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Lactobacillus rhamnosus RC007 intended for feed additive: immune-stimulatory properties and ameliorating effects on TNBS-induced colitis

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RESEARCH ARTICLE

Abstract

Lactobacillus rhamnosus RC007 is a potential probiotic bacterium that can exert beneficial effects as supplement for animal feed, by improving the immune status in healthy host, and by providing therapeutic benefits to infected/inflamed animals. The aim of the present work was to evaluate *in vivo* the beneficial properties of *L. rhamnosus* RC007, intended for animal feed, when administered to healthy and trinitro-benzene-sulfonic-acid (TNBS) colitis induced BALB/c mice. The administration of *L. rhamnosus* RC007 to healthy mice during 10 days increased the phagocytic activity of peritoneal macrophages and the number of immunoglobulin A+ cells in the lamina proper of the small intestine. Significant increases of monocyte chemotactic protein 1, interleukin (IL)-10 and tumour necrosis factor alpha (TNF-α) concentrations, and in the ratio between anti- and pro-inflammatory cytokines (IL-10/TNF-α) were observed in intestinal fluids after administration of bacteria. In the inflammation model, less body weight loss, macroscopic and histological damages in the large intestine were accompanied by increased IL-10/TNF-α ratio in the intestinal fluids of mice from the *L. rhamnosus*-TNBS group when compared to the TNBS group. In a healthy host, the oral administration of *L. rhamnosus* RC007 kept the gut immune system stimulated allowing a faster response to noxious stimulus. Mice that received *L. rhamnosus* RC007 also decreased the severity of the intestinal inflammation.

Keywords: lactic acid bacteria; immune system; gut inflammation; animal feed; immune modulation

1. Introduction

Lactic acid bacteria (LAB) constitute a group of microorganisms associated with plants, meat, and dairy, among other products. Many of them are normal microbiota of human beings and animals. They are used in the manufacture of dairy products, such as acidophilus milk, yoghurt, buttermilk, and cheeses. They are commercially important in the processing of meats (sausage, cured hams), alcoholic beverages (beer, fortified spirits), and vegetables (pickles and sauerkraut) (Lahtinen *et al.*, 2011). LAB also play an important role in the preservation process of moist forages for animal feeding (silage). The fresh forage from cultures like maize, pulses, alfalfa and wheat can be preserved by ensilage. The process is based on the fermentation of water-soluble carbohydrates by LAB and air exclusion. The fermentation lowers the pH due to lactic acid production, which in turn inhibits growth of many spoilage organisms (McDonald *et al.*, 1991). Previous studies demonstrated that *Lactobacillus rhamnosus* RC007 isolated from maize silage was able to inhibit toxicogenic fungi and to improve silage fermentation in laboratory scale-silos (Dogi *et al.*, 2015). In addition, it was reported that this strain has no antibiotic resistance genes of importance in veterinary medicine (Dogi *et al.*, 2013).

Some LAB not only contributes to the development of the organoleptic, physicochemical and rheological food properties, but also generate benefits to the health of consumers. So, the food industry has focused some

research in incorporating these microorganisms in food or feedstuff. These organisms are called probiotics, which are 'live microorganisms which, when administered in adequate amounts, confer health benefits the host' (FAO/WHO, 2001). The properties of LAB associated to health benefits or their roles in the food industry, are strain-specific. Numerous strains are continuously screened for desirable characteristics.

Therefore, the aim of this work was to evaluate *in vivo* the beneficial properties of *L. rhamnosus* RC007 when administered to healthy and trinitro-benzene-sulfonic-acid (TNBS) colitis induced BALB/c mice. The present study was carried out to the further development of a novel product that could improve silage fermentation and animal health after their consumption.

2. Materials and methods

Bacterial strains and growth conditions

L. rhamnosus RC007 was previously isolated form maize silage and maintained at the National University of Río Cuarto Collection Centre (Córdoba, Argentina). The LAB strain was identified from both the fermentation pattern (API 50 CHL test) and the 16S rRNA gene sequence. *L. rhamnosus* RC007 was grown at 37 °C for 16 h without agitation in De Man, Rogosa and Sharpe (MRS) broth (Britania, Buenos Aires, Argentina).

Evaluation of *Lactobacillus rhamnosus* RC007 effects on healthy mice

Animals and administration of bacteria

BALB/c mice (female, 5 weeks old, 20 to 25 g) were obtained from the animal facilities at CERELA (Centro de Referencia para Lactobacilos, San Miguel de Tucumán, Argentina). The Animal Protection Committee of CERELA (CRL-BIOT-LT-2010/1A) approved all animal protocols, and all experiments comply with the current laws of Argentina. Mice from *L. rhamnosus* group (n=5) orally received 0.1 ml of the LAB strain (1×10^8 cells/ml) resuspended in phosphate buffered saline (PBS) daily during 10 days. The Control group received orally 0.1 ml of PBS per day. After 10 days of bacterial or PBS administration, mice were euthanised by cervical dislocation. Live body weight was measured on day 0 (before LAB administration) and at the end of the experiment (day 10).

Evaluation of gut microbiota and bacterial translocation to liver

The caecum was aseptically removed, weighed and placed into sterile tubes containing 5 ml 0.1% (w/v) peptone solution. The samples were immediately homogenised under sterile conditions using a homogeniser (MSE, London, UK). Serial dilutions of the homogenised samples were obtained and aliquots (0.1 ml) of the appropriate dilution were spread onto the surface of the following media: Reinforced Clostridial agar (RCA; Britania, Buenos Aires, Argentina) for total anaerobic bacteria, Rogosa agar (Britania) for total lactobacilli, and MacConkey agar (Britania) for *Enterobacteriaceae*.

