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Protective effect of *Lactobacillus delbrueckii* subsp. *Lactis* CIDCA 133 in a model of 5 Fluorouracil-Induced intestinal mucositis

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ABSTRACT

Mucositis is a cytotoxic side effect caused by chemotherapy drugs, such as 5-Fluorouracil (5-FU), being a serious clinical issue. *Lactobacillus* spp. could be a helpful strategy to alleviate 5-FU chemotherapy-caused intestinal damage, due to their ability to contribute to intestinal homeostasis through improvement of microbiota balance and immunomodulation. In this work we evaluated the effect of *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 fermented milk in 5-FU-induced experimental mucositis. Intestinal histology, permeability and biochemical parameters showed that animals administrated with 5-FU and treated with CIDCA 133 fermented milk presented reduced intestinal IgA secretion and lower permeability in the small bowel. We showed that this strain preserves villus/crypt ratio, reduces the loss of goblet cells and inflammatory infiltration in ileum sections of 5-FU-treated animals. In conclusion, CIDCA 133 is able to prevent the intestinal mucosa damage caused by 5-FU revealing to be a promising strategy for intestinal mucositis treatment.

1. Introduction

Chemotherapy and radiotherapy are the principal treatments used in several types of cancer (Longley, Harkin, & Johnston, 2003; Sonis, 1998). The 5-Fluorouracil (5-FU) is one of the chemotherapeutic agents used in the clinical oncology practice. This drug acts on the proliferation of cancer cells through the inhibition of the thymidylate synthase (TS) enzyme (leading to unbalance of the nucleotide pool), as well as the incorporation of its metabolites into the DNA and/or RNA of these cells (Sonis, 2004), which impedes its normal functioning and induces apoptosis (Miura et al., 2010; Pinedo & Peters, 1988). However, this treatment, apart from destroying neoplastic cells, affect different cell populations of healthy tissue situated throughout the human body (Duncan & Grant, 2003), being mucositis one of the most prevalent adverse effects.

The mucositis is characterized by three phases (inflammation, epithelial degradation and ulceration) leading to quick loss of intestinal structure and functionality (Duncan & Grant, 2003) including damage

Abbreviations: 5-FU, 5-fluorouracil; CTL, control; CIDCA 133, *Lactobacillus delbrueckii* ssp. *lactis* CIDCA 133; CD, *crypt depths*; DMSO, dimethyl sulfoxide; DTPA, diethyleneaminopentacetic acid; EPO, eosinophil peroxidase; HTAB, hexadecyltrimethylammonium bromide; HE, hematoxylin and eosin; MPO, myeloperoxidase; MUC, mucositis; NAG, N-acetyl-beta-D-glucosaminidase; NO, nitric oxide; GIT, gastrointestinal tract; PBS, phosphate buffered saline; PMSF, phenylmethanesulfonyl fluoride; ROS, reactive oxygen species; SCFAs, short chain fatty acids; TS, thymidylate synthase; TER, trans, epithelial resistance; PAS, periodic acid schiff; VH, villus heights

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of the epithelial wall, increased intestinal permeability and bacterial translocation; disbalance of intestinal microbiota and reduced levels of mucin secretion (Stringer et al., 2009). Above effects lead to compromised food intake that in turn compromises cancer therapy (Logan, Gibson, Sonis, & Keefe, 2007; Soveri, Hermunen, de Gramont, Poussa, Quinaux, Bono, & Österlund, 2014).

Local anesthetics, analgesics and/or antibiotics are some proposed treatments for this disease. However, these palliative approaches present several side effects as short and temporary relief (Herbers, de Haan, van der Velden, Donnelly, & Blijlevens, 2014). Thus, the use of bacteria/yeast strains with probiotic potential constitute a promising perspective for the treatment of mucositis due to their ability to modify the composition of the intestinal microbial community, as well as their capability to improve the epithelial barrier function and the immunomodulating effect (Bastos et al., 2016; Carvalho et al., 2017; Justino et al., 2015; Oh, Lee, Lee, Lee, & Kim, 2017).

The probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (Hill et al., 2014). In this context, several studies conducted with probiotic microorganisms have demonstrated strain-dependent effects in the prevention/treatment of intestinal disorders. Indeed, beneficial effects have been demonstrated in (i) maintenance of the epithelial barrier in dextran sulphate-induced intestinal inflammation in mice (Mennigen et al., 2009), (ii) protection of the intestinal function in a model of hydrogen peroxide-induced epithelial barrier disruption (Seth, Yan, Polk, & Rao, 2008), (iii) increase in trans-epithelial resistance (TER), IL-10 deficient mice (Ewaschuk et al., 2008). The mechanisms behind these probiotics effects are related or to the reduction in IL-12 and IFN-γ levels, and increased of IL-10 (Martins et al., 2009; Sokol et al., 2008), diminution of cell apoptosis by inhibition of caspase 3 activation (Dalmasso et al., 2006; Mennigen et al., 2009), prevention of oxidative damage by induction of mucosal glutathione biosynthesis (Lutgendorff et al., 2009), enhancing of mucine gene expression (Caballero-Franco, Keller, De Simone, & Chadee, 2007) among others. For all these reasons, the probiotics represent a promising adjuvant strategy for mucositis amelioration.

Pioneering studies using *Lactobacillus delbrueckii* subsp. *lactis* CIDCA 133 strain (hereafter CIDCA 133) have shown its resistance to high acid and bile concentrations and the ability to inhibit the growth of food contaminants (Kociubinski, Pérez, & De Antoni, 1999; Kociubinski, Pérez, Añón, & De Antoni, 1996) and pathogenic microorganisms such as entero-hemorragic *E. coli* (Hugo, De Antoni, & Pérez, 2006). In addition, the CIDCA 133 strain, was able to decrease harmful bacterial enzymatic activities such as nitrate reductase (Hugo, Kakisu, De Antoni, & Pérez, 2008). Furthermore, CIDCA 133 strain has demonstrated to resist the inhibitory cationic effectors of the innate immune system in cultured human enterocytes (Hugo, De Antoni, & Pérez, 2010) and human ß-defensins (Hugo, Tymczyszyn, Gómez-Zavaglia, & Pérez, 2012). Another *in vitro* study on the interaction between CIDCA 133 strain with cultured murine macrophages (RAW 264.7 cells) infected with *C. rodentium* revealed that the presence of probiotic bacteria is able to stimulate phagocytosis, induces reactive oxygen species (ROS and NO), and promotes expression of surface markers related to antigen presentation (Hugo, Rolny, Romanin, & Pérez, 2017).

Altogether, these studies support the potential probiotic effect of CIDCA 133 strain and the promising activities that could be studied in different diseases models and their resulting response. Therefore, the aim of the present study was to investigate the effect of oral administration of *L. delbrueckii* subsp. *lactis* strain CIDCA 133 fermented milk in an *in vivo* model of 5-FU induced mucositis in BALB/c mice.

2. Materials and methods

2.1. Probiotic strain

Lactobacillus delbrueckii subsp. *lactis* CIDCA 133 strain was isolated from raw cow milk (Kociubinski et al., 1996), with probiotics characteristics described as was mentioned in previous section. This strain belongs to the culture collection of the Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina).

2.2. Dairy formulation and growth conditions

Frozen bacterial suspension (−80 °C) were reactivated in de Man, Rogosa and Sharpe (MRS) broth (Kasvi, Italia) at 37 °C for 16 h. Next, 50 µL of bacterial culture were inoculated into 15 mL of reconstituted skim milk (12% w/v) supplemented with glucose (2% w/v), yeast extract (1,2% w/v) (milk broth) for overnight growth at 37 °C. Afterwards, the culture was diluted 100 times in sterilized milk broth and administrated *ad libitum* for 12 h. Bacterial suspension were renewed every 12 h to avoid bacterial decantation and clogging. This beverage and standard chow diet were administered for 13 days *ad libitum* access. To confirm the administrated CFU to the mice, the number of colonies (viable bacteria) were counted by pour plate method (MRS-agar medium) by counting the colony forming unit (CFU) after incubation of 16 h.

2.3. Animal trial and experimental design

All experiments described were conducted on male BALB/c mice (4–6 weeks old, weight 21–24 g) obtained from Centro de Bioterismo (CEBIO) of the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil). Animals were kept in polycarbonate boxes under controlled conditions: temperature around 21 ± 2 °C, humidity of 55 ± 10%, photoperiod of 12 h light/dark. All procedures were in compliance with the Brazilian College of Animal Experimentation (COBEA) and were approved by the Local Animal Experimental Ethics Committee (CEUA-UFMG, Protocol no. 366/2012).

Mice were randomly split into 4 groups (8 animals per group): Control (CTL), Control + probiotic (CIDCA 133), Mucositis (MUC), and mucositis + probiotic (MUC + CIDCA 133). Animals were orally fed on a daily basis with non-fermented milk (CTL and MUC) or fermented milk by CIDCA 133 (7.5×10^7 CFU/mL) (CIDCA 133 and MUC + CIDCA 133) (the water was substituted by fermented milk or only milk broth) over a period of 13 days. In order to induce gastrointestinal mucositis on day 10, mice (MUC and MUC + CIDCA 133) received a single intraperitoneal injection (*i.p.*) of 5-FU (300 mg/kg) (Fauldfluor®, Libbs, São Paulo, Brazil) as previously reported (Carvalho et al., 2017). Control groups (CTL and CIDCA 133) received NaCl 0.9% (w/v) instead of 5-FU. At 72 h after *i.p.* administration either 5-FU or saline solution, the animals were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) mixture (Agener União) (Fig. 1). The blood and the small intestine were collected for analysis. Body weight, milk and feed intake were assessed daily.

