ACCEPTED VERSION

Atkins, Haydn Leslie; Geier, Mark Steven; Prisciandaro, Luca David; Pattanaik, Ashok K.; Forder, Rebecca Edith Anne; Turner, Mark S.; Howarth, Gordon Stanley

Effects of a lactobacillus reuteri BR11 mutant deficient in the cystine-transport system in a rat model of inflammatory bowel disease

Digestive Diseases and Sciences, 2012; 57(3):713-719

© Springer Science+Business Media, LLC 2011

The final publication is available at link.springer.com:
http://link.springer.com/article/10.1007%2Fs10620-011-1943-0

http://www.springer.com/open+access/authors+rights?SGWID=0-176704-12-683201-0

PERMISSIONS

Publishing in a subscription-based journal

If you publish an article in the traditional way, without open access our Copyright Transfer Statements reads (excerpt):

"Authors may self-archive the author's accepted manuscript of their articles on their own websites. Authors may also deposit this version of the article in any repository, provided it is only made publicly available 12 months after official publication or later. He/ she may not use the publisher's version (the final article), which is posted on SpringerLink and other Springer websites, for the purpose of self-archiving or deposit. Furthermore, the author may only post his/her version provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com"."

2nd April 2013

http://hdl.handle.net/2440/76166

Effects of a *Lactobacillus reuteri* BR11 mutant deficient in the cystine transport system in a rat model of inflammatory bowel disease

Haydn L Atkins, B Sc (Hons)[1], Mark S Geier, PhD[1,3], Luca D Prisciandaro, B Sc (Hons)[1,2], Ashok K Pattanaik, PhD[4], Rebecca EA Forder, PhD[1], Mark S Turner, PhD[5] and Gordon S Howarth, PhD[1,2]

[1]School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy Campus, University of Adelaide, South Australia

[2]Centre for Paediatric and Adolescent Gastroenterology; Children, Youth and Women's Health Service (CYWHS), North Adelaide, South Australia

[3]South Australian Research and Development Institute (SARDI), PPPI Nutrition Research Laboratory, Roseworthy, South Australia

[4]Clinical & Pet Nutrition Laboratory, Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, India

[5]School of Agriculture and Food Sciences, University of Queensland, St. Lucia Campus, Brisbane, Queensland, Australia

Authors' postal and email addresses

Haydn Atkins: School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy Campus, University of Adelaide atki0124@flinders.edu.au

Mark Geier: [3]South Australian Research and Development Institute (SARDI), PPPI Nutrition Research Laboratory, Roseworthy, South Australia Mark.Geier@sa.gov.au

Luca Prisciandaro: School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy Campus, University of Adelaide luca.prisciandaro@adelaide.edu.au

Ashok Pattanaik: Clinical & Pet Nutrition Laboratory, Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, India akpattanaik1@gmail.com

Rebecca Forder: School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy Campus, University of Adelaide bec.forder@adelaide.edu.au

Mark Turner: [5]School of Agriculture and Food Sciences, University of Queensland St. Lucia Campus, Brisbane, Queensland, Australia m.turner2@uq.edu.au

Gordon Howarth: School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy Campus, University of Adelaide gordon.howarth@adelaide.edu.au

Author for correspondence:

Professor Gordon S Howarth

School of Animal and Veterinary Sciences

University of Adelaide

Roseworthy Campus

South Australia

Australia 5371

Email: gordon.howarth@adelaide.edu.au

Tel: 8303 7885

Fax: 8303 7972

Grant support and financial disclosures:

This work was funded in-part by a grant from the Cancer Council South Australia and by the National Health and Medical Research Council of Australia. Professor Gordon Howarth is supported by the Sally Birch Cancer Council Australia Senior Research Fellowship in Cancer Control. None of the authors have any financial disclosures.

Abstract

Background: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract associated with altered composition of the gut microbiota. *Lactobacillus reuteri* BR11 (BR11) has recently been reported to reduce the severity of experimental IBD with its probiotic properties possibly attributed to a mechanism of thiol production via its unique cysteine/cystine transport system.

Aim: We compared BR11 and a BR11 mutant deficient in the cystine uptake system (PNG201), for their capacity to reduce the severity of experimental colitis.

Methods: Male Sprague Dawley rats (n=8 per group) were gavaged (1 ml/day) with skim milk, BR11 or PNG201 (1 x 10^9 CFU/ml) for 12 days. Rats consumed either water or 2% dextran sulfate sodium (DSS) in drinking water from days 6 to 12 to induce colitis. Metabolism data, disease activity index (DAI), intestinal mucin profile and histological analyses were assessed and compared by ANOVA.

Results: Assessed histologically, DSS administration resulted in significant colonic deterioration, including loss of crypt area and increased damage severity. BR11 administration only partially alleviated the DSS effects, with a minor improvement in crypt area ($p<0.05$). Administration of the PNG201 mutant strain to colitic animals failed to achieve significance ($p>0.05$) against the DSS control for any of the end-point parameters. However, the mutant strain induced a significantly greater ($p<0.05$) histological severity compared to BR11 treated colitic animals, representing a possible exacerbation of colitis.

