Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

# Folate-producing lactic acid bacteria reduce inflammation in mice with induced intestinal mucositis

R. Levit[1], G. Savoy de Giori[1,2], A. de Moreno de LeBlanc[1] and J.G. LeBlanc[1]

1 Centro de Referencia para Lactobacilos (CERELA-CONICET), San Miguel de Tucumán, Tucumán, Argentina
2 Cátedra de Microbiología Superior, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina

**Keywords**
5-fluorouracil, chemotherapy, folate, inflammation, lactic acid bacteria, mucositis, probiotic.

**Correspondence**
Jean Guy LeBlanc, CERELA-CONICET, Chacabuco 145, San Miguel de Tucumán, Tucumán (T4000ILC), Argentina.
E-mails: leblanc@cerela.org.ar; leblancjeanguy@gmail.com

Aljandra de Moreno de LeBlanc and Jean Guy LeBlanc are participated equally in this work.

2018/0761: received 11 April 2018, revised 31 May 2018 and accepted 25 June 2018

doi:10.1111/jam.14038

## Abstract

**Aim:** To evaluate two folate-producing strains, *Streptococcus* (*Strep.*) *thermophilus* CRL 808 and *Strep. thermophilus* CRL 415, against chemically induced mucositis in mice.
**Methods and Results:** *In vitro* assays with Caco-2 cells were performed to evaluate the effect of the bacteria in the presence of 5-fluorouracil (5-FU). For *in vivo* studies, mice were daily injected with 5-FU to induce intestinal mucositis (IM) and orally administered with folate-producing strains during 6 days. Clinical symptoms, histological parameters and cytokine profiles were assessed. The results showed that *Strep. thermophilus* CRL 808 increased the cytotoxicity of 5-FU against Caco-2 cells. Administration of this strain in mice with chemically induced IM resulted in a reduction in diarrhoea score and restoration of the intestinal architecture. Cytokine analysis showed that the anti-inflammatory effect by the bacterium is not associated with an immune mechanism. Regarding *Strep. thermophilus* CRL 415, no improvements were observed in any of the parameters evaluated.
**Conclusion:** The administration of the folate-producing *Strep. thermophilus* CRL 808 has the potential to prevent IM induced by 5-FU in mice.
**Significance and Impact of the Study:** Folate-producing LAB could be used in chemotherapy patients to reduce the symptoms of IM, improve their nutritional status and increase the effectiveness of 5-FU.

## Introduction

The drug 5-fluorouracil (5-FU) is one of the most commonly used chemotherapeutics in the treatment of various types of cancer, including colorectal cancer (Tucker *et al.* 2002). This agent acts by inhibiting the enzymes topoisomerase II and thymidylate synthase and thus interrupts the DNA synthesis (Duncan and Grant 2003). A problem with 5-FU is that it affects not only cancerous cells, but also normal proliferating cells such as those of the mucus membrane lining of the gastrointestinal tract resulting in intestinal mucositis (IM) (Smith *et al.* 2008). IM is characterized by damages mainly in the small intestine leading to a mucosal dysfunction (de Vasconcelos Generoso *et al.* 2015). Its development can be summarized in five stages: in the initial phase, the 5-FU causes DNA damage with generation of reactive oxygen species and death of the epithelial cells. In the second stage, the nuclear factor (NF)-κB pathway is activated with production of pro-inflammatory cytokines and may lead to cell apoptosis in the third phase. During the fourth stage, ulceration occurs, followed by the restoration of the mucosa in the last phase (Villa and Sonis 2015). Therefore, 5-FU affects both the structure and functionality of the small intestine; the villus atrophy and crypts loss produced by this agent lead to a disruption in the intestinal barrier function with risks of infections, and a reduction in the absorptive surface with a concomitant malnutrition (Araújo *et al.* 2015). For this reason, many patients under chemotherapy frequently take nutritional supplements and receive megavitamin therapy (Branda *et al.* 2004). Current therapies for IM are mostly ineffective; therefore,

developing new therapeutic options to manage IM induced by chemotherapy without compromising treatment efficacy has become essential. This would improve the quality of life of patients and avoid the dose reduction during chemotherapy or the discontinuation of treatments.

Folates (vitamin B9) belong to B-group vitamins. They are naturally found in leafy green vegetables, eggs, legumes, bran and dry fruit, whereas folic acid (the oxidized form) is chemically synthetized and used in dietary supplements and for food fortification (Cantarella *et al.* 2017). Members of the folate family participate in two types of reactions essential for the human body, the biosynthesis of nucleotides and the methylation reactions involved in DNA synthesis and repair (Ebara 2017).

Folic acid supplementation has demonstrated positive effects in attenuating the cisplatin-induced intestinal damage and apoptosis in a rat model (Bodiga *et al.* 2012); however, there are no precedents in the literature about the administration of natural folates. The use of natural folates such as those produced by lactic acid bacteria (LAB) would thus be an alternative to the use of the folic acid without the adverse effects associated with its consumption (LeBlanc *et al.* 2017). Numerous studies have demonstrated the ability of some LAB to produce B-group vitamins such as riboflavin, folate and thiamine (LeBlanc *et al.* 2011). Indeed, we previously studied the effect of administration of a riboflavin-producing lactic acid bacterium to mice with 5-FU-induced IM, and a significant attenuation in the pathological alterations induced by the chemotherapeutic drug has been observed (Levit *et al.* 2018).