Microbial translocation to liver was determined essentially as previously described (Del Carmen *et al.*, 2014). Briefly, the liver was aseptically removed, weighed and homogenised in 5.0 ml sterile 0.1% (w/v) peptone solution. Serial dilutions of the homogenates were plated in triplicate in MRS, MacConkey, and LAPTg (1% glucose, 1.5% peptone, 1% tryptone, 1% yeast extract and 0.1% Tween 80) media to detect a wide range of microorganisms. Bacterial growth was evaluated after incubation of the plates at 37 °C for 48 to 72 h.

Isolation of macrophages from peritoneum and determination of phagocytic activity

Peritoneal macrophages were obtained according to Valdez *et al.* (2001). Macrophages were extracted from peritoneal cavity with 5 ml of sterile PBS pH 7.4 containing 100 µg/ml of gentamicin. Phagocytosis assays were performed using *Saccharomyces boulardii* suspension (Hansen CBS 5926 from Floratil, MERCK Quimica, Argentina) at a concentration of 10^7 cells/ml. *S. boulardii* was opsonised by incubating the yeast suspension with blood serum obtained from a healthy mouse. Phagocytosis was performed by *ex vivo* assay using equal volumes of opsonised *S. boulardii* mixed with 10^6 cells/ml of macrophages. The mixture was incubated for 30 min at 37 °C. Phagocytosis was expressed as the percentage of phagocyting macrophages in 200 cells counted using an optical microscope.

Immunofluorescence assay for IgA^+ cells in small and large intestine

The tissues (small and large intestines) from mice were prepared for histological studies, fixed in formaldehyde, dehydrated using a graded series of ethanol and xylene and embedded in paraffin following standard methodology. For the small intestine, three portions (2 cm length each) were selected, one near to the stomach, the other from the middle and the last one at the end (before the cecum). For the large intestine, colon and rectum were removed and cut in pieces of approximately 2 cm of length. The number of immunoglobulin A (IgA) positive cells was determined on histological slices using a direct immunofluorescence assay. After deparaffinisation using xylene and rehydration in a decreasing gradient of ethanol, paraffin sections (4 µm) were incubated with a 1:100 dilution of anti-IgA (α-chain monospecific) antibody conjugated with fluorescein isothiocyanate (Sigma, St. Louis, MO, USA) for 30 min at 37 °C. The number of fluorescent cells was counted in

30 fields at 1000× magnification using a fluorescent light microscope and results were expressed as the average of the number of positive fluorescent cells in ten fields of vision.

Determination of cytokines in intestinal fluids

The intestinal contents were collected from the large and small intestines with 1 ml PBS and immediately centrifuged at 5,000×*g* for 15 min at 4 °C. The supernatants were recovered and stored at -80 °C until cytokine determination using the Cytometric Bead Array (CBA) mouse inflammation kit (BD Bioscience, San Diego, CA, USA). The concentration of each cytokine from the intestinal fluid of each mouse was obtained and the results were expressed in relation to the protein concentration measured in the sample. Total protein content of the samples was determined using the Bio-Rad Protein Assay (BioRad, Hercules, CA, USA) based on the method of Bradford (Bradford, 1976). Cytokine ratios for each mouse were also determined.

Evaluation of *Lactobacillus rhamnosus* RC007 on colitis-induced mice

Colitis induction and bacterial administration

Induction of colitis with TNBS was achieved essentially as previously described (LeBlanc *et al.*, 2011). Briefly, BALB/c mice (female, 5 weeks old) were fully anesthetised with an intraperitoneal injection of ketamine hydrochloride (Holliday-Scott S.A., Buenos Aires, Argentina; 100 µg g/body weight) mixed with xylazine hydrochloride (Rompun; Bayer, Buenos Aires, Argentina; 5 µg g/body weight). Colitis was then induced by intrarectal inoculation of TNBS solution (Sigma; 2 mg/mouse) dissolved in 0.01 M PBS (pH 7.4) and mixed with an equal volume of ethanol (50% ethanol), using a 4 cm length catheter. Control mice (mock group) received only PBS mixed with ethanol (without TNBS), using the same technique. TNBS-treated mice were subdivided into 2 groups (n=10): (1) inflammation control group (or TNBS group), (2) mice receiving *L. rhamnosus* RC007 (*L. rhamnosus* RC007-TNBS group). LAB were orally administered to mice (as was described above for healthy mice) 10 days before TNBS injection and continued until the end of the experiment (4 consecutive days after colitis induction). All groups were fed *ad libitum* with balanced rodent diet and maintained in a room with a 12 h light/dark cycle at 18±2 °C. Body weight and animal mortality rates were controlled daily.

Assessment of colonic inflammation

Four days after TNBS injection, 3 mice per group were sacrificed. Large intestines and cecum were removed, visually inspected for macroscopic evaluation, and prepared for histological analysis using standard methods. Serial paraffin sections of 4 µm were made and stained with haematoxylin-eosin for light microscopy examination. Macroscopic lesions and extent of colonic damage and inflammation (histologic observations) were assessed using previously described grading systems (Del Carmen *et al.*, 2013). For macroscopic lesions, each parameter (erythema, haemorrhage, oedema, stricture formation, ulceration, faecal blood, presence of mucus, diarrhoea and adhesions) was awarded 1 point if observed in tissue examination. Microscopically, the extent of colonic damage and inflammation was assessed using a standard histopathological grading system: histological findings identical to normal mice (grade 0); mild mucosal and/or submucosal inflammatory infiltrate (admixture of neutrophils) and oedema, punctate mucosal erosions often associated with capillary proliferation, muscularis mucosae intact (grade 1); grade 1 changes involving 50% of the specimen (grade 2); prominent inflammatory infiltrate and oedema (neutrophils usually predominating) frequently with deeper areas of ulceration extending through the muscularis mucosae into the submucosa; rare inflammatory cells invading the muscularis propria but without muscle necrosis (grade 3); grade 3 changes involving 50% of the specimen (grade 4); 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); grade 5 changes involving 50% of the specimen (grade 6). Blind analyses were performed by two different scientists. High macroscopic or histological damage scores indicate increased damage in the intestines.

