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Original Article

Therapeutic effects of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 powder on dextran sulfate sodium-induced colitis in mice

Chien-Li Chen [a], *Pei-Yu Hsu* [b], *Tzu-Ming Pan* [a,b,*]

[a] *Department of Research and Development Division, SunWay Biotech Co., Ltd., Taipei, Taiwan*
[b] *Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei, Taiwan*

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ABSTRACT

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) whose exact cause is still unclear. Disruption of the intestinal microflora is considered one of the main causes of the disease. *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (NTU 101) is a multifunctional strain that has been shown in previous studies to possess anti-inflammatory properties and to exert a modulatory effect on intestinal bacteria associated with certain pathogenic mechanisms of IBD. In the current study, we investigated the effects of NTU 101 on dextran sulfate sodium (DSS)-induced colitis in a mouse model. Colitis was induced in male C57BL/6 mice (total number $n = 60$) via dissolved DSS in drinking water on days 15–21 of the experiment. The effects of continuous 25 d feeding (days 0–25) of either a half or a full dose [2.3×10^9 colony-forming units (CFU)/kg body weight (BW)/d and 4.5×10^9 CFU/kg BW/d, respectively] of NTU 101 was evaluated. *Lactobacillus rhamnosus* BCRC 16000 (BCRC 16000) and *L. paracasei* subsp. *paracasei* BCRC 14023 (BCRC 14023) strains were given to control groups. The results indicated that NTU 101 powder improved anti-oxidant capacity, reduced pro-inflammatory cytokine levels, increased anti-inflammatory cytokine levels, and slightly ameliorated body weight loss in DSS-treated mice during the final days of the study. This indicated that NTU 101 powder can relieve the clinical symptoms of DSS-induced colitis in mice.

1. Introduction

Inflammatory bowel disease (IBD) refers mainly to Crohn's disease (CD) and ulcerative colitis (UC), both intestinal inflammatory diseases of unknown etiology [1]. Although their mechanisms of pathogenicity and correlation are not yet fully understood [2], they are linked to various factors, including changes in the environment, genetic susceptibility, imbalances in the intestinal microflora, and immune system

* *Corresponding author*. Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan. Fax: +886 2 33663838.
E-mail address: tmpan@ntu.edu.tw (T.-M. Pan).

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disorders. Therefore, further study is required to fully understand IBD and achieve a clinical cure [3].

At present, treatment of UC focuses on steroid-free clinical remission, the postponement of hospitalization and surgery, mucosal repair, improving quality of life, and avoiding disability. Mesalazine, containing the active ingredient 5-aminosalicylic acid (5-ASA), is usually used as a first-line treatment for patients with mild to moderate UC [4], achieving an approximately 50% remission rate [5]. For patients not responding to mesalazine, treatment with corticosteroids, immunosuppressive agents (such as azathioprine, 6-mercaptopurine, cyclosporin A, and methotrexate) [6], and various biologics [7] are considered. Most of the common clinical therapeutic drugs used can cause side effects or discomfort. Alternatively, the intake of probiotic-related products affects the intestinal microflora and has shown to be associated with human health [8].

One of the causes of IBD is a change in the composition of the symbiotic microflora in the intestinal tract. This stimulates activation of the immune system and promotes inflammation of the intestinal mucosa [9]. The concept of using probiotics to maintain consistent intestinal microflora and regulate the immune system has therefore been suggested as a possible intervention for IBD [10]. It has been shown that patients with UC taking certain probiotics are able to achieve clinical remission comparable to 5-ASA treatment [11], and that the synergy of probiotics and 5-ASA in patients with mild to moderate UC promotes clinical remission [12]. These prior clinical trials with patients with UC indicated that certain lactic acid bacteria may reduce the inflammatory response and alleviate clinical symptoms of the disease, suggesting their potential as an adjuvant therapy in clinical settings.

Lactobacillus paracasei subsp. *paracasei* NTU 101 (NTU 101) has recently been isolated from neonatal feces in our laboratory [13]. It is a multifunctional strain that tolerates gastric acid pH and bile salts and can affect the intestinal microflora [14]. In animal models, dextran sulfate sodium (DSS)-induced colitis is accompanied by pathological changes that mirror UC [15]. These clinical changes include disruption of epithelial integrity in the colon, increased intestinal permeability, continued chronic inflammation, and intestinal bleeding. In the current study, we investigated the pathogenesis of DSS-induced colitis in C57BL/6 mice, and evaluated the effects and UC-preventive potential of three probiotic preparations, NTU 101, *Lactobacillus rhamnosus* BCRC 16000 (BCRC 16000), and *L. paracasei* subsp. *paracasei* BCRC 14023 (BCRC 14023).

2. Methods

2.1. *Preparation of probiotics*

Three strains were used in the current study, NTU 101 (lyophilized powdered, Vigilis101; probiotic powder from SunWay Biotech Co., Ltd., Taipei, Taiwan), *L. rhamnosus* BCRC 16000, and *L. paracasei* subsp. *paracasei* BCRC 14023 obtained from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan).

2.2. *Design of animal experiments*

Six-week old C57BL/6 male mice were used in this study and were purchased from BioLASCO Taiwan Co., Ltd. (Ilan, Taiwan). Animals were maintained in 12 cages (5 animals/cage), in an environment with a relative humidity of 50–60%, temperature of 25 ± 2 °C, and a light/dark cycle of 12 h (illumination between 0700 and 1900 h). All animals received humane care according to guidelines laid out by the Institutional Animal Care and Use Committee of National Taiwan University (Taiwan, ROC). To assess the effect of probiotics on DSS-induced colitis in a mouse model, animals were divided into experimental six groups. These consisted of a control group (control; $n = 10$), a DSS-induced colitis group with no probiotic treatment (DSS), a DSS-induced colitis group with BCRC 16000 treatment group, a DSS-induced colitis group with BCRC 14023 treatment group, a DSS-induced colitis group with NTU 101 group administered half a dose (NTU 101 0.5 ×), and a NTU 101 group administered a full dose (NTU 101 1.0 ×). The experiment was performed over 25 d. Mice in each group were fed different doses and strains daily. Colitis was induced by DSS added to drinking water on days 15–21. The disease activity index (DAI) was recorded from day 15 onwards. Animals were sacrificed by CO_2 asphyxiation on day 25.

