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ORIGINAL ARTICLE

Development of a potential probiotic yoghurt using selected anti-inflammatory lactic acid bacteria for prevention of colitis and carcinogenesis in mice

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Abstract

Aims: To evaluate the beneficial properties of a potentially probiotic yoghurt obtained by the fermentation of two selected anti-inflammatory bacterial strains using *in vivo* mouse models of intestinal inflammation and colon carcinogenesis.

Methods and Results: Yoghurt was administered to mice suffering chemically induced intestinal inflammation or colon carcinogenesis. It was shown that this novel yoghurt was able to prevent local inflammation in the intestines of mice through a regulation of the immune response, prevent macroscopic and histological damages, and prevent colon carcinogenesis through an anti-inflammatory response.

Conclusions: The developed yoghurt showed *in vivo* anti-inflammatory properties by modulation of the host immune response for the prevention of colon inflammation and carcinogenesis.

Significance and Impact of the Study: This new yoghurt could thus be considered a probiotic food and be useful as a complement to current treatment protocols for inflammatory bowel diseases and colon cancer, a first since there are no current functional foods specifically oriented for these patients.

Introduction

During the last decades, the increasing demand for healthier foods, which not only provide nutritional value, but also confer additional health benefits has led to developing of many functional foods. In recent years, the global market for functional foods represented the fastest growing food market worldwide (Bimbo *et al.* 2016). This is associated to a concomitant upturn in the consumption of probiotics, defined as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO 2001). Although these micro-organisms can currently be acquired as dietary supplements in pharmacies, fermented foods continue to be the most popular source of intake of probiotics. In this sense, food matrix can contribute to the probiotic delivery (Sanders and Marco 2010). Yoghurt has been produced and consumed by humans for thousands of years and several studies, including very recent ones, have shown its potential as a health promoting food (Wang *et al.* 2013; Astrup 2014; Webb *et al.* 2014). According to the Codex-Alimentarius (2003), yoghurt is fermented milk resulting from the protocooperation of two micro-organisms (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in which both bacterial species must remain live in the final product (Codex-Alimentarius 2003). Because of the numerous beneficial effects attributed to yoghurt consumption, some studies have proposed to consider yoghurt as a functional food with the certain starter cultures being considered probiotics if they remain alive during consumption (Guarner *et al.* 2005). However, probiotic characteristics of micro-organisms are strain dependent, so the potential benefits associated to specific yoghurt bacterial strains need to be adequately evaluated before making such a claim (Arena *et al.* 2015).

The International Scientific Association for Probiotics and Prebiotics (ISAPP) has recently published a consensus document where the appropriate use and scope of the term probiotic is defined (Hill *et al.* 2014). These experts discussed about the benefits attributed to certain traditional fermented foods containing live micro-organisms (especially dairy products); however, they also highlighted that these potentially beneficial micro-organisms are frequently not adequately identified.

Inflammatory bowel diseases (IBD) constitute a group of chronic inflammatory disorders of the intestine. Conventional IBD therapies often cause negative side effects that impact social and economic aspects of the patients and on health care systems (Annahazi and Molnar 2015). It has been reported that chronic intestinal inflammation is associated with an increased incidence of colon cancer (Herszenyi *et al.* 2015). The major challenges in developing new therapeutic options for the treatment of IBD are to (i) maintain the remission, (ii) avoid relapses and (iii) improve the patient's quality of life (Sales-Campos *et al.* 2015). In this sense, probiotics, used as dietary supplements or pharmaceutical preparations, have been evaluated as an alternative (Celiberto *et al.* 2015).

It has been previously reported that a yoghurt, prepared with a pool of 10 strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lact. bulgaricus*) and *Streptococcus* (*Strep.*) *thermophilus* from the Culture Collection of Centro de Referencia para Lactobacilos (Argentina), inhibited the initiation and promotion of colon cancer in mice (de Moreno de Leblanc and Perdigon 2004; de Moreno de LeBlanc *et al.* 2004). This effect was attributed to different mechanisms; the most important being its anti-inflammatory potential. This yoghurt also decreased the severity of inflammation in acute and chronic models of colitis in mice. Mice that received yoghurt showed a modulated immune response as shown by increases in the production of the anti-inflammatory cytokine IL-10 and decreased IL-17 and IFNγ (pro-inflammatory cytokines) production at the intestinal level. (de Moreno de LeBlanc *et al.* 2009; Chaves *et al.* 2011). Based on the new probiotic guidelines, individual bacterial strains from this yoghurt have been evaluated *in vitro* and *in vivo* to select lactic acid bacteria with intrinsic anti-inflammatory potential. Two strains (*Strep. thermophilus* CRL807 and *Lact. bulgaricus* CRL864) were shown to modulate immune responses (del Carmen *et al.* 2014a).