Conclusions: The cystine uptake system only minimally influences the biological effects of BR11, as evidenced by histological and macroscopic colitic changes.

Keywords: colon; dextran sulfate sodium; inflammatory bowel disease; Lactobacillus reuteri; probiotic; ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) refers to an assemblage of chronic intestinal inflammatory disorders with an incidence of approximately 1 in 9-10000 [1]. The etiology of IBD is not fully understood. However, aside from a genetic predisposition, contributing factors include environmental, immunological and microbial influences [2]. The pathology of IBD is characterized by damage to the gastrointestinal epithelium and mucosa, resulting in ulceration, inflammation and hemorrhage.

Current pharmaceutical treatment options for IBD are relatively ineffective; consequently investigations into alternative treatment options have increased. Probiotics, strains of living bacteria that confer positive effects when administered in sufficient quantities [3], represent one such option. A wide array of probiotic strains, and combinations thereof, have been investigated with varied degrees of success to identify the most effective treatment options for IBD, and also to better understand their modes of action [4-6]. These bacterial strains are believed to exert their beneficence through a number of different mechanisms, most of which are not completely understood [7,8]. These mechanisms include competition with pathogens, modulation of the intestinal immune system, maintaining homeostasis of the intestinal epithelium, promoting crypt cell proliferation, inhibition of cell apoptosis and reduction of pro-inflammatory cytokine expression [4,7,9-12].

Lactobacillus reuteri BR11 (BR11) is a bacterial strain ((formerly *Lactobacillus fermentum* BR11) with unique antioxidant properties [13] that has shown promise in the treatment of experimental colitis [14]. BR11 efficacy is proposed to be mediated through its unique

cystine transport system [13,15,16]. This transport system is responsible for transporting exogenous cystine into the bacterial cell and expelling the thiol products, which diminish the effects of oxidative stress by detoxifying reactive oxygen species present [17]. Oxidative stress is one of the main factors influencing intestinal injury in IBD [18], with recent studies suggesting that probiotic administration can attenuate oxidative stress in rats [19]. The uptake system of *L. reuteri* BR11 comprises a high affinity cystine binding protein termed Cystine Uptake C (CyuC), a component of the ATP binding cassette cystine transport system [20]. In order to determine the role of this unique system, the *cyuC* gene was inactivated using homologous recombination to produce a *L. reuteri* mutant (PNG201) deficient in cystine uptake [13,21]. Utilizing a rat model of colitis induced by ingesting dextran sulfate sodium (DSS) it was hypothesized that elimination of the CyuC protein, which effectively inactivates the entire transport of cystine, would result in a decreased ability of the bacteria to prevent colonic injury.

Materials and Methods

Bacterial preparations

L. reuteri BR11, and the CyuC deficient strain PNG201, were both kind gifts from Professor Philip Giffard and Dr Mark Turner, Queensland University of Technology (Queensland, Australia). *L. reuteri* BR11, originally isolated from the vaginal tract of a guinea pig [22], and *L. reuteri* PNG201, a CyuC deficient mutant developed using homologous recombination [13], were grown on Mann Rogosa Sharp (MRS) agar and broths (Oxoid, Hampshire, England) with or without the antibiotic, erythromycin (10 µg/ml). As PNG201 was developed to be resistant to erythromycin, culturing the mutant in the presence of this antibiotic ensured the mutant had not reverted to wild-type, and allowed for an uncontaminated specimen. Both were incubated under anaerobic conditions and incubated at 37°C. The bacteria were grown to concentrations of approximately 1×10^9 colony forming units (CFU)/ml, dissolved in 10% skim milk powder and stored at -20°C until required.

Animal trial

The animal trial was performed in compliance with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and was approved by the Animal Care and Ethics Committee of the University of Adelaide. Male Sprague Dawley rats (n=48; 120-160 g) were housed individually in metabolism cages (Tecniplast, PA, USA) at a room temperature of 22°C with a 12 light:dark cycle. Throughout the duration of the trial the rats were provided *ad libitum* access to a standard 18% casein diet [23] and water. All animals were acclimatized for two days prior to experimentation. Metabolism parameters including

body weight, food and water intake, and fecal and urine output were monitored and measured daily.

Rats were randomly allocated to one of six treatment groups (n=8 rats/treatment); Water + Vehicle, Water + BR11, Water + PNG201, dextran sulfate sodium (DSS) + Vehicle, DSS + BR11 and DSS + PNG201. *L. reuteri* BR11 solution (1 ml) or vehicle (skim milk) was administered once daily via oral gavage from day 1 to 12. Between days 6 and 12, experimental colitis was induced via the addition of 2% DSS (ICN Biomedical, CA, USA) to the drinking water.

Disease severity was monitored daily, based on a disease activity index (DAI), scoring overall animal condition, weight loss, stool consistency and rectal bleeding on a scale from 0-3 for each parameter between days 7 and 12 (0 being healthy and 3 being severely affected) [24]. At day 12, all rats were sacrificed by carbon dioxide asphyxiation and cervical dislocation prior to dissection. Following sacrifice, the lengths of the gastrointestinal organs were measured un-stretched, and all internal organs were emptied of contents, if necessary, and weighed. Separate 2 cm segments of proximal and distal colon were removed and placed in 10% buffered formalin for histological analysis and 4 cm segments were snap-frozen in liquid nitrogen and stored at -80°C for biochemical analysis.

