

## Probiotic Effects on 5-Fluorouracil-Induced Mucositis Assessed by the Sucrose Breath Test in Rats

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**Abstract** The sucrose breath test (SBT) was employed to noninvasively assess the efficacy of probiotics in 5-fluorouracil (5-FU)-induced intestinal mucositis. Dark Agouti rats were allocated to 5 groups ( $n = 10$ ): 5-FU + *L. fermentum* BR11, 5-FU + *L. rhamnosus* GG, 5-FU + *B. lactis* BB12, 5-FU + skim milk (SM), and saline + SM. Probiotics were administered by oral gavage for 10 days. Mucositis was induced on day 7 by intraperitoneal injection of 5-FU (150 mg/kg) or vehicle (saline). Rats were sacrificed 72 h after 5-FU injection. The SBT measured breath  $^{13}\text{CO}_2$  (expressed as percentage cumulative dose at 90 min; %CD90) on days 0, 7, and 10. %CD90 was significantly lower in 5-FU-treated controls compared with that in saline-treated controls on day 10. 5-FU caused an 83% reduction in sucrose and a 510% increase in MPO activity. The SBT

detected damage induced by 5-FU and is a simple, noninvasive indicator of small bowel injury. The probiotics assessed offered no protection from mucositis at the dose tested.

**Keywords** 5-Fluorouracil · *Bifidobacterium* · *Lactobacillus* · Mucositis · Probiotic · Sucrose breath test

### Introduction

Mucositis is a debilitating side effect of chemotherapy associated with common treatment regimens for malignancy. Approximately 40% of all patients undergoing chemotherapy develop mucositis [1, 2]. Systemic effects of mucositis include diminished oral ingestion of fluids and solids which leads to dehydration, malnutrition, nausea, vomiting, abdominal pain, bloating, and diarrhea [3, 4]. Currently there is a lack of effective prophylactic or therapeutic treatments for mucositis [5, 6]. Furthermore, current approaches focus on symptoms and have been limited to rinses, barrier protectants, topical antimicrobials, cryotherapy (ice), and analgesics. A number of alternative strategies are currently being pursued, including new pharmaceuticals and bioactives such as keratinocyte growth factor [7, 8], insulin-like growth factor [9], milk-derived growth factors [10, 11], and, more recently, probiotics.

Probiotics are defined as bacteria or bacterial products that have a significant health benefit for the recipient [12]. It has become apparent that bacterial strains such as bifidobacteria and lactobacilli play an important role in maintaining barrier function within the intestine [13–15]. Bifidobacteria are capable of enhancing specific immune functions [16] and have also been demonstrated to prevent adhesion of *Escherichia coli* to mucosal surfaces [17] and *Bifidobacterium lactis* BB12 has been shown to trigger gene expression of

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the inflammatory mediator interleukin-6 [18]. *Lactobacillus* species possess beneficial physiologic effects, including antimicrobial activity, enhancement of immune potency, anti-tumorigenic activities, and antioxidative activity [19]. *Lactobacillus rhamnosus* GG has been shown to be effective in the control of diarrhea [17] and *Lactobacillus fermentum* BR11, a newly described organism isolated from guinea pig [20, 21], possesses antioxidant properties [21]. *Lactobacillus fermentum* BR11 has also been shown to provide partial protection from dextran sulfate sodium-induced colitis in rats [22].

The sucrose breath test (SBT) has been developed as a noninvasive method for determining total intestinal sucrase activity [23]. Sucrase is an enzyme found in the brush border membrane of mucosal cells lining the lumen of the small intestine [24]. It catalyzes the breakdown of sucrose into its constituent monosaccharides, glucose and fructose. Following ingestion of  $^{13}\text{C}$ -sucrose, these monosaccharides are transported to the liver and metabolized to liberate  $^{13}\text{CO}_2$ , which is exhaled from the lungs and quantified using an isotope ratio mass spectrometer (IRMS). These *in vivo* determinations of sucrase activity are indicative of mucosal damage [23, 25]. Carcinomata and hematologic malignancies are treated primarily with antimetabolite chemotherapeutic agents such as 5-fluorouracil (5-FU) and methotrexate (MTX), which cause cell death by interfering with DNA synthesis [26]. Applicability of the SBT to the detection of intestinal mucositis induced by MTX has been demonstrated previously by Pelton *et al.* [23].

