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Effects of Pretreatment with *Bifidobacterium bifidum* Using 16S Ribosomal RNA Gene Sequencing in a Mouse Model of Acute Colitis Induced by Dextran Sulfate Sodium

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Background: *Bifidobacterium* is a potentially effective and safe treatment for patients with inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease. However, information on the influence of *B. bifidum* on gut microbial diversity of treated and pretreated IBD patients is limited.

Material/Methods: Our study investigated therapeutic and preventive effects of *B. bifidum* ATCC 29521 on C57BL/6 mice with dextran sulfate sodium (DSS)-induced acute colitis via 16S ribosomal ribonucleic acid (rRNA) gene sequencing.

Results: Treatment and pretreatment of mice with *B. bifidum* ATCC 29521 significantly alleviated the severity of acute colitis on the basis of clinical and pathologic indicators. 16S rRNA gene sequencing showed that administration of *B. bifidum* shifted composition of the gut microbiome in mice with DSS-induced colitis in both treated and pretreated groups. Mice pretreated with *B. bifidum* ATCC 29521 for 21 days exhibited a significant increase in diversity of the gut microbiome. Principal coordinate analysis showed that gut microbiota structure was shaped by different treatments and time points. On the basis of linear discriminant analysis of effect size, the abundance of the genus *Escherichia-Shigella*, belonging to the family Enterobacteriaceae, was reduced in the *B. bifidum*-treated group, indicating that pathogens were inhibited by the *B. bifidum* treatment. Furthermore, the genera *Intestinimonas* and *Bacteroides* were significantly associated with the *B. bifidum*-pretreated group.

Conclusions: 16S rRNA gene sequencing showed that pretreatment with *B. bifidum* ATCC 29521 reduced intestinal inflammation and altered the gut microbiota to favor the genera *Intestinimonas* and *Bacteroides*.

Keywords: **Colitis, Ulcerative • Encephalocele • Inflammatory Bowel Diseases • Microbiota • Sequence Analysis, DNA**

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Background

Inflammatory bowel disease (IBD) describes a group of chronic and recurrent intestinal inflammatory diseases, including ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology remains largely unknown, a reasonable hypothesis is that the intestinal microbiota or gut microbiome activates abnormal and hyperactive immune responses in a genetically susceptible host, resulting in chronic inflammation [1,2]. Gut microbiota, consisting of many microbial species and bacteria, makes up a protective system for the gastrointestinal tract [3]. The gut microbiome, as a microbial community, mainly includes viruses, bacteria, and fungi [4]. Current treatment paradigms for IBD are based on the use of 5-aminosalicylic acid, corticosteroids, thiopurines, and infliximab, among other agents, but these standard protocols are not satisfactory; that is, a drug treatment with a nonrelapsing cure rate is not available for IBD. Therefore, nontoxic treatment choices have been increasingly used to improve IBD-associated intestinal inflammation.

Probiotics are defined as live microorganisms that provide health benefits to the host when administered in adequate amounts (Food and Agriculture Organization/World Health Organization, 2002). Different probiotic strains have been used as selective medications to alleviate the symptoms of IBD [5-8]. The mechanisms involved in the host-beneficial effects are numerous, including inhibiting the growth of pathogenic microorganisms, competing with pathogens for nutrients and niches to prevent colonization of the host by pathogens, rectifying intestinal microbial imbalance, regulating the immune system, and producing substances that contribute to cell proliferation and maturation [9-11]. Increasing the number of probiotics in the gut, such as mucosa-associated *Bifidobacterium* spp and *Lactobacillus* spp, can alleviate dysbiosis by inducing a decrease in the levels of pathogens such as *Escherichia coli* in patients with IBD [12-14]. Because IBD has multiple correlations with the gut microbiota, preventive strategies that target abnormalities in the composition of the gut microbiota may inspire new possibilities for effective treatments that may support the use of specific probiotics in patients with IBD [15].