Histological analysis

Segments (2 cm) of the proximal and distal colon were routinely processed and embedded in paraffin wax. Sections (4 μm) were then cut and stained with hematoxylin and eosin. Severity

of intestinal damage was assessed using a quantitative analysis based on the parameters of surface enterocyte disruption, goblet cell numbers, crypt disruption, crypt abscess formation, polymorphonuclear cell infiltration, submucosal thickening and submuscularis externa thickening [25]. A score of 0 (unaffected) to 3 (severe damage) was recorded for each parameter to give an overall disease severity score, which was expressed as a median score (range). Both crypt loss and lengthening are common features of the DSS-colitis model [26]. Measurements of crypt depth in the proximal and distal colon were also determined, using 40 crypts per tissue section per rat to obtain a mean value [27]. Crypt area was calculated for the distal colon using three tissue sections per rat to obtain a mean value. All analyses were performed in a blinded fashion, using an Olympus BH-2 light microscope (Tokyo, Japan) with Sony digital camera (Tokyo, Japan) and Image Pro Plus Software Package Version 4.5.1.27 (Media Cybernetics, MD, USA).

Mucin characterization

The heterogeneous oligosaccharide chains of mucin glycoproteins provide potential binding sites for both commensal and pathogenic organisms, and may perform a defensive role as a protective barrier during periods of mucosal injury [28]. Derangement of this barrier has been linked to the pathophysiology of ulcerative colitis [29]. Colonic sections from each animal were stained with periodic acid-Schiff (PAS) [30]. Sections were subjected to mild acid hydrolysis to eliminate the contribution of sialic acid residues before PAS staining. Sections were immersed in periodic acid solution (Sigma, St. Louis, MO, USA) for 20 min, washed, and immersed in Schiff's Reagent (Sigma) for a further 20 min. Sections were rinsed in tap water for 10 min, dehydrated, and mounted in Entellan (ProSciTech, Kirwan, Queensland, Australia). Stained sections were examined by light microscopy with an Olympus CX41

microscope (Olympus, Tokyo, Japan) using a 20× objective and digital colour images [super high quality (2080 × 1544 pixels)] captured using an Olympus U-CAMD3 wide-zoom camera. Image analysis program analySIS® FIVE (Olympus Soft Imaging Solutions GmBH, Germany) was used to measure the parameters for each of the stained sections. The number of goblet cells per unit of epithelial area (mm^2) was calculated.

Statistical analysis

Statistical analysis was performed using the SPSS 15.0 software package (SPSS Inc. Chicago, IL, USA). Histological damage severity was expressed as a median value while all other data was expressed as a mean ± SEM. Comparisons of data between treatment groups was performed using a one-way ANOVA, with a Tukey's *post hoc* test, while DAI data was analyzed using repeated measure ANOVA with a Tukey's *post hoc* test. Qualitative analysis of histology data were analyzed using a Kruskal-Wallis test. For all analyses, $p<0.05$ was considered significant.

Results

Metabolism data

No significant difference ($p>0.05$) in weight gain was evident among the water or DSS treated groups, except for DSS + PNG201 in which the weight gain was significantly reduced ($p<0.05$) compared to healthy control rats (Table 1). Food and water consumption remained similar ($p>0.05$) across the groups over the course of the experimental period, with no influence of treatments. While there was no significant difference in urine or fecal output in the water-treated groups ($p>0.05$, Table 1), DSS consumption increased fecal production

($p<0.05$) for BR11 (29%) and PNG201 (25%) treated groups compared to healthy controls (water + skim milk). Urine output for all DSS treated animals decreased ($p<0.001$) remarkably (by ~40%) compared to water controls.

Disease activity index (DAI)

An increase in DAI is usually evident in the DSS-induced model of colitis [14]. On days 8, 10 and 11 DAI was significantly greater ($p<0.05$) in DSS + PNG201 compared to healthy controls. On day 12, DAI was significantly greater in DSS-treated rats receiving BR11 compared to healthy control rats, with DSS + PNG201 highly significant ($p<0.001$) compared to healthy controls (Figure 1).

Organ weights and lengths

Organ weights were expressed as a proportion of total body weight to correct for differences in body weight. There was no significant difference ($p>0.05$) observed in the fractional weights of any of the visceral organs (heart, lungs, left kidney, right kidney, liver, spleen and thymus) among the treatment groups as a result of BR11, PNG201 or DSS administration compared to normal controls (data not shown). Similarly, administration of neither bacterial strain nor DSS failed to impart any significant influence ($p>0.05$) on the lengths of the duodenum and small intestine. However, the length of the colon was significantly reduced ($p<0.05$) in DSS-treated rats receiving BR11 (18%) and PNG201 (20%) compared to healthy controls (Table 2). This was accompanied by a significantly greater (28%; $p<0.05$) weight of the colon in DSS-treated rats receiving PNG201 when compared to water drinking controls.

Nonetheless, weights of all other gastrointestinal organs (stomach, duodenum, small intestine and cecum) were not altered significantly in any of the treatment groups (Table 2).