2.3. Determination of DAI and fecal *Bifidobacterium* spp. content

Following the induction of colitis, mouse body mass was determined and recorded every day, and the presence of occult blood in mouse feces and fecal hardness were assessed. The scores for these three factors were used to calculate the daily DAI, according to the disease activity scoring system proposed by Wirtz et al. for the evaluation of colitis in animal models [15]. Fresh feces were collected in centrifuge tubes and were immediately filled with CO_2 to maintain anaerobic conditions. To 1 g of feces, 9 mL saline of anaerobic dilution and several glass beads (0.8 mm in diameter) were added in the centrifuge tube and mixed. Selective medium BIM-25 (*Bifidobacterium* iodoacetate medium 25) was used to culture *Bifidobacterium* spp., under anaerobic conditions at 37 °C, for 72 h.

2.4. *Intestinal tissue homogenization*

The intestines were collected from experimental animals immediately after culling, washed with phosphate-buffered saline (PBS), sliced into three 4–5 mm fragments, placed into microcentrifuge tubes, and kept on ice. Portions of the intestinal tissue were then transferred to microcentrifuge tubes for homogenization (BioMasher II, Laboratory & Medical Supplies Co. Ltd., Tokyo, Japan), and placed on ice after the addition of 580 μL of PBS. Homogenization was performed for approximately 10 min using a homogenizer and a battery-powered motor (Power Masher II, Nippi. Inc., Tokyo, Japan). The samples were then centrifuged at 100,000 × *g* for 10 min at 4 °C. The supernatant was removed and stored at −80 °C for later use.

2.5. *Detection of antioxidative enzymes in the colonic tissue*

The total antioxidative capacity of colonic tissue was measured using a commercially available antioxidant assay kit. Glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities were determined using specific assay kits. Malondialdehyde (MDA) was detected using the commercial thiobarbituric acid reactive substance (TBARS) assay kit. All assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and determinations were performed following the manufacturer's instruction. To calculate the antioxidative capacity, GSH, and MDA contents, and GPx, GR, CAT, and SOD activities, the absorption of each sample from the final processing step was compared to the respective standard curve and the value divided by the total protein concentration of the assayed homogenate.

2.6. *Quantification of biochemical markers associated with the inflammatory response in the colonic tissue*

Interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12 (p70), and IL-10 were each measured in colonic tissue using commercially available Murine ELISA MAX™ Standard Sets (Biolegend, San Diego, CA, USA), following the manufacturer's instructions. The nitric oxide (NO) content was determined using a commercial nitrate/nitrite colorimetric assay kit (Cayman Chemical Company), according to the manufacturer's recommendations.

2.7. *Histological examination*

To evaluate histological damage of DSS-induced colitis severity, cut a small fragment of the colon, place in a tissue cassette and submerge in buffered 10% neutral formalin solution, alcohol-dehydrated, paraffin-embedded, and sectioned to a mean thickness of 4–5 μm. Colon fragments can be taken from the proximal, mid-colon, or distal section of the colon. The histological examination by the above conventional methods was evaluated for the index of DSS-induced colitis by assessing the morphological changes with hematoxylin and eosin (H&E) stain.

2.8. *Statistical analysis*

Data are expressed as means ± standard deviation (SD). The statistical significance of differences between samples was determined by one-way analysis of variance (ANOVA) using a general linear model in SPSS version 10.0 software (SPSS Institute, Inc., Chicago, IL, USA), followed by a one-way ANOVA with a Newman–Keuls post-hoc test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. *Effects of probiotic intake on the body weight and DAI of DSS-treated mice*

Animal weights were recorded every day at a specific time, to enable the evaluation of daily weight change and the change of body weight after disease induction on day 15 of the experiment. Before the induction of colitis (days 1–14), the weights of animals in all groups were similar (Fig. 1a). After induction of colitis, no obvious changes were initially observed in animal body weight until day 18, when the weights in the DSS group began to decline. The weights of animals in other groups were relatively constant at this point. A significant change in body weight ($p < 0.05$) was measured on days 20 and 21 in the NTU 101 1.0 × , BCRC 16000, BCRC 14023, and DSS group animals (Fig. 1b). After the induction of colitis, the change in the body weight of each mouse, fecal hardness, and fecal occult blood were assessed every day to evaluate the DAI, as shown in Fig. 1c. The DSS group began to show significantly higher DAI values compared to the control group ($p < 0.05$). The DAI values of the remaining NTU 101,