From all of these observations, we hypothesized that specific lactic acid bacteria (LAB) strains with proven immunomodulatory properties could be used to prepare a potentially probiotic yoghurt with anti-inflammatory activities. Therefore, the aim of this study was to evaluate the beneficial properties of yoghurt obtained through the fermentation of two selected anti-inflammatory strains using *in vivo* mouse models of intestinal inflammation and colon carcinogenesis.

Materials and methods

Bacterial strains, growth conditions and preparation of yoghurt

Streptococcus thermophilus CRL807 and *Lact. delbrueckii* subsp. *bulgaricus* CRL864 were obtained from the CERELA Culture Collection (Tucumán, Argentina) and grown for 16 h at 37°C without agitation in LAPTg (1% glucose, 1·5% peptone, 1% tryptone, 1% yeast extract and 0·1% Tween 80) or Man Rogosa and Sharpe (MRS, Britania, Buenos Aires, Argentina) broths respectively.

For yoghurt preparation, bacterial strains were growth overnight in reconstituted sterile (autoclaved at 110°C during 10 min) nonfat milk (Milkaut, Buenos Aires, Argentina). These cultures were then used to inoculate the sterile milk at a concentration of 0·5% (v/v) each strain suspension (total inoculum of 1%, v/v), and incubated without agitation for 16 h at 37°C.

Colitis induction and yoghurt administration

Induction of colitis with trinitrobenzenesulfonic acid (TNBS) was performed as previously described (del Carmen *et al.* 2011). Mock group was composed by control mice that received only PBS mixed with ethanol (without TNBS), using the same protocol.

TNBS-injected mice were subdivided into two groups (containing $n = 6$ each): (i) TNBS group that was the inflammation control group, and (ii) mice receiving yoghurt (Yoghurt-TNBS group). Yoghurt was administered *ad libitum* to mice 1 day before TNBS injection and for 4 consecutive days after colitis induction. TNBS and mock groups received milk *ad libitum,* the same milk used as food matrix for the bacterial fermentation in yoghurt.

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 daily controlled.

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.

Assessment of colonic inflammation

Four days after TNBS injection, three mice per group were killed by cervical dislocation. Large intestines were removed, inspected for macroscopic evaluation according

to the grading system described previously (Cenac *et al.* 2002) and their lengths were measured. Then, the intestines were prepared for histological analysis. Microscopic lesions and extent of colonic damage and inflammation were assessed using previously described score (LeBlanc *et al.* 2011). The microscopic scoring analyses were performed by two different scientists. High macroscopic or histological damage scores indicated increased damage in the intestines. Microbial translocation to liver was also determined as previously described (LeBlanc *et al.* 2011).

Cytokine analysis in colitis model

Cytokine producing cells were also analyzed in the intestinal tissue samples. IL-10 and IL-17 positive cells were detected by indirect immunofluorescence (del Carmen *et al.* 2011). Rabbit anti-mouse IL-10 (ProSci Inc. Poway, CA) or goat anti-mouse IL-17 (BD Bioscience, San Diego, CA) polyclonal antibodies were used as primary antibodies, and fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit or rabbit anti-goat antibodies (Jackson Immuno Research Laboratories Inc., West Grove, PA) were used as secondary antibodies. The number of fluorescent cells was counted (two individual blinded counts per sample) and the results were expressed as the number of positive cells in ten fields of vision as seen at 1000× using a fluorescence light microscope (Carl Zeizz, Germany).

DMH-colon carcinogenesis model and yoghurt feeding protocol

BALB/c mice (female, 6-week-old, weighing 22–25 g) obtained from the inbred closed colony were maintained in a room with a 12-h light/dark cycle at 18 ± 2°C at CERELA-CONICET. Animal protocol was approved by the Animal Protection Committee of CERELA (CRL-BIOT-LI-20141A), and all experiments comply with the current laws of Argentina.

To induce the tumours, mice were injected subcutaneously with the carcinogen 1,2-dimethylhydrazine (DMH, Sigma, St. Louis, MO) at a weekly dose of 20 mg kg^{-1} $week^{-1}$ (in 100 μl of sterile PBS) during 10 consecutive weeks. Approximately 60% of mice from tumour control group (DMH group) developed tumours 5–6 months after the first injection.

For the feeding protocol, unfermented nonfat milk (DMH group) or yoghurt (DMH-yoghurt group) were administered *ad libitum* during six months, starting with the first DMH injection and until the end of the experiment. Both groups were fed *ad libitum* with a balanced rodent diet. Each experimental group consisted of 30–35 mice.