Histological analysis

The damage severity score in the proximal and distal colon was significantly greater ($p<0.001$) following DSS consumption compared to the healthy controls (Table 3). Interestingly, the severity scoring for DSS + PNG201 was significantly greater ($p<0.05$) than DSS + BR11 in the distal colon. There was no significant ($p>0.05$) effect exerted by either bacterial strain (BR11: 215 ± 24 µm, PNG201: 198 ± 23 µm) or DSS consumption (252 ± 11 µm) on crypt depth compared to the water treated controls (210 ± 11 µm) in the distal colon. Crypt area in the distal colon was not affected by BR11 or PNG201 administration in healthy animals, however it was significantly ($p<0.05$) decreased in the DSS control and DSS + PNG201 compared to the water treated controls (Figure 2) such that there was no significant difference between the DSS + BR11 treatment group and healthy controls ($p>0.05$).

Mucin characterization

Numbers of neutral mucin containing goblet cells in the colon were not significantly influenced by bacterial administration or DSS consumption (Water + Vehicle: 5.32 ± 0.36 goblet cells ($x10^3$)/mm^2; Water + BR11: 4.74 ± 0.40; Water + PNG201: 4.96 ± 0.70; DSS + Vehicle: 4.82 ± 0.40; DSS + BR11: 5.17 ± 0.40; DSS + PNG201: 4.58 ± 0.43; $p>0.05$).

Discussion

While the novel antioxidant mechanism of L. reuteri BR11 has been examined extensively in vitro, the contribution of the cystine uptake transport system to probiotic activity has not been investigated in vivo. BR11 has been tested for therapeutic efficacy primarily using the DSS-colitis model. As such, we utilized this model to investigate the role of the cystine uptake system to better define its contribution to the effects conferred by BR11.

In the present study, DSS consumption resulted in moderate damage to the proximal and distal colon, observed through histological scoring, increased fecal production and a progressively increasing DAI score during the period of DSS consumption, including the presence of diarrhea, and rectal bleeding in later stages. Consumption of DSS is known to impart histological changes in the colon, including an elevated damage severity score, lengthening of crypts and crypt loss [26], often in conjunction with significant shortening of the colon [31]. Crypt lengthening and crypt loss are also commonly observed indicators of DSS-induced colitis [32], however, only crypt loss was observed in the current study. Lengthening of crypts is believed to be a compensatory mechanism to maximize absorptive function during periods of damage and repair, the absence of this feature in the current study may therefore have been related to the severity of DSS-induced damage or the timing of the measurement.

In the current study, administration of BR11 did not significantly alter any assessed parameters compared to the DSS-treated colitic control, whilst administration of PNG201 produced minimal changes compared to wild-type BR11, namely, an elevated intestinal

severity score in the distal colon. Based on this finding, we can speculate that the presence or absence of CyuC has minimal impact on the mode of action of BR11 in the current setting. It should be noted however that rats treated with PNG201 displayed increased colon weight, reduced body weight and reduced crypt area compared to healthy control rats, findings that were not reflected in the BR11 treated rats. This may provide some indication that the PNG201 treated rats were affected by DSS to a greater extent than their BR11 treated counterparts. Since the DSS-colitis control group failed to reach significance against the healthy controls for many of the measured parameters it could be surmised that the challenge provided by the DSS in the current trial was reflective of very low grade colitis. As such, the significance of the cystine uptake system to the possible probiotic properties of BR11 could not be determined with certainty. As the severity of colitis was relatively low, it was difficult to discern whether the bacterial strains acted as hypothesized, with BR11 partially normalizing crypt area, but failing to reduce overall damage severity in the colon.

As responses to DSS are dose-dependent [33], in future studies a higher dosage of DSS in the drinking water could induce colitis of greater severity to fully evaluate the role of the cystine uptake system. Previously, an appropriate disease severity was achieved by administering DSS at 2% to Sprague Dawley rats for seven days [14]. This DSS concentration was consistent with the current study; however, rats were exposed to DSS for only six days. Although some animals responded as expected, with progressively worsening diarrhea and anal bleeding, others displayed no overt clinical signs of disease for the duration of the animal trial; however, they proved to be affected after observing histological sections.

While BR11 in the current study was able to partially prevent crypt loss, there was no significant ameliorating effect exerted across any of the other parameters. To this end, both the BR11 wild-type and the PNG201 mutant failed to achieve statistical significance compared to the colitis control for all measured parameters. Probiotics, unlike conventional treatments, are living organisms, and thus their effects within the body can be variable. The reduced bioactivity of the BR11 wild-type in the current study was in contrast to the study conducted by Geier *et al*. [14], in which BR11 was found to reduce disease activity and histological severity, prevent colon shortening and was able to normalize weight gain and food intake, all of which were significantly affected in the DSS-treated controls. A possible reason for these differences may have been the concentration of bacteria administered to the animals. In the study by Geier *et al.* [14] the bacteria were grown to a concentration of 1-2×10^{10} CFU/ml, while in the current study bacterial concentrations were tenfold lower (1×10^{9} CFU/ml). In support of this hypothesis, animal studies using BR11 in a model of 5-FU induced intestinal mucositis have failed to achieve beneficial results using lower or similar BR11 concentrations. One study comparing three different bacterial strains using concentrations of 1×10^{6} CFU/ml failed to improve any of the assessed parameters, despite previous studies indicating their potential [34]. Another study investigating BR11 at concentrations of 1×10^{9} CFU/ml, also failed to achieve any significant beneficial results [35]. However, it should be noted that a higher DSS dose (3%) was also used by Geier *et al.* [35].