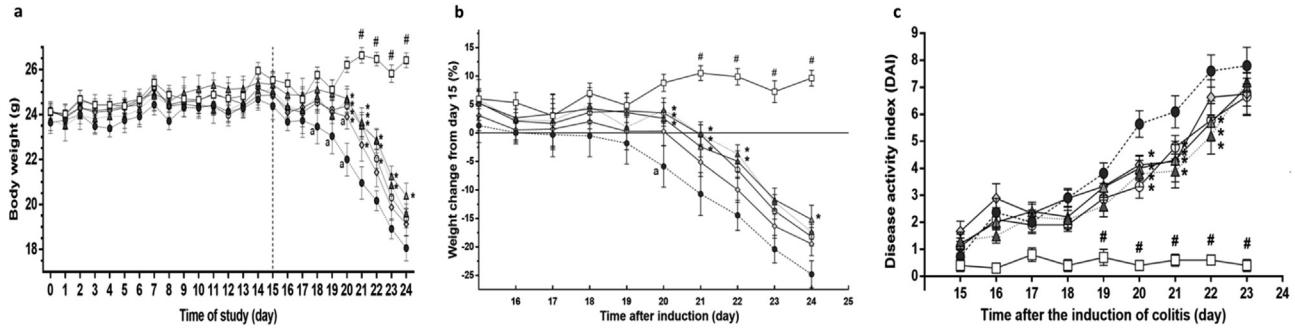

Fig. 1 – The effect of probiotics on weight changes after dextran sulfate sodium-induced colitis in mice. (a) Weight change of mice during study. (b) Percentage change in body weight of mice after induction of colitis. (c) Daily disease activity index (DAI) of mice after induction of colitis. Values are means ± SEMs (n = 10). Statistical analysis was performed by two-way ANOVA with a Newman–Keuls post-hoc test. [a] $p < 0.05$, compared with control group; [#] $p < 0.05$, control group compared with other groups; [*] $p < 0.05$, probiotic groups compared with DSS group. NTU 101 (0.5 ×) and (1.0 ×): 2.3 × 10^9 and 4.6 × 10^9 CFU of NTU 101/kg BW of mice/day. –□–: control group; –●–: DSS group; –▲–: *L. rhamnosus* BCRC 16000 group; –△–: *Lactobacillus paracasei* subsp. *paracasei* BCRC 14023 group; –◇–: NTU 101 (0.5 ×) group; –○–: NTU 101 (1.0 ×) group. DSS: dextran sulfate sodium; NTU 101: *Lactobacillus paracasei* subsp. *paracasei* NTU 101.

BCRC 16000, and BCRC 14023 groups were significantly lower ($p < 0.05$) than in the DSS group on day 20.

3.2. Effects of probiotic intake on colon length, spleen weight, and the intestinal microflora of DSS-treated mice

During continued colonic inflammation, the length of the mouse colon decreases due to ulceration. This is accompanied by colon tissue adhesion [16]. The colon lengths for each group of mice are shown in Fig. 2a. In the DSS group, colon length was significantly shorter than in controls ($p < 0.05$). Mice in groups fed probiotics showed a significant recovery in colon length in comparison with the DSS group ($p < 0.05$), despite colon shortening and tissue adhesion due to inflammation and ulceration. Systemic inflammatory responses, as assessed by spleen enlargement, are shown in Fig. 2b. The spleen weights of mice in the DSS group were also significantly higher than in the control group ($p < 0.05$). In groups fed probiotics, mean spleen weight was lower than the DSS group animals ($p < 0.05$) and was more similar to control group animals.

As shown in Fig. 2c, the bacterial counts of *Bifidobacterium* spp. were similar in the DSS and control groups. The bacterial counts in the NTU 101 1.0 × , BCRC 16000, and BCRC 14023 groups were significantly higher than the control and DSS groups ($p < 0.05$), while the bacterial count did not significantly change in the NTU 101 0.5 × group.

3.3. Effects of probiotic intake on the total antioxidative capacity and GSH redox cycle of the colonic tissues in DSS-treated mice

In addition to the induction of colitis, the proinflammatory agent DSS also causes excessive production of oxidative metabolites at the sites of inflammation [17]. As shown in Fig. 3a, the total antioxidative capacity of the colonic tissues of the DSS group was significantly lower than in the control group ($p < 0.05$). Conversely, the antioxidative capacities of the probiotic groups were significantly higher than in the DSS group ($p < 0.05$), with higher values in the NTU 101 1.0 × group animals than in the BCRC 16000 and BCRC 14023 groups. In addition to total antioxidative capacity, antioxidative enzymes and antioxidation also play key roles in offsetting oxidative stress in an organism [18]. One example is the GSH redox cycle that includes GPx, GR, and the non-enzymatic compound, GSH. As shown in Fig. 3b–d, GPx activity in the DSS group was significantly lower than in the control group ($p < 0.05$). In all animals taking a full dose of probiotics, GPx activity was significantly higher than in the DSS group ($p < 0.05$). In the NTU 101 0.5 × group, the increase was not statistically significant. The GR activity in the DSS group animals was also significantly lower than in the control group ($p < 0.05$) and was significantly enhanced in the probiotic-fed groups, with the NTU 101 1.0 × animals showing greater activity than the BCRC 16000 and BCRC 14023 group animals. The GSH levels in the DSS group were significantly lower than in the control group ($p < 0.05$). In the probiotic-fed groups, the GSH levels were higher than in the DSS group, with the highest levels found in the NTU 101 1.0 × group ($p < 0.05$). Based on these data, it is apparent that during the DSS-induced colitis, feeding animals a full dose of NTU 101 enhances total antioxidative capacity and enzyme activity in colonic tissues.

3.4. Effects of probiotic intake on CAT and SOD activities, and MDA content in the colonic tissues of DSS-treated mice

In addition to the GSH redox cycle, organisms possess several other antioxidant enzyme systems, such as CAT and SOD, that decompose reactive oxygen metabolites and minimize the damage to body cells and tissues [18]. CAT activity determinations for each group are shown in Fig. 4a. This shows that CAT activity in the DSS group animals was lower than in the control group but the difference was not significant. The animals in groups that were fed probiotics showed increased CAT activity, but the difference was only significant in NTU 101 the 1.0 × versus DSS group comparison ($p < 0.05$). SOD activity measurements are shown in Fig. 4b. SOD activity in the DSS group was significantly lower than in the control

