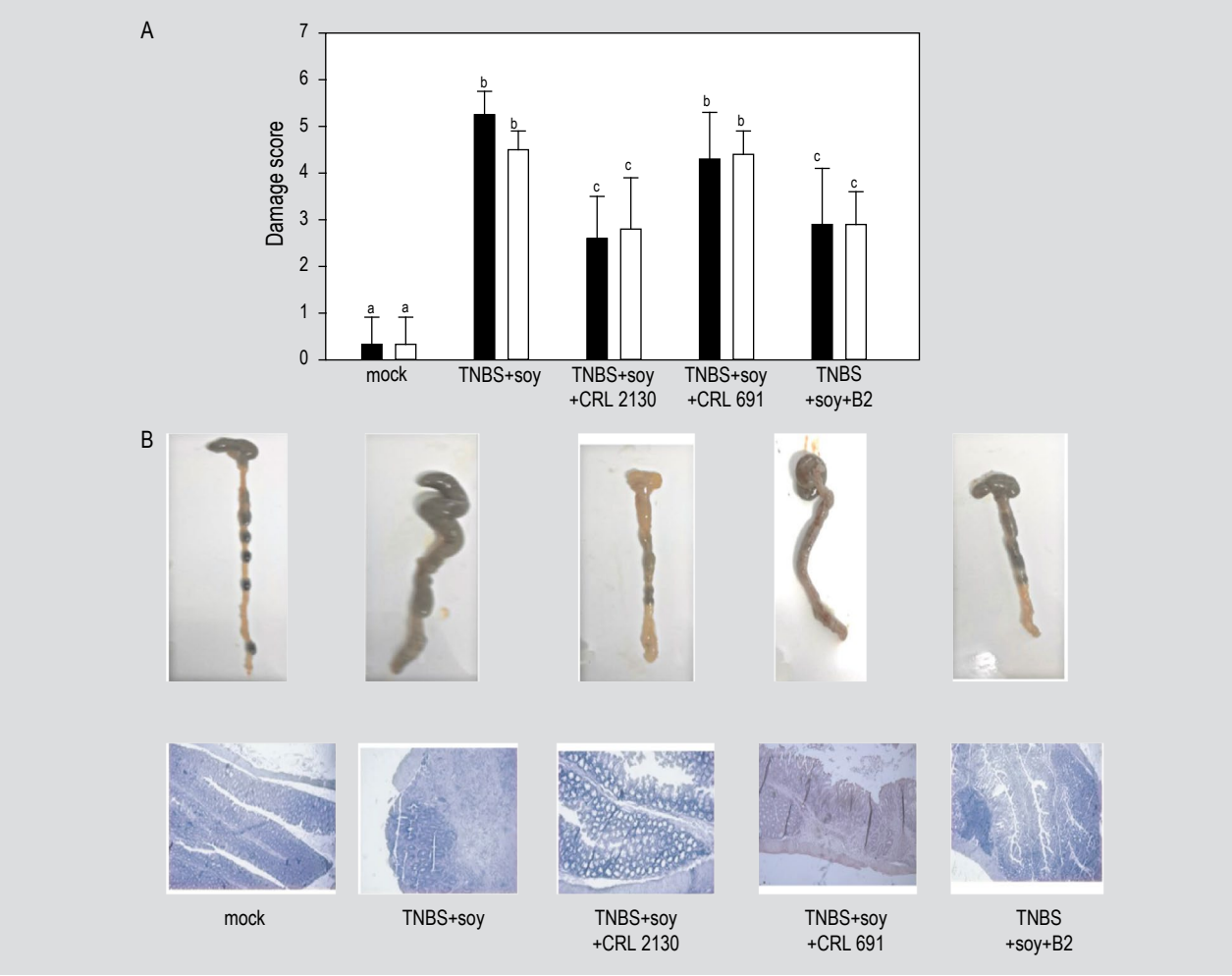

Figure 2. Effects of soymilk fermented by the riboflavin producing lactic acid bacteria on intestinal damages induced by trinitrobenzene sulfonic (TNBS). (A) Macroscopic (black bars) and microscopic (white bars) damage score of samples taken 4 days post-TNBS. Each value represents the mean of n=10 ± SD from 2 individual trials. (B) Photographs of the large intestine representative for each group (100× magnification).

were not included in this figure because their levels were below the detection limit in all experimental groups.