Bifidobacterium species have been widely used as probiotics to prevent or treat gastrointestinal diseases. Dextran sulfate sodium (DSS)-induced colitis is the most widely used chemical model of intestinal inflammation, with DSS causing the death of epithelial cells, which in turn compromises barrier functions and leads to subsequent inflammation [16]. The DSS model of acute colitis is beneficial for studying epithelial healing and the development of regulatory immune responses, which are part of the spontaneous recovery after termination of DSS treatment [17]. Reportedly, the enteric microbiota is involved in the suppression of acute colitis in DSS models [17]. The beneficial effects of *Bifidobacterium breve* administration in IBD

have been shown in both acute and chronic DSS-induced colitis [18,19]. *Bifidobacterium bifidum* 231 can also facilitate the recovery of damaged tissue in rats with trinitrobenzenesulfonic acid-induced UC, resulting in beneficial effects on cell adhesion and immunomodulatory properties [6]. *Bifidobacterium bifidum* ATCC 29521, a representative strain of *Bifidobacterium* spp., has been reported to modulate the composition of the microbiota or microbiome in the intestinal tract by increasing the number of beneficial bacteria and reducing harmful bacterial populations in healthy BALB/c mice on the basis of polymerase chain reaction-denaturing gradient gel electrophoresis data [20]. Acknowledgment of the 16S ribosomal ribonucleic acid (rRNA) gene as a critical standard for identifying bacteria has been widely applied for determining the gene sequence of bacterial species [21].

Therefore, this study aimed to use 16S rRNA gene sequencing to investigate effects of pretreatment for 21 days with *B. bifidum* ATCC 29521 in a C57BL/6 mouse model of acute colitis induced by DSS.

Material and Methods

Animals

Eighty female specific-pathogen-free C57BL/6 mice (9-10 weeks old, 20±2 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were maintained under the same conditions, with a constant temperature (20-25°C) and humidity (40-70%). Food (chow; Teklad 7964 rodent diet, Harlan, Madison, WI), water, bedding, and cages were autoclaved.

All experiments were approved by the Guangdong Medical University Animal Experimental Ethics Committee.

Bacterial Strains and Culture Conditions

Bifidobacterium bifidum ATCC 29521 was purchased from the American Type Culture Collection (Manassas, VA). Before the experiments, the microorganisms were revived through 2 successive transfers in de Man-Rogosa-Sharpe medium (Oxoid, Hampshire, UK) supplemented with 0.05% L-cysteine hydrochloride at 37°C for 48 h in an anaerobic incubator.

Induction of Experimental Colitis and Bacterial Colonization in Mice

DSS (36-50 kDa; MP Biomedicals, Aurora, OH) was dissolved in distilled water at a concentration of 3% (w/v) and was administered to the mice for 7 days to induce acute colitis. *Bifidobacterium bifidum* ATCC 29521 pellets were collected by