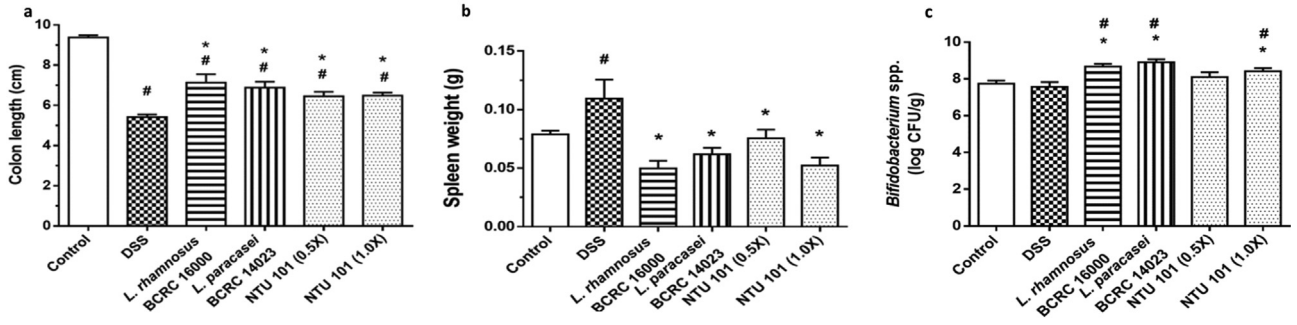

Fig. 2 – The effect of probiotics on colon length, spleen weight, and bacterial counts after dextran sulfate sodium-induced colitis in mice. Shown are the mean (a) colon lengths, (b) spleen weights, and (c) cecum bacterial counts for groups with or without probiotic treatment after induction of colitis. Values are means ± SEMs (n = 10). Statistical analysis was performed by one-way ANOVA with a Newman–Keuls post-hoc test. $^{\#}p < 0.05$, compared with control group; $^{*}p < 0.05$, probiotic groups compared with DSS group. NTU 101 (0.5 ×) and (1.0 ×): 2.3 × 10^9 and 4.6 × 10^9 CFU of NTU 101/kg BW of mouse/day, respectively. DSS: dextran sulfate sodium; *L. rhamnosus* BCRC 16000: *L. rhamnosus* BCRC 16000; *L. paracasei* BCRC 14023: *Lactobacillus paracasei* subsp. *paracasei* BCRC 14023; NTU 101: *Lactobacillus paracasei* subsp. *paracasei* NTU 101.

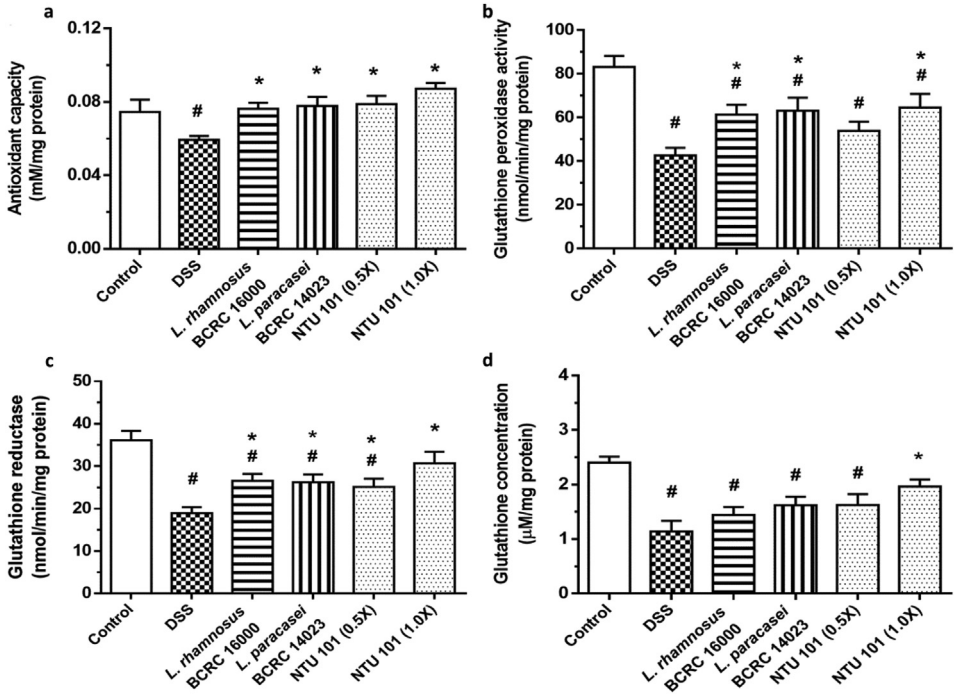

Fig. 3 – The effects of probiotics on antioxidant capacity and the GSH redox cycle after dextran sulfate sodium-induced colitis in mice. (a) The total antioxidant capacity of mice after induction of colitis. (b) The activities of glutathione peroxidase (GPx), and (c) glutathione reductase (GR) in colonic tissue after induction of colitis. (d) Concentration of glutathione in colonic tissue. Abbreviations for each group are shown in Fig. 2. Values are means ± SEMs (n = 10). Statistical analysis was performed by one-way ANOVA with a Newman–Keuls post-hoc test. $^{\#}p < 0.05$, compared with control group; $^{*}p < 0.05$, probiotic groups compared with DSS group.

group ($p < 0.05$), although the SOD activities in the probiotic groups were significantly higher than in the DSS group ($p < 0.05$).

Excess accumulation of reactive oxygen species *in vivo* leads to oxidative damage. For example, MDA is formed after oxidation of unsaturated fatty acids as compounds containing free radicals are released and attack the cell membrane or other lipids (Fig. 4c). After the induction of colitis by DSS, the colonic tissues of the DSS group contained significantly higher amounts of the lipid peroxidation product MDA than the control group animals ($p < 0.05$). The amount of MDA in groups fed probiotics was significantly lower than in the DSS group ($p < 0.05$), returning to almost the same level as that found in the control group animals.