Microbial translocation to liver was determined essentially as described above for healthy mice. The intestinal content from large intestine were recovered and stored at -80 °C until cytokine determination by using the CBA mouse inflammation kit (BD Bioscience).

Statistical analyses

Three or five mice of each group were sacrificed according to the experimental protocols and samples were collected. The experimental protocols were performed in duplo. Determinations to be carried out immediately after sampling (microbial translocation, microbiota analysis and phagocytosis assay) were performed separately in each trial. Considering that no interactions were observed between these two independent assays, results were analysed together (n=10 or n=6). The evaluation of cytokines was performed at the same time for the samples obtained from both trials. Statistical analyses were performed using MINITAB 15 software (Minitab, Inc., State College, PA, USA). Comparisons were accomplished by an ANOVA general linear model followed by a Tukey's post hoc test and, unless otherwise specified, $P<0.05$ was considered significant.

3. Results

Effects of *Lactobacillus rhamnosus* RC007 on healthy mice

Variations of body weight, caecum microbiota and microbial translocation

The administration of *L. rhamnosus* RC007 during 10 days did not affect the body weight of the mice. Similar increases (near to 3%) were observed in both *L. rhamnosus* and control groups when the body weight at days 0 and 10 were compared (Figure 1A). The analysis of gut microbiota in caecum did not show significant differences for total coliforms, lactobacilli and total anaerobes between the assayed groups (Figure 1B). Administration of LAB was not associated to increased microbial translocation to liver compared to control mice (Figure 1C).

Determination of peritoneal macrophage phagocytic activity and IgA+ cells in small and large intestine

The phagocytic activity using *S. boulardii* as antigen was analysed in peritoneal macrophages. The administration of *L. rhamnosus* RC007 to healthy mice during 10 days increased the phagocytic activity of these macrophages significantly ($P<0.05$), compared to mice that did not receive the LAB strain (Figure 1D). Administration of *L. rhamnosus* RC007 during ten days significantly ($P<0.05$) increased the number of IgA+ cells in the lamina propria of the small intestine compared to the control group (Figure 2). However, no significant differences were observed in the number of IgA+ cells in the large intestine between mice from the control group and those receiving the LAB strain (Figure 2 + Supplementary Figure S1).

Determination of cytokine in intestinal fluids

Monocyte chemotactic protein 1 (MCP-1), IL-10, IL-12 and TNF-α were evaluated in intestinal fluids from small and large intestines (Figure 3). The chemokine MCP-1 was significantly increased in the large intestine of mice receiving *L. rhamnosus*, but not in the small intestinal fluids (Figure 3A). No significant differences were observed for IL-12 levels between control group and mice given LAB strain in both small and large intestine fluids (Figure 3B). Significant increases in IL-10 and TNF-α concentrations were observed in small intestine fluids of mice that received LAB during 10 days (Figure 3C). These cytokines could not be evaluated in the large intestinal fluids because they were under the detection limit in the samples obtained from most mice