Sample collection and evaluation of intestinal damages in DMH induced carcinogenesis model

Five animals from each group were killed monthly by cervical dislocation. Large intestines were removed and their contents collected with 500 μl of PBS containing Complete Mini EDTA-free Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Buenos Aires, Argentina), centrifuged (4000 ***g***, 10 min, 4°C) and supernatants were stored at −80°C until further cytokine analysis.

Multiple plaque lesions (MPL) in the large intestine were observed macroscopically and counted. Intestinal tissues were then prepared for histological evaluation as previously above for the inflammation model. MPL were observed in the microscopy, measured and their area were calculated and separated in two categories, <0·1 μm^2 and >0·1 μm^2.

Tissues were analyzed and scored microscopically as previously described (Santiago *et al.* 2007) with the considering of the tumour presence (tumour absent (0); present (1)). This score was added to those obtained for the other variables observed.

Determination of cytokines in the DMH induced carcinogenesis model

Samples obtained from the intestinal contents were assayed with the Cytometric Bead Array (CBA) Mouse Inflammation Kit (BD Bioscience) following the manufacturer's instructions. The concentration of each cytokine from the intestinal fluid of each mouse was obtained and the results were expressed in relation to the total protein concentration measured in the sample, determined using the Bio-Rad Protein Assay based on the method of Bradford (Bradford 1976). IL-10/TNF ratio for each mouse was also determined.

Statistical analysis

All data are expressed as mean values and standard deviations and they were analyzed using MINITAB 16 Statistical Software (Minitab, State College, PA). For the colitis model, the experiment was repeated three times with three animals per group. No interactions were observed between the repetitions and the results obtained from the three trials were analyzed together ($n = 9$). For the colon carcinogenesis model, the experiment was repeated twice with five animals per sample. No interactions were observed between the repetitions and the results obtained from the two trials were analyzed together ($n = 10$). Comparisons were performed by an ANOVA general linear model followed by Tukey's *post hoc* test. Unless otherwise specified, $P < 0{\cdot}05$ was considered significant.

Results

Yoghurt shows anti-inflammatory activity in a TNBS induced acute colitis model

As shown in Fig. 1, mice that received yoghurt resulted in a significant improvement in body weight loss, and even started to gain weight at day 3 post-TNBS, compared to TNBS control group (Fig. 1a). Macroscopic observations of the large intestines showed significant less damage score in mice that received yoghurt compared to the animals from TNBS group (Fig. 1b). The inflammation in the TNBS group was also accompanied by a decreased length of the large intestine, this was not observed in the mice supplemented with yoghurt whose intestinal lengths were similar to the mock (normal) group (Fig. 1b). At the histological level, mice receiving yoghurt showed a decrease in intestinal damage scores compared to the TNBS group (Fig. 1b). Most of the mice that received yoghurt presented in their large intestines a lower number of infiltrating cells and less thickening of the muscle layer than the inflamed control animals (TNBS group), accompanied by intact crypt architecture in large areas of intestinal tissue (Fig. 1c).

The intestinal damages were associated with microbial translocation to liver. Mice that received yoghurt showed a significantly reduced microbial growth in their livers compared to TNBS group (Fig. 1d).