3.5. *Effects of probiotics on changes to cytokine and NO levels that associate with the inflammatory response of distal colonic tissue in DSS-treated mice*

DSS causes colon ulceration and increases intestinal permeability, which leads to inflammation [16]. TNF-α levels were significantly higher in the DSS group than in the control group

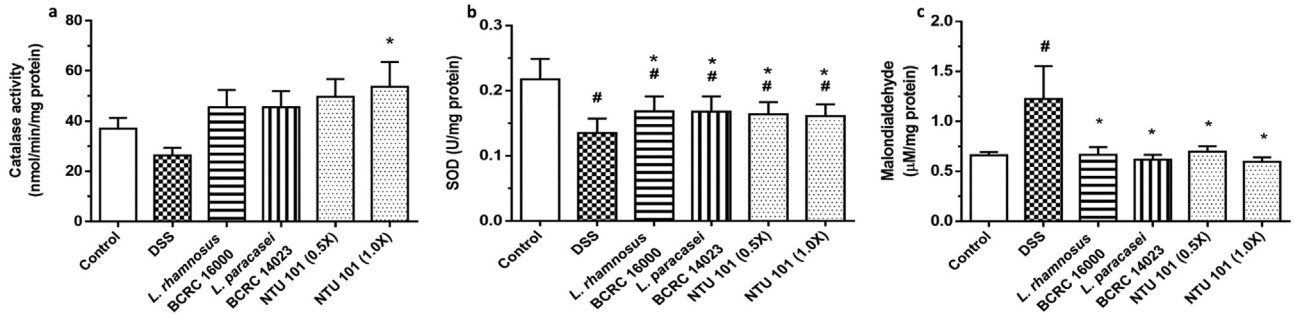

Fig. 4 – The effect of probiotics on antioxidative enzymes of dextran sulfate sodium-induced colitis in mice. (a) The activities of catalase (CAT), (b) superoxide dismutase (SOD), and (c) malondialdehyde (MDA) in colonic tissue after induction of colitis. Abbreviations for each group are shown in Fig. 2. Values are means ± SEMs (n = 10). Statistical analysis was performed by one-way ANOVA with a Newman–Keuls post-hoc test. $^{\#}p < 0.05$, compared with control group; $^{*}p < 0.05$, probiotic groups compared with DSS group.

($p < 0.05$), reflecting elevated inflammation and a greater immune response by the host. Secretion of TNF-α in the four animal groups that were fed probiotics was significantly reduced in comparison with the DSS group ($p < 0.05$) (Fig. 5a). Compared to the control group, IL-6 levels were significantly increased in the DSS group ($p < 0.05$). IL-6 secretion was significantly lower in all groups fed probiotics relative to the DSS group ($p < 0.05$), with the exception of the NTU 101 0.5 × group where the decrease was not significant (Fig. 5b).

In patients with IBD and experimental animals, elevated IFN-γ levels are linked to activation of inflammatory cells, vascular structure disorder in inflamed tissue, vascular hyperpermeability, and epithelial cell apoptosis [19]. Fig. 5c demonstrates DSS-induced colonic inflammation and immune responses in the employed animal model. In the DSS group, IFN-γ secretion was significantly higher than in control animals ($p < 0.05$). In the four groups fed probiotics, IFN-γ levels were significantly lower than the DSS group ($p < 0.05$).

An increase in IL-12 (p70) can lead to an increase in other proinflammatory cytokine levels [20]. In the DSS group, IL-12 (p70) secretion was significantly higher than in the control group ($p < 0.05$). In all of the animals fed probiotics, IL-12 (p70) was significantly lower than in the DSS group ($p < 0.05$), with the exception of the NTU 101 0.5 × group where the decrease was not significant (Fig. 5d).

As shown in Fig. 5e, IL-10 secretion in the DSS group was significantly lower than in the control ($p < 0.05$). With the exception of the BCRC 14023 group animals, where no significant increase in IL-10 levels was noted, IL-10 secretion in all other groups that were fed probiotics was significantly higher than in the DSS group ($p < 0.05$). Finally, inflammation of DSS-induced colonic ulcers often leads to the secretion of large quantities of NO, an excess of which leads to free radical formation and causes oxidative damage by oxidizing proteins in other tissues [16]. As shown in Fig. 5f, the DSS group animals secreted significantly more NO than control animals ($p < 0.05$). This indicated a more effective attenuation of NO secretion in the distal colon during DSS-induced inflammation by a full dose of probiotics than by the half dose, minimizing free radical formation and oxidative damage.

3.6. *Histopathologic alterations in inflamed colonic tissues in DSS-induced colitis mice*

Damage to the colonic mucosa is an integral feature of the DSS model, reflected by increased histological damage severity scores, crypt hyperplasia and decreased crypt area. The Fig. 6 showed overall severity following NTU 101, BCRC 16000, and BCRC 14023 treatment was significantly lower than in DSS-treated mice.

4. Discussion

In 2007, Wirtz et al. reported that certain colitis symptoms could be stimulated in C57BL/6 mice fed DSS-supplemented drinking water for seven consecutive days, without causing severe colitis [15]. In the current study, the weights of all DSS induced groups were decreased, supporting this hypothesis. Weight loss and changes in daily DAI were alleviated by feeding