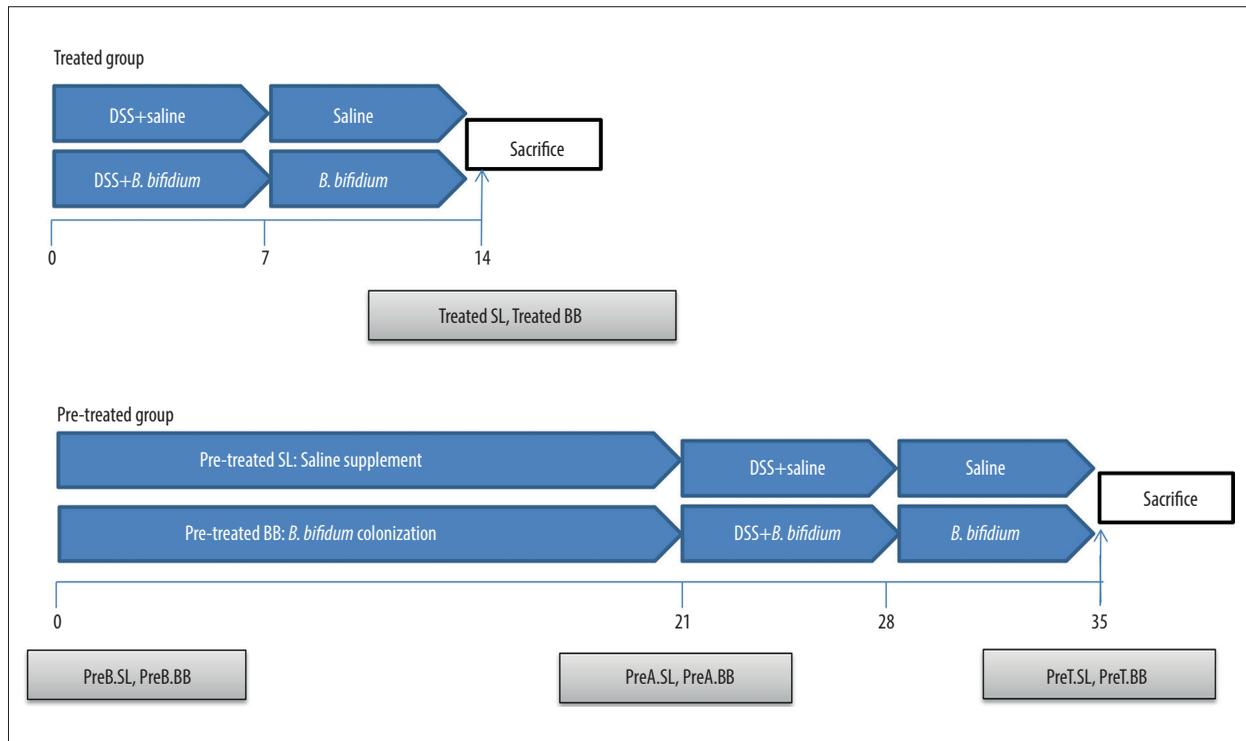


Figure 1. Experimental design for this study. (A) Mice were treated with saline or *Bifidobacterium bifidum* ATCC 29521 from days 0 to 14 (2 weeks) and received dextran sulfate sodium (DSS) from days 0 to 7 in the treated groups. (B) Mice were treated with saline or *B. bifidum* ATCC 29521 from days 21 to 35 (5 weeks) and received DSS from days 0 to 7 in the pretreated groups.

centrifugation, after which the cells were washed and resuspended in saline. As shown in **Figure 1**, the 80 mice were randomly divided into 2 groups, a treated group and a pretreated group, with each group then being randomly divided into 2 subgroups of 20 mice each; these subgroups were treated with saline or *B. bifidum* ATCC 29521. The subgroups treated with saline or *B. bifidum* were named the treatment-with-saline (treated SL) group and treatment-with-*B. bifidum* ATCC 29521 (treated BB) group, respectively, whereas in the pretreated group, the respective groups were named the pretreatment-with-saline (pretreated SL) group and pretreatment-with-*B. bifidum* ATCC 29521 (pretreated BB) group. The mice in the treated BB group were treated with *B. bifidum* for 2 weeks, starting at the time of DSS administration (which lasted for 7 days), with the pretreated BB group receiving treatment for 3 weeks before DSS administration; the respective number of days of treatment thus amounted to 14 and 35 days. The mice in the test groups were also colonized with *B. bifidum* ATCC 29521 at a dose of 1×10^9 colony-forming units resuspended in 0.5 mL of saline, administered once daily by oral gavage. The mice in the control groups were given 0.5 mL of saline by oral gavage.

The feces of the mice in the pretreated group were collected at days 0, 21, and 35 (before *B. bifidum* colonization [preB BB] and before the saline group [preB SL]) after 3 weeks of *B. bifidum* colonization (preA BB) and saline (preA SL) and on

the day of sacrifice (preT BB, preT SL). However, the feces of the mice in the treated group were only collected at day 14, the day of sacrifice. The mice were anesthetized with diethyl ether via intraperitoneal injection (3 mL/kg) and were euthanized at day 14 or 35 in the treated and pretreated groups, respectively (**Figure 1**).

Weight and Clinical Score Assessment

The mice were weighed every day, and their clinical scores were evaluated accordingly. Weight data are shown as the percentage of the initial weight. The clinical activity score (CAS) [22] was determined on the basis of body weight, stool consistency, and occult blood. Weight loss was scored as 0, no weight loss; 1, 1-5%; 2, 5-10%; 3, 10-15%; or 4, >15%. Stool consistency was scored as 0, well-formed pellets; 2, pasty and semi-formed stools that did not stick to the anus; or 4, watery diarrhea that did stick to the anus. Intestinal bleeding was scored as 0, hemocult negative; 2, hemocult positive; or 4, gross bleeding. The individual scores were added and divided by 3, resulting in a CAS that ranged from 0 to 4.