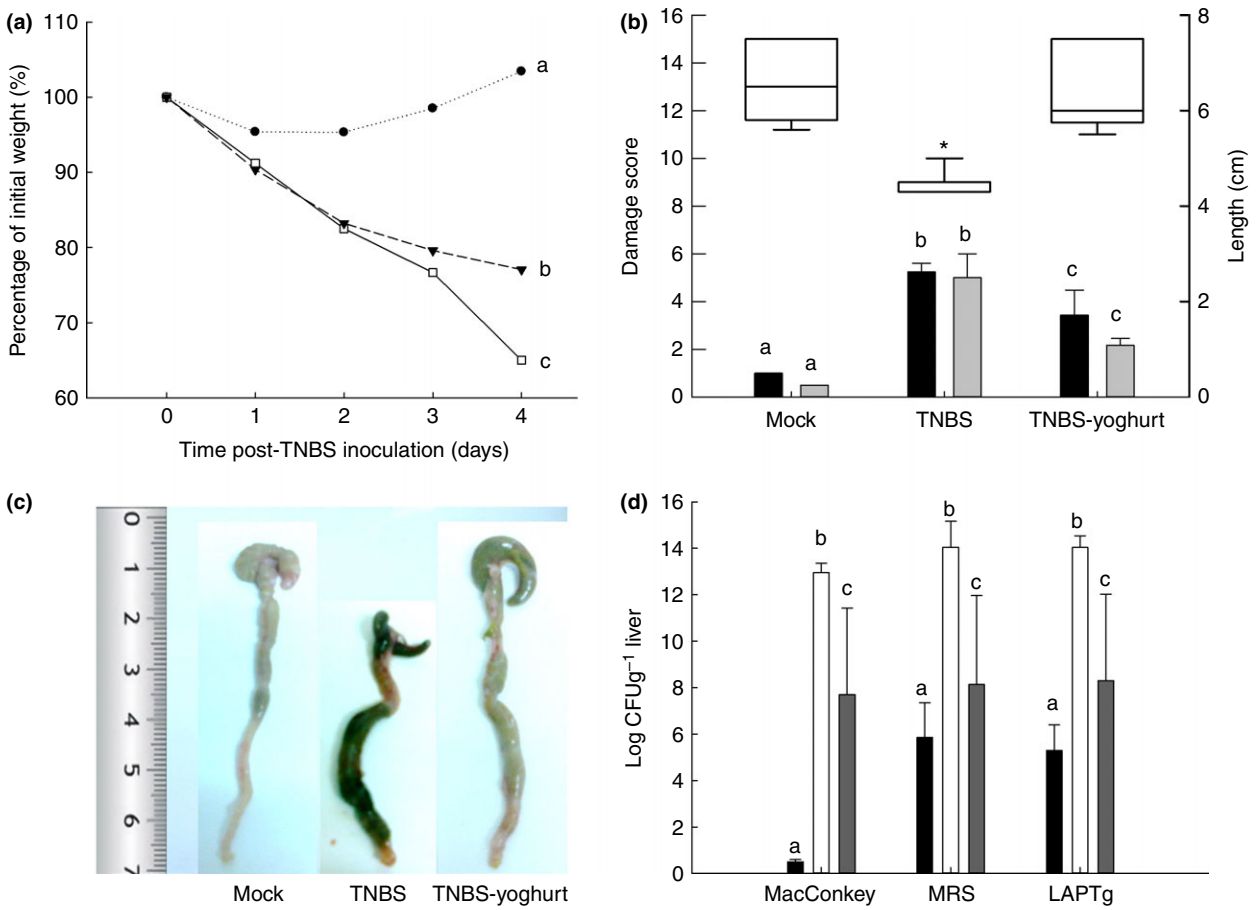

Figure 1 Effects of yoghurt on TNBS induced inflammation in mice. The loss of body weight (a), macro and microscopic damage scores (b and c), with the difference of colon length (box diagram, b) and liver microbial translocation (d) were evaluated in mice from the different groups Body weight was measured from the day of TNBS inoculation up to 4 days post-TNBS and they are represented as a percentage of the initial mice body weight for Mock group (black circles and dotted line), TNBS group (white square and black line), and TNB-yoghurt group (black triangle and dashed line). Microscopic (black bars) and macroscopic (grey bars) damage score correspond to samples taken 4 days post TNBS. Each value represents the mean of $n = 9 \pm$ SD from three individual trials. Photographs of large intestine are representative for each group. Microbial growth in MacConkey, MRS or LAPTg of liver samples obtained from Mock group (black bars), TNBS group (white bars), and TNBS-yoghurt group (gray bars) were evaluated. Results are expressed as means ± SD of the log CFU g^{-1} liver. ^{a,b,c} Means for each value without a common letter differ significantly ($P < 0{\cdot}05$); * shows a significant difference compared Mock treated group in (d).

The production of two cytokines was also evaluated in the intestinal tissues. The results showed that mice from TNBS group increased the number of the IL-17+ cells compared to the mock group. In contrast, the cells producing this pro-inflammatory cytokine were not increased in the mice that received yoghurt; where they remained similar to those of mock group. As for the regulatory cytokine IL-10 positive cells, it was shown that these were increased significantly in both TNBS and TNBS-yoghurt groups compared to mock group (Fig. 2a). An anti-inflammatory cytokine profile was observed in the animals that received yoghurt after TNBS induction since the IL-10 + cells/IL-17+ cells ratio was increased significantly in these mice compared to both mock and TNBS groups (Fig. 2b).