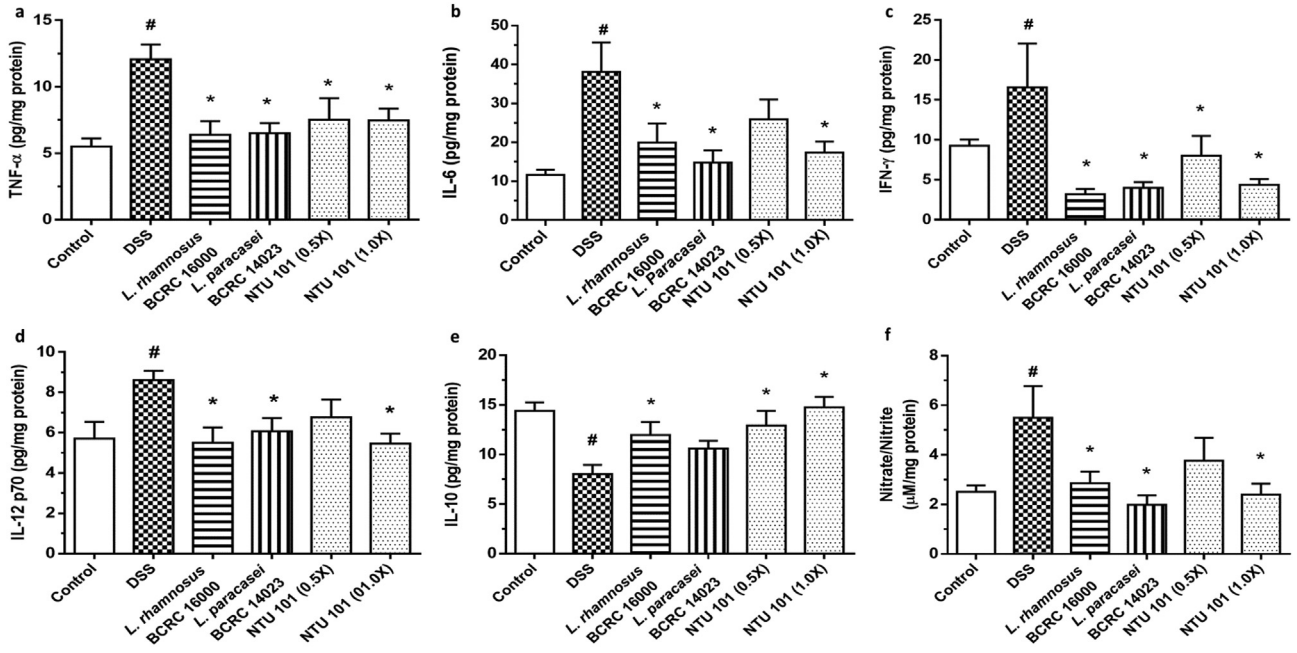

Fig. 5 – The effects of probiotics on pro-inflammatory cytokines after dextran sulfate sodium-induced colitis in mice. (a) The levels of TNF-α and (b) immunoregulatory IL-6, (c) IFN-γ, and (d) IL-12 (p70) in colonic tissue. (e) Levels of the anti-inflammatory cytokine IL-10 in colonic tissue. (f) Levels of nitric oxide (NO) in colonic tissue. Abbreviations for each group are shown in Fig. 2. Values are means ± SEMs (n = 10). Statistical analysis was performed by one-way ANOVA with a Newman–Keuls post-hoc test. #p < 0.05, compared with control group; *p < 0.05, probiotic groups compared with DSS group.

the animals NTU 101 powder during the induction stage, which indicated an attenuation of clinical UC symptoms. Moreover, a full dose of NTU 101 powder was more effective than half a dose, and feeding mice other probiotic powders (BCRC 16000 and BCRC 14023) had a comparable effect to NTU 101 1.0 × .

In terms of the intestinal microflora, the bacterial count of *Bifidobacterium* spp. did not decrease in the ceca of the DSS group animals, which might be because the colitis induced by DSS largely localizes to the distal colon and does not significantly impact the cecal microflora [21]. Feeding mice NTU 101 1.0 × , BCRC 16000, and BCRC 14023 associated with increased *Bifidobacterium* spp. counts in the cecum. This supports the observation that NTU 101 stimulates the amount of *Bifidobacterium* spp. reported in a previous study [14]. Since *Bifidobacterium* spp. in the intestinal tract also aids regulation of immune function [22], we speculate that the NTU 101 1.0 × , BCRC 16000, and BCRC 14023 doses used in the current study could regulate the immune system of the host by promoting the growth of other beneficial bacteria.

On the other hand, in patients with IBD, reduced GSH synthesis has been demonstrated [23]. Oxidative stress can cause DNA damage, leading to lipid peroxidation and protein carbonylation, resulting in epithelial cell injury and intestinal barrier dysfunction [24]. This in turn leads to increased intestinal permeability and mucosal ulceration. As such, oxidative stress may aggravate mucosal inflammation.

In the current study, antioxidant enzymes and compounds were evaluated in mice as an indicator of oxidative stress. Feeding mice a half dose of NTU 101 increased GR activity in the GSH redox system but failed to significantly elevate GSH levels and GPx activity. Conversely, NTU 101 1.0 × increased the activity of GPx and GSH levels. As shown in the current study, the NTU 101 1.0 × dosage effectively improved the scavenging of free radicals by the GSH redox system when compared to the DSS group. Feeding mice BCRC 16000 and BCRC 14023 did not increase the GSH levels. This difference supports the observation that various probiotic strains have differing antioxidative capacities [25]. However, the increase