2.4. Intestinal histology, morphology and goblet cells analysis

After euthanasia, the entire small intestine was removed and its length was measured, ileum sections (approximately 5 cm) were longitudinally opened, carefully washed and rolls were excised and handled for histological analysis. Tissue samples were placed in 10% buffered formaldehyde for 24 h. Then, samples were embedded in paraffin wax and slides of 4 µm sections were mounted on glass slides and stained with hematoxylin and eosin (HE) or Periodic Acid Schiff (PAS). In HE-stained slides, alterations of the mucosal architecture and polymorphonuclear cells infiltrate were analyzed using a histopathological grading system (Soares et al., 2008). Ten images per specimen were captured with a BX41 optical microscope (Olympus, Tokyo, Japan) and digital images were processed using *ImageJ 1.51j.8* software (NIH, Bethesda, MD, USA), for morphological examination. Image acquisition was performed with a 40× magnification objective. Villus heights (VH)

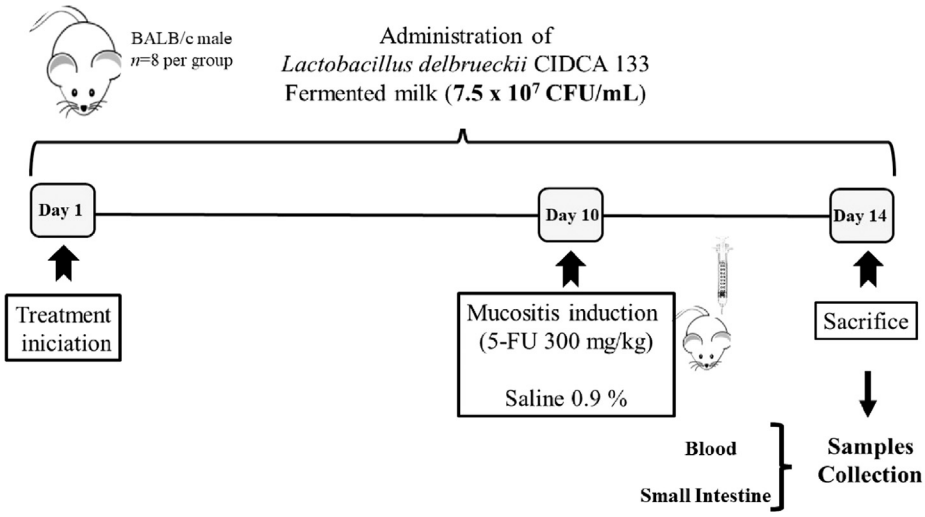

Fig. 1. Experimental protocol of mucositis induced in a murine model. BALB/c mice (n = 8 animals per group) were treated with *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk for 13 days. 300 mg/kg of 5-FU by intraperitoneal injection was administrated, on day 10, to induce the mucositis. Control groups received 0.9% (w/v) NaCl (*i.p.*).

measurements (from villus tip to villus- crypt junction) and crypt depths (CD) (defined as invagination depth between adjacent villi) per small intestinal tissue section (10 sections/mouse) were measured. The values were averaged. The ratio of villus height/crypt depth from the intestinal epithelium was also acquired. The study of goblet cells was done in PAS-stained samples.

2.5. Intestinal permeability

In order to study the epithelial permeability from lumen to blood, intestinal permeability was determined by blood radioactivity of diethylenetriamine pentaacetic acid (DTPA) labelled with technecium-99 m (^{99m}Tc). The DTPA probe is larger enough (molecular weight: 500–700 Da) to allow for the study of intestinal permeability through the paracellular pathway (Jørgensen, Nielsen, Espersen, & Perner, 2006). Briefly, at the end of the experimental design, all mice received 0.1 mL of ^{99m}Tc-DTPA solution containing 18.5 MBq of activity by gavage. After 4 h, mice were anesthetized and blood samples were collected, weighted, and placed in appropriate tubes for radioactivity determination using a Wallac Wizard 1470-020 automated gamma counter (Perkin Elmer, Waltham, MA, USA). A standard dosage containing the same injected amount was counted simultaneously in a separate tube, which was defined as 100% radioactivity. The results were expressed as the percentage of injected dose per gram (%ID/g) of blood.

2.6. Leukocyte count

Blood samples were collected by axial plexus. The total number of white cells was measured by an automatic hematological counter (Bio-2900 Vet, Bioeasy, EUA). Results were expressed as number of leukocytes per μL of sample.

2.7. Enzyme assay: Intestinal myeloperoxidase (MPO) and eosinophil peroxidase (EPO) activity

To evaluate the extent of neutrophil and eosinophil accumulation in the intestinal mucosa the MPO and EPO activity assays were performed (Bradley, Christensen, & Rothstein, 1982; Vieira et al., 2012). Enzyme activity values are considered appropriate and reliable markers for neutrophil and eosinophil infiltration, respectively (Vieira et al., 2009). Briefly, 100 mg of tissue was homogenized in 1.9 mL phosphate buffered saline (PBS, pH 7.4) using a tissue homogenizer. The homogenate was centrifuged (3000*g* for 10 min), then the pellets were subjected to hypotonic lysis (1.5 mL of 0.2% NaCl) and osmolarity was restored with 1.5 mL of a 1.6% NaCl solution containing 5% glucose. Then, samples were centrifuged (3000*g* for 10 min) and the pellet was resuspended in 0.5% hexadecyltrimethylammonium bromide (HTAB-Sigma-Aldrich, USA) in phosphate buffer. The tissue suspension was homogenized, freeze-thawed three times in liquid nitrogen and centrifuged for 15 min at 3000*g*. The resulting supernatant was used in the colorimetric assay to measure EPO and MPO activities.

For EPO assessment, 75 μL of supernatant was added to 75 μL of 1.5 mM *o*-phenylenediamine (OPD-Sigma-Aldrich, USA), diluted in 0.075 mM Tris–HCl and 6.6 mM H_2O_2 and incubated at 37 °C during 30 min. For MPO quantification, 25 μL of supernatant was added to 25 μL of 1.6 mM 3,3,5,5′-Tetramethylbenzidine (TMB-Sigma-Aldrich, USA) in dimethyl sulfoxide (DMSO-Sigma-Aldrich, USA). After the addition of 100 μL 0.5 mM H_2O_2, the solution was incubated at 37 °C for 5 min. Both reactions were stopped by adding 50 μL of 1 M H_2SO_4. Absorbance was measured at 492 nm (EPO) and 450 nm (MPO) on a microplate spectrophotometer (Bio-Rad 450 model, Bio-Rad Laboratories, Hercules, CA, USA). Results were reported as MPO or EPO arbitrary units/mg of tissue.

2.8. Intestinal secretory IgA (sIgA)

Levels of sIgA were determined by enzyme-linked immunosorbent assay (ELISA), as described by Martins et al. (2009). To this end, the small intestine of each mouse was removed by an incision at the gastroduodenal and ileocecal junctions. The content was withdrawn, weighed, and supplemented with PBS 0.1 M (pH 7.2) with protease inhibitors cocktails [1 μM aprotinin, 25 μM leupeptin, 1 μM pepstatin and 1 mM Phenylmethanesulfonyl fluoride (PMSF)], this mixture was added in the ratio of 500 mg of intestinal contents per 2.0 mL PBS. Afterwards, samples were centrifuged (5.000 rpm for 30 min at 4 °C), and the supernatant was collected and frozen at −80 °C for further dosage of the immunoglobulin.

To determinate total sIgA, microtiter plates (Nunc-Immuno Plates, MaxiSorp) were coated with goat anti-mouse IgA antibody (M-8769, Sigma, St. Louis, USA) in coat buffer (1 M Na_2CO_3; 0,1M $NaHCO_3$; pH 9.6) for 18 h at 4 °C. Plates were washed 5 times with washing solution (0.1 M PBS containing 0.05% Tween 20) and blocked with 200 μL of blocking solution (1% albumin in PBS Tween 20) for 1 h at room temperature. The plate was then washed 5 times and the pre-diluted intestinal fluids (1:1000) in 0.1 M PBS containing 0.05% Tween 20 were added and incubated at room temperature for 1 h. After this time, the plates were washed 5 times and biotin-conjugated anti-mouse IgA antibody (A 4789-Sigma, St. Louis, MO, USA) in PBS-0.05% Tween 20 (1:1000) was added and incubated for 1 h. After washing 100 μL/well of OPD (1 mg/mL) and 0.04% H_2O_2 substrates were added and

incubated for 10 min at room temperature. The reaction was stopped by the addition of 20 µL/well of 1 M H_2SO_4. The absorbance was determined at 492 nm using on a microplate spectrophotometer (Bio-Rad model 450, Bio-Rad Laboratories, Hercules, CA, USA). The results of the concentration of sIgA were expressed in µg/mL of intestinal contents.

2.9. Statistical analysis

Data normality was assessed by Kolmogorov-Smirnov test. Normal data (body weight loss, small bowel length, MPO activity, leukocytes count, intestinal permeability, villus/crypt ratio, histological score and globet cells count) were evaluated by analysis of variance (ANOVA) followed by the Bonferroni post-test (parametric distribution). Non-normal data (non-parametric distribution) (food and milk intake, EPO activity and sIgA levels) were evaluated by Kruskal-Wallis test followed by the Dunn's post-test. Two-ANOVA followed by the Bonferroni test was performed to compare the variation of body weight between all experimental groups. Mann Whitney test was performed to compare food and milk intake before and after mucositis induction. All data were analyzed using GraphPad Prism 5.0 software, and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Weight loss, shortening intestinal length and leucopenia was relieved in 5-FU induced mucositis mice by CIDCA 133 fermented milk treatment

Total milk and food intake was similar in all analyzed groups before the induction of mucositis. Animals of all groups consumed on average the same amount of food and drink (3 g and 5 mL/day/animal respectively). There were no significant statistical differences between the experimental groups. After induction of mucositis, the MUC group reduced food and milk intake, as expected (2.18 ± 1.29 g, and 2.81 ± 1.48 mL, $p < 0.01$). Nevertheless, administration of CIDCA 133 strain (7.5×10^7 CFU/mL) did not modify food and milk intake (1.90 ± 1.14 g and 2.65 ± 1.41 mL) (Fig. 2A and B). Accordingly, the analysis of time-course weight of mice showed a significant reduction in the 5-FU-treated variation from day 12 (MUC and MUC + CIDCA 133 groups). However, body weight variation was significantly lower in CIDCA 133-treated mice (Fig. 2C). In contrast, body weight of mice injected with 5-FU were significantly lower ($p < 0.001$). Noteworthy, the body weight loss of the mice in MUC + CIDCA 133 group was significantly lower (approximately 3%) than those in the MUC group (about 9%) ($p < 0.001$) (Fig. 2D). These results showed that treatment with CIDCA 133 fermented milk prevents the loss of body mass induced by chemotherapy accompanied by less intake of food and milk. It is also observed that treatment with CIDCA 133 without mucositis induction does not have any influence on the weight of the animals during the experimental period (Fig. 2C and D). It is worth noting that no mortality was observed during the experiment.