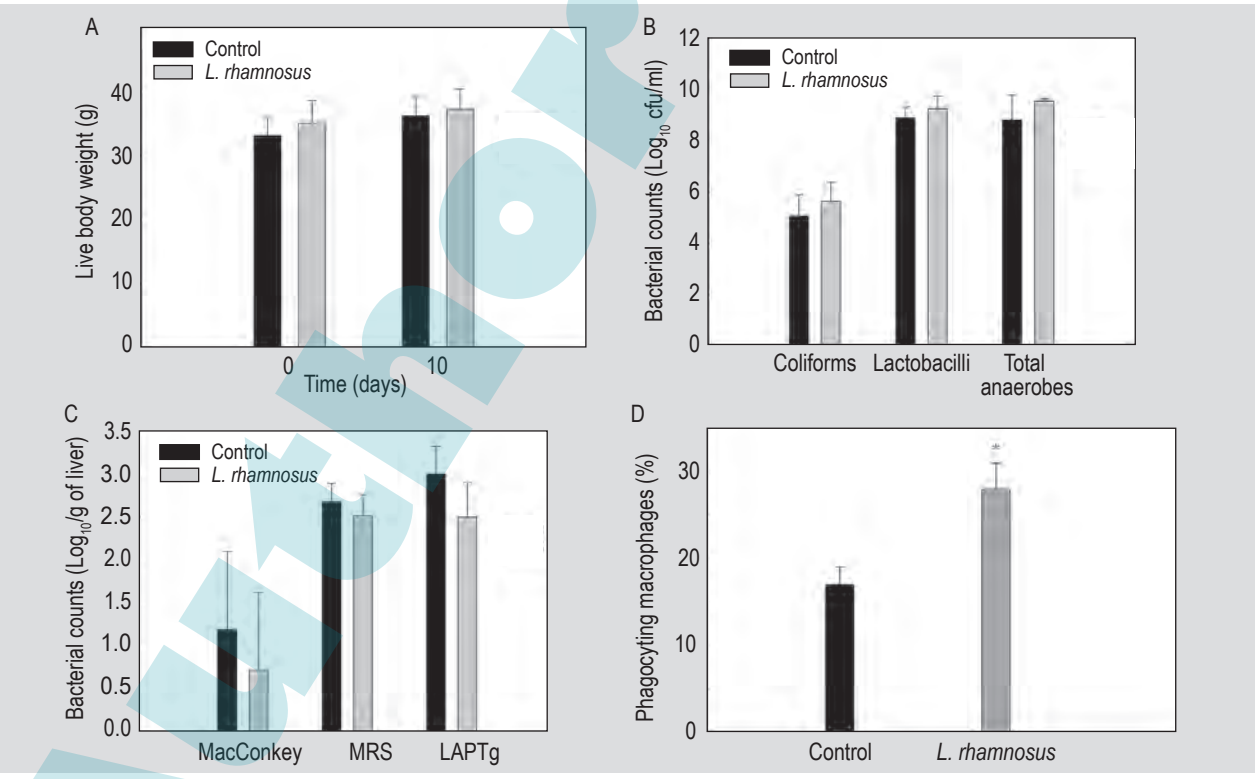

Figure 1. Variations of (A) live body weight, (B) caecum microbiota at the end of the feeding period, (C) microbial translocation to liver by plating samples in different media, and (D) phagocytosis of peritoneal macrophages from healthy mice (% phagocyting macrophages in 200 cells). Mice received *Lactobacillus rhamnosus* RC007 (*L. rhamnosus* group) or phosphate buffered saline (Control) orally during 10 days. Values are expressed as average (n=10 mice from 2 independent experiments) ± standard deviation. * *P*<0.05 from control.

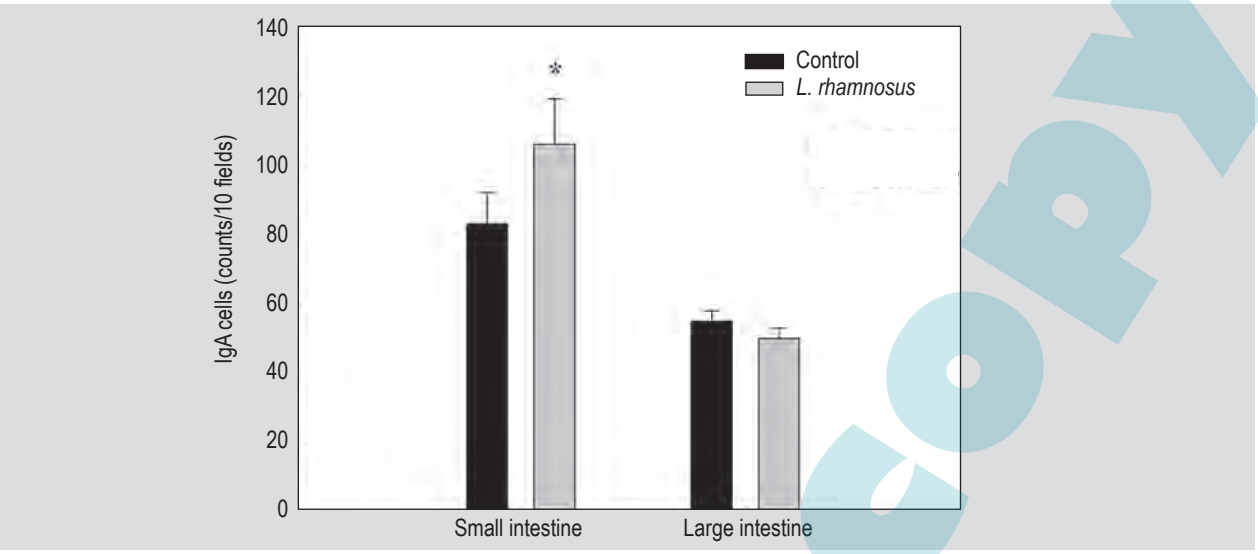

Figure 2. IgA+ cells in intestinal tissues of mice that received *Lactobacillus rhamnosus* RC007 or phosphate buffered saline (Control) orally during 10 days. Results are expressed as the number of positive cells counted in 10 fields of vision at 1000× magnification. Each bar represents the mean ± standard deviation (n=10). * Mean values differ significantly with the control (*P*<0.05).