The aim of this study was to evaluate the effect of folate-producing LAB in a mouse model of 5-FU-induced IM.

## Materials and methods

### Microbial strains and growth conditions

The two folate-producing strains employed in this study, *Streptococcus* (*Strep.*) *thermophilus* CRL 808 and *Strep. thermophilus* CRL 415, were obtained from the culture collection of CERELA (Centro de Referencia para Lactobacilos, San Miguel de Tucumán, Argentina). These strains were selected from previous studies because of their high vitamin production (Laiño *et al.* 2012). The first of them was reported to have an important intracellular folate producer, and the latter strain showed mainly extracellular folate production. A non-folate-producing strain (*Strep. thermophilus* CRL 807) was used as a control. Streptococci were grown for 16 h at 37°C without agitation in LAPTg broth containing (w/v) 1·5% peptone, 1% tryptone, 1% yeast extract, 0·1% glucose, 2% lactose and 0·1% Tween-80.

Before the *in vitro* and *in vivo* studies, the folate-producing bacteria were inoculated at 4% (v/v) in 10 ml of folate-free culture medium (Folic Acid Casei Medium; Difco, Becton, Dickinson, and Co., Sparks, MD) and incubated without agitation at 37°C for 16 h. The cultures in folate-free culture medium or LAPTg medium (for *Strep. thermophilus* CRL 807) were centrifuged (5000×***g*** for 10 min), washed using sterile saline solution (0·85% m/v NaCl) and resuspended in 1 ml of this solution.

### *In vitro* studies

*Cell culture*

Caco-2 human colorectal adenocarcinoma cells (ATCC HTB37), passage 57, were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Gran Island, NY) containing 10% foetal bovine serum (FBS; NATOCOR, Córdoba, Argentina) 100 IU $ml^{-1}$ penicillin, 100 $\mu g$ $ml^{-1}$ streptomycin and 0·25 $\mu g$ $ml^{-1}$ amphotericin B (Gibco, Gran Island, NY). The cells were cultured in 25-$cm^2$ vented tissue culture flasks at 37°C in a humidified incubator with 5% $CO_2$.

*Incubation of Caco-2 cells in the presence of LAB*

The cells were seeded in a 96-well plate at an initial cell density of $5 \times 10^4$ cells per ml. After overnight incubation, the medium was discarded and replaced by DMEM without antibiotic containing the folate-producing strains (*Strep. thermophilus* CRL 808 or *Strep. thermophilus* CRL 415) or a non-folate-producing strain (*Strep. thermophilus* CRL 807) in a cell : bacterium ratio of $1 : 10^3$ or commercial folic acid solution (HPLC grade, Fluka BioChemika, Sigma-Aldrich, Buchs, Switzerland) at a final concentration of 100 ng $ml^{-1}$ (similar to the concentration produced by the strains). After 24 h, the cells were exposed for an additional 24 h to 5-FU (100 $\mu g$ $ml^{-1}$).

*Viability assay*

Cell viability was evaluated by performing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Gentamicin (20 mg $l^{-1}$) was first added to kill bacteria, and 10 $\mu l$ of MTT (Sigma-Aldrich, St. Louis, MO) solution at a concentration of 5 mg $ml^{-1}$ in phosphate-buffered solution (PBS) was added to each well and incubated at 37°C for 4 h. Then, the medium was discarded and 100 $\mu l$ of dimethyl sulfoxide (DMSO) was added to each well, and the plate was shaken for 5 min. Finally, the absorbance was measured at 570 nm using a microplate reader (VersaMax; Molecular Devices, San Jose, CA, USA). Results were expressed as percentages relative to control reading (100%).

### *In vivo* studies

*Animals and bacterial or folic acid supplementations*

The study was performed using female BALB/c mice (6 weeks old) obtained from the inbred closed colony maintained at CERELA. Mice were randomized in different groups into appropriate cages under the same standard conditions (light/dark cycle of 12/12 hours and temperatures of 18–20°C). Animals were provided free access to conventional rodent food and water.

Animal experiments were pre-approved by the Animal Protection Committee of CERELA (CRL-BIOT-LT-2016/1A) in accordance with the current laws of Argentina and international organizations for the use of experimental animals.

Bacteria were grown in folate-free culture medium, washed and resuspended in 0·85% m/v NaCl to obtain a suspension of $7 \pm 1 \times 10^8$ colony-forming units (CFU) per ml.

A pure solution of folic acid was also prepared at a concentration equivalent to that produced by the strains (100 ng $ml^{-1}$) to be administered as a control.