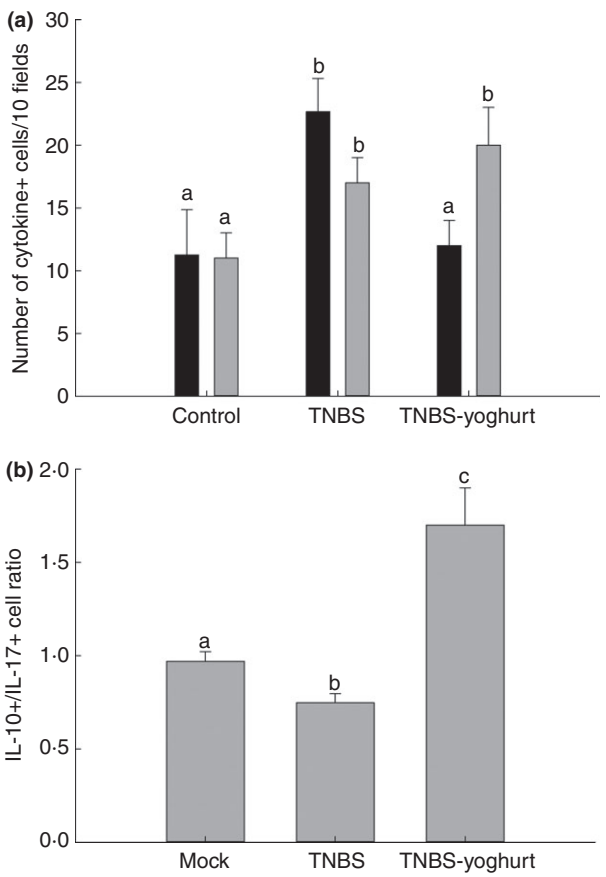

Figure 2 IL-17 and IL-10-producing cells in the large intestine tissues. IL-17 (black bars) and IL-10 (grey bars) positive cells were evaluated by immunofluorescence in mice from mock group, TNBS group, and mice that received yoghurt (a). The results are expressed as the means of the total number of positive cells counted in 10 fields at 1000× magnification. The ratio between the number of IL-10 + / IL-17 + cells is also represented for each group (b). Data correspond to the means of $n = 9 \pm$ SD. ^{a,b,c}Means for each value without a common letter differ significantly ($P < 0{\cdot}05$).

Yoghurt shows anti-cancer properties in a DMH induced colon carcinogenesis model

MPL were observed macroscopically and counted. The number of MPL was significantly different between the groups of mice that received yoghurt and the control that received unfermented milk (DMH group) (Fig. 3a). When the sizes of the MPL were measured in the histological sections, significant differences between the two groups were observed in all the samples taken throughout the experiment. The microscopic observation of MPL showed the predominance of mononuclear cells. Mice from DMH group showed more than 50% of the MPL with areas higher than 0·01 μm^2 (Fig. 3a). Some of the MPL occupied areas of 0·4–0·5 μm^2 (Fig. 3a). It was also observed that microscopic counts of MPL were higher than the counts performed macroscopically; however, some of them were smaller than 0·1 μm^2. Differing from the animals in the DMH group, the mice that received yoghurt showed a predominance of smaller MPL (area <0·01 μm^2).

The analysis of histologic damages showed the highest scores, with the inflammation of the large intestine characteristic of the model, in the samples obtained from DMH group (Fig. 3b). Mice from this group increased the intestinal damages throughout the time of the experiment. In the last two samples (months 5 and 6) mice showed severe loss of mucosal architecture, important cellular inflammation and thickness of muscle, depletion of goblet cells, and the presence of crypt abscess formation (Fig. 3c). Only three mice from this group developed tumours between months 5 and 6; however, we cannot discard that mice killed at months 3 and 4, with important histological lesions in their intestine could have developed tumour in the future. Mice that received yoghurt showed decreased damage score, especially in the sample obtained at the end of the experiment (month 6) compared to the DMH group. The lack of significant differences in the samples from months 4 and 5 was due to one mouse that increased also the standard deviations for the values obtained at these time points. Besides this particular mouse, the rest of the animals showed less inflammation with a lower predominance of MPL than those observed in the DMH group and increased goblet cells (Fig. 3c).

The analysis of cytokines in the intestinal fluids showed a pro-inflammatory status in mice from DMH group. They showed highest concentrations of the monocyte chemoattractant protein-1 (MCP-1) and the pro-inflammatory cytokine TNFα (Fig. 4a). Mice that received yoghurt showed decreased levels of MCP-1 and increased IL-10, compared to the DMH group (Fig. 4a). These results correlated with the significant difference obtained for IL-10/TNF ratio that was increased in the mice from