The current study aimed to further characterize the SBT's ability to detect intestinal damage induced by 5-FU, and to assess the effects of *Lactobacillus fermentum* BR11, *Lactobacillus rhamnosus* GG, and *Bifidobacterium lactis* BB12 administration on the severity of 5-FU-induced mucositis in rats.

## Methods

### Animals and experimental design

Female Dark Agouti (DA) rats (body weight =  $146 \pm 1$  g) were maintained in metabolism cages (Tecniplast USA Inc., Exton, PA, USA) for the duration of the experimental period and maintained at 25°C with a 12-h light/dark cycle. Rats were allocated to 5 groups: saline + skim milk (SM), 5-FU + SM, 5-FU + *Lactobacillus fermentum* BR11, 5-FU + *Lactobacillus rhamnosus* GG, and 5-FU + *Bifidobacterium lactis* Bb12 ( $n = 10$ ). Metabolism data, including water and food intake and urine and fecal output, were collected for 11 days. All animals were provided *ad libitum* access to drinking water and a standard 18% casein-based diet [27] except during fasting periods. All animal studies were carried out in compliance with the Australian Code of

Practice for the Care and Use of Animals and were approved by the Animal Ethics Committee of the Children, Youth and Women's Health Service, Adelaide, South Australia.

### 5-FU-induced mucositis

Rats were administered a single dose of 5-FU (150 mg/kg; Fluorouracil Injection, Mayne Pharma Pty Ltd, Melbourne, Australia) or saline control on day 7 by intraperitoneal injection. This dose was determined in a previous study [28] in Sprague-Dawley rats in which intestinal damage was evident within 2 days of treatment, and gastrointestinal symptoms were maximal 3 days after chemotherapy administration. On this basis, rats were sacrificed on day 10 of the experimental period, 3 days after 5-FU injection.

### Probiotic preparations

*L. fermentum* BR11 and *L. rhamnosus* GG were grown in De Mann/Rogosa/Sharpe (MRS) broth (Oxoid Ltd, Hampshire, England). *B. lactis* BB12 was grown in Brain Heart Infusion (BHI) broth (Oxoid). All bacterial broths were incubated at 37°C, 5% CO<sub>2</sub>, for 48 h. The broths were pooled and centrifuged at 1000 rpm for 10 min and the supernatant was removed. The pellet was then resuspended in a 10% skim milk/1% glucose solution, reconstituted from Bonlac® skim milk powder (Inpak Foods, Adelaide, South Australia, Australia). The concentration of bacteria in skim milk suspensions was approximately  $1 \times 10^6$  CFU/ml, determined by viable count. The skim milk suspensions were separated into 10 ml aliquots and stored at  $-80^\circ\text{C}$ .

Probiotic bacteria were administered daily by orogastric gavage. Administration began on day 0 of the trial period and continued until day 9. All rats were sacrificed on day 10. The orogastric gavage consisted of 1 ml of skim milk solution alone as a control, or 1 ml of bacteria in skim milk solution. Both the saline-injected and 5-FU-injected control groups were administered skim milk alone.

### Sucrose breath test

Breath collection was performed by placing rats in a sealed 600 ml perspex container, allowing breath to accumulate for 2 min, and drawing 20 ml of breath into a syringe attached to a two-way outlet in the lid of the container [23, 25]. Rats were fasted overnight and a baseline breath sample was collected at  $t = 0$ . Rats were then gavaged with 1 ml of a sucrose solution (250 mg/ml) and breath was collected every 15 min for a total of 2 h. Samples were collected in evacuated 10 ml glass tubes (Exetainer, Labco Ltd, High Wycombe, UK) and analyzed using an IRMS (Europa Scientific Automated Breath  $^{13}\text{C}$  Carbon Analyser, Crewe, UK) for breath

$^{13}\text{CO}_2$ , expressed as percentage cumulative dose at 90 min (%CD90). The SBT was performed before commencement of the trial (day 0), on day 7 before injection with saline or 5-FU, and before sacrifice on day 10.