### Mouse model of IM induced by 5-FU

The animals were divided into five experimental groups, each containing five animals. IM-induced mice received intraperitoneal injections of 5-FU (50 mg $kg^{-1}$ animal weight) daily for 6 consecutive days and were divided into four groups according to their treatment. The 5-FU + CRL 808 group and the 5-FU + CRL 415 group were administered 100 $\mu$l of suspension of *Strep. thermophilus* CRL 808 or *Strep. thermophilus* CRL 415 orally twice a day during the 6 days. The 5-FU + B9 group received commercial folic acid (100 ng $ml^{-1}$) orally twice a day during the 6 days, and the 5-FU + Saline group received saline orally twice a day during the 6 days. The mock group was injected intraperitoneally with saline daily for 6 consecutive days and received saline orally twice a day during the 6 days.

Mice were monitored daily to register stool consistency which was assigned a score from 0 to 3 according to a previously described scoring system (Huang *et al.* 2009). At the end of the experiment, animals were anaesthetized using a mixture of ketamine hydrochloride (König Laboratories, Buenos Aires, Argentina) 100 $\mu$g $g^{-1}$ body weight and xylazine at 2% (Bayer: División Sanidad Animal, Buenos Aires, Argentina) 5 $\mu$g $g^{-1}$ body weight and bled out by cardiac puncture. Small intestines were excised and washed with 500 $\mu$l of PBS containing Complete Mini EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany) to recover the intestinal contents. Jejunum samples were fixed in formaldehyde solution (10% v/v in PBS) for histological observations.

### Histological analysis

The damage to the small intestine was evaluated in segments of jejunum embedded in paraffin, sectioned (4 $\mu$m) and stained with haematoxylin and eosin (H&E). The histological sections were examined under a light microscope (Carl Zeiss Axio Scope.A1, Jena, Germany), and the measurement of villus height and crypt depth was performed using the AxioVision Release 4.8 software (Carl Zeiss, Jena, Germany). Five villi and crypts were measured per mouse, and the values were averaged for each group.

The inflammation grade of the small intestine was determined by observation of different histological findings that indicate mucosal damage described in a previously reported scoring system (Justino *et al.* 2014). The samples were scored from 0 to 3 according to the presence of low (0) or high (3) histological damage.

### Cytokine analysis

Blood samples were incubated at 37°C during 1 h, centrifuged 5 min at 1000 ***g*** to obtain serum and then stored at −80°C. Measurements of cytokines were also performed in intestinal contents which were centrifuged (3000 ***g***, 10 min, 4°C), and the supernatants were collected and stored at −80°C. The levels of interleukin-10 (IL-10), IL-17, tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-6, IL-4 and IL-2 were assessed by the cytometric bead array mouse inflammation kit (BD Bioscience, San Diego, CA) according to the manufacturer's instructions.

### Statistical analysis

Data were analysed using MINITAB 15 software (Minitab, State College, PA) and presented as mean ± SD. The differences between groups were determined using ANOVA general linear model followed by Tukey's post hoc test with $P < 0{\cdot}05$ indicating a statistically significant difference.

## Results

### Effect of the administration of folate-producing LAB on the cytotoxic action of 5-FU on Caco-2 cells

The data depicted in Fig. 1 show a cytotoxic effect when the cells were cocultured with *Strep. thermophilus* CRL 808 alone. However, when incubated with *Strep. thermophilus* CRL 415, *Strep. thermophilus* CRL 807 or commercial folic acid, no decrease in cell viability was observed. The combination of 5-FU and *Strep.*

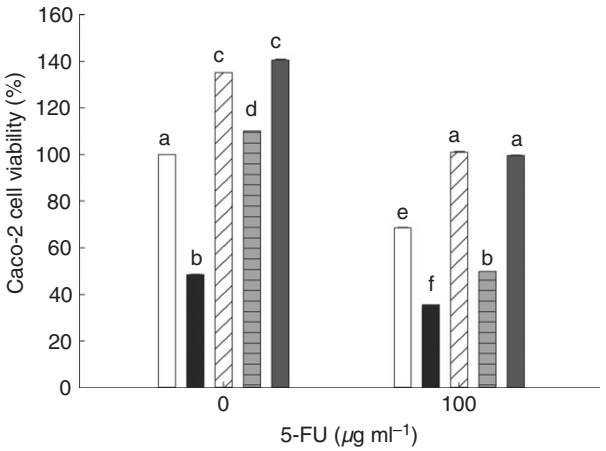


**Figure 1** Effect of folate-producing strains on 5-FU-induced cytotoxicity in Caco-2 cells. Caco-2 cells were grown in DMEM with FBS (white), and some of them were incubated with *Streptococcus thermophilus* CRL 808 (black), *Strep. thermophilus* CRL 415 (white with diagonal lines), *Strep. thermophilus* CRL 807 (dark grey) or commercial folic acid (grey with horizontal lines) with and without 5-FU (100 $\mu$g ml$^{-1}$). The effects on cell viability of Caco-2 cells were analysed by MTT assay. Data represent the mean ± SD from an experiment conducted in triplicate. Data with different letters (a–f) are significantly different ($P < 0{\cdot}05$).