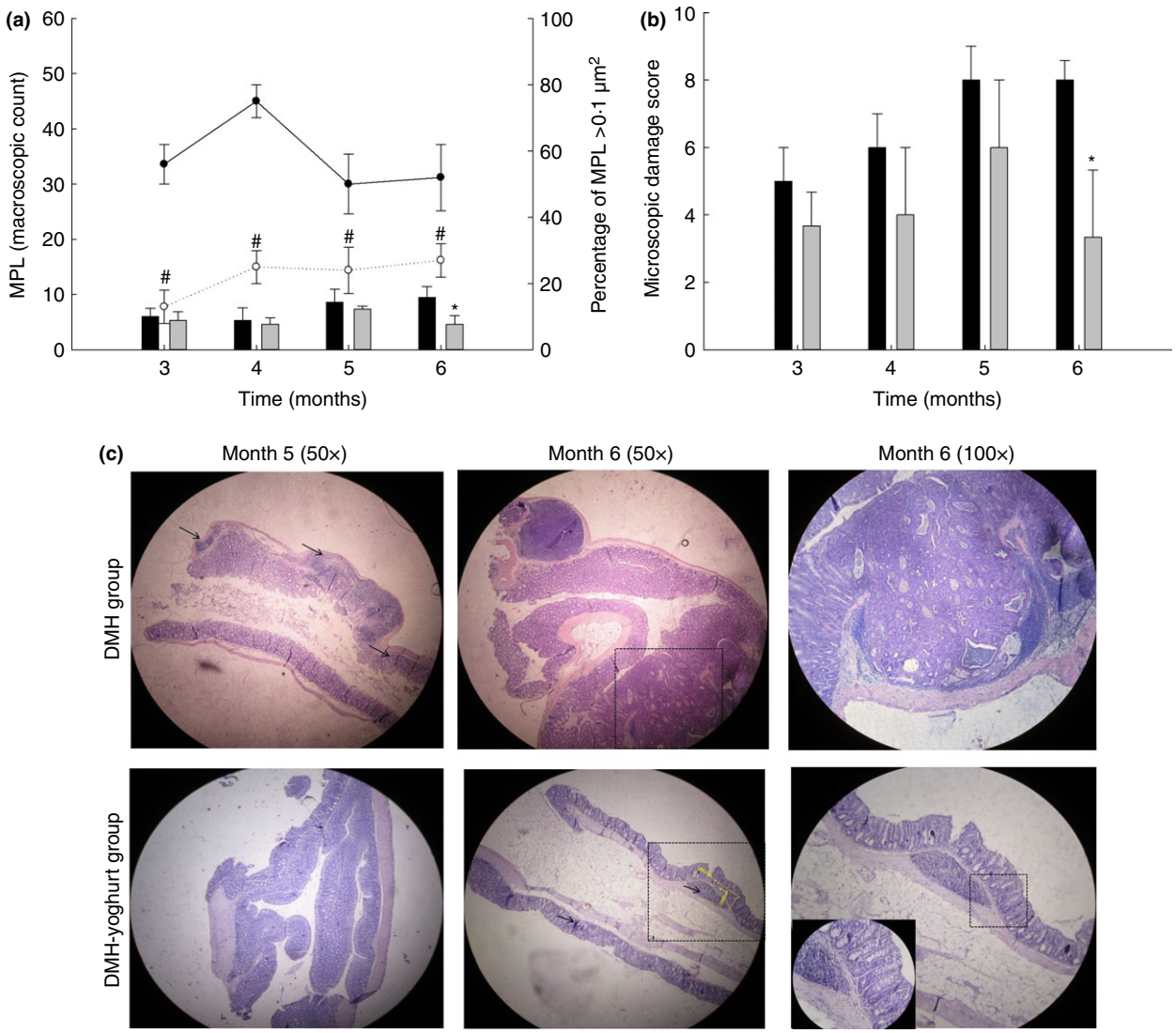

Figure 3 Effects of yoghurt on DMH induced colon cancer in mice. (a) Mice (n = 10 per group) were killed every month, multiple plaque lesions (MPL) were macroscopically counted (black and gray bars for DMH and DMH-yoghurt groups respectively) and intestinal tissues were prepared for histological evaluation. Tissues were analyzed under microscopy, the size of MPLs was measured and grouped according their areas (< or >0·1 μm^2). The percentage of MPLs > 0·1 μm^2 is represented with black circles and lines for DMH group and white circles and dotted line for DMH-yoghurt group. (b) Tissue damages were scored (black and gray bars for DMH and DMH-yoghurt groups respectively). Data are expressed as mean values and SD. * and # show significant differences compared to DMH group (P < 0·05). Figure (c) shows representative histological pictures observed in most animals from each group. Small and big infiltrates are shown with arrows. For both groups at 6 months, a representative area was selected (dotted square) at 50× and showed at higher magnification (100X). An area selected (dotted area) from the picture obtained at 100× for the mouse of DMH-yoghurt group is showed at 400× in the bottom of the picture to show the presence of goblet cells in the animals from this group.