Intestinal shortening, was observed in MUC group (~46.00 ± 1.38 cm). Interestingly, we observed that animals treated with CIDCA 133 fermented milk were able to prevent the shortening of the intestinal length caused by the 5-FU (50.75 ± 2.31 cm, $p < 0.01$) as shown in Fig. 3A. As expected, the negative control groups (CTL and CIDCA 133) showed normal intestinal length (~55.00 ± 2.43 cm).

Significant leukopenia was observed after 5-FU administration (0.627 ± 0.13 cells × 10^3/µL) compared to the negative control (CTL) (4.350 ± 1.06 cells × 10^3/µL, $p < 0.001$) (Fig. 3B). However, this effect of 5-FU was minimized in animals treated with CIDCA 133 fermented milk (MUC + CIDCA 133 group) (2.043 ± 0.93 cells × 10^3/µL), which significantly ($p < 0.05$) prevented the reduction of the total leukocyte blood rate induced by chemotherapy. No statistical differences were observed between CTL and CICDA 133 groups (4.769 ± 1.49 cells × 10^3/µL) that were not treated for mucositis induction (Fig. 3B).

3.2. CIDCA 133 fermented milk treatment reduces inflammatory parameters in intestinal mucosa

To verify whether treatment with CIDCA 133 fermented milk could have an effect on reducing the infiltration of neutrophils and eosinophils in the intestinal mucosal layer, the activity of myeloperoxidase (MPO) and eosinophil peroxidase (EPO) were measured in ileum cell lysates. As shown in Fig. 3(C and D), mucositis induced significant recruitment of intestinal neutrophils and eosinophils. These parameters were significantly reduced (1.02 ± 0.20 U/mg for MPO, $p < 0.001$; 0.40 ± 0.07 U/mg for EPO, $p < 0.01$) by administration of CIDCA 133 fermented milk in inflamed animals.

3.3. Reduced IgA levels and intestinal permeability in mice with mucositis treated with CIDCA 133 fermented milk

Secretory IgA (sIgA) in the small intestine was investigated. As shown in Fig. 3E, 5-FU induced significant increase of sIgA levels in the intestinal fluid (MUC group: 3.684 ± 303.9 µg/mL) as compared with negative controls (CTL group: 2.402 ± 230.0 µg/mL, $p < 0.001$) and CIDCA 133 group (without mucositis induction: 2.439 ± 296.0 µg/mL, $p < 0.001$). The treatment with CIDCA 133 fermented milk (MUC + CIDCA 133 group) had significantly reduced levels of sIgA (2.347 ± 247 µg/mL) ($p < 0.001$) in the intestinal fluid of the animals which received the chemotherapy treatment.

Alteration of mucosal permeability is another side effect of 5-FU treatment. To determine whether the administration of CIDCA 133 fermented milk prevents mucosal damage, the intestinal permeability was evaluated. At 72 h after the 5-FU injection, intestinal permeability was significantly increased in the MUC group (0.13 ± 0.021 %ID/g) compared to the CTL (0.019 ± 0.005 %ID/g) and CIDCA 133 (0.014 ± 0.006 %ID/g) groups (without mucositis induction) ($p < 0.001$). Interestingly the animals that received CIDCA 133 fermented milk (MUC + CIDCA 133) group were able to reduce significantly the effect of 5-FU treatment on intestinal permeability (0.036 ± 0.015 %ID/g) as compared to the MUC group mice ($p < 0.001$) (Fig. 3F).

3.4. Mucosal damage and loss of goblet cells were reduced by CIDCA 133 fermented milk administration

Alterations such as villus shortening, increase in crypt depth, intense inflammatory cell infiltrate in the villi, *lamina propria* and submucosa, ulceration, edema, vacuolization, and decrease in goblet cells number, were clearly observed in inflamed animals (Fig. 4A), which correlated with histological scores (Fig. 4B). Our results showed that feeding of inflamed mice with CIDCA 133 fermented milk (MUC + CIDCA 133group) was able to ameliorate 5-FU-induced intestinal mucosal damage. Indeed, crypts depth, villus height, as well as villus/crypt ratio were significantly restored. In addition, inflammatory infiltrate was also decreased when compared with inflamed group (MUC) ($p < 0.05$) (Fig. 4C and D). Consequently, the villus/crypt ratio was increased (Fig. 4E). The histological scores correlated with above results demonstrating a reduction in scores in the MUC + CIDCA 133 group as compared with the MUC group (Fig. 4B and E).

As expected the MUC group presented a significant decrease in goblet cells (11.80 ± 3.47 cell/field). However, feeding of 5-FU-treated animals with milk containing CIDCA 133 strain (MUC + CIDCA 133) was able to prevent the loss of goblet cells (23.85 ± 5.53 cell/field) ($p < 0.05$) as compared with MUC group (Fig. 5). In addition, no histopathological nor morphological changes were observed for the CTL and CIDCA 133 groups (without mucositis induction) (Fig. 4).

4. Discussion

Intestinal mucositis, as a side effect of chemotherapy or

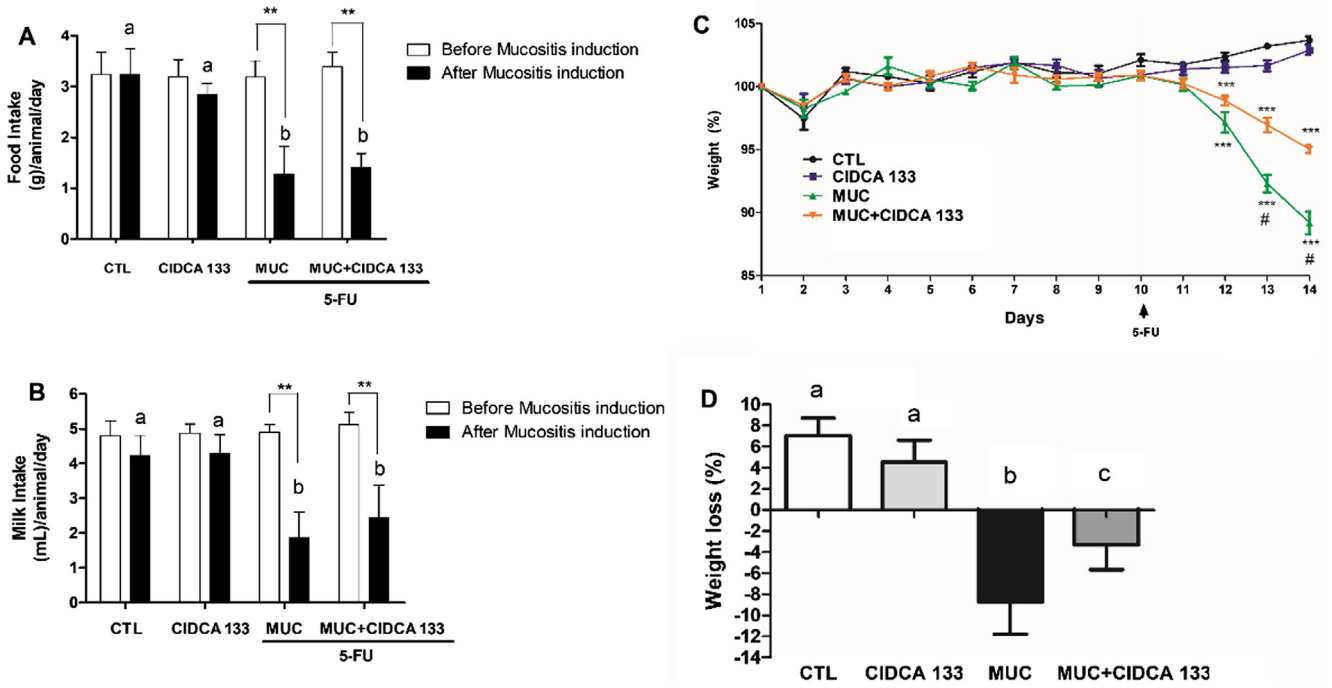

Fig. 2. Food, milk intake and body weight of animals: (A) Food and (B) Milk intake, (C) Body weight variation and (D) (%) Body weight loss measured daily. Mice received intraperitoneal 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline solution (CTL and CIDCA 133 groups) and were treated with non-fermented milk or *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (n = 8 animals per group). * indicates a statistically significant difference ($p < 0.05$) between MUC and MUC + CIDCA 133 group before and after mucositis induction by Mann Whitney test (A and B).* indicates a statistically significant difference ($p < 0.05$) between CTL, CIDCA 133, MUC and MUC + CIDCA 133 groups by Two-ANOVA followed by the Bonferroni post-test (C). # indicates a statistically significant difference ($p < 0.05$) between MUC and MUC + CIDCA 133 group by Two-ANOVA followed by the Bonferroni post-test (C). Different letters (a–c) indicate statistically significant differences ($p < 0.05$) by Kruskal-Wallis test followed by the Dunn's post-test (A and B), and ANOVA followed by the Bonferroni post-test (D).

radiotherapy for cancer treatment, is one of the most relevant gastrointestinal inflammatory conditions in humans. This disease leads a considerable reduction in doses of chemotropic drugs administrated (45%) and delays in therapy (71%) of patients undergoing treatment, and statistically, 3% of them discontinue the therapy (Arnold et al., 2005; Lalla & Peterson, 2006). The 5-FU is one of the most commonly used chemotherapy drugs in oncological practice (Fata et al., 1999). To prevent, reduce and/or treat the intestinal mucosal damage caused by anticancer drugs and/or radiotherapy, many investigations about therapeutic alternatives are being considered.