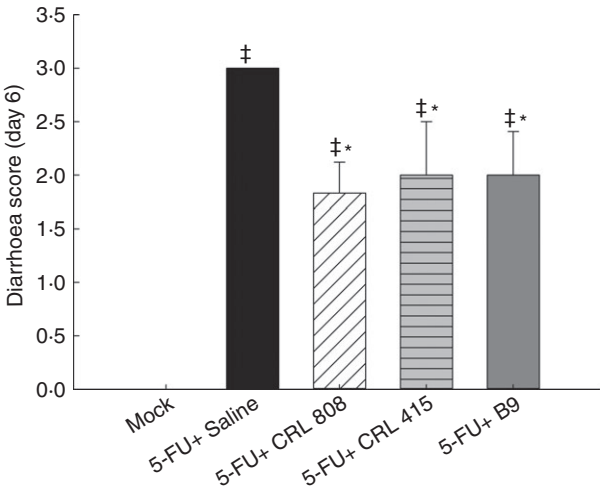


**Figure 2** Effect of folate-producing strains on diarrhoea induced by 5-FU. Stool consistency of mice was evaluated on Day 6 and scored from 0 to 3. Values were expressed as mean ± SD ($n$ = 5/group). ‡$P < 0{\cdot}05$ *vs* mock group. *$P < 0{\cdot}05$ *vs* 5-FU+ Saline group. Mock (white), 5-FU+Saline (black), 5-FU+CRL 808 (white with diagonal lines), 5-FU+CRL 415 (light grey with horizontal lines) and 5-FU+B9 (dark grey).

*thermophilus* CRL 808 enhanced significantly ($P < 0{\cdot}05$) the cytotoxic effect of 5-FU showing a reduction in cell viability of 65%. The cytotoxic action of 5-FU was also affected by commercial folic acid, but the effect was lower. The incubation of *Strep. thermophilus* CRL 415 with intestinal cells exposed to 5-FU showed significantly lower cytotoxicity than the control, and similar results were observed with the non-folate-producing control strain (*Strep. thermophilus* CRL 807).

### Folate-producing LAB decreased diarrhoea on 5-FU-injected mice

The shape and appearance of faeces was monitored daily in animals to detect the onset of diarrhoea. Figure 2 shows that animals injected with 5-FU presented diarrhoea when compared to the mock group, whereas the severity of diarrhoea was significantly attenuated ($P < 0{\cdot}05$) in those mice that received the folate-producing strains (5-FU+CRL 808 and 5-FU+CRL 415) or commercial folic acid (5-FU+B9) after 5-FU administration.

### Folate-producing LAB improve the histopathological changes induced by 5-FU in jejunum

Animals that received daily injections of 5-FU showed a significant ($P < 0{\cdot}05$) shortening of the villi and an increasing crypt depth when compared to the mock group (Fig. 3a). However, mice administered *Strep. thermophilus* CRL 808 or commercial folic acid showed a mild histological damage with significantly higher values ($P < 0{\cdot}05$) of villus height/crypt depth ratio compared to the 5-FU+Saline group. On the other hand, the villus height/crypt depth ratio remained without significant differences in mice that received *Strep. thermophilus* CRL 415. The evaluation of histological alterations in jejunal sections showed that repeated 5-FU administration resulted in villus blunting with vacuolated cells, crypt necrosis and intense inflammatory cell infiltration in the intestines of mice which was reflected in a high inflammation score in animals from the 5-FU+Saline group (Fig. 3b). Administration of *Strep. thermophilus* CRL 808 as well as of commercial folic acid resulted in a significantly decrease ($P < 0{\cdot}05$) in the inflammation score. Regarding the animals that received *Strep. thermophilus* CRL 415, there was no statistically significant improvement compared to those in the 5-FU control group.

### Effect of administration of folate-producing LAB on cytokine levels

Taking into account that in the other parameters evaluated the administration of *Strep. thermophilus* CRL 415 did not produce significant improvements in mice injected with 5-FU, cytokines were not determined in the 5-FU+CRL 415 group.