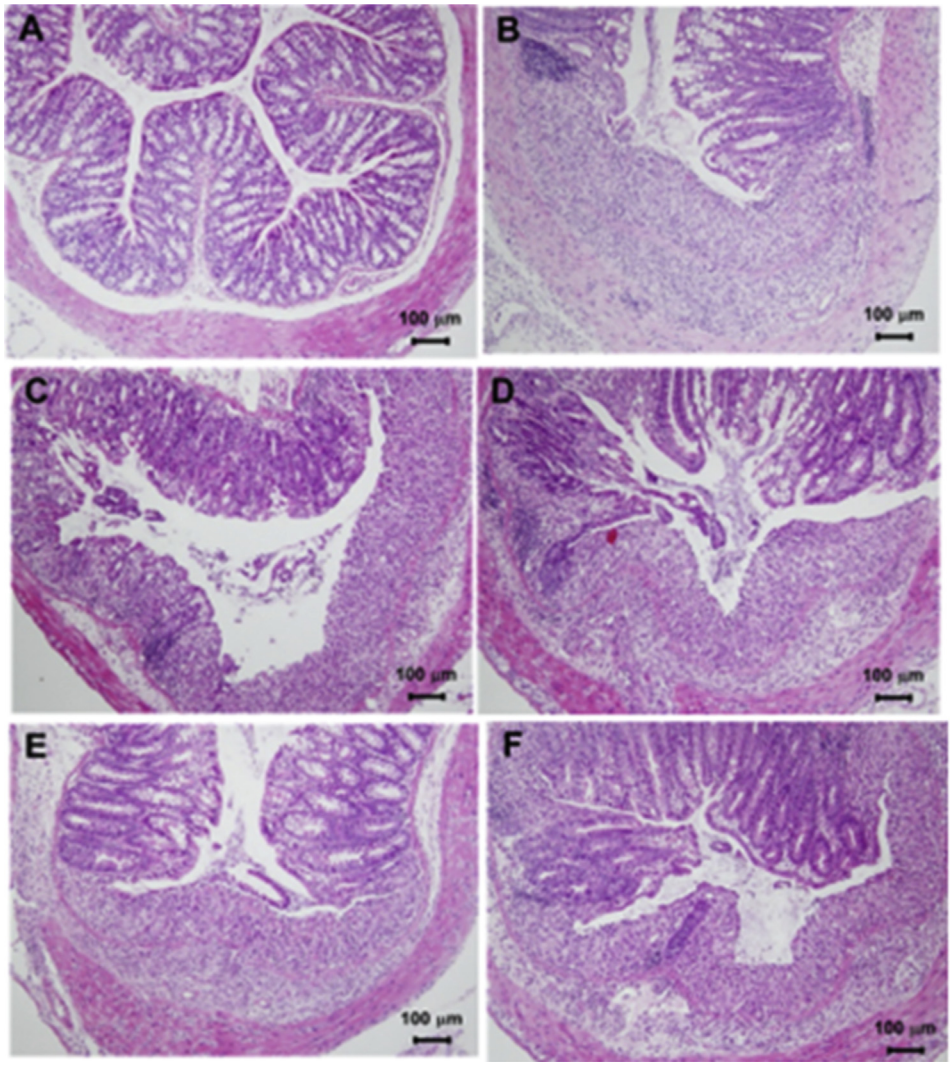

Fig. 6 – Histopathologic alterations in inflamed colonic tissues in DSS-induced colitis mice. A: control group; B: DSS-induced colitis group; C: *L. rhamnosus* BCRC 16000 group; D: *Lactobacillus paracasei* subsp. *paracasei* BCRC 14023; E: NTU 101 (0.5 ×); F: NTU 101 (1.0 ×). H&E stain scale: 100 μm. NTU 101: *Lactobacillus paracasei* subsp. *paracasei* NTU 101. Values are means ± SEMs (n = 10).

in GSH levels by the probiotic strain could simply be a result of the promotion of GSH synthesis [26]. NTU 101 feeding increased SOD activity and reduced the accumulation of the lipid peroxidation product MDA to neutralize the injury of the intestinal mucosa caused by oxidative stress. This has also been suggested by previous studies [27]. These results indicate that, in addition to enhancing SOD activity, NTU 101 feeding also stimulates the activity of the redox enzymes CAT and GSH, improving the scavenging efficiency of free radicals and thereby inhibiting the promotion of inflammatory response by oxidative stress.

NTU 101 1.0 × , BCRC 16000, and BCRC 14023 also reduced the secretion of proinflammatory cytokines TNF-α, IL-6, and IFN-γ. The acute stage of DSS-induced colitis involves an immune response dominated by Th1 cells. A key defining feature of Th1 and antigen-presenting cells is that both secrete IFN-γ. It has previously been shown that blocking IFN-γ using antibodies can reduce the occurrence of colitis in mice [28]. Feeding mice NTU 101 1.0 × , BCRC 16000, and BCRC 14023 reduced the secretion of IFN-γ. A similar effect was observed in the NTU 101 0.5 × group, pointing to an effective inhibition of IFN-γ by probiotic intake. Increased secretion of IL-6 by macrophages and $CD4^+$ T cells has also been observed in patients with IBD and animals with colitis [29]. IL-6 enhances the activation of T cells and other antigen-presenting cells to promote the inflammatory response [7].

Higher amounts of TNF-α are secreted by patients with IBD than by healthy individuals [7], especially by macrophages, adipocytes, fibroblasts, and lymphocytes [29]. Besides the activation of nuclear factor (NF)-κB and triggering of the proinflammatory response, the binding of TNF-α to its receptor also leads to cell apoptosis, enhanced angiogenesis, intestinal Paneth cell necrosis, release of matrix metalloproteinases, immune cell activation, and epithelial cell injury. Infliximab, an anti-TNF-α antibody commonly used in a clinical setting, can effectively alleviate UC in patients. As shown in the current study, NTU 101, BCRC 16000, and BCRC 14023 effectively reduced the secretion of TNF-α in colonic tissue.