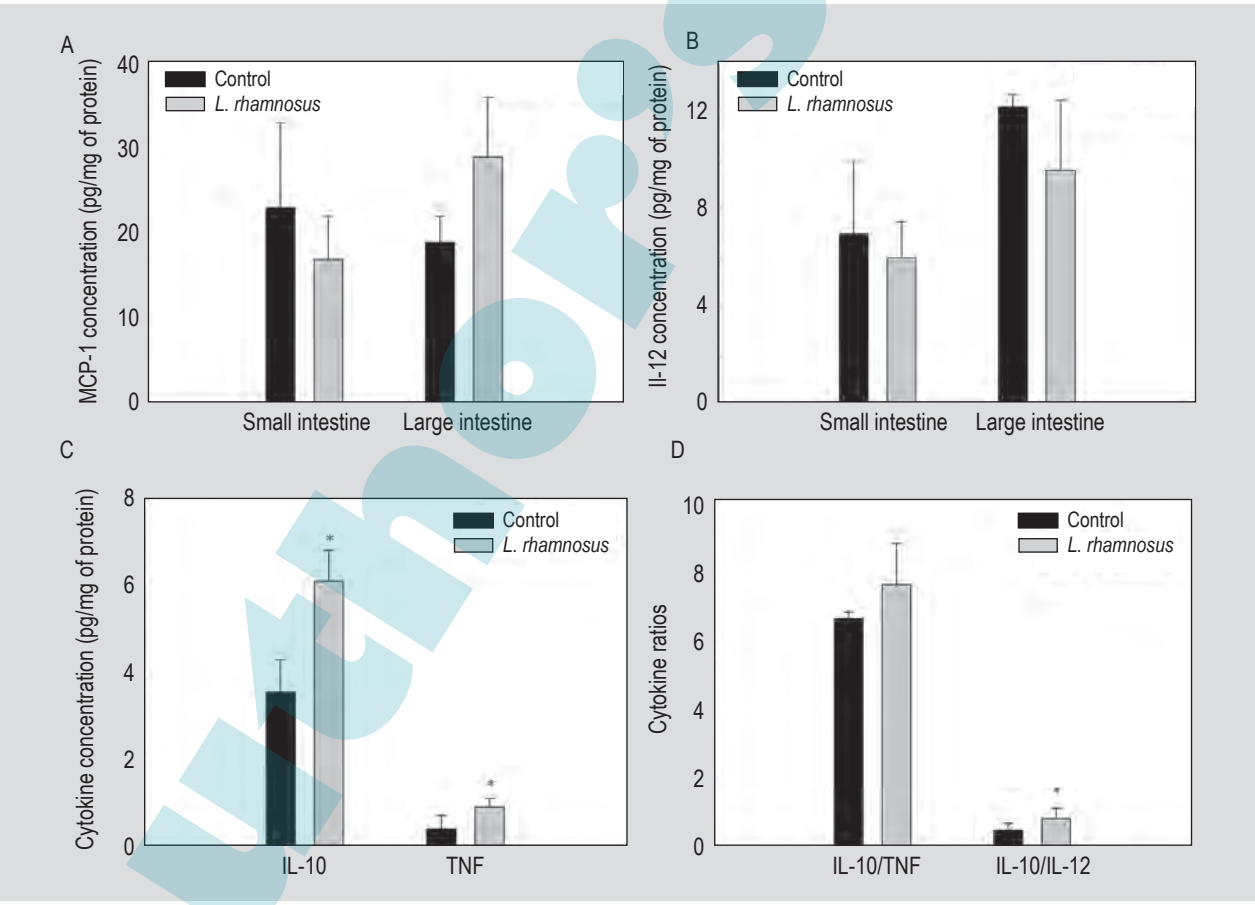

Figure 3. Cytokine concentrations in intestinal fluids of mice that received *Lactobacillus rhamnosus* RC007 or phosphate buffered saline (Control) orally during 10 days. (A) Monocyte chemotactic protein 1 (MCP-1), (B) interleukin (IL)-12, (C) IL-10 and tumour necrosis factor alpha (TNF-α) concentrations. Results are expressed as concentration of each cytokine per mg of proteins in the intestinal fluid (pg/mg protein). (D) The ratio IL-10/pro-inflammatory cytokines and IL-10/IL-12 for small intestinal contents. Each bar represents the mean ± standard deviation (n=10 from 2 independent experiments). * Means differ significantly from the control (*P*<0.05).

(independent of group). The ratio between the anti- and pro-inflammatory cytokines was also evaluated in the small intestinal fluids. The results showed that the mean values for IL-10/IL-12 increased significantly in mice that received *L. rhamnosus* compared to the control group (Figure 3D).

Effects of *Lactobacillus rhamnosus* RC007 on colitis-induced mice

Body weight, intestinal damage and microbial translocation to liver

The evaluation of body weight showed that inflammation in control mice (TNBS group) decreased body weight after TNBS inoculation. Mice that received *L. rhamnosus* RC007a started to recover the body weight the day 3 post-TNBS (Figure 4A). This finding was related with less macroscopic and histologic damages in the large intestine of mice from *L. rhamnosus*-TNBS group compared to TNBS group (Figure 4C and 4D). Some animals that received LAB showed a score similar to the control mice without inflammation (mock group). Microbial translocation to the liver was also analysed as a consequence of the intestinal damage caused by the inflammatory drug. The results showed that TNBS inoculation increased the microbial translocation to the liver, however, LAB administration decreased the counts of different microorganisms in this organ, compared to the control mice (TNBS group, Figure 4B).

Large intestine cytokine profiles associated with inflammation and Lactobacillus rhamnosus administration

The evaluation of MCP-1 showed that the inflamed control mice significantly increased the concentration of this chemokine in the large intestinal fluid compared to the mock group. This increase was not observed in mice that received LAB (Figure 5A). The pro-inflammatory cytokines IL-12 and TNF-α also significantly increased in the intestinal fluids of mice from TNBS group compared to the mock group. However, the administration of *L. rhamnosus* to TNBS inoculated mice significantly decreased the concentrations of these cytokines in the intestinal fluids