Fermented milk products are considered as a "functional foods" (foods with health-promoting properties) (Balthazar et al., 2018; Lollo et al., 2013) having high relevance for the industrial and food sector. During the fermentation process the microorganisms generate metabolites, products coming from the hydrolysis of the components of the food matrix, capable to modify the organoleptic characteristics of the foods, as well as promote the conservation of nutrients and consequently improve their shelf-life (Champagne, Gomes da Cruz, & Daga, 2018; Silva et al., 2018).

The probiotic potential of *L. delbrueckii* subsp. *lactis* CIDCA 133 has been analyzed *in vitro*, where authors showed its ability to resist high concentrations of acids and bile salts (Kociubinski et al., 1999), and its capability to resist antimicrobial peptides derived from enterocytes (Hugo et al., 2010) and human ß -defensins (Hugo et al., 2012). It was also described that this strain exhibits immunomodulatory properties by stimulating TNF-α production in dendritic cells (DC) in a co-culture system with *B. cereus*-infected epithelial cells (Rolny, Tiscornia, Racedo, Pérez, & Bollati-Fogolín, 2016), as this cytokine promotes the recruitment of immune cells helps to control of pathogens multiplication (Ramadan, Moyer, & Callegan, 2008). Furthermore, this strain was able to activate murine macrophages (RAW 264.7) infected with *C. rodentium* through increase of phagocytic activity, expression of surface markers associated to antigen presentation, and also induction of ROS activity (Hugo et al., 2017).

Taking into account the immunomodulatory potential of lactobacilli and particularly the characteristics of CIDCA 133 strain, we hypothesized that the orally administration of *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (7.5×10^7 CFU/mL) could alleviate the intestinal damage associated to the administration of 5-FU in a murine model of mucositis.

In the present study, it was demonstrated that the daily administration of the probiotic strain CIDCA 133 significantly reduced the harshness of 5-FU intestinal induced mucositis in a murine model. One of the mucositis features is the shortening of small intestine length, as was demonstrated in animals of MUC group that presented 16% shortening of the small intestine. A lower percentage of bowel shortening was observed in mice treated with the probiotic CIDCA 133 fermented milk (7.2%). This finding is important because a larger area of the intestine provides enough absorption surface for nutrient uptake, as well as lower compromise of the loss of water and electrolytes which, consequently, acts positively on the energetic balance of the animals, which makes attractive the use of CIDCA 133 fermented milk. The shortening of the small intestine correlates with the decrease in body weight observed in mucositis disease (de Barros et al., 2018; Kato et al., 2017; Maioli et al., 2014; Vieira et al., 2012). In agreement with these authors, our work shows that animals which received 5-FU (MUC group) exhibit a considerable weight loss (around 9%). These findings are related to lower food consumption since the inflamed groups (MUC and MUC + CIDCA 133) showed significantly less food and liquid intake than the controls groups. In contrast, animals treated with the probiotic CIDCA 133 were able to recover around 6% of this weight loss. The same effect was reported by Maioli et al. (2014). Interestingly, the administration of probiotic to 5-FU-treated mice reduces weight loss and this finding can be related to the prevention of intestine shortening.

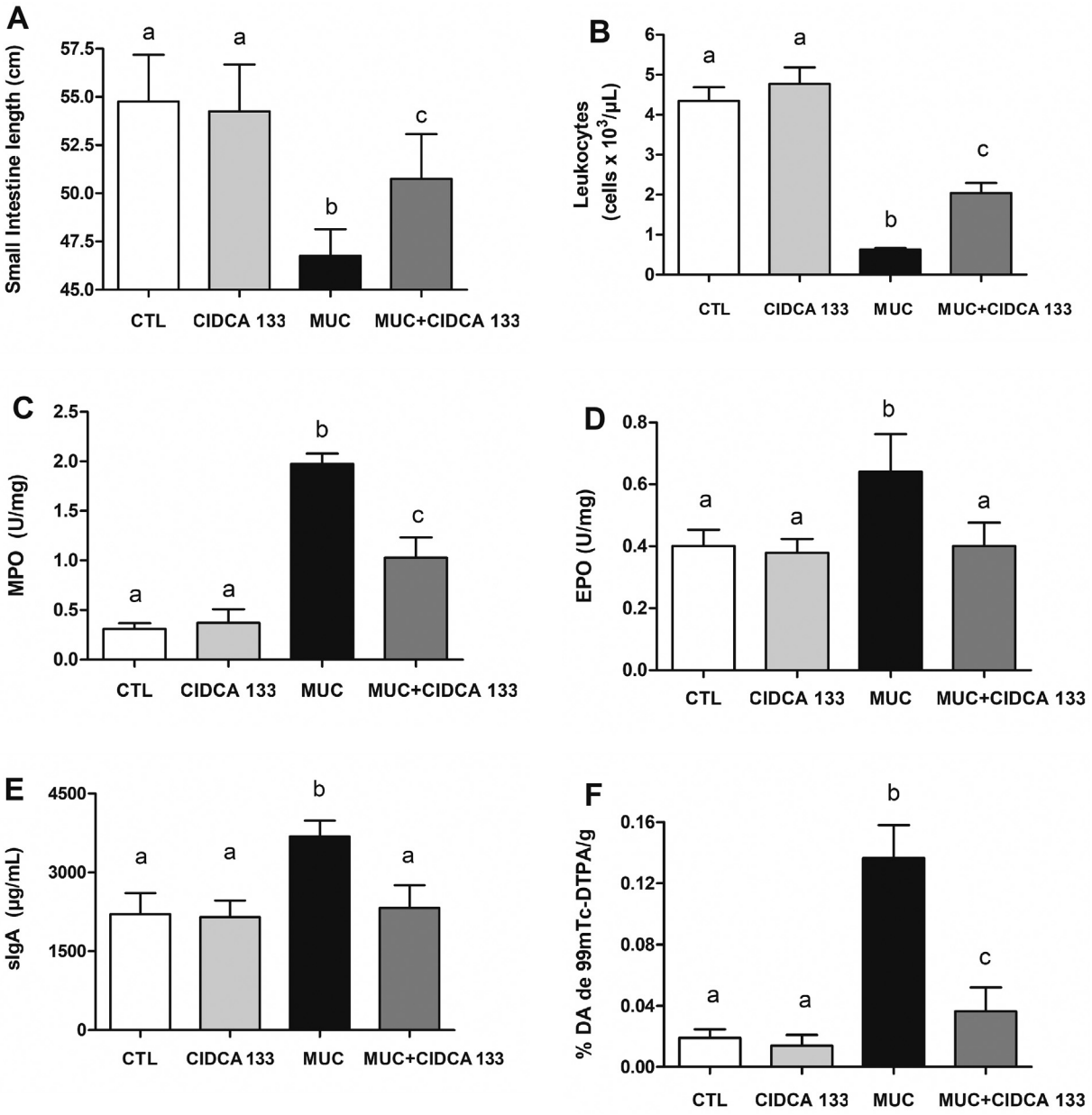

Fig. 3. Effect of CIDCA 133 fermented milk in mouse small intestines with induced mucositis by 5-fluorouracil (5-FU): (A) small intestine length, (B) number of leukocytes (cells $\times 10^3/\mu L$), study of (C) MPO activity and (D) EPO activity, (E) levels of sIgA (µg/mL) and (F) percentage of injected dose per gram (%ID/g) of blood to measure the intestinal permeability. Mice received intraperitoneal 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% (w/v) NaCl (CTL and CIDCA 133 group) injection and were fed daily with milk or *L. delbrueckii* subsp. *lactis* CIDCA 133 fermented milk (n = 8 animals per group). Different letters (a, b, and c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (A, C, E), and Kruskal-Wallis test followed by the Dunn's post-test (B, D, F).

Another important feature of intestinal mucositis is the alteration of the small intestine architecture and integrity, causing villus flattening, inflammatory cell infiltrates in the *lamina propria* and cell damage. These effects let to diminished villus/crypt ratio (Duncan & Grant, 2003; Lee, Ryan, & Doherty, 2014; Soares et al., 2013; Sonis, 2004), and also to an increased production of pro-inflammatory cytokine. In the present study, animals injected with 5-FU without probiotic administration showed loss of architecture of the ileum mucosa. Interestingly, mice inflamed with 5-FU and given *L. delbrueckii* CIDCA 133 fermented milk were able to circumvent mucosa inflammation, showing decrease of mucosal inflammation scores, with maintenance of the length villus and the depth crypt and also, preserving mucosal architecture and total thickness when compared to those that only received 5-FU. These findings also are correlated with signs of improvement of the intestinal dysbiosis induced by 5-FU. Thus, CIDCA 133 fermented milk administration was able to attenuate the mucosal damage in inflamed mice.