The lack of efficacy observed in other studies indicates that BR11 may only prove to be an effective probiotic at high (~1×10^{10} CFU/ml) concentrations, which would suggest the bacterial concentration levels in the current study may have been insufficient. Additionally, any decreased function in the PNG201 mutant would only be observed at higher concentrations. As such, future studies could benefit from the administration of higher

concentrations of BR11 and the PNG201 mutant strain. Although it was routinely cultured in the presence of erythromycin, the possibility still exists that the PNG201 mutant may have reverted back to the wild-type in the animals in the absence of erythromycin selection. In future studies, culturing the bacterial population in the feces, on MRS agar + erythromycin, could be utilized to monitor the stability and/or survival of the mutant.

In conclusion, in the current study, neither the wild-type BR11, nor a CyuC deficient strain of *L. reuteri* could prevent the development of experimental colitis in rats. However, rats administered the PNG201 BR11 mutant strain displayed some indications of a minor exacerbation of colitis compared to the wild-type strain. Nevertheless, the challenge provided by the DSS, and possibly the concentration of the bacterial treatments, may have been sub-optimal to provide conclusive evidence regarding the contribution of the cystine uptake system to the mechanism of action of BR11. Further studies utilizing BR11 are required to clarify the conflicting results observed in previous studies of BR11 in the contexts of colitis and mucositis to definitively classify BR11 as a probiotic.

Acknowledgements:

Dr Ashis Samanta and Kerry Lymn assisted in conducting the animal trials. Dr Roger Yazbek assisted in interpretation of results.

References

1 Hanauer SB: Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. *Inflammatory Bowel Diseases* 2006;12:S3-S9.

2 Amit-Romach E, Uni Z, Reifen R: Therapeutic potential of two probiotics in inflammatory bowel disease as observed in the trinitrobenzene sulfonic acid model of colitis. *Diseases of the Colon & Rectum* 2008;51:1828-1836.

3 Shanahan F: Probiotics in inflammatory bowel disease - therapeutic rationale and role. *Advanced Drug Delivery Reviews* 2004;56:809-818.

4 Boudeau J, Glasser AL, Julien S, Colombel JF, Darfeuille-Michaud A: Inhibitory effect of probiotic escherichia coli strain nissle 1917 on adhesion to and invasion of intestinal epithelial cells by adherent-invasive e-coli strains isolated from patients with crohn's disease. *Alimentary Pharmacology & Therapeutics* 2003;18:45-56.

5 Chapman TM, Plosker GL, Figgitt DP: Vsl#3 probiotic mixture - a review of its use in chronic inflammatory bowel diseases. *Drugs* 2006;66:1371-1387.

6 Peran L, Sierra S, Comalada M, Lara-Villoslada F, Bailon E, Nieto A, Concha A, Olivares M, Zarzuelo A, Xaus J, Galvez J: A comparative study of the preventative effects exerted by two probiotics, *Lactobacillus reuteri* and *Lactobacillus fermentum*, in the trinitrobenzenesulfonic acid model of rat colitis. *British Journal of Nutrition* 2007;97:96-103.

7 Seksik P, Dray X, Sokol H, Marteau P: Is there any place for alimentary probiotics, prebiotics or synbiotics, for patients with inflammatory bowel disease? *Molecular Nutrition & Food Research* 2008;52:906-912.

8 Vanderpool C, Yan F, Polk DB: Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory Bowel Diseases* 2008;14:1585-1596.

9 Geier MS, Butler RN, Howarth GS: Inflammatory bowel disease: Current insights into pathogenesis and new therapeutic options; probiotics, prebiotics and synbiotics. *International Journal of Food Microbiology* 2007;115:1-11.

10 Mahida YR, Rolfe VE: Host-bacterial interactions in inflammatory bowel disease. *Clinical Science* 2004;107:331-341.

11 Rioux KP, Madsen KL, Fedorak RN: The role of enteric microflora in inflammatory bowel disease: Human and animal studies with probiotics and prebiotics. *Gastroenterology Clinics of North America* 2005;34:465-+.

12 Amit-Romach E, Uni Z, Reifen R: Mulistep mechanism of probiotic bacterium, the effect on innate immune system. *Molecular Nutrition & Food Research* 2010;54:277-284.

13 Turner MS, Woodberry T, Hafner LM, Giffard PM: The *bspA* locus of *Lactobacillus fermentum* BR11 encodes an L-cystine uptake system. *Journal of Bacteriology* 1999;181:2192-2198.

14 Geier MS, Butler RN, Giffard PM, Howarth GS: *Lactobacillus fermentum* BR11, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulfate sodium (dss) in rats. *International Journal of Food Microbiology* 2007;114:267-274.