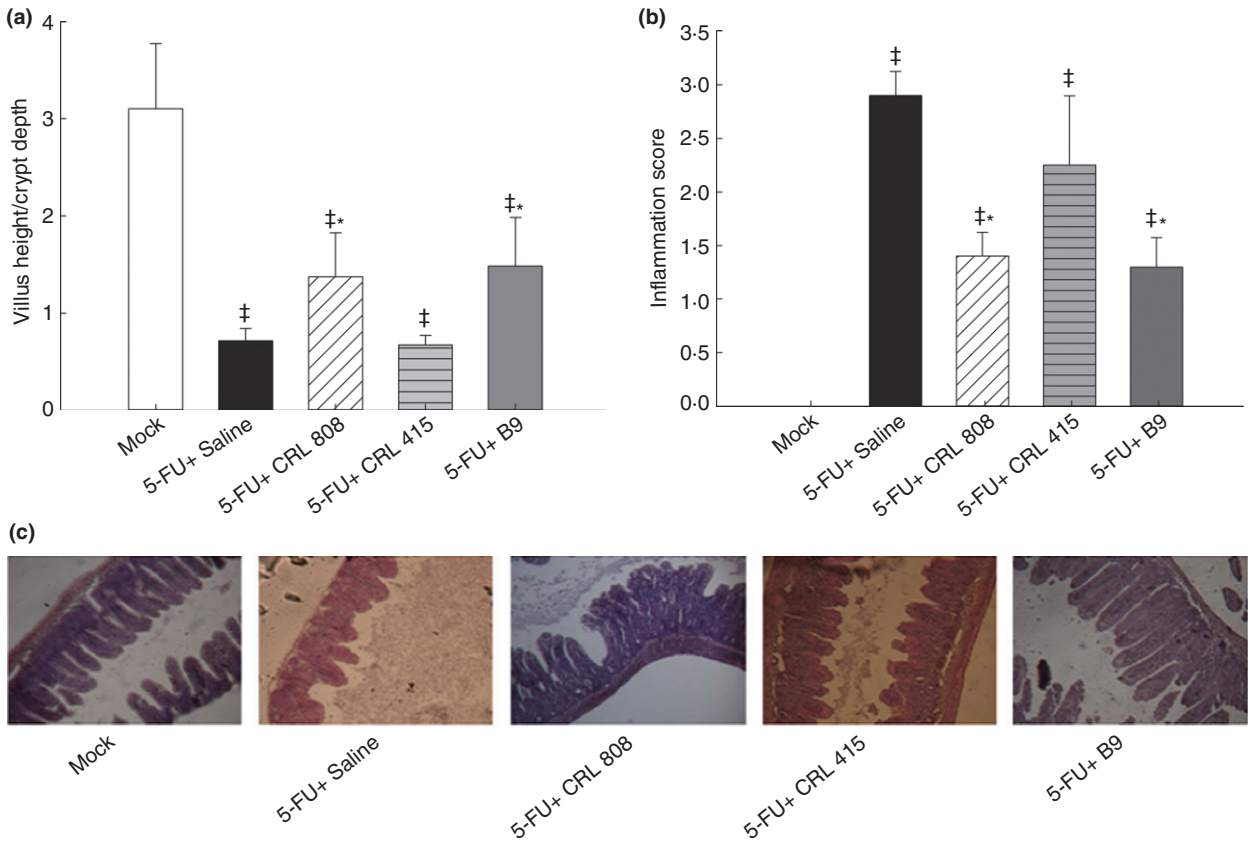


**Figure 3** Effect of folate-producing strains on morphometric and histopathological intestinal changes induced by 5-FU. (a) The length of the intestinal villi and depth of the crypts were measured in samples of jejunum. (b) Histological grading (0–3) of intestinal inflammation on jejunum sections. (c) Representative photomicrographs for each group of jejunum sections stained with haematoxylin and eosin (magnification ×100). Values are represented as mean ± SD ($n$ = 5 per group). $^{‡}P < 0{\cdot}05$ *vs* mock group. $^{*}P < 0{\cdot}05$ *vs* 5-FU+Saline group. Mock (white), 5-FU+Saline (black), 5-FU+CRL 808 (white with diagonal lines), 5-FU+CRL 415 (light grey with horizontal lines) and 5-FU+B9 (dark grey). [Colour figure can be viewed at wileyonlinelibrary.com]

Daily injections of 5-FU resulted in a significant increase ($P < 0{\cdot}05$) of IL-6 in serum of animals from the 5-FU+Saline group compared to the mock group. This was not modified by folic acid administration. The levels of IL-6 decreased in animals from the 5-FU+CRL 808 group, and no significant differences were observed when compared to the mock group. No significant variations were observed in the other tested cytokines evaluated in serum (Fig. 4a).

As shown in Fig. 4b, the levels of IL-17, TNF-α, INF-γ, IL-6, IL-4 and IL-2 detected in samples of intestinal contests were significant higher in mice from the 5-FU+Saline group compared with the mock group. Similarly, animals that received *Strep. thermophilus* CRL 808 maintained the increases in the concentration of these cytokines; however, the anti-inflammatory cytokine IL-10 also increased significantly ($P < 0{\cdot}05$). A similar profile of cytokines was found in samples from 5-FU+B9 group, but IL-10 levels presented a lower increase, without significant difference when compared to the 5-FU control group.

## Discussion

The reduction in toxic side effects of chemotherapeutic agents during cancer treatments without affecting their efficacy has stimulated the research for novel adjunct agents. These agents would not only improve the quality of life of oncological patients, but also avoid discontinuing cancer treatment because of their additive side effects (Mi *et al.* 2017; Whittaker *et al.* 2017). In this sense, IM is a common side effect that causes intestinal damage and a state of malnutrition in patients undergoing anticancer therapy (Decker-Baumann *et al.* 1999).