The use of antibodies to block IL-12 in animal experiments, and the subsequent suppression of colitis symptoms in mice, has previously been reported [30]. We demonstrated an effective inhibition of IL-12 secretion by a full dose of each of the probiotic strains tested, suggesting an effect on T cell differentiation and a reduction in IFN-γ secretion. Dysregulation of proinflammatory cytokines expression in IBD could contribute to the inflammatory response [31]. In the present work, we observed that application of the BCRC 14023, BCRC16000 and NTU 101 correlated with a significant down-regulation cytokines expression in the DSS-induced colitis model compared to the DSS-treated group. This specific down-regulation of toll like receptor by treatment with the BCRC 14023, BCRC16000 and NTU 101 could explain the lower expression of the proinflammatory cytokines IL-12 and IFN-γ [32]. The lower expression of IL-12 suggests that probiotic induces fewer proinflammatory cytokines in macrophages and dendritic cells, as IL-12 is a proinflammatory cytokine that is produced mainly by these cell types [33]. DSS induced colitis also involves the adaptive immune system, especially in more chronic experimental set-ups [34]. IFN-γ, a proinflammatory cytokine typically expressed by T helper 1 cells and known to be up-regulated in chronic DSS induced colitis [34,35], was also suppressed in the probiotic treated group compared to the DSS-treated group. The data from our experiments with BCRC 14023, BCRC16000 and NTU 101 in the DSS-induced murine colitis model cannot be translated easily to the clinical setting, as introducing bacterial mutants in humans is not straightforward. This seems to be especially important when the intestinal epithelial barrier function is impaired, as BCRC 14023, BCRC16000 and NTU 101 could then show increased proinflammatory activation of macrophages and fewer modulatory signaling effects on intestinal epithelial cells,

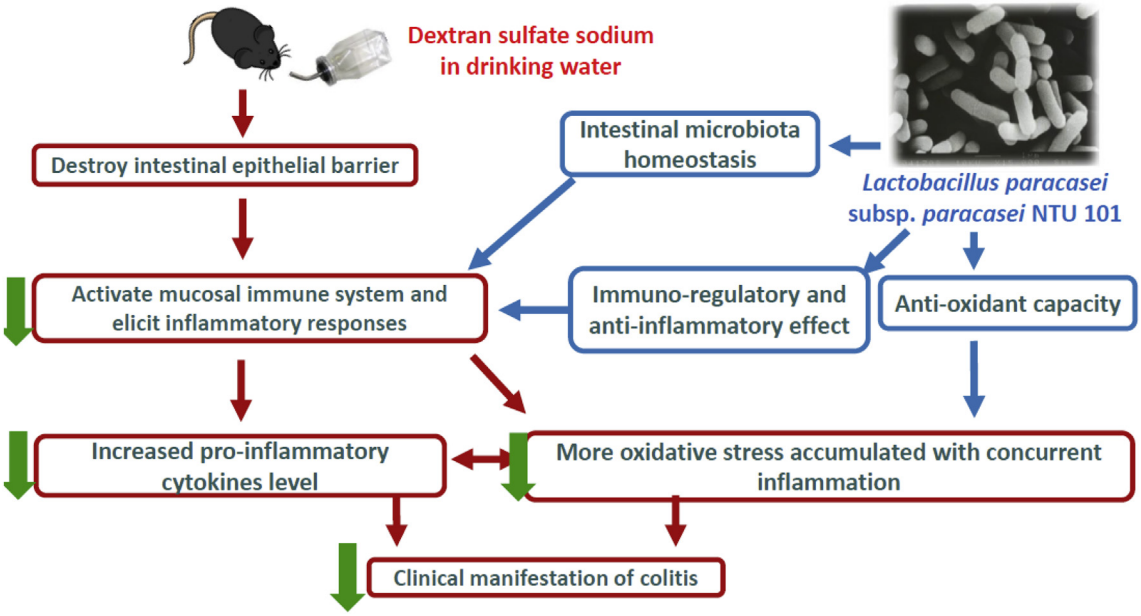

Fig. 7 – The possible pathways of relieving symptoms.

such as by proteins p 70 and decreased nitrate/nitrite IL-10 [36].

The mechanisms by which probiotics protect against intestinal disease include optimizing microbial balance, competitive exclusion of pathogens, promotion of mucus secretion, production of bacteriocins, enhancement of barrier integrity, and maturation of intestinal immunity [36]. The mechanism of protection depends upon the specific probiotic strain and disease model used in investigation [37]. In clinical trials investigating probiotics as a preventive strategy for commensal strains known not to translocate or cause mucosal injury to the host are typically chosen [38]. Not all probiotic strains have the same immunologic effects, even between bacteria of the same species [39,40]. Finally, NO can combine with the superoxide anion to form peroxynitrite that causes cell death and activates the NF-κB inflammatory pathway [41]. A full dose of any of the probiotic strains tested could lower the level of NO in colitic mice, suggesting that these bacteria could also reduce the production of proinflammatory cytokines and limit tissue damage.

5. Conclusion

In order to explore the improvement of colitis clinical symptoms by the probiotic NTU 101, previously isolated by our group, DSS was used to induce symptoms similar to UC in mice, with equivalent prevention and treatment. Colitis symptoms, such as weight loss, diarrhea, and anal bleeding, were induced in mice after 7 d of induction with DSS-supplemented drinking water. Feeding of NTU 101 alleviated the weight loss caused by DSS and inhibited DAI, while increasing the amount of *Bifidobacterium* spp. in the cecum, and modulating immune activity. The probiotic strains also reduced the oxidative stress caused by DSS by enhancing the antioxidative capacity of total antioxidants capacity (GR, GSH, CAT, SOD, MDA), and hindering the secretion of proinflammatory cytokines, such as TNF-α, IL-6, IFN-γ, and IL-12. Based on our study, continuous feeding of NTU 101 powder could reduce oxidative stress and the inflammatory response, slowing down the aggravation of clinical symptoms during UC. The possible mechanisms relieving symptoms of UC by NTU 101 are shown as Fig. 7.

In conclusion, the difference in therapeutic effect between BCRC 14023, BCRC16000 and NTU 101 *in vivo* suggests a role for the cell surface in determining its therapeutic efficacy. Given the current data, our attitude regarding the use of probiotics in IBD is that they should be considered as adjunctive therapies. We encourage patients who want to take a probiotic to continue their pharmaceutical-based treatment regimen; they can then use probiotics as a potential adjunct, as the probiotics likely will not cause any severe side effects and could provide some benefit.

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Conflicts of interest

All authors declare no conflicts of interest.

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