15 Lo R, Turner MS, Barry DG, Sreekumar R, Walsh TP, Giffard PM: Cystathionine gamma-lyase is a component of cystine-mediated oxidative defense in *Lactobacillus reuteri* BR11. *Journal of Bacteriology* 2009;191:1827-1837.

16 Turner MS, Timms P, Hafner LM, Giffard PM: Identification and characterization of a basic cell surface-located protein from *Lactobacillus fermentum* BR11. *Journal of Bacteriology* 1997;179:3310-3316.

17 Javed S, Howard A, Hirst B: Protection of intestinal cells against reactive oxygen species (ros) by amino acids. *Mutagenesis* 2009;24:38.

18 Damiani CR, Benetton CAF, Stoffel C, Bardini KC, Cardoso VH, Di Giunta G, Pinho RA, Dal-Pizzol F, Streck EL: Oxidative stress and metabolism in animal model of colitis induced by dextran sulfate sodium. *Journal of Gastroenterology and Hepatology* 2007;22:1846-1851.

19 Sengul N, Isik S, Aslim B, Ucar G, Demirbag AE: The effect of exopolysaccharide-producing probiotic strains on gut oxidative damage in experimental colitis. *Digestive Diseases and Sciences* 2011;56:707-714.

20 Hung J, Turner MS, Walsh T, Giffard PM: Bspa (CyuC) in *Lactobacillus reuteri* BR11 is a highly expressed high-affinity L-cystine-binding protein. *Current Microbiology* 2005;50:33-37.

21 Hung J, Cooper D, Turner MS, Walsh T, Giffard PM: Cystine uptake prevents production of hydrogen peroxide by *Lactobacillus fermentum* BR11. *FEMS Microbiology Letters* 2003;227:93-99.

22 Rush CM, Hafner LM, Timms P: Genetic-modification of a vaginal strain of lactobacillus fermentum and its maintenance within the reproductive-tract after intravaginal administration. *Journal of Medical Microbiology* 1994;41:272-278.

23 Tomas FM, Knowles SE, Owens PC, Read LC, Chandler CS, Gargosky SE, Ballard FJ: Effects of full-length and truncated insulin-like growth factor-i on nitrogen-balance and muscle protein-metabolism in nitrogen-restricted rats. *Journal of Endocrinology* 1991;128:97-105.

24 Howarth GS, Xian CJ, Read LC: Predisposition to colonic dysplasia is unaffected by continuous administration of insulin-like growth factor-i for twenty weeks in a rat model of chronic inflammatory bowel disease. *Growth Factors* 2000;18:119-133.

25 Howarth GS, Xian CJ, Read LC: Insulin-like growth factor-1 partially attenuates colonic damage in rats with experimental colitis induced by oral dextran sulphate sodium. *Scandinavian Journal of Gastroenterology* 1998;33:180-190.

26 Gaudio E, Taddei G, Vetuschi A, Sferra R, Frieri G, Ricciardi G, Caprilli R: Dextran sulfate sodium (dss) colitis in rats - clinical, structural, and ultrastructural aspects. *Digestive Diseases and Sciences* 1999;44:1458-1475.

27 Howarth GS, Francis GL, Cool JC, Xu X, Byard RW, Read LC: Milk growth factors enriched from cheese whey ameliorate intestinal damage by methotrexate when administered orally to rats. *Journal of Nutrition* 1996;126:2519-2530.

28 Longman RJ, Poulson R, Corfield AP, Warren BF, Wright NA, Thomas MG: Alterations in the composition of the supramucosal defense barrier in relation to disease severity of ulcerative colitis. *Journal of Histochemistry & Cytochemistry* 2006;54:1335-1348.

29 Corfield AP, Myerscough N, Bradfield N, Corfield CDA, Gough M, Clamp JR, Durdey P, Warren BF, Bartolo DCC, King KR, Williams JM: Colonic mucins in ulcerative colitis: Evidence for loss of sulfation. *Glycoconjugate Journal* 1996;13:809-822.

30 Forder REA, Howarth GS, Tivey DR, Hughes RJ: Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. *Poultry Science* 2007;86:2396-2403.

31 Yan YT, Kolachala V, Dalmasso G, Nguyen H, Laroui H, Sitaraman SV, Merlin D: Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. *Plos One* 2009;4

32 Vetuschi A, Latella G, Sferra R, Caprilli R, Gaudio E: Increased proliferation and apoptosis of colonic epithelial cells in dextran sulfate sodium-induced colitis in rats. *Digestive Diseases and Sciences* 2002;47:1447-1457.

33 Shimizu T, Suzuki M, Fujimura J, Hisada K, Yoshikazu O, Obinata K, Yamashiro Y: The relationship between the concentration of dextran sodium sulfate and the degree of induced experimental colitis in weanling rats. *Journal of Pediatric Gastroenterology and Nutrition* 2003;37:481-486.

34 Mauger CA, Butler RN, Geier MS, Tooley KL, Howarth GS: Probiotic effects on 5-fluorouracil-induced mucositis assessed by the sucrose breath test in rats. *Digestive Diseases and Sciences* 2007;52:612-619.