#### Tissue collection

Animals were sacrificed by  $\text{CO}_2$  asphyxiation and cervical dislocation on day 10. The abdomen was opened by a midline surgical incision and the jejunum separated from the duodenum at the ligament of Treitz, as described previously [10]. The gut, from stomach to anus, was removed and placed on an ice-cold glass slab. The length of the duodenum, small intestine, and colon was recorded. All gastrointestinal organs were flushed of contents and weighed. Two centimeter segments of duodenum, small intestine, and colon were collected and fixed in 10% buffered formalin for histologic analysis. Segments (4 cm) directly adjacent to the histologic samples were snap-frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for later homogenization and biochemical analyses. Sampling sites in the small intestine corresponded to 10% (proximal jejunum), 25%, 50%, 75%, and 90% (distal ileum) of the intestinal length. Heart, lungs, kidneys, spleen, liver, and thymus were removed, weighed, and discarded.

#### Biochemical analyses

Intestinal samples from the proximal jejunum and distal ileum were thawed in 1.5 ml of 10 mM phosphate buffer (pH 6.0) and homogenized for 2 min at 17,000 rpm. Aliquots of homogenate (200  $\mu\text{l}$ ) were stored at  $-80^\circ\text{C}$  until required for sucrase and myeloperoxidase assays. *In vitro* determination of active intestinal sucrase allows direct analysis of brush border integrity [25, 29] in different regions of the intestine. Endogenous sucrase activity was assessed by a modification of the method described previously by Dahlqvist [30]. Intestinal homogenates were thawed on ice and a minimum of two dilutions were performed for each sample, which were plated in triplicate and corrected for background absorbance. Glucose standards were used to obtain a standard curve. Plates were read using a microtiter plate reader (MR7000, Dynatech Laboratories Inc., Chantilly, VA, USA) with the test filter set at 490 nm and the reference filter set at 690 nm. Tablecurve 2D software (Systat Software Inc., Point Richmond, CA, USA) was used to calculate glucose concentration.

Myeloperoxidase (MPO) is an intracellular enzyme found in neutrophils [31], cells which are the principal component of acute inflammatory cell infiltrates. Levels of MPO were determined by a modification of the procedure described by Krawisz *et al.* [31]. Intestinal homogenates were thawed and centrifuged at 15,000 rpm for 12 min (Heraeus Biofuge Pico, Kendro Laboratory Products, Langensfeld, Germany). The

supernatant was discarded and the pellet was resuspended in 200  $\mu\text{l}$  of 0.5% hexadecyltrimethylammonium bromide (HTAB) buffer (pH 6.0) (Sigma Chemical Co., St Louis, MO, USA) to release MPO from neutrophil primary granules. Samples were then centrifuged at 15,000 rpm for 5 min to remove cellular debris and 50  $\mu\text{l}$  of each supernatant was plated in duplicate. Optical density was read immediately following addition of a freshly prepared O-dianisidine-based reaction mixture (200  $\mu\text{l}$ /well) at 1 min intervals for 15 min using a microplate reader (MR7000) with the test filter set at 450 nm.

#### Histological analysis

Tissue samples were transferred into 70% alcohol after 24 h in 10% buffered formalin, routinely processed, and embedded in paraffin wax. Transverse sections (5  $\mu\text{m}$ ) of proximal jejunum were stained using hematoxylin and eosin. A light microscope (SM-Lux, Leitz Wetzlar, Germany) was used to perform semiquantitative histologic examination and images were acquired using a digital camera (Sony, Tokyo, Japan). Sections were assessed in a blinded manner using a set of 11 parameters to obtain an overall score of damage severity (semiquantitative; maximum score = 33) as described previously [32, 33]. These parameters included villus atrophy, crypt loss or disruption, and inflammatory cell infiltration.

#### Statistical analysis

Statistical analyses were performed using the GraphPad Prism 4.0 software package (GraphPad Software, San Diego, CA, USA). Parametric data, including organ wet weights and lengths, breath  $^{13}\text{CO}_2$  levels (%CD90), sucrase levels, and MPO levels, were analyzed using one-way analysis of variance (ANOVA) and Tukey's *post-hoc* tests. Daily metabolic data were analyzed using two-way ANOVA and Bonferroni *post-hoc* tests. Nonparametric data, including severity scores, were analyzed using the Kruskal – Wallis test with Dunn's *post-hoc* tests comparing all data sets. Observed differences were considered significant if  $P < 0.05$ .