4. Discussion

Soymilk is a beverage increasingly chosen to be consumed in several countries due to the increasing discovery of health-associated benefits; however, it contains very low quantities of certain B-group vitamins such as riboflavin. *L. plantarum* CRL 2130 is a roseoflavin resistant variant selected in a previous work because it was shown to be able to increase 6-fold the initial riboflavin levels of soymilk when used as a starter culture (Juarez del Valle *et al.*, 2014). This bio-enriched food represents a natural alternative to the fortification with chemically synthesised riboflavin, which is also less expensive and in line with the increase consumer demand for healthier foods.

In the present study, the anti-inflammatory potential of soymilk fermented by the riboflavin producing *L. plantarum* strain was evaluated in a TNBS induced colitis mouse model. The riboflavin concentration in the soymilk used in this study (fermented by *L. plantarum* CRL 2130) was the same as that obtained previously 1.6 µg/ml (Juarez del Valle *et al.*, 2014). The anti-oxidant and anti-inflammatory effects of this vitamin were recently reported (Mazur-Bialy *et al.*, 2015); however, there are no reports demonstrating these benefits in animal models. TNBS induces in mice injuries similar to those described in human suffering IBD (especially Crohn's disease). These damages are shown by a continuous decrease in animal live body weight, high macroscopic and histologic inflammatory scores in the large intestine, elevated microbial translocation to liver and increased levels of cytokines in the intestinal lumen. Increases in pro-inflammatory cytokines, such as TNF-α, IL-12, IFN-γ, IL-17 and MCP-1 were associated to the uncontrolled gut inflammatory response observed in

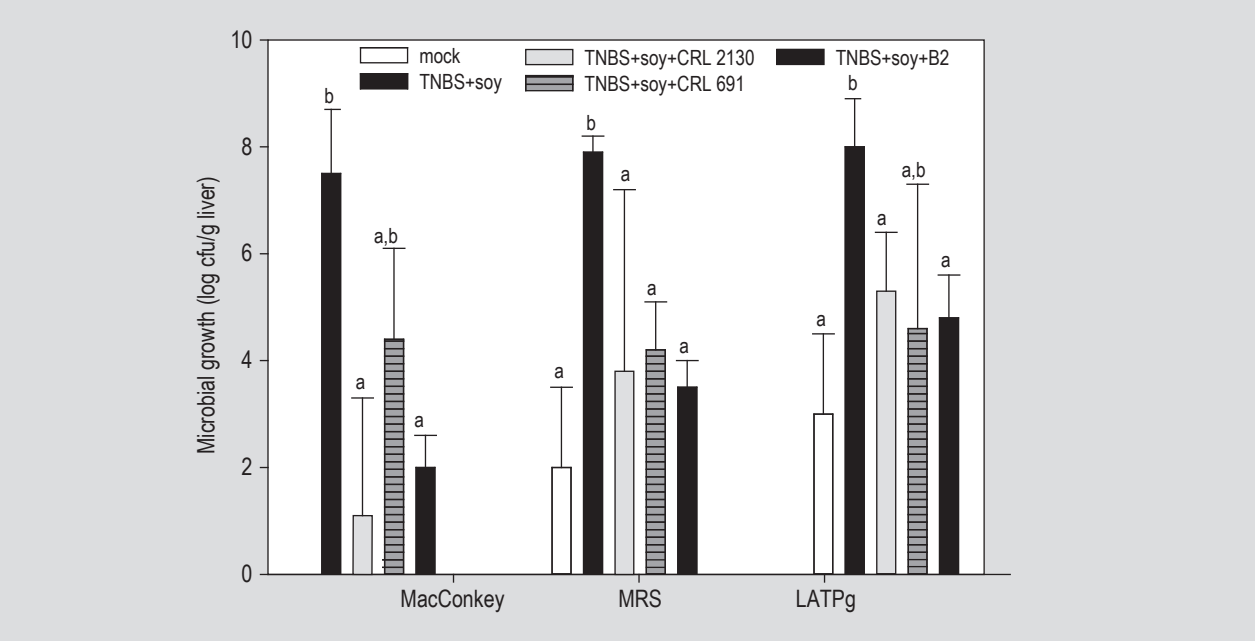

Figure 3. Soymilk fermented by the riboflavin producing lactic acid bacteria reduced microbial traslocation to liver in the trinitrobenzene sulfonic (TNBS) colitis model. Microbial growth in MacConkey, MRS or LAPTg of liver samples obtained from different groups were evaluated. Results are expressed as means (n=10) ± standard deviation of the log cfu/g liver. For each medium, different letters (a,b) show that the values differ significantly (*P*<0.05).