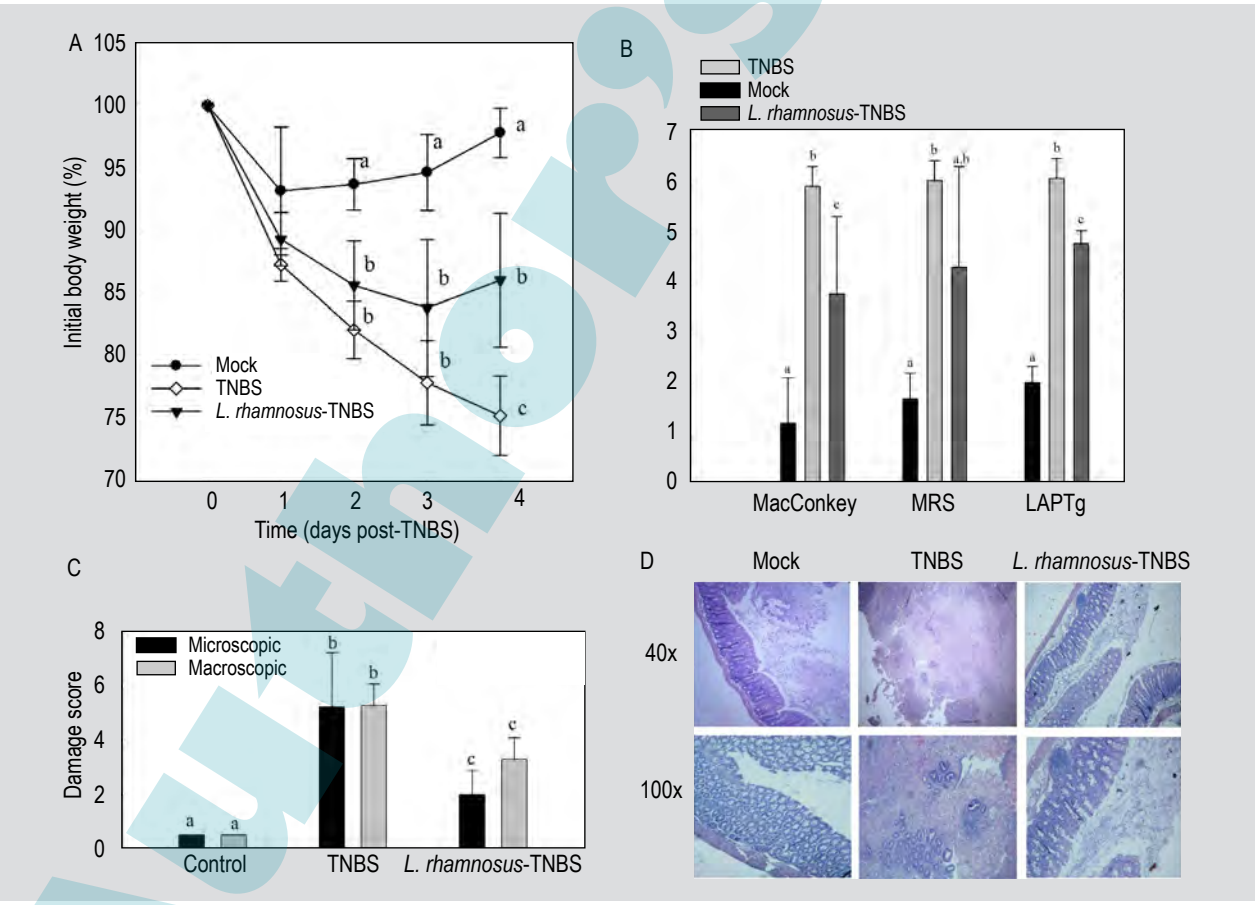

Figure 4. (A) Body weight variations, (B) microbial translocation to liver evaluated in different agar media, and (C) damage scores of the large intestines 4 days post-treatment of trinitro-benzene-sulphonic acid (TNBS)-treated mice receiving *Lactobacillus rhamnosus* RC007 or phosphate buffered saline orally during 10 days. Mock is the control group without TNBS treatment. Each value represents the mean of n=6 (from 2 independent experiments) ± standard deviation. Different letters mean significant differences (*P*<0.05) between the values. (D) Representative microphotographs of the large intestine at 400× and 1000× from each group.

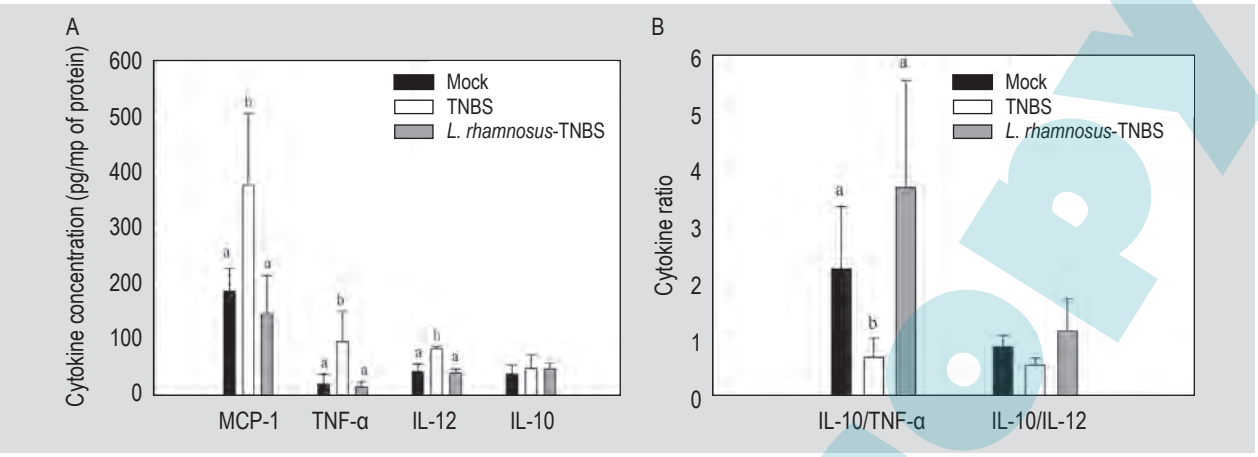

Figure 5. (A) Cytokine concentrations in the large intestinal contents from TNBS-induced mice. Results are expressed as concentration of each cytokine per mg of proteins. (B) Ratios between IL-10 and the pro-inflammatory cytokines TNF-α or IL-12. Each bar represents the mean ± standard deviation (n=6, from 2 independent experiments). Means values without a common letter differ significantly (*P*<0.05).

and maintained similar levels to those observed in the mock group (Figure 5A). No significant differences were observed for IL-10 when compared mice from *L. rhamnosus*-TNBS group with mice from both TNBS and mock groups (Figure 5A). The analysis of anti-/pro-inflammatory cytokine ratios was also performed (Figure 5B). The results showed that the IL-10/TNF-α ratio was significantly higher in the mice that received LAB than in the inflamed control mice (TNBS group).