## Results

#### Body weight and gastrointestinal organs

Between days 0 and 10, rats administered 5-FU + SM showed a significant loss in body weight ( $P < 0.05$ ) compared with saline + SM-treated controls (Table 1). Body weight loss in all three probiotic-treated groups was significantly greater than in saline + SM-treated rats ( $P < 0.05$ ) but not different than in 5-FU + SM-treated rats (Table 1).

**Table 1** Body weight change and adjusted gastrointestinal organ weights in saline + SM-, 5-FU+ SM-, and 5-FU+ probiotic-treated groups

	Treatment group				
	Saline + SM	5-FU + SM	5-FU+ <i>L. fermentum</i>	5-FU+ <i>L. rhamnosus</i>	5-FU + <i>B. lactis</i>
Body weight change (g)	+1.8 ± 0.7	-2.8 ± 0.9*	-6.1 ± 1.4***	-2.4 ± 0.5*	-4.7 ± 1.2***
Duodenum	0.272 ± 0.01	0.263 ± 0.01	0.257 ± 0.01	0.235 ± 0.05	0.262 ± 0.06
Small intestine	1.797 ± 0.03	1.499 ± 0.03***	1.477 ± 0.04***	1.612 ± 0.04**	1.467 ± 0.03***
Cecum	0.351 ± 0.02	0.509 ± 0.02***	0.475 ± 0.02**	0.426 ± 0.02	0.511 ± 0.03***
Colon	0.535 ± 0.01	0.583 ± 0.02	0.621 ± 0.02*	0.549 ± 0.01	0.657 ± 0.03**

Note. Body weight change values are expressed as mean change (g) ± SEM. Organ weights are expressed as mean % organ weight/body weight ± SEM. A significant difference compared with saline + SM is denoted by \* $P < 0.05$ , \*\* $P < 0.01$ , or \*\*\* $P < 0.001$ .

All organ weights were adjusted for body weight to eliminate this parameter as a source of variation. Small intestine weight was reduced by 17% in 5-FU + SM-treated rats compared with saline + SM-treated rats ( $P < 0.001$ ; Table 1). Small intestine weight was also reduced in 5-FU + *L. fermentum*- (18%), 5-FU + *L. rhamnosus*- (10%), and 5-FU + *B. lactis*-treated (18%) rats. Cecum weight increased following 5-FU + SM (45%), 5-FU + *L. fermentum* (35%), and 5-FU + *B. lactis* (46%) treatment compared with saline + SM treatment. 5-FU + *L. fermentum* and 5-FU + *B. lactis* treatment displayed significant increases in colon weight compared with saline + SM, 16% and 23%, respectively. 5-FU-treated rats did not display a decrease in small intestine length compared with saline + SM-treated rats ( $P > 0.05$ ; Table 2). 5-FU + *L. fermentum*- and 5-FU + *B. lactis*-treated rats did display a decrease in small intestine length compared with saline + SM-treated rats, 8% and 6%, respectively. Colon length was decreased in 5-FU + SM- (16%), 5-FU-*L. fermentum*- (9%), 5-FU + *L. rhamnosus*- (9%), and 5-FU + *B. lactis*-treated (12%) rats compared with saline + SM-treated controls (Table 2). There was no significant difference in small intestine and colon length between any of the three probiotic-treated groups and the 5-FU + SM-treated controls (Table 2).

Adjusted thymus weight was significantly decreased in 5-FU + SM-treated rats compared with saline + SM (0.050 ± 0.003 vs. 0.136 ± 0.016, % thymus weight/body weight;  $P < 0.001$ ). Thymus weight was also significantly decreased in 5-FU + *L. fermentum*- (0.056 ± 0.003), 5-FU + *L. rhamnosus*- (0.062 ± 0.004), and 5-FU + *B. lactis*-treated (0.053 ± 0.003) rats. Spleen weight was signif-

icantly decreased in 5-FU + SM-treated rats compared with saline + SM (0.168 ± 0.005 vs. 0.233 ± 0.004, % spleen weight/body weight;  $P < 0.001$ ). Spleen weight was also significantly decreased in 5-FU + *L. fermentum*- (0.172 ± 0.005), 5-FU + *L. rhamnosus*- (0.163 ± 0.004), and 5-FU + *B. lactis*-treated (0.158 ± 0.005) rats.