Histopathology

Mouse colons were fixed with 10% paraformaldehyde, embedded in paraffin using a standard protocol, cut into 4- μ m sections,

stained with hematoxylin and eosin, and assessed under a light microscope. Histopathologic changes were scored as follows: depth of the ulcer: 0, no ulcer; 1, mucosal involvement; 2, mucosal-submucosal involvement; 3, penetration of the muscularis propria; or 4, full-thickness involvement. Extent of the ulcer: 0, no ulcer; 1, punctate; 2, minimal; 3, moderate; or 4, widespread. Presence of inflammation: 0, none; 1, minimal; 2, mild; 3, moderate; or 4, severe. Extent of inflammation: 0, none; 1, mucosal; 2, mucosal-submucosal involvement; 3, mucosal-submucosal muscle penetration; or 4, full-thickness involvement. Location of fibrosis: 0, none; 1, mucosa only; 2, mucosa and submucosa; 3, including the muscle layer; or 4, full-thickness fibrosis. For each mouse, the scores from the 5 parts were added to obtain histologic scores that ranged from 0 to 20 [23].

Deoxyribonucleic Acid (DNA) Extraction and 16S rRNA Gene Sequencing

Total DNA was isolated from the feces using a stool DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 16S rRNA gene sequencing was carried out as described by a previous study [24], with a few modifications. The 16S rRNA gene was sequenced in a region that targeted the V4 variable region, and the bacterial genome was sequenced on an Illumina HiSeq instrument with the barcoded primers 515F (GTGCCAGCMGCCGCGTAA) and 806R (GGACTACHVGGGTWTCTA AT) according to protocols provided by Novogene Bioinformatics Technology (Beijing, China). Paired-end reads, 250 base pairs in length in each direction, were generated, and the overlapping reads were assembled.

Statistical Analysis

Weight data are shown as the percentage of the initial weight. Differences in weight loss and histologic scores obtained from the groups were examined using an independent-sample t test. Single-factor repeated measures and analysis of variance were performed to evaluate CAS. After low-quality sequences were filtered out, the high-quality sequences were analyzed. The assembled reads were clustered into operational taxonomic units (OTUs) using scripts from UPARSE software (v7.0.1090), defined at a 97% similarity level. The Shannon, Chao 1, and ACE indexes were used to evaluate the α diversity, and the β diversity was assessed by principal coordinate analysis (PCoA). A comparison of the relative abundances between groups was performed by the nonparametric Mann-Whitney U test. Microbial diversity and statistical analyses were implemented in Quantitative Insights into Microbial Ecology (QIIME) and SPSS 19.0 version. $P < 0.05$ was considered statistically significant. Differentially abundant features were determined using linear discriminant analysis effect size (LEfSe). LEfSe is an algorithm for high-dimensional biomarker discovery and explanation that identifies genomic features by characterizing

the differences between 2 or more biological conditions. The threshold on the logarithmic local-density approximation score for discriminative features was 4.0.

Results

Fecal Condition and Body Weight

All mice survived throughout the whole process of the experiment. All mice showed weight loss and hematochezia, and several had diarrhea after the DSS treatment. According to the weight curve, DSS treatment led to a peak decrease in body weight on days 10 and 11, followed by a gradual return to baseline levels. After 2 weeks of *B. bifidum* administration, no significant difference was observed between control and *B. bifidum*-treated mice in the treated group. In the pretreated group, from days 31 to 34, the DSS-induced decrease in body weight was significantly less in mice treated with *B. bifidum* compared with the control groups (Figure 2A, 2B). Clinical symptoms were also assessed using CAS. Compared with the control groups, the mice treated with *B. bifidum* showed better-formed stools with less evident blood in both the treated and the pretreated groups. After exposure to DSS, the CAS curve notably increased on day 4 and then gradually decreased on day 9 after reaching its peak. After treatment with *B. bifidum*, the CASs for mice in both the treated and the pretreated groups were significantly decreased compared with the values for the control mice with colitis ($P < 0.05$). Particularly, mice pretreated with *B. bifidum* exhibited a more significant decrease in CAS compared with the control group ($P = 0.000$) (Figure 2C, 2D).

Effect of *B. bifidum* ATCC 29521 on Histologic Inflammation

Mice with DSS-induced colitis displayed a shorter intestinal length due to intestinal inflammation. Intestinal shortening was curtailed in mice treated or pretreated with *B. bifidum* when compared with that in the control groups, although significant differences were not observed (treated SL group [6.9±0.6 cm] vs treated BB group [7.1±0.4 cm] and pretreated SL group [7.0±0.3 cm] vs pretreated BB group [7.3±0.5 cm]) (Figure 3A).