The pathogenesis of 5-FU induced intestinal mucositis involves intestinal injury which is associated with inflammatory infiltration in the small intestine (Soares et al., 2008), which can be verified by increase in myeloperoxidase (indirect neutrophil infiltration), eosinophils peroxidase (indirect eosinophils infiltration), and NAG (macrophage infiltration) activity. In the present study, the administration of 300 mg/kg of 5-FU induces increased MPO and EPO activities in the mouse ileum mucosa. Of note, animals which received the probiotic bacteria showed reduced levels of neutrophil and eosinophil infiltration. Moreover, these inflammatory parameters are intrinsically related to our results referring to the increased leukocytes number in the blood of animals with intestinal mucositis treated with CIDCA 133, showing that

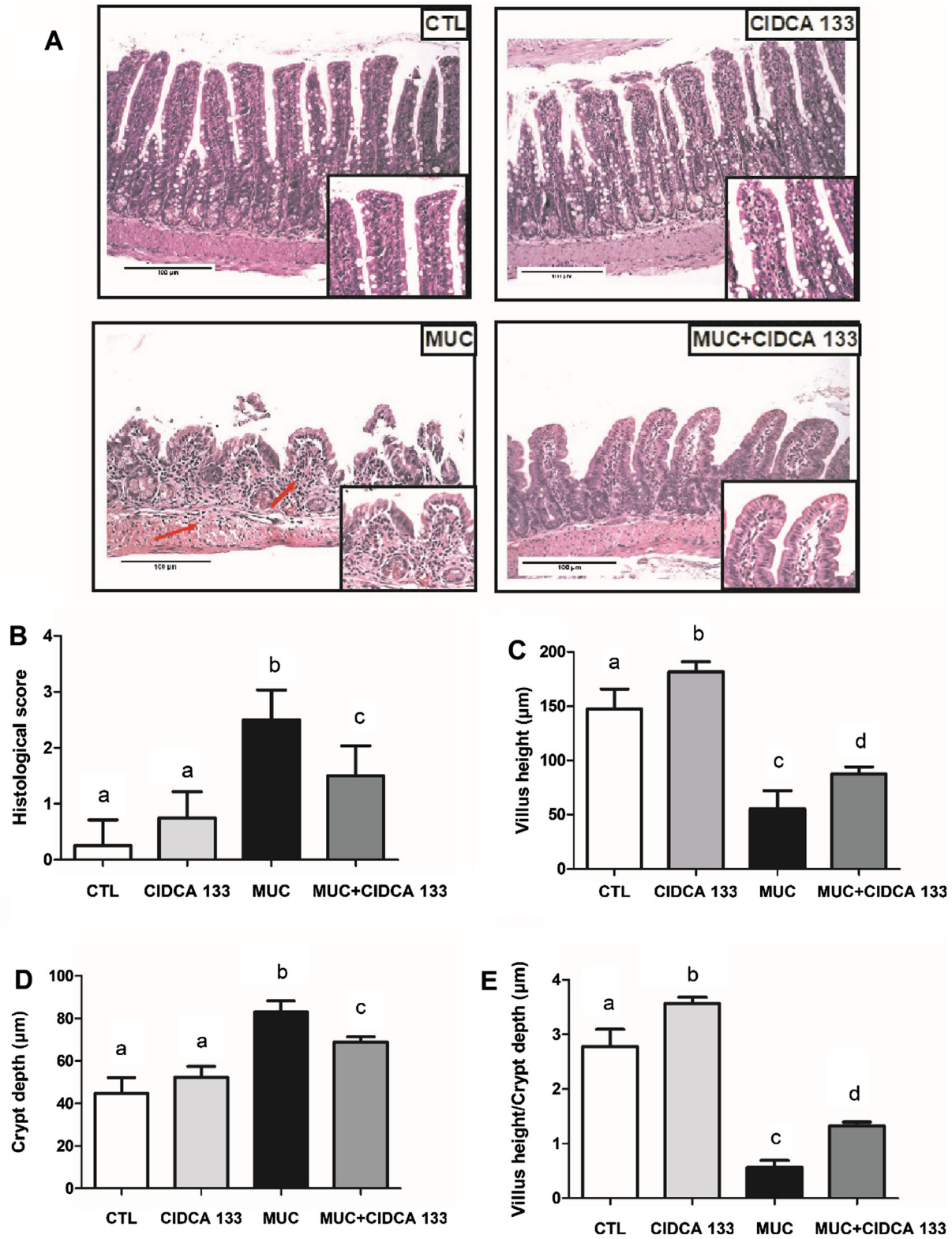

Fig. 4. Representative photomicrographs from: (A) mucosal histopathology, (B) Histopathological scores, (C) morphometrical analysis of villus height, (D) crypt depth and (E) villus height-to-crypt depth ratio of the ileum of animals with mucositis and administrated with strain CIDCA 133 fermented milk (objective: ×20, scale 100 µm). Mice received 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline (CTL and CIDCA 133 group) *i.p.* injection, (n = 8 animals per group). Different letters (a–d) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (B–E).

the lower migration of these cells to the affected tissues is a result of the administration of this probiotic strain improving the severity of intestinal mucositis.

As reported by Sonis (2004), late manifestations of intestinal mucositis encompass an increase in intestinal permeability, villus and crypts atrophy, thus leading to severe loss of function of the epithelial barrier (Daniele et al., 2001; Song, Park, & Sung, 2013). One of the causes of these effects could be attributed to the reduction in the

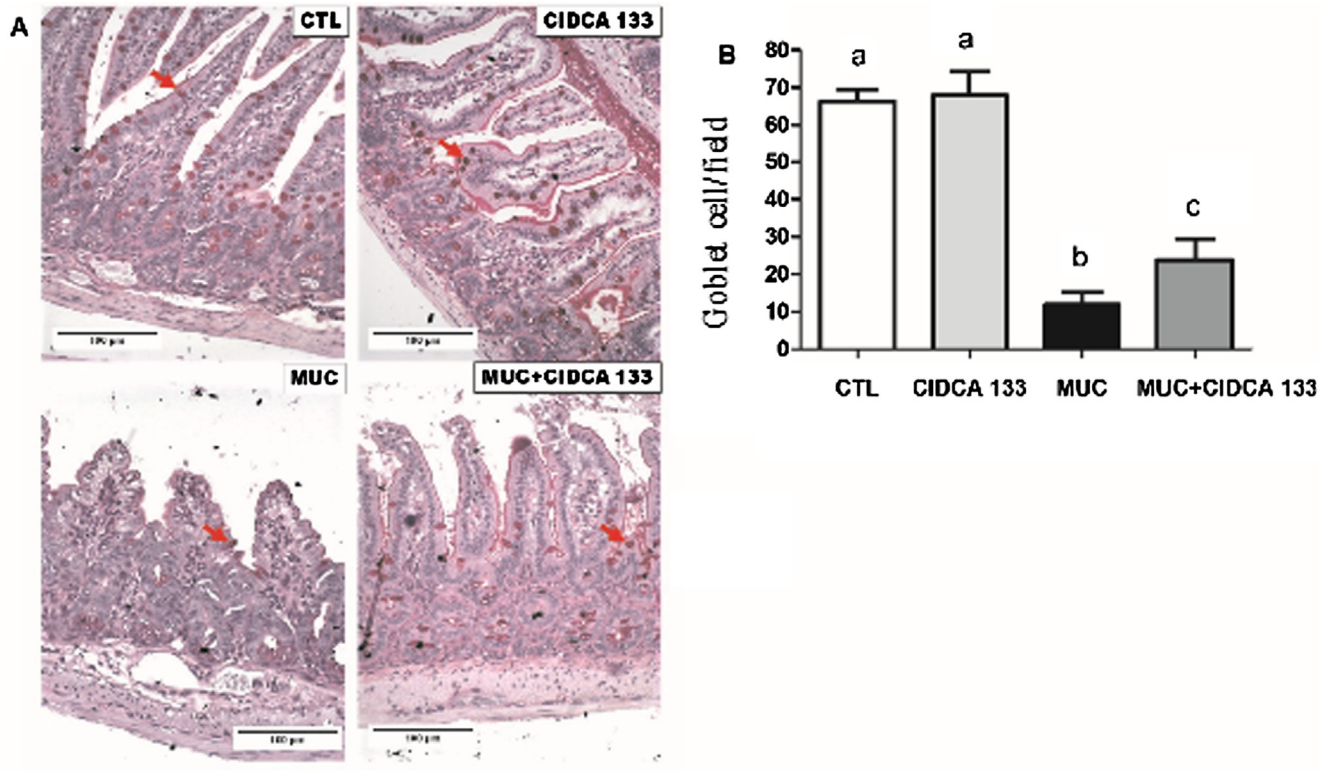

Fig. 5. Representative photomicrographs from: (A) ileum section stained with PAS: the arrows shows the Goblet cells (objective: ×20, scale 100 μm), (B) Number of Goblet cells/field obtained for experimental groups: Mice received 5-FU (300 mg/kg) (MUC and MUC + CIDCA 133 group) or 0.9% saline (CTL and CIDCA 133 group) *i.p* injection and were fed daily either with milk or with CIDCA 133 fermented milk (n = 8 animals per group). Different letters (a, b, and c) indicate statistically significant differences ($p < 0.05$) by ANOVA followed by the Bonferroni post-test (B).

expression of genes encoding tight junction proteins (Youmba, Belmonte, Galas, Boukhettala, Bôle-Feysot, Déchelotte, Coëffier, 2012). In our work, as in other reports (Ferreira et al., 2012; Li et al., 2017; Song et al., 2013) it was demonstrated the 5-FU drug increases the intestinal permeability. However, it was observed that mice injected with 5-FU and treated with CIDCA 133 fermented milk greatly reduced the intestinal permeability as it was detected by the ^{99m}Tc-DTPA administration. This is in agreement with other reports which related the probiotics administration with the reduction of the intestinal permeability induced by chemotherapy drugs (Bastos et al., 2016; Favaro-Trinidade & Grosso, 2000; Justino et al., 2014).

According to Yu, Yuan, Deng, and Yang (2015), microorganisms belonging to the genus *Lactobacillus* are able to improve the integrity of epithelia due to its ability antagonize the adhesion of pathogens to intestinal cells. Therefore, these pathogens cannot cause damage to the cellular junctions (Yu et al., 2015), and also might reduce the paracellular permeability by modulating the gene expression of tight junction proteins (Ahrne & Hagslatt, 2011).