#### Noninvasive sucrose breath test

On days 0 and 7 there was no significant difference in breath  $^{13}\text{CO}_2$  (%CD90) between 5-FU + SM- and saline + SM-treated rats (Fig. 1A). On day 10, however, breath  $^{13}\text{CO}_2$  in the 5-FU + SM-treated rats exhibited a 63% reduction compared with that in saline + SM-treated controls ( $P < 0.001$ ; Fig. 1B). There was no significant difference in %CD90 values between any of the probiotic-treated groups and 5-FU + SM-treated rats on days 0 and 7 (Fig. 1A). On day 10, the %CD90 of all three probiotic-treated groups were significantly lower ( $P < 0.001$ ) than saline + SM-treated controls although they were not significantly different than that of 5-FU + SM-treated rats (Fig. 1B).

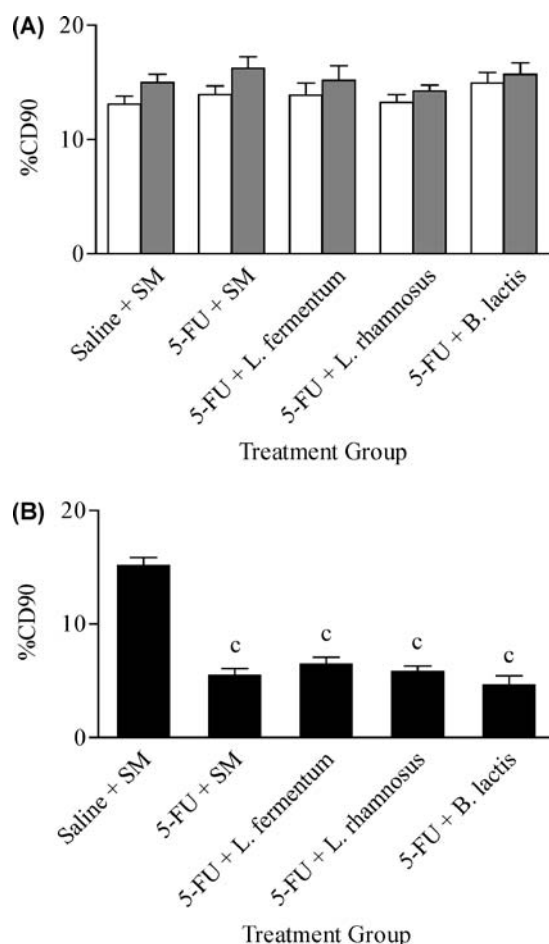
#### Biochemically assessed sucrase activity

Proximal jejunum sucrase activity in 5-FU + SM-treated rats was 83% lower compared with saline + SM-treated controls ( $P < 0.001$ ; Fig. 2A). Distal ileum sucrase activity in 5-FU + SM-treated rats was below the sensitivity of the assay. In saline + SM-treated rats, sucrase activity in the distal ileum was 11% of proximal jejunum values ( $P < 0.001$ ). There was no significant difference in jejunal sucrase

**Table 2** Intestinal lengths (cm) in saline + SM-, 5-FU+ SM-, and 5-FU+ probiotic-treated groups

	Treatment group				
	Saline + SM	5-FU + SM	5-FU+ <i>L. fermentum</i>	5-FU+ <i>L. rhamnosus</i>	5-FU + <i>B. lactis</i>
Duodenum	7.2 ± 0.1	6.4 ± 0.2	6.2 ± 0.2	6.5 ± 0.2	6.5 ± 0.2
Small intestine	70.4 ± 0.7	67.1 ± 1.0	64.5 ± 0.8**	67.9 ± 1.2	66.2 ± 0.9*
Colon	13.1 ± 0.2	11.0 ± 0.3***	11.9 ± 0.3*	11.9 ± 0.3*	11.5 ± 0.3**

Note. Values are expressed as mean (cm) ± SEM. A significant difference compared with saline + SM is denoted by \* $P < 0.05$ , \*\* $P < 0.01$ , or \*\*\* $P < 0.001$ .