35 Geier MS, Butler RN, Giffard PM, Howarth GS: Prebiotic and synbiotic fructooligosaccharide administration fails to reduce the severity of experimental colitis in rats. *Diseases of the Colon & Rectum* 2007;50:1061-1069.

Table 1. Effect of *Lactobacillus reuteri* BR11 and its mutant PNG201 on metabolic attributes of rats following DSS administration

	Water+SM	Water+BR11	Water+PNG201	DSS+SM	DSS+BR11	DSS+PNG201
Weight gain (g)	49.1 ± 1.0	47.7 ± 1.6	47.9 ± 0.9	42.0 ± 1.3	40.6 ± 3.7	39.2 ± 2.5*
Food intake (g)	144.1 ± 3.4	143.3 ± 3.8	140.0 ± 1.9	130.4 ± 2.7	133.1 ± 4.3	130.6 ± 3.2
Water intake (ml)	267 ± 23	240 ± 18	253 ± 18	210 ± 15	230 ± 16	220 ± 18
Fecal output (g)	11.2 ± 0.3	11.6 ± 0.7	11.3 ± 0.6	13.6 ± 0.6	14.4 ± 0.8*	14.0 ± 0.9*
Urine output (ml)	129 ± 8	123 ± 9	129 ± 7	77 ± 3***	79 ± 4***	78 ± 2***

n=8 rats/treatment. Data are expressed as mean ± SEM. Skim milk (SM) as vehicle.

*Indicates p<0.05 compared to water+SM. ***Indicates p<0.001 compared to Water+SM.

Table 2. Effect of *Lactobacillus reuteri* BR11 and its mutant PNG201 on gastrointestinal organ lengths and weights of rats following DSS administration

	Water+SM	Water+BR11	Water+PNG201	DSS+SM	DSS+BR11	DSS+PNG201
Length (cm)						
Duodenum	7.8 ± 0.2	7.4 ± 0.1	7.3 ± 0.2	7.7 ± 0.3	7.7 ± 0.1	7.9 ± 0.4
Small Intestine	83.1 ± 2.7	81.4 ± 1.3	82.1 ± 1.6	82.5 ± 2.0	80.8 ± 1.8	83.4 ± 1.7
Colon	14.5 ± 0.6	14.3 ± 0.4	14.6 ± 0.4	12.8 ± 0.4	11.9 ± 0.4*	11.7 ± 0.4*
Weight						
Stomach	54.8 ± 1.0	58.4 ± 1.5	56.3 ± 2.0	59.5 ± 1.2	61.7 ± 3.1	58.2 ± 1.4
Duodenum	28.8 ± 1.2	28.5 ± 1.0	26.9 ± 1.4	29.8 ± 1.2	28.4 ± 1.2	28.3 ± 1.4
Small Intestine	220 ± 4	217 ± 5	216 ± 4	221 ± 3	230 ± 5	225 ± 2
Cecum	41.9 ± 2.6	44.6 ± 2.8	39.0 ± 1.8	38.2 ± 2.1	40.7 ± 2.3	42.9 ± 2.8
Colon	38.3 ± 1.2	39.2 ± 1.7	37.4 ± 1.8	45.6 ± 1.9	47.6 ± 2.8	49.0 ± 4.1*

n=8 rats/treatment. Length data are expressed as mean (cm) ± SEM. Weight data are expressed as mean ($x10^{-2}$% relative to body weight) ± SEM. Skim milk (SM) as vehicle. *Indicates $p<0.05$ significance to Water+SM.

Table 3. Effect of *Lactobacillus reuteri* BR11 and its mutant PNG201 on histological damage severity scores in the proximal colon of rats following DSS administration

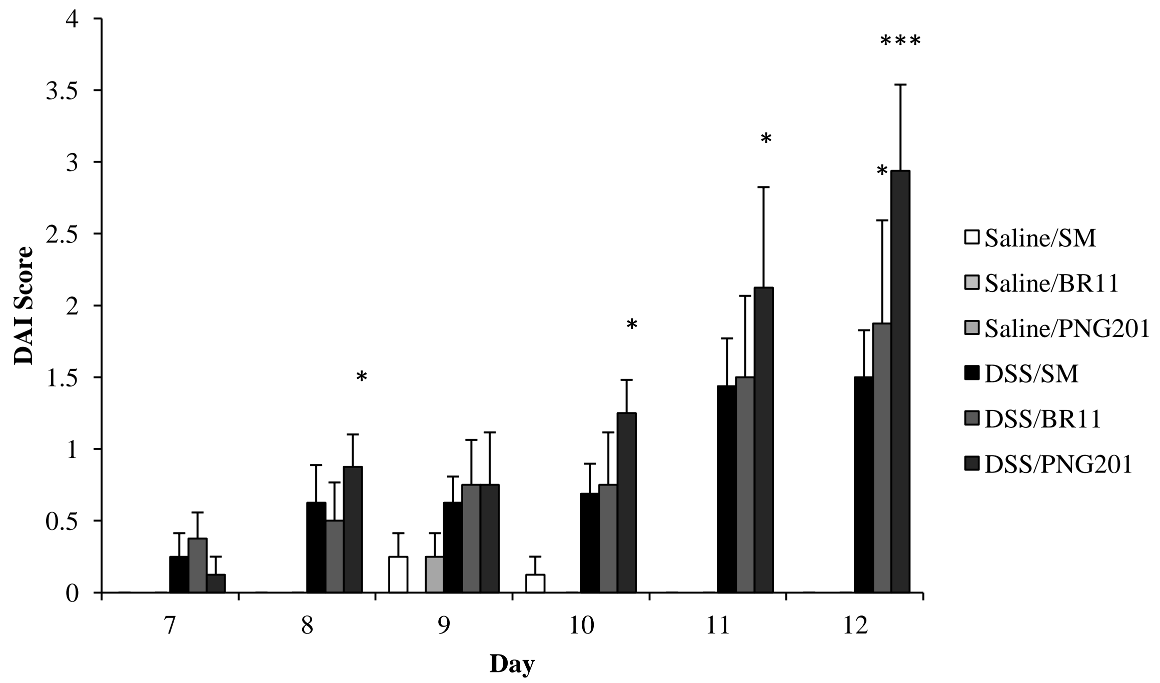
	Water+SM	Water+BR11	Water+PNG201	DSS+SM	DSS+BR11	DSS+PNG201
Proximal Colon	0 (0-0)	0 (0-1)	0 (0-0)	4.5 (2-6)***	3.5 (1-5)***	5 (2-6)***
Distal Colon	0.5 (0-3)	1 (0-2)	0.5 (0-2)	6 (4-8)***	5 (1-10)***	9 (4-12)***†