It is also know that administration of 5-FU lead to a marked decrease in goblet cell number (Ciobanu et al., 2016; Stringer et al., 2009; Yeung et al., 2015). These cells are responsible of mucins secretion that along with trefoil factor are important components for the protection of the epithelium (van Vliet, Harmsen, de Bont, & Tissing, 2010). Our results revealed that the number of goblet cells dramatically decrease in 5-FU-treated mice. However, mice administrated with CIDCA 133 (MUC + CIDCA 133) presented significantly higher goblet cell numbers that mice administrated only with 5-FU (MUC group).

Our results with CIDCA 133 fermented milk are in agreement with previous reports that showed that probiotic bacteria and yeast are beneficial for both the structure and the function of the intestinal epithelium (Bastos et al., 2016; Oh et al., 2017; Yeung et al., 2015). We can hypothesize that the effect of strain CIDCA 133 fermented milk in preventing loss of goblet cells number could be related to its ability to protect the cells from intestinal crypts that contain stem cells that in turn differentiate into cells with different functions in the intestinal epithelium, including goblet cells.

Level of secretory IgA is another parameter related to the intestinal barrier which have relevant importance in the maintenance of mucosal homeostasis (Mantis, Rol, & Corthésy, 2011; Monteiro, 2014) both controlling harmful effects of intestinal pathogens and containing potential inflammatory processes (Schmucker, Owen, Outenreath, & Thoreux, 2003). Mucositis is characterized by high levels of sIgA and this pattern was observed in animals that did not receive CIDCA 133 strain (MUC group). Interesting, the administration of probiotic fermented milk was able to decrease the levels of sIgA to similar values found in the negative controls, demonstrating a reduced severity of 5-FU-induced intestinal mucositis in the mice ileum. Similar results were observed in mice administrated with *L. lactis* NZ9000 in a model of experimental mucositis (Carvalho et al., 2017). In different experimental models, it has been demonstrated that probiotics are able to increase sIgA production and this ability is considered a positive effect (Kawashima et al., 2018; Kikuchi et al., 2014; Santos Rocha et al., 2014). Interestingly, in our model of severe 5-FU-induced intestinal inflammation, the strain CIDCA 133 normalized levels of sIgA that were increased in the MUC group. Also, our model shows that reduced levels of sIgA as compared with inflamed animals, could be related to the improvement of intestinal mucosal barrier that in turns reduces to the improvement in the general state of inflammation.

Many researches are being done to clarify the underlying mechanisms related to the beneficial effect of probiotic bacteria in the bowel in different IBDs models. *L. acidophilus* (Justino et al., 2015; Oh et al., 2017), *L. fermentum* (Smith et al., 2008), *Saccharomyces boulardii* (Justino et al., 2014) and *L. plantarum CRL 2130* (Levit, Savoy de Giori, de Moreno de LeBlanc, & LeBlanc, 2018) are able to modulate the

inflammatory infiltrate generated by chemotherapy drugs. Furthermore, the capacity of some probiotics to prevent production of pro-inflammatory cytokines (Justino et al., 2015; Yeung et al., 2015) could be related to the inhibition of the NF-κB pathway (Dai et al., 2013) and the restoration of the Th17/Treg cells balance (Jeong, Lee, Jang, Han, & Kim, 2018). In addition, as suggested by other studies, probiotic bacteria are able to produce anti-oxidant compounds that contribute to ameliorate oxidative stress associated to inflammatory bowel disease (Juarez del Valle, Laiño, Savoy de Giori, & LeBlanc, 2014; Levit, Savoy de Giori, de Moreno de LeBlanc, & LeBlanc, 2018).

It is also known that fermented milk by Lactic Acid Bacteria (LAB) could produce extracellular factors. In addition it has been demonstrated that bacterial grow in milk are able to hydrolyze the large proteins contained in milk (LeBlanc, Matar, Valdéz, LeBlanc, & Perdigon, 2002), that could contribute to their immunomodulating activity (Cordeiro et al., 2018). Thus, we can hypothesize that soluble factors present in suspensions of CIDCA 133 fermented milk, contribute to the effects reported in our study. Indeed, it is important to highlight that even short chain fatty acids produced during growth of lactic acid bacteria have demonstrated beneficial effects to the host (Garrote, Abraham, & Rumbo, 2015; Iraporda et al., 2015).

Additionally, it is important to highlight that the immunomodulatory/regulatory effects reported for CIDCA 133 fermented milk can be enhanced with the use of prebiotic, because these compounds stimulate growth activating bacteria metabolism and promote protection of bacteria beneficial to the host organism, as reported in previous studies (Galdino et al., 2018; Trindade et al., 2018).

5. Conclusion

The present work demonstrates for the first time the protective effect of *L. delbrueckii* subsp. *lactis* CIDCA 133 on the damage of the intestinal mucosa in a murine model of inflammation induced by a chemotherapeutic drug. Our findings showed that fermented milk produced in this work serve as efficient matrix for delivery of CIDCA 133 in GIT, and enlarge the perspectives of application of this strain and support novel probiotic-based treatments for gastrointestinal toxicity associated with anticancer therapy.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ahrne, S., & Hagslatt, M.-L. J. (2011). Effect of lactobacilli on paracellular permeability in the gut. *Nutrients, 3*(1), 104–117. https://doi.org/10.3390/nu3010104.

Arnold, R. J. G., Gabrail, N., Raut, M., Kim, R., Sung, J. C. Y., & Zhou, Y. (2005). Clinical implications of chemotherapy-induced diarrhea in patients with cancer. *The Journal of Supportive Oncology, 3*(3), 227–232. http://www.ncbi.nlm.nih.gov/pubmed/15915825.

Balthazar, C. F., Silva, H. L. A., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A. V., ... Cruz, A. G. (2018). The addition of inulin and Lactobacillus casei 01 in sheep milk ice cream. *Food Chemistry, 246*, 464–472. https://doi.org/10.1016/j.foodchem.2017.12.002.

Bastos, R. W., Pedroso, S. H. S. P., Vieira, A. T., Moreira, L. M. C., França, C. S., Cartelle, C. T., ... Martins, F. S. (2016). Saccharomyces cerevisiae UFMG A-905 treatment reduces intestinal damage in a murine model of irinotecan-induced mucositis. *Beneficial Microbes, 7*(4), 549–557. https://doi.org/10.3920/BM2015.0190.

Bradley, P. P., Christensen, R. D., & Rothstein, G. (1982). Cellular and extracellular myeloperoxidase in pyogenic inflammation. *Blood, 60*(3), 618–622. http://www.ncbi.nlm.nih.gov/pubmed/6286012.

Caballero-Franco, C., Keller, K., De Simone, C., & Chadee, K. (2007). The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 292*(1), G315–G322. https://doi.org/10.1152/ajpgi.00265.2006.

Carvalho, R. D., Breyner, N., Menezes-Garcia, Z., Rodrigues, N. M., Lemos, L., Maioli, T. U., ... de Azevedo, M. S. (2017). Secretion of biologically active pancreatitis-associated protein I (PAP) by genetically modified dairy Lactococcus lactis NZ9000 in the prevention of intestinal mucositis. *Microbial Cell Factories, 16*(1), 27. https://doi.org/10.1186/s12934-017-0624-x.

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science, 22*, 160–166. https://doi.org/10.1016/j.cofs.2018.04.008.

Ciobanu, L., Tantau, M., Valean, S., Parau, A., Bedecean, I., Mîrleneanu, R., ... Taulescu, M. (2016). Rifaximin modulates 5-fluorouracil-induced gastrointestinal mucositis in rats. *European Review for Medical and Pharmacological Sciences, 20*(23), 4993–5001. http://www.ncbi.nlm.nih.gov/pubmed/27981532.

Cordeiro, B. F., Oliveira, E. R., da Silva, S. H., Savassi, B. M., Acurcio, L. B., Lemos, L., ... do Carmo, F. L. R. (2018). Whey protein isolate-supplemented beverage, fermented by lactobacillus casei BL23 and propionibacterium freudenreichii 138, in the prevention of mucositis in mice. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.02035.

Dai, C., Zheng, C.-Q., Meng, F.-J., Zhou, Z., Sang, L.-X., & Jiang, M. (2013). VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. *Molecular and Cellular Biochemistry, 374*(1–2), 1–11. https://doi.org/10.1007/s11010-012-1488-3.

Dalmasso, G., Loubat, A., Dahan, S., Calle, G., Rampal, P., & Czerucka, D. (2006). Saccharomyces boulardii prevents TNF-alpha-induced apoptosis in EHEC-infected T84 cells. *Research in Microbiology, 157*(5), 456–465. https://doi.org/10.1016/j.resmic.2005.11.007.

Daniele, B., Secondulfo, M., De Vivo, R., Pignata, S., De Magistris, L., Delrio, P., ... Carratù, R. (2001). Effect of chemotherapy with 5-fluorouracil on intestinal permeability and absorption in patients with advanced colorectal cancer. *Journal of Clinical Gastroenterology, 32*(3), 228–230. http://www.ncbi.nlm.nih.gov/pubmed/11246350.

de Barros, P. A. V., Rabelo Andrade, M. E., de Vasconcelos Generoso, S., Mendes Miranda, S. E., dos Reis, D. C., Lacerda Leocádio, P. C., ... Cardoso, V. N. (2018). Conjugated linoleic acid prevents damage caused by intestinal mucositis induced by 5-fluorouracil in an experimental model. *Biomedicine & Pharmacotherapy, 103*, 1567–1576. https://doi.org/10.1016/j.biopha.2018.04.133.

Duncan, M., & Grant, G. (2003). Oral and intestinal mucositis - causes and possible treatments. *Alimentary Pharmacology & Therapeutics, 18*(9), 853–874. http://www.ncbi.nlm.nih.gov/pubmed/14616150.

Ewaschuk, J. B., Diaz, H., Meddings, L., Diederichs, B., Dmytrash, A., Backer, J., ... Madsen, K. L. (2008). Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 295*(5), G1025–G1034. https://doi.org/10.1152/ajpgi.90227.2008.