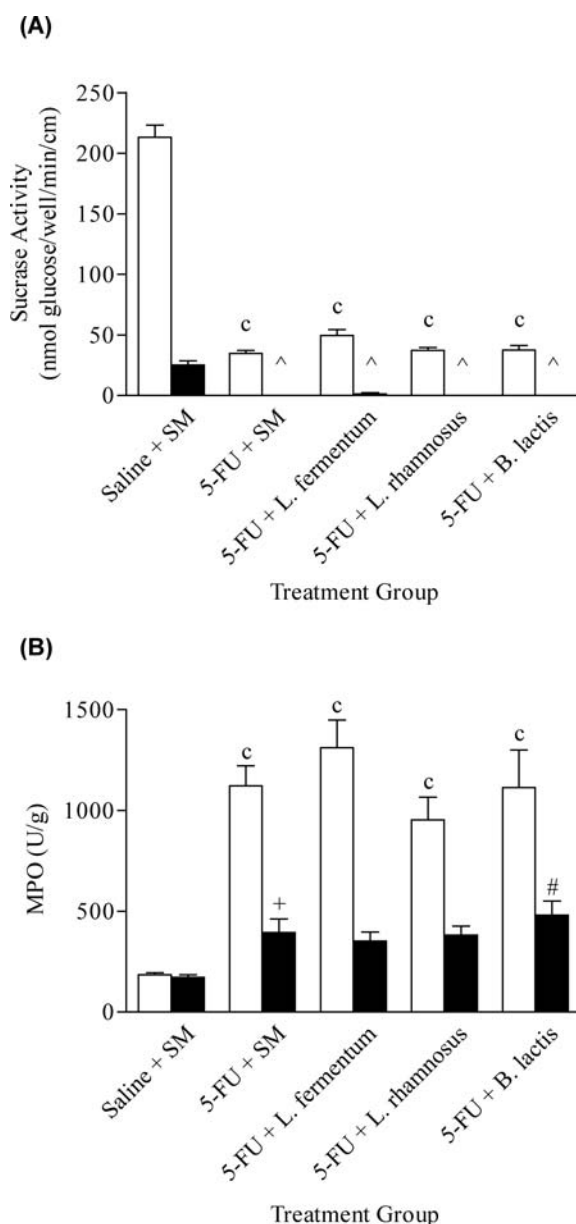


**Fig. 1** Sucrose breath test results [expressed as percentage cumulative dose at 90 min (%CD90)] in female Dark Agouti rats (A) on day 0 (white columns) and day 7 (grey columns), and (B) on day 10. Columns represent means + SEM. A significant difference compared with saline + SM is denoted by c ( $P < 0.001$ )

activity between any of the three probiotic-treated groups and 5-FU + SM-treated controls (Fig. 2A). Breath  $^{13}\text{CO}_2$  levels (%CD90) and sucrase activity values from proximal jejunum homogenates were compared directly by linear regression and a strong correlation was found between these two parameters ( $r^2 = 0.77$ ,  $P < 0.0001$ ).