Considering the importance that the proposed adjunct treatments should not interfere with the anticancer activity of 5-FU, *in vitro* assays were performed. The results showed that similar to other LAB, *Strep. thermophilus* CRL 808 exerted by itself a cytotoxic effect on Caco-2 cells (Sevda *et al.* 2015). This folate-producing strain also enhanced the antiproliferative effect of 5-FU. This was also observed with commercial folic acid. It is known that one of the

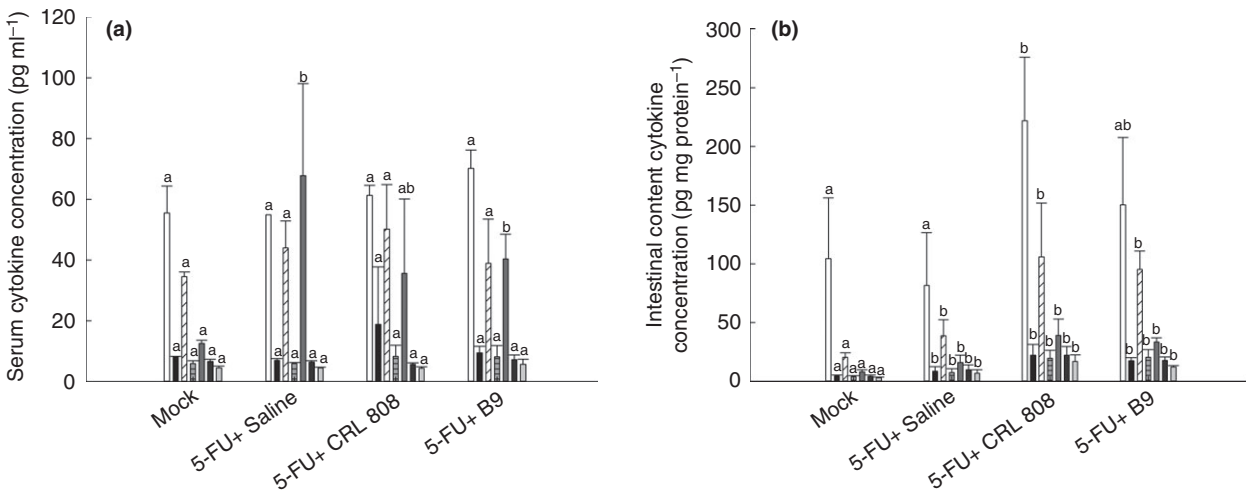


**Figure 4** Effect of folate-producing strains on cytokines profile in mice with 5-FU-induced intestinal mucositis. The levels of cytokines IL-10 (white), IL-17 (black), TNF-α (white with diagonal lines), IFN-γ (light grey with horizontal lines), IL-6 (dark grey), IL-4 (dotted white) and IL-2 (dotted black) in (a) serum and (b) intestinal contents were measured by Cytometric bead array. Bars represent the mean ± SD ($n = 5$ per group). Different letters (a, b) statistically differ ($P < 0{\cdot}05$).

mechanisms of action of 5-FU includes the inhibition of the enzyme thymidylate synthase by the formation of a ternary complex with 5,10-methylenetetrahydrofolate (5,10-CH2FH4), a compound that is produced from folic acid or folates by different pathways (Tsukihara *et al.* 2016). The increased effectiveness of 5-FU together with *Strep. thermophilus* CRL 808 when compared to folic acid alone could be due to the fact that this strain produces tetrahydrofolates which are more effective than folic acid for the formation of the ternary complex. The lack of effect observed with *Strep. thermophilus* CRL 415 can be explained because this strain produces mainly high concentrations of extracellular folates, that would be lost after washing the bacteria, and under these *in vitro* conditions, folate production and release would not occur. In this regard, the folate concentrations in the DMEM culture media with *Strep. thermophilus* CRL 415 were increased to a lesser extent compared to when *Strep. thermophilus* CRL 808 was used (38 ± 4 and 108 ± 9 ng $ml^{-1}$, respectively). However, the use of *Strep. thermophilus* CRL 415 cannot be ruled out as it could be useful to increase the concentrations of this vitamin in fermented foods, as has already been reported to obtain naturally fortified foods (Laiño *et al.* 2012).

The *in vivo* model of IM induced by 5-FU in mice also showed the beneficial effect associated with the administration of the folate-producing *Strep. thermophilus* CRL 808. The results indicated that *Strep. thermophilus* CRL 808 decreased diarrhoea scores, improved histological changes and reduced jejunal inflammation. Regarding cytokines, this effect was accompanied by decreases in pro-inflammatory IL-6 in serum, and increases in anti-inflammatory IL-10 were only observed in intestinal contents. In contrast to the anti-inflammatory effect observed with the administration of the riboflavin-overproducing strain *Lact. plantarum* CRL 2130 to mice injected with 5-FU (Levit *et al.* 2018), in the present study the increased levels of IL-10 were not associated with decreases in pro-inflammatory cytokines. In this sense, other nonimmune mechanisms would be involved in the benefits observed with *Strep. thermophilus* CRL 808. The lack of a beneficial effect in mice given *Strep. thermophilus* CRL 415 could be explained by the fact that this strain contains lower intracellular folate concentrations compared with *Strep. thermophilus* CRL 808 (15 ± 1 and 55 ± 2 ng $ml^{-1}$, respectively), and assuming that the strains might not be able to produce this vitamin in the gastrointestinal tract, the LAB would serve as folate carriers.