Fata, F., Ron, I. G., Kemeny, N., O'Reilly, E., Klimstra, D., & Kelsen, D. P. (1999). 5-fluorouracil-induced small bowel toxicity in patients with colorectal carcinoma. *Cancer, 86*(7), 1129–1134. http://www.ncbi.nlm.nih.gov/pubmed/10506695.

Favaro-Trinidade, C., & Grosso, C. (2000). The effect of the immobilization of L. acidophilus and B. lactis in alginate on their tolerance to gastrointestinal secretions. *Milchwissenschaft, 55*, 496–499.

Ferreira, T. M., Leonel, A. J., Melo, M. A., Santos, R. R. G., Cara, D. C., Cardoso, V. N., ... Alvarez-Leite, J. I. (2012). Oral supplementation of butyrate reduces mucositis and intestinal permeability associated with 5-Fluorouracil administration. *Lipids, 47*(7), 669–678. https://doi.org/10.1007/s11745-012-3680-3.

Galdino, F. M. P., Andrade, M. E. R., de Barros, P. A. V., de Generoso, S. V., Alvarez-Leite, J. I., Almeida-Leite, C. M., ... de Cardoso, V. N. (2018). Pretreatment and treatment with fructo-oligosaccharides attenuate intestinal mucositis induced by 5-FU in mice. *Journal of Functional Foods, 49*, 485–492. https://doi.org/10.1016/j.jff.2018.09.012.

Garrote, G. L., Abraham, A. G., & Rumbo, M. (2015). Is lactate an undervalued functional component of fermented food products? *Frontiers in Microbiology, 6*, 629. https://doi.org/10.3389/fmicb.2015.00629.

Herbers, A. H. E., de Haan, A. F. J., van der Velden, W. J. F. M., Donnelly, J. P., & Blijlevens, N. M. A. (2014). Mucositis not neutropenia determines bacteremia among hematopoietic stem cell transplant recipients. *Transplant Infectious Disease : An Official Journal of the Transplantation Society, 16*(2), 279–285. https://doi.org/10.1111/tid.12195.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66.

Hugo, A. A., De Antoni, G. L., & Pérez, P. F. (2006). Lactobacillus delbrueckii subsp lactis strain CIDCA 133 inhibits nitrate reductase activity of Escherichia coli. *International Journal of Food Microbiology, 111*(3), 191–196. https://doi.org/10.1016/j.ijfoodmicro.2006.04.024.

Hugo, A. A., De Antoni, G. L., & Pérez, P. F. (2010). Lactobacillus delbrueckii subsp lactis (strain CIDCA 133) resists the antimicrobial activity triggered by molecules derived from enterocyte-like Caco-2 cells. *Letters in Applied Microbiology, 50*(4), 335–340. https://doi.org/10.1111/j.1472-765X.2010.02796.x.

Hugo, A. A., Kakisu, E., De Antoni, G. L., & Pérez, P. F. (2008). Lactobacilli antagonize biological effects of enterohaemorrhagic Escherichia coli in vitro. *Letters in Applied Microbiology, 46*(6), 613–619. https://doi.org/10.1111/j.1472-765X.2008.02363.x.

Hugo, A. A., Rolny, I. S., Romanin, D., & Pérez, P. F. (2017). Lactobacillus delbrueckii subsp. lactis (strain CIDCA 133) stimulates murine macrophages infected with Citrobacter rodentium. *World Journal of Microbiology & Biotechnology, 33*(3), 48. https://doi.org/10.1007/s11274-017-2219-4.

Hugo, A. A., Tymczyszyn, E. E., Gómez-Zavaglia, A., & Pérez, P. F. (2012). Effect of human defensins on lactobacilli and liposomes. *Journal of Applied Microbiology, 113*(6), 1491–1497. https://doi.org/10.1111/j.1365-2672.2012.05433.x.

Iraporda, C., Errea, A., Romanin, D. E., Cayet, D., Pereyra, E., Pignataro, O., ... Rumbo, M. (2015). Lactate and short chain fatty acids produced by microbial fermentation downregulate proinflammatory responses in intestinal epithelial cells and myeloid cells. *Immunobiology, 220*(10), 1161–1169. https://doi.org/10.1016/j.imbio.2015.06.004.

Jeong, J.-J., Lee, H.-J., Jang, S.-E., Han, M. J., & Kim, D.-H. (2018). Lactobacillus plantarum C29 alleviates NF-κB activation and Th17/Treg imbalance in mice with TNBS-induced colitis. *Food and Agricultural Immunology, 29*(1), 577–589. https://doi.org/10.1080/09540105.2017.1418841.

Jørgensen, V. L., Nielsen, S. L., Espersen, K., & Perner, A. (2006). Increased colorectal permeability in patients with severe sepsis and septic shock. *Intensive Care Medicine, 32*(11), 1790–1796. https://doi.org/10.1007/s00134-006-0356-6.

Juarez del Valle, M., Laiño, J. E., Savoy de Giori, G., & LeBlanc, J. G. (2014). Riboflavin producing lactic acid bacteria as a biotechnological strategy to obtain bio-enriched soymilk. *Food Research International, 62*, 1015–1019. https://doi.org/10.1016/j.foodres.2014.05.029.

Justino, P. F. C., Melo, L. F. M., Nogueira, A. F., Costa, J. V. G., Silva, L. M. N., Santos, C. M., ... Soares, P. M. G. (2014). Treatment with Saccharomyces boulardii reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *The British Journal of Nutrition, 111*(9), 1611–1621. https://doi.org/10.1017/S0007114513004248.

Justino, P. F. C., Melo, L. F. M., Nogueira, A. F., Morais, C. M., Mendes, W. O., Franco, A. X., ... Soares, P. M. G. (2015). Regulatory role of Lactobacillus acidophilus on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice. *Cancer Chemotherapy and Pharmacology, 75*(3), 559–567. https://doi.org/10.1007/s00280-014-2663-x.

Kato, S., Hamouda, N., Kano, Y., Oikawa, Y., Tanaka, Y., Matsumoto, K., ... Shimakawa, M. (2017). Probiotic Bifidobacterium bifidum G9–1 attenuates 5-fluorouracil-induced intestinal mucositis in mice via suppression of dysbiosis-related secondary inflammatory responses. *Clinical and Experimental Pharmacology & Physiology, 44*(10), 1017–1025. https://doi.org/10.1111/1440-1681.12792.

Kawashima, T., Ikari, N., Kouchi, T., Kowatari, Y., Kubota, Y., Shimojo, N., & Tsuji, N. M. (2018). The molecular mechanism for activating IgA production by Pediococcus acidilactici K15 and the clinical impact in a randomized trial. *Scientific Reports, 8*(1), 5065. https://doi.org/10.1038/s41598-018-23404-4.

Kikuchi, Y., Kunitoh-Asari, A., Hayakawa, K., Imai, S., Kasuya, K., Abe, K., ... Hachimura, S. (2014). Oral administration of Lactobacillus plantarum strain AYA enhances IgA secretion and provides survival protection against influenza virus infection in mice. *PloS One, 9*(1), e86416. https://doi.org/10.1371/journal.pone.0086416.

Kociubinski, G. L., Pérez, P. F., Añón, M. C., & De Antoni, G. L. (1996). A method of screening for highly inhibitory lactic acid bacteria. *Journal of Food Protection, 59*(7), 739–745. https://doi.org/10.4315/0362-028X-59.7.739.

Kociubinski, G., Pérez, P., & De Antoni, G. (1999). Screening of bile resistance and bile precipitation in lactic acid bacteria and bifidobacteria. *Journal of Food Protection, 62*(8), 905–912. http://www.ncbi.nlm.nih.gov/pubmed/10456745.

Lalla, R. V., & Peterson, D. E. (2006). Treatment of mucositis, including new medications. *Cancer Journal (Sudbury, Mass.), 12*(5), 348–354. http://www.ncbi.nlm.nih.gov/pubmed/17034671.

LeBlanc, J. G., Matar, C., Valdéz, J. C., LeBlanc, J., & Perdigon, G. (2002). Immunomodulating effects of peptidic fractions issued from milk fermented with Lactobacillus helveticus. *Journal of Dairy Science, 85*(11), 2733–2742. https://doi.org/10.3168/jds.S0022-0302(02)74360-9.

Lee, C. S., Ryan, E. J., & Doherty, G. A. (2014). Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: The role of inflammation. *World Journal of Gastroenterology, 20*(14), 3751–3761. https://doi.org/10.3748/wjg.v20.i14.3751.

Levit, R., Savoy de Giori, G., de Moreno de LeBlanc, A., & LeBlanc, J. G. (2018). Protective effect of the riboflavin-overproducing strain Lactobacillus plantarum CRL2130 on intestinal mucositis in mice. *Nutrition, 54*, 165–172. https://doi.org/10.1016/j.nut.2018.03.056.

Li, H.-L., Lu, L., Wang, X.-S., Qin, L.-Y., Wang, P., Qiu, S.-P., ... Wu, X.-J. (2017). Alteration of gut microbiota and inflammatory cytokine/chemokine profiles in 5-fluorouracil induced intestinal mucositis. *Frontiers in Cellular and Infection Microbiology, 7*, 455. https://doi.org/10.3389/fcimb.2017.00455.

Logan, R. M., Gibson, R. J., Sonis, S. T., & Keefe, D. M. K. (2007). Nuclear factor-kappaB (NF-kappaB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. *Oral Oncology, 43*(4), 395–401. https://doi.org/10.1016/j.oraloncology.2006.04.011.

Lollo, P. C. B., de Moura, C. S., Morato, P. N., Cruz, A. G., de Castro, W. F., Betim, C. B., ... Amaya-Farfan, J. (2013). Probiotic yogurt offers higher immune-protection than probiotic whey beverage. *Food Research International, 54*(1), 118–124. https://doi.org/10.1016/j.foodres.2013.06.003.