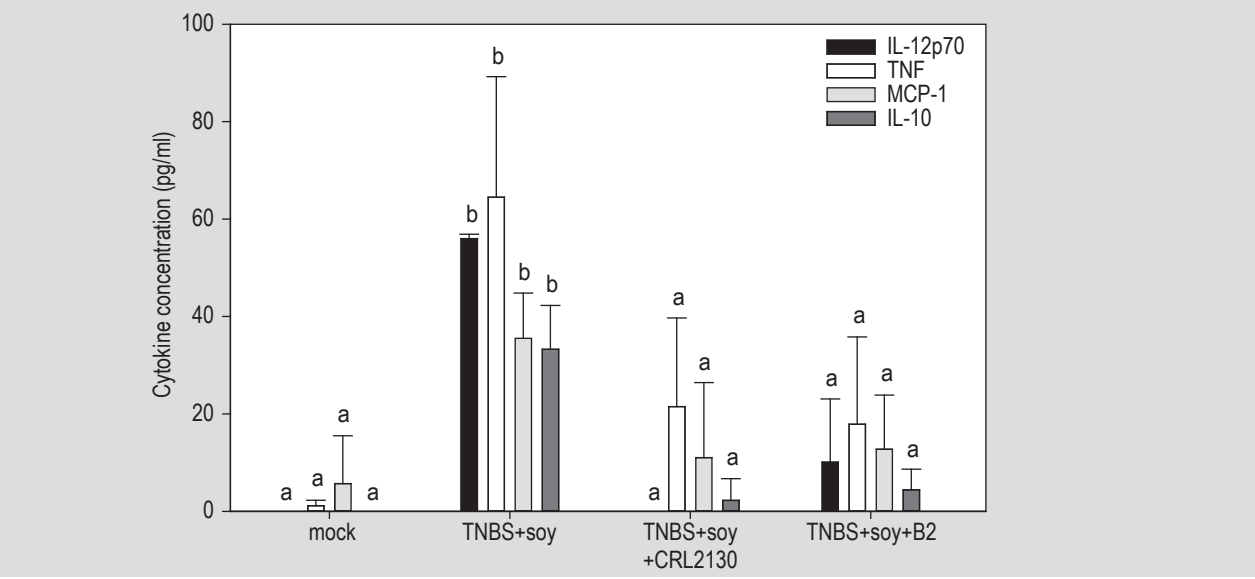

Figure 4. Cytokine analysis from the intestinal contents of mice. The concentration (pg/ml) of interleukin (IL)-12p70, tumour necrosis factor (TNF), monocyte chemoattractant protein (MCP)-1 and IL-10 were measured in the large intestine content of mice from each group. Results are expressed as means (n=10) ± standard deviation. For each cytokine, different letters (a,b) show significant differences (*P*<0.05) between the values.

this colitis model (Del Carmen *et al.*, 2013, 2015). It was previously reported that in addition to the pro-inflammatory cytokines, IL-10 (an anti-inflammatory cytokine) was also increased in acute TNBS induced colitis as an innate mechanism to revert the unbalance immune response (LeBlanc *et al.,* 2011b). The addition of anti-inflammatory or anti-oxidant agents by LAB to increase intestinal IL-10 or to diminish the gut inflammatory environment was also demonstrated previously (Del Carmen *et al.*, 2011, 2015). In this context, oxidative stress has been suggested as an etiologic factor for IBD. The gastrointestinal tract is a key source of ROS generation due to the presence of a plethora

of microbes, food ingredients and the interactions between immune cells. The enhanced/unbalanced production of ROS is associated with chronic intestinal inflammation in the early stages of IBD, and their destructive effects on DNA, proteins and lipids may contribute to initiation and progression of IBD. Intestinal cells are equipped with an intricate antioxidant defence system, including enzymes, such as superoxide dismutase (SOD) and catalase (CAT), and non-enzymatic compounds, such as β-carotene, vitamin C and reduced glutathione. However, an excessive generation of ROS can deplete antioxidant defences (Moura *et al.*, 2015). For this reason, foods rich in antioxidants or antioxidants administered as supplements can be applied in an attempt to alleviate ROS induced damage. In this sense the relationship between antioxidant vitamins and IBD was studied and it was shown that vitamin E and C (anti-oxidant vitamins) supplementation resulted in a significant reduction of oxidative stress in patients with Crohn's disease (CD) (Aghdassi *et al.*, 2003; Roggenbuck *et al.*, 2008). Furthermore, preclinical and clinical data suggested that vitamin D has therapeutic potential in IBD, particularly in CD patients (Hlavaty *et al.*, 2015).