Studies provided evidences of the positive effects of folate supplementation in attenuating cisplatin-induced intestinal damage in rats (Bodiga *et al.* 2012), and also to increase the efficacy of fluorouracil in a cancer model (Raghunathan and Priest 1999). A study to investigate whether different supplemental levels of folic acid could modulate the sensitivity of human colon cancer cells to 5-FU using a xenograft model showed that the administration of the equivalent level to the recommended daily allowance of folic acid (2 mg) was not in detriment of the chemotherapy action of 5-FU (Ishiguro *et al.* 2016). However, in this previous study, when mice received four times this amount of folic acid, 5-FU-treated xenografts grew faster. This can be explained by the accumulation of nonactive forms of folates. Studies have reported that leucovorin ((6R,S)-5-formyl-THF) increases the concentrations of 5,10-CH2FH4 and improves the effectiveness of the chemotherapeutic action of 5-FU, and for this reason, they are usually used in combination (Tsukihara

*et al.* 2016). This drug has the advantage that it is active by itself, an advantage over folic acid that needs be converted to its active form (dihydrofolate) in the liver (Danenberg *et al.* 2016). Similarly, we suggest that the positive effect observed when mice received *Strep. thermophilus* CRL 808 compared to the group that received folic acid could be associated with the production of 5,10-CH2FH4 and 5-MTHF produced by this strain that is active when it reaches the intestines. Also, unlike folic acid, the natural folates such as those produced by LAB have not been shown to cause adverse effects associated with their consumption (LeBlanc *et al.* 2007). Folates produced by the selected LAB would be one of the mechanisms, but not necessarily the only mechanism, involved in the beneficial effects observed *in vitro* and *in vivo;* further studies using isogenic non-folate-producing strains would be necessary to assert this claim.

Considering these findings, we suggest that it could be possible to design a potentially adjunct therapeutic product based on a combination of different LAB. A mixture of probiotic strains that act through different anti-inflammatory mechanisms could be effective for the treatment of different intestinal inflammatory pathologies and be used together with traditional oncological therapies to reduce their adverse side effects on nontarget cells without interfering with their anticancer effects.

In conclusion, the current study demonstrated that the administration of folate-producing strain *Strep. thermophilus* CRL 808 reduced the damages associated with 5-FU-induced IM in a mouse model. *In vitro* studies showed an enhanced efficacy of 5-FU in combination with *Strep. thermophilus* CRL 808 on colon cancer cells. Therefore, we suggest that the administration of selected folate-producing bacterium could be useful to attenuate the symptoms of intestinal mucositis in patients undergoing chemotherapy, improve their nutritional status and potentially improve the efficacy of 5-FU as an anticancer drug.

## Acknowledgements

This work was financially supported by CONICET (projects PIP006 and 0697) and ANPCyT (projects 0301, 2554, 2859 and 3045).

## Conflict of Interest

No conflict of interest declared.

## References

Araújo, C.V., Lazzarotto, C.R., Aquino, C.C., Figueiredo, I.L., Costa, T.B., de Oliveira Alves, L.A., Ribeiro, R.A., Bertolini, L.R. *et al.* (2015) Alanyl-glutamine attenuates 5-fluorouracil-induced intestinal mucositis in apolipoprotein E-deficient mice. *Braz J Med Bioll Res* **48**, 493–501.

Bodiga, V.L., Bodiga, S., Surampudi, S., Boindala, S., Putcha, U., Nagalla, B., Subramaniam, K. and Manchala, R. (2012) Effect of vitamin supplementation on cisplatin-induced intestinal epithelial cell apoptosis in Wistar/NIN rats. *Nutrition* **28**, 572–580.

Branda, R.F., Naud, S.J., Brooks, E.M., Chen, Z. and Muss, H. (2004) Effect of vitamin B12, folate, and dietary supplements on breast carcinoma chemotherapy-induced mucositis and neutropenia. *Cancer* **101**, 1058–1064.

Cantarella, C.D., Ragusa, D., Giammanco, M. and Tosi, S. (2017) Folate deficiency as predisposing factor for childhood leukaemia: a review of the literature. *Gen Nutr* **12**, 14.

Danenberg, P.V., Gustavsson, B., Johnston, P., Lindberg, P., Moser, R., Odin, E., Peters, G.J. and Petrelli, N. (2016) Folates as adjuvants to anticancer agents: Chemical rationale and mechanism of action. *Critl Rev Oncol/ Hematol* **106**, 118–131.

Decker-Baumann, C., Buhl, K., Frohmuller, S., Herbay, A.V., Dueck, M. and Schlag, P.M. (1999) Reduction of chemotherapy-induced side-effects by parenteral glutamine supplementation in patients with metastatic colorectal cancer. *Eur J Cancer* **35**, 202–207.