4. Discussion

The use of probiotic microorganisms to improve human and animal health has been investigated since many years. *In vitro* and *in vivo* studies using experimental models showed that modulation of the host's immune response is one of the most important properties attributed to probiotics (Galdeano *et al.*, 2007). In this sense, the ability of certain probiotic bacteria to beneficially modulate the immune response against animal gut pathogens by reducing tissue damages associated to the inflammation has also been demonstrated (Castillo *et al.*, 2013). Taken these considerations into account, as well as the importance of maintaining the animal health without the excessive use of antibiotics; the current study on *L. rhamnosus* RC007, a LAB that has interesting properties to be used as a probiotic supplement for animal feeds, was evaluated. Mice were used as experimental models to assess the effect of LAB administration on both healthy and intestinal inflamed hosts.

The evaluation of general health parameters, such as body weight and microbial translocation to the liver showed that *L. rhamnosus* RC007 administration was safe. The lack of bacterial translocation from the intestine to other organs after probiotic consumption is related to their safety, and associated with the maintenance of the intestinal microbial balance. Probiotic translocation in a healthy host is not common, however, some reports have shown detrimental effects of probiotic translocation in immunocompromised hosts. Therefore it needs to be investigated as part of the safety assessment of potential probiotics (Liong, 2008). The acceptance of probiotics as a safe food adjunct is related to the GRAS (generally regarded as safe) status of the strains. In this sense, some studies have described the use of probiotic bacteria as growth promoters in calves instead of antibiotics to avoid the negative consequences associated to their widespread use (Abe *et al.*, 1995; Mokhber-Dezfouli *et al.*, 2007).

LAB have been also used as a tool to maintain the intestinal microbial balance and to prevent the colonisation of opportunistic pathogens. Increases of lactobacilli in the gut were associated with beneficial effects of probiotics in different hosts (Riboulet-Bisson *et al.*, 2012). In our work, the lack of significant differences in the mean values of *Lactobacillus* counts in the caecum of mice that received *L. rhamnosus* RC007 compared to the control mice could have been caused by the relative short time period of the experiment (10 days). Previous studies demonstrated that this LAB strain was able to resist simulated gastrointestinal conditions without loss of viability (data not shown). New studies are currently underway to analyse the establishment of *L. rhamnosus* RC007 in the gastrointestinal tract. A meta-analysis of randomised controlled trials by Signorini *et al.* (2012) showed that LAB supplementation can exert a protective effect and reduce the incidence of diarrhoea in calves; however, also many probiotics did not improve the faecal characteristics and were unable to change the LAB:coliforms ratio.

The results of this study showed that *L. rhamnosus* RC007 was able to stimulate the mouse immune system not only in the intestine but also by activating peritoneal macrophage's phagocytosis. Similar results were observed for other probiotics, such as *L. casei* CRL431 where increased phagocytic activity of peritoneal macrophages was related with the potential protection against *Salmonella enterica* serovar Typhimurium (De Moreno de LeBlanc *et al.*, 2010). Recently, comparable beneficial effects were described for the potential probiotic yeast *S. cerevisiae* RC016 (García *et al.*, 2015). Secretory IgA (s-IgA) is the main mechanism of protection given by the gut associated lymphoid tissue (GALT) that prevents the entry of potentially harmful antigens, and interacts with mucosal pathogens without potentiating damages. Many probiotic strains have shown to increase s-IgA (Delcenserie *et al.*, 2008; Thomas and Versalovic, 2010), therefore, the stimulation of IgA producing cells is often considered a desirable property in the screening of probiotic microorganisms (O'Sullivan, 2001).

The modulation of the immune response in the gut was also assessed by analysing certain cytokines in the intestinal fluids. It was observed that LAB administration stimulated the release of cytokines, such as TNF-α and IL-10 in the intestine. However, it is important to remark that the ratio between these cytokines showed an anti-inflammatory potential for LAB administration. The observed increase for MCP-1 can be related with the recruitment of macrophages in the intestinal tissues, as was reported for other probiotics. We propose that administration of *L. rhamnosus* RC007 to a healthy host keeps the gut immune system stimulated allowing a faster response to noxious stimuli, such as a pathogenic microorganisms or agents causing inflammation. The results obtained in healthy mice conducted us to analyse the anti-inflammatory potential of *L. rhamnosus* RC007 using a murine acute inflammation model as proof of concept. Mice that received the LAB decreased the severity of the inflammation. They showed less intestinal damage, which is related with less microbial translocation to the liver as intestinal damage can facilitate the spread from the intestinal lumen to distant organs.

Changes in the intestinal cytokine profiles were also analysed. IL-10 is an anti-inflammatory cytokine that was increased after TNBS treatment in the inflamed control mice. Similar results were reported previously for IL-10 producer cells, which were increased in the lamina propria of the large intestine of TNBS-treated mice in the acute episode as a normal immune response against the inflammatory process (LeBlanc *et al.*, 2011).

However, during the experiment, the pro-inflammatory cytokines remained elevated in the TNBS control group and the IL-10/TNF-α ratio was lower than that observed in the mock group (without inflammation). In contrast, in mice that received LAB (*L. rhamnosus*-TNBS group) IL-10 concentrations in the intestinal fluids maintained values between those obtained in TNBS and mock groups, but the concentrations of pro-inflammatory cytokines were similar to that of the mock group without inflammation, confirming the anti-inflammatory effect. It is important to note that LAB administration conserved an important release of IL-10 to the intestinal fluid demonstrating its anti-inflammatory properties by maintaining increased ratio of anti-/pro-inflammatory cytokines.

5. Conclusions

Lactobacillus rhamnosus RC007 is a potential probiotic bacterium for use as a supplement in animal feed, which may exert beneficial effects, such as improving the immune status in healthy hosts. This LAB also has the potential to be used in infected/inflamed animals, however, studies with certain specific pathogens are needed to confirm this potential.

Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/BM2015.0147.