Longley, D. B., Harkin, D. P., & Johnston, P. G. (2003). 5-fluorouracil: Mechanisms of action and clinical strategies. *Nature Reviews. Cancer, 3*(5), 330–338. https://doi.org/10.1038/nrc1074.

Lutgendorff, F., Nijmeijer, R. M., Sandström, P. A., Trulsson, L. M., Magnusson, K.-E., Timmerman, H. M., ... Söderholm, J. D. (2009). Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. *PloS One, 4*(2), e4512. https://doi.org/10.1371/journal.pone.0004512.

Maioli, T. U., de Melo Silva, B., Dias, M. N., Paiva, N. C., Cardoso, V. N., Fernandes, S. O., ... de Vasconcelos Generoso, S. (2014). Pretreatment with Saccharomyces boulardii does not prevent the experimental mucositis in Swiss mice. *Journal of Negative Results in Biomedicine, 13*, 6. https://doi.org/10.1186/1477-5751-13-6.

Mantis, N. J., Rol, N., & Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology, 4*(6), 603–611. https://doi.org/10.1038/mi.2011.41.

Martins, F. S., Silva, A. A., Vieira, A. T., Barbosa, F. H. F., Arantes, R. M. E., Teixeira, M. M., & Nicoli, J. R. (2009). Comparative study of Bifidobacterium animalis, Escherichia coli, Lactobacillus casei and Saccharomyces boulardii probiotic properties. *Archives of Microbiology, 191*(8), 623–630. https://doi.org/10.1007/s00203-009-0491-x.

Mennigen, R., Nolte, K., Rijcken, E., Utech, M., Loeffler, B., Senninger, N., & Bruewer, M. (2009). Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 296*(5), G1140–G1149. https://doi.org/10.1152/ajpgi.90534.2008.

Miura, K., Kinouchi, M., Ishida, K., Fujibuchi, W., Naitoh, T., Ogawa, H., ... Sasaki, I. (2010). 5-fu metabolism in cancer and orally-administrable 5-fu drugs. *Cancers, 2*(3), 1717–1730. https://doi.org/10.3390/cancers2031717.

Monteiro, R. C. (2014). Immunoglobulin A as an anti-inflammatory agent. *Clinical and Experimental Immunology, 178*(Suppl), 108–110. https://doi.org/10.1111/cei.12531.

Oh, N. S., Lee, J. Y., Lee, J. M., Lee, K. W., & Kim, Y. (2017). Mulberry leaf extract fermented with Lactobacillus acidophilus A4 ameliorates 5-fluorouracil-induced intestinal mucositis in rats. *Letters in Applied Microbiology, 64*(6), 459–468. https://doi.org/10.1111/lam.12741.

Pinedo, H. M., & Peters, G. F. (1988). Fluorouracil: Biochemistry and pharmacology. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, 6*(10), 1653–1664. https://doi.org/10.1200/JCO.1988.6.10.1653.

Ramadan, R. T., Moyer, A. L., & Callegan, M. C. (2008). A role for tumor necrosis factor-alpha in experimental Bacillus cereus endophthalmitis pathogenesis. *Investigative Ophthalmology & Visual Science, 49*(10), 4482–4489. https://doi.org/10.1167/iovs.08-2085.

Rolny, I. S., Tiscornia, I., Racedo, S. M., Pérez, P. F., & Bollati-Fogolín, M. (2016). Lactobacillus delbrueckii subsp lactis CIDCA 133 modulates response of human epithelial and dendritic cells infected with Bacillus cereus. *Beneficial Microbes, 7*(5), 749–760. https://doi.org/10.3920/BM2015.0191.

Santos Rocha, C., Gomes-Santos, A. C., Garcias Moreira, T., de Azevedo, M., Diniz Luerce, T., Mariadassou, M., ... van de Guchte, M. (2014). Local and systemic immune mechanisms underlying the anti-colitis effects of the dairy bacterium Lactobacillus delbrueckii. *PloS One, 9*(1), e85923. https://doi.org/10.1371/journal.pone.0085923.

Schmucker, D. L., Owen, R. L., Outenreath, R., & Thoreux, K. (2003). Basis for the age-related decline in intestinal mucosal immunity. *Clinical and Developmental Immunology, 10*(2–4), 167–172. https://doi.org/10.1080/10446670310001642168.

Seth, A., Yan, F., Polk, D. B., & Rao, R. K. (2008). Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 294*(4), G1060–G1069. https://doi.org/10.1152/ajpgi.00202.2007.

Silva, H. L. A., Balthazar, C. F., Silva, R., Vieira, A. H., Costa, R. G. B., Esmerino, E. A., ... Cruz, A. G. (2018). Sodium reduction and flavor enhancer addition in probiotic prato cheese: Contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. *Journal of Dairy Science, 101*(10), 8837–8846. https://doi.org/10.3168/jds.2018-14819.

Smith, C. L., Geier, M. S., Yazbeck, R., Torres, D. M., Butler, R. N., & Howarth, G. S. (2008). Lactobacillus fermentum BR11 and fructo-oligosaccharide partially reduce jejunal inflammation in a model of intestinal mucositis in rats. *Nutrition and Cancer, 60*(6), 757–767. https://doi.org/10.1080/01635580802192841.

Soares, P. M. G., Mota, J. M. S. C., Gomes, A. S., Oliveira, R. B., Assreuy, A. M. S., Brito, G. A. C., ... Souza, M. H. L. P. (2008). Gastrointestinal dysmotility in 5-fluorouracil-induced intestinal mucositis outlasts inflammatory process resolution. *Cancer Chemotherapy and Pharmacology, 63*(1), 91–98. https://doi.org/10.1007/s00280-008-0715-9.

Soares, P. M. G., Mota, J. M. S. C., Souza, E. P., Justino, P. F. C., Franco, A. X., Cunha, F. Q., ... Souza, M. H. L. P. (2013). Inflammatory intestinal damage induced by 5-fluorouracil requires IL-4. *Cytokine, 61*(1), 46–49. https://doi.org/10.1016/j.cyto.2012.10.003.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J.-J., ... Langella, P. (2008). Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America, 105*(43), 16731–16736. https://doi.org/10.1073/pnas.0804812105.

Song, M.-K., Park, M.-Y., & Sung, M.-K. (2013). 5-Fluorouracil-induced changes of intestinal integrity biomarkers in BALB/c mice. *Journal of Cancer Prevention, 18*(4), 322–329. http://www.ncbi.nlm.nih.gov/pubmed/25337561.

Sonis, S. T. (1998). Mucositis as a biological process: A new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncology, 34*(1), 39–43. http://www.ncbi.nlm.nih.gov/pubmed/9659518.

Sonis, S. T. (2004). The pathobiology of mucositis. *Nature Reviews. Cancer, 4*(4), 277–284. https://doi.org/10.1038/nrc1318.

Soveri, L. M., Hermunen, K., de Gramont, A., Poussa, T., Quinaux, E., Bono, P., ... Österlund, P. (2014). Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity? *European Journal of Cancer (Oxford, England : 1990), 50*(17), 2966–2974. https://doi.org/10.1016/j.ejca.2014.08.017.

Stringer, A. M., Gibson, R. J., Logan, R. M., Bowen, J. M., Yeoh, A. S. J., Hamilton, J., & Keefe, D. M. K. (2009). Gastrointestinal microflora and mucins may play a critical role in the development of 5-Fluorouracil-induced gastrointestinal mucositis.

Experimental Biology and Medicine (Maywood, N.J.), 234(4), 430–441. https://doi.org/10.3181/0810-RM-301.

Trindade, L. M., Martins, V. D., Rodrigues, N. M., Souza, E. L. S., Martins, F. S., Costa, G. M. F., ... Generoso, S. V. (2018). Oral administration of Simbioflora® (synbiotic) attenuates intestinal damage in a mouse model of 5-fluorouracil-induced mucositis. *Beneficial Microbes, 9*(3), 477–486. https://doi.org/10.3920/BM2017.0082.

van Vliet, M. J., Harmsen, H. J. M., de Bont, E. S. J. M., & Tissing, W. J. E. (2010). The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. *PLoS Pathogens, 6*(5), e1000879. https://doi.org/10.1371/journal.ppat.1000879.

Vieira, A. T., Fagundes, C. T., Alessandri, A. L., Castor, M. G. M., Guabiraba, R., Borges, V. O., ... Teixeira, M. M. (2009). Treatment with a novel chemokine-binding protein or eosinophil lineage-ablation protects mice from experimental colitis. *The American Journal of Pathology, 175*(6), 2382–2391. https://doi.org/10.2353/ajpath.2009.090093.

Vieira, E. L. M., Leonel, A. J., Sad, A. P., Beltrão, N. R. M., Costa, T. F., Ferreira, T. M. R., ... Alvarez-Leite, J. I. (2012). Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. *The Journal of Nutritional Biochemistry, 23*(5), 430–436. https://doi.org/10.1016/j.jnutbio.2011.01.007.

Yeung, C.-Y., Chan, W.-T., Jiang, C.-B., Cheng, M.-L., Liu, C.-Y., Chang, S.-W., ... Lee, H.-C. (2015). Amelioration of Chemotherapy-Induced Intestinal Mucositis by Orally Administered Probiotics in a Mouse Model. *PloS One, 10*(9), e0138746. https://doi.org/10.1371/journal.pone.0138746.

Youmba, S. B., Belmonte, L., Galas, L., Boukhettala, N., Bôle-Feysot, C., Déchelotte, P., & Coëffier, M. (2012). Methotrexate Modulates Tight Junctions Through NF-κB, MEK, and JNK Pathways. *Journal of Pediatric Gastroenterology and Nutrition, 4*(54), 463–470.

Yu, Q., Yuan, L., Deng, J., & Yang, Q. (2015). Lactobacillus protects the integrity of intestinal epithelial barrier damaged by pathogenic bacteria. *Frontiers in Cellular and Infection Microbiology, 5*, 26. https://doi.org/10.3389/fcimb.2015.00026.