DMH-yoghurt group compared to the DMH group (Fig. 4b).

Discussion

Fermented products like yoghurt have been consumed for thousands of years, and numerous beneficial properties have been attributed to them. It is also common for patients suffering from IBD to look for natural therapies that can improve their quality of life, especially considering that their current treatment options are prolonged and cause many adverse secondary effects. Fermented milks and probiotics appear to be very promising for IBD patients since several studies have shown that these can

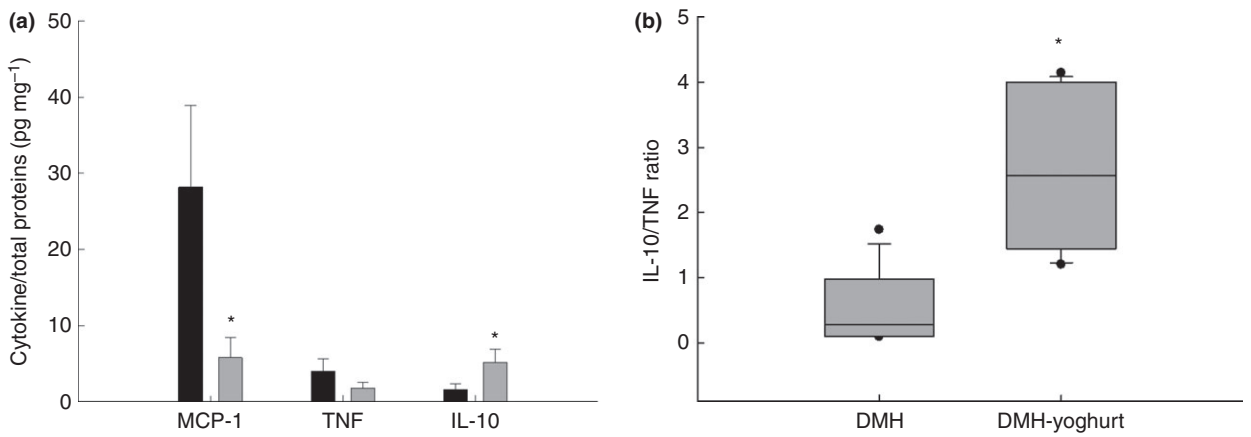

Figure 4 Cytokine analysis from the intestinal contents of mice. MCP-1, TNF and IL-10 are expressed as cytokine concentration in relation to total protein concentration in the large intestine content of mice from DMH group (black bars) and DMH-yoghurt (group gray bars) (a). Figure (b) shows a ratio between IL-10 concentration and TNF-α concentration. Results were obtained from samples of months 5 and 6 analyzed together ($n = 10$). *Shows a significant difference ($P < 0{\cdot}05$) with DMH group.

possess anti-inflammatory properties (de Moreno de LeBlanc and LeBlanc 2014; Celiberto *et al.* 2015; Wasilewski *et al.* 2015). Probiotic yoghurts are by definition a fermented milk product elaborated using *Lact. bulgaricus* and *Strep. thermophilus,* but these micro-organisms must be considered probiotics and not simply live starter cultures as is the case for conventional (traditional) yoghurts (Shadnoush *et al.* 2013). There are many studies that have shown the health promoting benefits associated to the consumption of traditional yoghurts; however, these are not consider probiotics just because of these beneficial properties (Shamir and Donovan 2015). We also have previous studies with a yoghurt composed of 10 different bacterial strains for which its benefices were demonstrated in animal models (de Moreno de LeBlanc *et al.* 2009). However, we cannot be sure that the effect was associated with some particular bacteria or if all bacterial pool is responsible for the results obtained. Our hypothesis was that some strains of *Strep. thermophillus* and *Lact. bulgaricus* (normal starter cultures for the preparation of yoghurts, cheeses and other fermented products) have the potential to exert beneficial effects on their own, and could thus be considered as probiotics. With this in mind, we decided to prepare a yoghurt with two bacteria that were previously selected for showing *in vitro* anti-inflammatory properties in cell culture lines and also in primary cell cultures from human and mice (del Carmen *et al.* 2014a). In the present work, yoghurt was elaborated with the selected strains and evaluated in two mouse models that mimic human colitis and colon carcinogenesis.