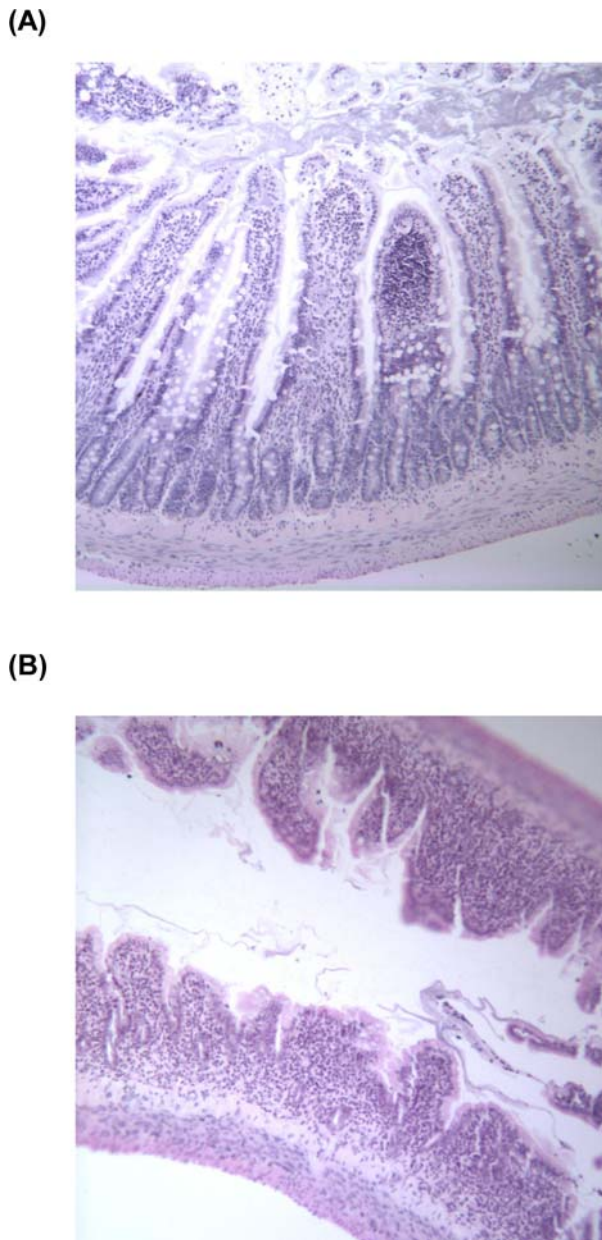
#### MPO activity and small intestine histology

MPO activity in proximal jejunum was not significantly different from the distal ileum in saline + SM-treated rats (Fig. 2B). MPO activity in the proximal jejunum and distal ileum of 5-FU + SM-treated rats was increased by 510% ( $P < 0.001$ ) and 129% ( $P < 0.05$ ), respectively, compared with saline + SM-treated rats (Fig. 2B). MPO activity in both the proximal jejunum and distal ileum did not vary significantly in any of the three probiotic treatment groups when compared with 5-FU + SM-treated rats (Fig. 2B).



**Fig. 2** A Sucrase activity and B MPO activity in the proximal jejunum (white columns) and the distal ileum (black columns) of female Dark Agouti rats. Columns represent means + SEM. A significant difference compared with saline + SM in the jejunum is denoted by c ( $P < 0.001$ ), and +, #, and ^ denote a significant difference compared with saline + SM in the ileum,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively

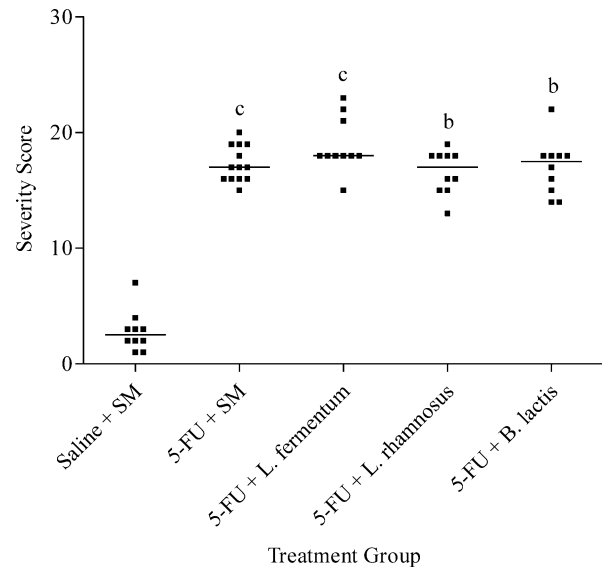
Examples of histologic images from normal (saline + SM) and damaged (5-FU + SM) proximal jejunum are shown in Fig. 3. The median severity score increased by 14.5 in 5-FU + SM-treated rats ( $P < 0.001$ ) compared with saline + SM-treated controls, but none of the three probiotic-treated groups displayed a significant difference in severity score compared with 5-FU + SM-treated rats (Fig. 4).



**Fig. 3** Hematoxylin and eosin-stained sections (magnification 100×) of (A) proximal jejunum of saline and (B) 5-FU-treated (150 mg/kg intraperitoneal) rats

**Discussion**

There is currently a lack of effective therapeutic interventions for the treatment of chemotherapy-induced mucositis. We used the noninvasive sucrose breath test to assess three potential probiotic strains for their capacity to reduce the severity of small intestinal mucositis. Consistent with previous studies in rats, 5-FU caused a significant decrease in body weight and weights of the thymus, spleen, and small intestine [29], further causing a significant increase in damage severity in the jejunum.