The anti-inflammatory properties associated to riboflavin were also described; however, studies about the effect of riboflavin in IBD are lacking. Studies have reported that this vitamin alleviated oxidative injuries by scavenging the free radicals which improved early cardiac ischemia-reperfusion injury and reduced the development of coronary allograft vasculopathy. Furthermore, riboflavin administered in the setting of cardiac allograft transplantation appears to be a powerful means to reduce early graft lipid peroxidation, leukocytic infiltration, and cytokine production (Iwanaga *et al.*, 2007). Moreover, riboflavin treatment reduces the risk of diabetes complications by reducing inflammation caused by oxidative stress (Alam *et al.*, 2015). It has also been shown that the depletion of riboflavin can affect the intestinal cells (Powers, 2003).

In this present study it was observed that the administration of soymilk fermented by the riboflavin producing LAB strain *L. plantarum* CRL 2130 was able to significantly attenuate TNBS-induced intestinal damages, similar to the effect obtained with the administration of the commercial vitamin; however, mice that received soymilk fermented by a non-riboflavin-producing LAB strain (*L. plantarum* CRL 691) did not show this beneficial effect. This was evidenced by decreased loss of body weight and decreased macroscopic and histologic inflammation scores in the large intestine which was also associated to lower microbial translocation to the liver. Considering that other mechanisms, such as an immunomodulatory capacity, can be associated to the anti-inflammatory effect of LAB, the cytokine response was evaluated in the intestinal fluids. The non-inflammatory status was corroborated with the analysis of cytokines released in the intestinal fluid. Cytokines concentrations were maintained without significant differences with the mock group (non-inflamed mice) in the mice that received the soymilk fermented by *L. plantarum* CRL 2130 or in those that received the soymilk fortified with riboflavin. Similar results were obtained by our research group when *Lactobacillus casei* BL23 was genetically modified to produce the anti-oxidant enzymes CAT and SOD and evaluated in a murine model of Crohn's disease (LeBlanc *et al.*, 2011b). Our findings are also in agreement with the antioxidant and anti-inflammatory actions reported for riboflavin as responsible for the normalisation of hepatic function at the biochemical and structural level in rats with carbon tetrachloride (CCl_4) induced hepatic injury (Al-Harbi *et al.*, 2014). However, in contrast to the immune modulation associated with riboflavin previously (Mazur-Bialy, 2015), in our acute colitis model, decreases of pro-inflammatory cytokines in the riboflavin or fermented soymilk supplemented mice were not related to increased levels of the anti-inflammatory IL-10. This demonstrated that an anti-inflammatory property associated to the host immune modulation was not observed in the mice that received *L. plantarum* CRL 2130. Our hypothesis is that riboflavin obtained by fermenting soymilk with *L. plantarum* CRL 2130 as well as the fortification with commercial riboflavin act by an anti-oxidant mechanism in the acute phase of the inflammation. As stated above, some antioxidant and anti-inflammatory effects have been reported using purified riboflavin; however, this is the first report where a product resulting from the fermentation with a riboflavin-producing *L. plantarum* was shown to have anti-inflammatory effects in an animal model. It must be clarified that the non-riboflavin-producing LAB strain (*L. plantarum* CRL 691) used in this study is not an isogenic strain of *L. plantarum* CRL 2130, thus it is possible that these might be genetically and phenotypically different. These differences might explain why the non-riboflavin strain did not show beneficial effects in the colitis induced animals. A strain where the riboflavin biosynthesis genes in *L. plantarum* CRL 2130 are knocked-out should be used in future studies to avoid the possible effect of genetic backgrounds. It is however clear that *L. plantarum* CRL 2130 produces riboflavin, and that mice that received the same concentration of riboflavin as produced by this strain show the same beneficial effect.

In conclusion, soymilk fermented by the riboflavin producing *L. plantarum* strain attenuates experimental colitis in mice similar to the use of the commercial vitamin by itself. This is the first study where the antioxidant/anti-inflammatory effect of a riboflavin-producing LAB was demonstrated. This fermented beverage could be proposed as a candidate to prevent acute intestinal inflammation in sensible host and also to maintain the remission in patients suffering chronic IBD. Studies are currently being performed to understand the mechanisms involved in the anti-inflammatory effect of B_2 against IBD. The riboflavin

bio-enriched soymilk could also be useful for people who do not consume dairy products because of specific eating habits (such as vegetarianism) or are intolerant to lactose who are more prone to have an inadequate intake of this essential vitamin.

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