The anti-inflammatory effect of the new potential probiotic yoghurt was demonstrated using a TNBS-induced IBD model. Mice that received yoghurt decreased the severity of the inflammation compared to the animals given unfermented milk. These results were similar to those obtained with a yoghurt prepared with the pool of 10 bacterial strains (from which the strains used in this study were selected) (de Moreno de LeBlanc *et al.* 2009), and to others obtained with other probiotic micro-organisms (Toumi *et al.* 2014; Srutkova *et al.* 2015).

Different mechanisms can be involved in the beneficial effect observed; however, considering the immunomodulatory properties reported for the individual strains (del Carmen *et al.* 2014a), some immune populations involved in the inflammatory process were evaluated. In the present work, the evaluation of IL-17+ cells confirmed the anti-inflammatory effect of our potentially probiotic yoghurt. Mice that received yoghurt maintained a number of IL-17 + cells that was similar to that observed in the mock group (without inflammation). In contrast, mice from TNBS group, showed a significantly increased number of cells producing this inflammatory cytokine, which was demonstrated that play an important role in some T cell-mediated diseases, including IBD (Catana *et al.* 2015). In addition, IL-10 was analysed as a regulatory cytokine. The role of IL-10 in intestinal inflammation and carcinogenesis was reported in different models (Sydora *et al.* 2003; de Moreno de Leblanc *et al.* 2011). Lactic acid bacteria were genetically modified to produce IL-10 protein or to deliver IL-10 cDNA and they have been effectively used to decrease the severity of the inflammation in animal models (del Carmen *et al.* 2011, 2013, 2014b). In this study, both groups of mice treated with TNBS increased the number of IL-10+ cells compared to the mock group. However, this increase was not sufficient to prevent the increase of pro-inflammatory cytokines that remained elevated in the TNBS group. In

contrast, those mice which received yoghurt maintained an elevated production of IL-10 but down regulated the production of the pro-inflammatory cytokines. This anti-inflammatory profile in the intestines of these animals was confirmed with the increased IL-10/IL-17 ratio. Thus, the induction of the host anti-inflammatory response through IL-10 production could be one of the mechanisms by which this new potential probiotic yoghurt can exert its anti-inflammatory effect.

Considering the relation between inflammation and cancer development, especially between IBD and increased risk of colorectal cancer (Herszenyi *et al.* 2015), the new yoghurt was also evaluated in a mouse colorectal carcinogenesis model. The results obtained showed the anti-carcinogenesis potential of our probiotic yoghurt. Mice receiving yoghurt reduced the severity of the inflammation in colon tissues which is related with tumour development. This effect was also associated with the host immunomodulation exerted by yoghurt consumption. In this model, macrophages were evaluated because they are the immune cells with a key role in the immune response against tumours (Van Ginderachter *et al.* 2006). An exacerbated inflammation with increased macrophage infiltration was observed in the mice from DMH group and it was associated with the elevated levels of MCP-1 in the intestinal fluid of these mice. This agrees with reports from histological observation of samples from patients with colon adenocarcinoma in which macrophages were the most numerous inflammatory cells found (Mogoanta *et al.* 2014). In contrast, mice given our potential probiotic yoghurt decreased the concentration of MCP-1 and also decreased the levels of the pro-inflammatory cytokine TNFα. For intestinal cancer, the inflammatory environment improves tumour growth (Wang and Karin; Tong *et al.* 2011) and probiotic microorganisms with intestinal anti-inflammatory potential have also shown protection against colorectal cancer (de Moreno de Leblanc and Perdigon 2010; Bassaganya-Riera *et al.* 2012). Finally, IL-10 was analyzed as an anti-inflammatory cytokine. And the regulated profile of cytokines (increased IL-10/TNFα ratio) observed in the intestinal fluids of mice that received yoghurt was associated to the anti-tumour effect of yoghurt. However, we cannot discard that other mechanisms such as the production of short chain fatty acids (SCFA) can be also involved in the beneficial effect of the yoghurt alone or in combination with the low pH generated in the fermented product, as was recently demonstrated (Matsuki *et al.* 2013).

Finally, considering that a functional food was developed, it is important to evaluate the acceptability of consumers. In this sense, the assessment of volatiles/sensory acceptance of this yoghurt will be performed.

The results obtained in this study show the importance to select starter cultures with desirable properties as a strategy to maximize health effect in the final product. The new potential probiotic yoghurt could be recommended as a functional food with anti-inflammatory and anti-tumour properties at intestinal level. Although it is a natural food that can be ingested by the general population, because of its specific beneficial properties it could be of interest to accompany the therapy of patients with chronic inflammatory diseases in the intestine. However, considering the current guidelines for the appointment of probiotics, future human studies are needed to substantiate these results and to name it as probiotic yoghurt.

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Conflict of Interest

No conflict of interest declared.

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