Figure S1. Representative microphotographs of the small (duodenum, jejunum and ileum) and large intestine (colon and rectum) of mice from the Control and *Lactobacillus rhamnosus* group.

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References

Abe, F., Ishibashi, N. and Shimamura, S., 1995. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. Journal of Dairy Science 78: 2838-2846.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Castillo, N.A., De Moreno de LeBlanc, A., M. Galdeano, C. and Perdigón, G., 2013. Comparative study of the protective capacity against Salmonella infection between probiotic and non probiotic lactobacilli. Journal of Applied Microbiology 114: 861-876.

De Moreno de LeBlanc, A., Castillo, N.A. and Perdigon, G., 2010. Anti-infective mechanisms induced by a probiotic Lactobacillus strain against Salmonella enterica serovar Typhimurium infection. International Journal of Food Microbiology 138: 223-231.

Del Carmen, S., De Moreno de LeBlanc, A., Martin, R., Chain, F., Langella, P., Bermúdez-Humarán, L.G. and LeBlanc, J.G., 2014. Genetically engineered immunomodulatory Streptococcus thermophilus strains producing antioxidant enzymes exhibit enhanced anti-inflammatory activities. Applied and Environmental Microbiology 80: 869-877.

Del Carmen, S., Zurita-Turk, M., Alvarenga Lima, F., Coelho Dos Santos, J.S., Leclercq, S.Y., Chatel, J.M., Azevedo, V., De Moreno de LeBlanc, A., Miyoshi, A. and LeBlanc, J.G., 2013. A novel interleukin-10 DNA mucosal delivery system attenuates intestinal inflammation in a mouse model. European Journal of Inflammation 11: 25-38.

Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y. and Roy, D., 2008. Immunomodulatory effects of probiotics in the intestinal tract. Current Issues in Molecular Biology 10: 37-54.

Dogi, C.A., Fochesato, A., Armando, R., Pribull, B., De Souza, M.M., Da Silva Coelho, I., Araújo de Melo, D., Dalcero, A. and Cavaglieri, L., 2013. Selection of lactic acid bacteria to promote an efficient silage fermentation capable of inhibiting the activity of Aspergillus parasiticus and Fusarium gramineraum and mycotoxin production. Journal of Applied Microbiology 114: 1650-1660.

Dogi, C.A., Pellegrino, M., Poloni, V., Poloni, L., Pereyra, C.M., Sanabria, A., Pianzzola, M.J., Dalcero, A. and Cavaglieri, L., 2015. Efficacy of corn silage inoculants on the fermentation quality under farm conditions and their influence on Aspergillus parasiticus, A. flavus and A. fumigatus determined by q-PCR. Food Additives and Contaminants Part A 32: 229-235.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), 2001. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Available at: http://tinyurl.com/8bccc3r.

Galdeano, C.M., De Moreno de LeBlanc, A., Vinderola, G., Bonet, M.E. and Perdigón, G., 2007. Proposed model: mechanisms of immunomodulation induced by probiotic bacteria. Clinical and Vaccine Immunology 14: 485-492.

García G., Dogi, C., De Moreno de LeBlanc, A., Greco C. and Cavaglieri L., 2015. Lactobacillus rhamnosus RC007 with immune-modulatory properties in an experimental model of TNBS-induced colitis in mice. Potential use as a feed additive for pig production. In: Frontiers in Immunology, Conference Abstract, Immunocolombia. 11th Congress of the Latin American Association of Immunology. November 26-27, 2015. Buenos Aires, Argentina. DOI: http://dx.doi.org/10.3389/conf.fimmu.2015.05.00232.

Lahtinen, S., Salminen, S., Ouwehand, A. and Wright, A.V., 2011. Lactic acid bacteria: Microbiological and functional aspects. CRC Press, Boca Raton, FL, USA.

LeBlanc, J.G., Del Carmen, S., Miyoshi, A., Azevedo, V., Sesma, F., Langella, P., Bermúdez-Humarán, L.G., Watterlot, L. Perdigon, G. and De Moreno de LeBlanc, A., 2011. Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice. Journal of Biotechnology 51: 287-293.

Liong, M.T., 2008. Safety of probiotics: translocation and infection. Nutrition Research 66: 192-202.

McDonald, P., Henderson, A.R. and Heron, S.J.E., 1991. The biochemistry of Silage Kingston. Chalcombe Publications, Canterbury, UK.

Mokhber-Dezfouli, M.R., Tajik, P., Bolourchi, M. and Mahmoudzadeh, H., 2007. Effects of probiotics supplementation in daily milk intake of newborn calves on body weight gain, body height, diarrhea occurrence and health condition. Pakistan Journal of Biological Sciences 10: 3136-3140.

O'Sullivan, G.C., 2001. Probiotics. British Journal of Surgery 88: 161-162.

Riboulet-Bisson, E., Sturme, M.H., Jeffery, I.B., O'Donnell, M.M., Neville, B.A., Forde, B.M., Claesson, M.J., Harris, H., Gardiner, G.E., Casey, P.G., Lawlor, P.G., O'Toole, P.W. and Ross, R.P., 2012. Effect of Lactobacillus salivarius bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One 7: e31113.

Signorini, M.L., Soto, L.P., Zbrun, M.V., Sequeira, G.J., Rosmini, M.R. and Frizzo, L.S., 2012. Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. Research in Veterinary Science 93: 250-258.

Thomas, C. and Versalovic, J., 2010. Probiotics-host communication: modulation of signaling pathways in the intestine. Gut Microbes 1: 148-163.

Valdez, J., Rachid, M., Gobbato, N. and Perdigon, G., 2001. Lactic acid bacteria induce apoptosis inhibition in Salmonella typhimurium infected macrophages. Food and Agricultural Immunology 13: 189-197.