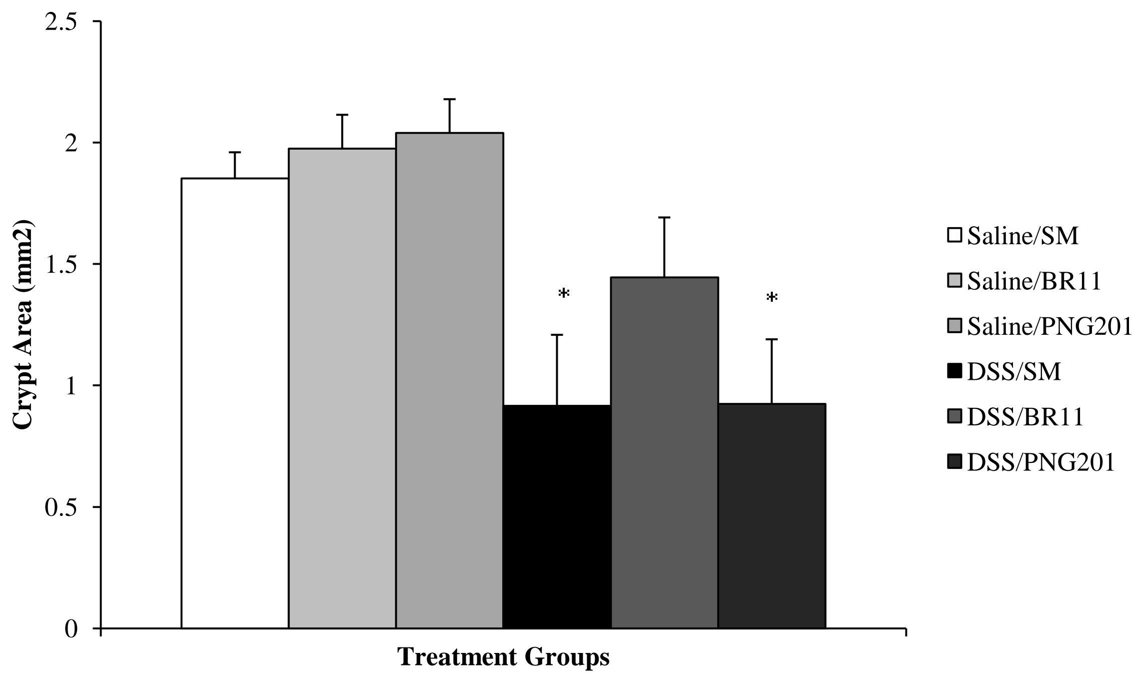
Data are expressed as median score with range in parentheses. Skim milk (SM) as vehicle. n=8 rats/treatment. ***p<0.001 compared to Water+SM (skim milk); †p<0.05 compared to DSS+BR11.

Figure Legends

Figure 1. Disease Activity Index (DAI) between days 7-12 during the development of DSS-induced colitis in rats (n=8 rats/treatment). Data are expressed as mean ± SEM. *p<0.05 and ***p<0.001 compared to water+Vehicle (skim milk).

Figure 2. Measurements of crypt area in distal colon in rats (n=8 rats/treatment). Data are expressed as mean (mm^2) ± SEM. *p<0.05 compared to water+ Vehicle (skim milk).


4
3.5
3
2.5
2
1.5
1
0.5
0
DAI Score
*
*
*
*
***
7
8
9
10
11
12
Day
Saline/SM
Saline/BR11
Saline/PNG201
DSS/SM
DSS/BR11
DSS/PNG201


2.5
2
1.5
1
0.5
0
Crypt Area (mm2)
*
*
Treatment Groups
Saline/SM
Saline/BR11
Saline/PNG201
DSS/SM
DSS/BR11
DSS/PNG201