Duncan, M. and Grant, G. (2003) Oral and intestinal mucositis–causes and possible treatments. *Alimentary Pharmacol Therap* **18**, 853–874.

Ebara, S. (2017) The nutritional role of folate. *Congenit Anomal*, **57**, 138–141

Huang, T.-Y., Chu, H.-C., Lin, Y.-L., Ho, W.-H., Hou, H.-S., Chao, Y.-C. and Liao, C.-L. (2009) Minocycline attenuates 5-fluorouracil-induced small intestinal mucositis in mouse model. *Biochem Biophys Res Commun* **389**, 634–639.

Ishiguro, L., Yang, M., Sohn, K.-J., Streutker, C.J., Grin, A., Croxford, R. and Kim, Y.-I. (2016) Folic acid supplementation adversely affects chemosensitivity of colon cancer cells to 5-fluorouracil. *Nutr Cancer*, **68**, 780–790.

Justino, P.F., Melo, L.F., Nogueira, A.F., Costa, J.V., Silva, L.M., Santos, C.M., Mendes, W.O., Costa, M.R. *et al.* (2014) Treatment with *Saccharomyces boulardii* reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *Br J Nutr* **111**, 1611–1621.

Laiño, J.E., LeBlanc, J.G. and Savoy de Giori, G. (2012) Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Can J Microbiol* **58**, 581–588.

LeBlanc, J.G., de Giori, G.S., Smid, E.J., Hugenholtz, J. and Sesma, F. (2007) Folate production by lactic acid bacteria and other food-grade microorganisms. *Communicat Curr Res Educat Topics Trends Appl Microbiol* **1**, 329–339.

LeBlanc, J.G., Laiño, J.E., del Valle, M.J., Vannini, V., van Sinderen, D., Taranto, M.P., de Valdez, G.F., de Giori, G.S. *et al.* (2011) B-group vitamin production by lactic

acid bacteria–current knowledge and potential applications. *J Appl Microbiol* **111**, 1297–1309.

LeBlanc, J.G., Chain, F., Martín, R., Bermúdez-Humarán, L.G., Courau, S. and Langella, P. (2017) Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact* **16**, 79.

Levit, R., De Savoy Giori, G., de Moreno LeBlanc, A. and LeBlanc, J.G. (2018). Protective effect of the riboflavin-overproducing strain *Lactobacillus plantarum* CRL 2130 on 5-fluorouracil-induced intestinal mucositis in mice. *Nutrition*, **54**, 165–172.

Mi, H., Dong, Y., Zhang, B., Wang, H., Peter, C.C.K., Gao, P., Fu, H. and Gao, Y. (2017) *Bifidobacterium infantis* ameliorates chemotherapy-induced intestinal mucositis via regulating t cell immunity in colorectal cancer rats. *Cell Physiol Biochem* **42**, 2330–2341.

Raghunathan, K. and Priest, D.G. (1999) Modulation of fluorouracil antitumor activity by folic acid in a murine model system. *Biochem Pharmacol* **58**, 835–839.

Sevda, E.R., Ayşe Tansu, K. and Merih, K. (2015) Cytotoxic effects of various lactic acid bacteria on Caco-2 cells. *Turk J Biol* **39**, 23–30.

Smith, C.L., Geier, M.S., Yazbeck, R., Torres, D.M., Butler, R.N. and Howarth, G.S. (2008) Lactobacillus fermentum BR11 and fructo-oligosaccharide partially reduce jejunal inflammation in a model of intestinal mucositis in rats. *Nutr Cancer* **60**, 757–767.

Tsukihara, H., Tsunekuni, K. and Takechi, T. (2016) Folic acid-metabolizing enzymes regulate the antitumor effect of 5-fluoro-2'-deoxyuridine in colorectal cancer cell lines. *PLoS ONE* **11**, e0163961.

Tucker, J.M., Davis, C., Kitchens, M.E., Bunni, M.A., Priest, D.G., Spencer, H.T. and Berger, F.G. (2002) Response to 5-fluorouracil chemotherapy is modified by dietary folic acid deficiency in ApcMin/+ mice. *Cancer Lett* **187**, 153–162.

de Vasconcelos Generoso, S., Rodrigues, N.M., Trindade, L.M., Paiva, N.C., Cardoso, V.N., Carneiro, C.M., de Matos Ferreira, A.V., Faria, A.M.C. *et al.* (2015) Dietary supplementation with omega-3 fatty acid attenuates 5-fluorouracil induced mucositis in mice. *Lip Health Dis* **14**, 1.

Villa, A. and Sonis, S.T. (2015) Mucositis: pathobiology and management. *Cur Opinion Oncol* **27**, 159–164.

Whittaker, A.L., Zhu, Y., Howarth, G.S., Loung, C.S., Bastian, S.E.P. and Wirthensohn, M.G. (2017) Effects of commercially produced almond by-products on chemotherapy-induced mucositis in rats. *W J Gastrointestinal Pathophysiol* **8**, 176.