**Fig. 4** Proximal jejunum histologic severity score (maximum score = 33) in female Dark Agouti rats. Each point represents a single value and medians are represented by horizontal bars. A significant difference compared with saline + SM is denoted by b ( $P < 0.01$ ) or c ( $P < 0.001$ )

In the current study, 5-FU-induced intestinal damage was readily detectable by the SBT. Pelton *et al.* [23], using the SBT, reported diminished sucrase activity in rats subcutaneously injected with the related antimetabolite methotrexate as indicated by a significant reduction in breath <sup>13</sup>CO<sub>2</sub> and biochemically determined sucrase activity. Breath <sup>13</sup>CO<sub>2</sub> in the present study was strongly correlated with biochemically assessed sucrase activity. Clarke *et al.* [25] used the SBT to investigate the effects of methotrexate in rats and found that breath <sup>13</sup>CO<sub>2</sub> was significantly lower 72 h after methotrexate injection. Moreover, these investigators also reported that sucrase activity, assessed biochemically, was significantly decreased in the proximal jejunum [25], consistent with the current study. These investigators further reported a strong correlation between jejunal sucrase activity and breath <sup>13</sup>CO<sub>2</sub> ( $r^2 = 0.89$ ) [25], again consistent with the current study, further supporting the SBT as a reliable, noninvasive marker of intestinal damage.

The probiotic *Lactobacillus rhamnosus* GG and probiotic compound VSL#3 have previously been shown to reduce levels of MPO in the colitis setting [34] and prevent recurrence of colitis after antibiotic treatment [35], and the probiotic *Bifidobacterium infantis* has been shown to be effective in the treatment of irritable bowel syndrome [12]. At the doses tested, the probiotics investigated in the current study could not prevent the intestinal damage caused by administration of 5-FU. The probiotics were ineffective at preventing overall body weight loss and thymus, spleen, and small intestine weight loss, and they could not prevent small intestine damage as assessed by the SBT, sucrase, and myeloperoxidase

activity. Possible explanations for the lack of efficacy may have included the dosage, timing and duration of delivery, or lack of suitability of the strains used for the mucositis setting.

There has been extensive investigation of the probiotics *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12. Administration of probiotic mixtures containing *Lactobacillus rhamnosus* GG to humans has resulted in a reduction of abdominal bloating [36], symptom score in patients with irritable bowel syndrome [37], risk of antibiotic-associated diarrhea [38], and treatment-related symptoms in addition to a greater success of eradication in *H. pylori* treatment [39]. However, the current study was unable to reproduce the positive gastrointestinal effects in humans exhibited by *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12.

A recent study by Tooley *et al.* [40] showed that treatment with the probiotic *Streptococcus thermophilus* TH-4 reduced the severity of MTX-induced mucositis in Dark Agouti rats with SBT and sucrase activity values comparable to that of normal rats, while MTX treatment alone decreased these parameters. These investigators reported that *Streptococcus thermophilus* TH-4-treated rats also had a lower MPO activity compared with MTX-treated rats, indicating less inflammation in the small intestine. The study by Tooley *et al.* highlighted the capability for the SBT to detect the preventative effects of *Streptococcus thermophilus* TH-4 in a mucositis model, confirming that the SBT could be used to rapidly and noninvasively screen novel therapies for mucositis [40]. Coupled with the current study, this emphasizes the importance of screening large numbers of bacterial strains, since not all strains would necessarily be beneficial in mucositis setting.

In conclusion, the sucrose breath test is an effective indicator of 5-FU-induced intestinal damage. However, at the doses tested, none of the three probiotic strains examined exhibited any demonstrable protective effect on the mucosa of the chemotherapy-damaged small intestine. To detect any potential beneficial effects of *Lactobacillus fermentum* BR11, *Lactobacillus rhamnosus* GG, or *Bifidobacterium lactis* BB12 in the treatment of chemotherapy-induced mucositis, additional studies could investigate the use of viable bacteria at a higher dosage, differing timing regimens, and combinations of the three probiotics.

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