Pathologic inflammation was assessed using histologic colonic severity scores that combined the extent and depth of inflammation and ulceration. In both the treated and the pretreated groups, the histologic colonic severity scores were higher in mice that received saline than in those that received *B. bifidum* (treated SL group [9.3±2.4] vs treated BB group [7.4±2.9] and pretreated SL group [9.5±1.9] vs pretreated BB group [5.9±2.1]) (Figure 3B). In addition, the histologic scores were significantly lower in mice pretreated with *B. bifidum* than those in control mice ($P < 0.05$).

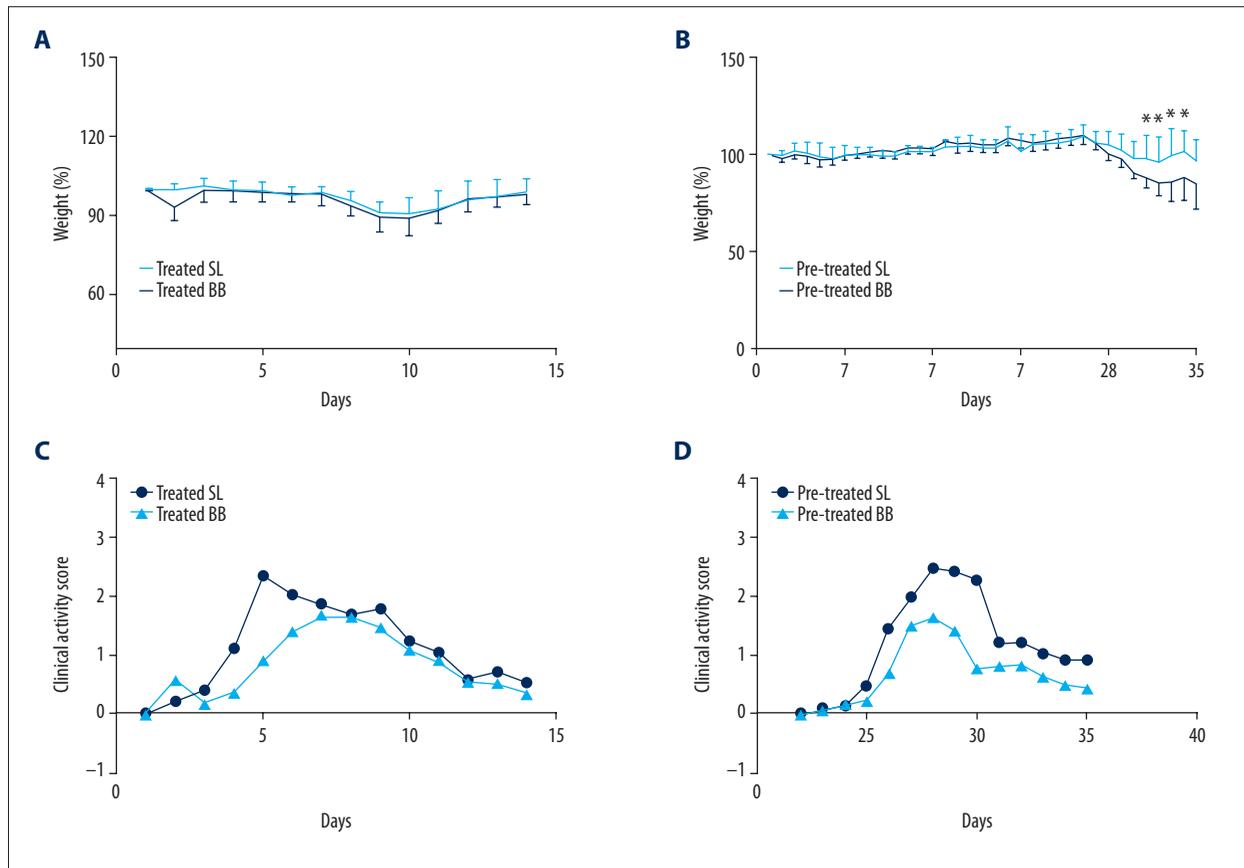


Figure 2. Weight loss and clinical activity score (CAS) of mice in the treated and pretreated groups. **(A)** No significant difference in weight loss was observed between the treated SL and treated BB groups. **(B)** Significantly less weight loss was observed in the pretreated BB group from days 31 to 34. **(C)** CASs of the treated BB group were remarkably reduced compared with those of the treated TL group ($P=0.001$). **(D)** CASs of the pretreated BB group were significantly decreased compared with those of the pretreated TL group ($P=0.000$). * $P<0.05$.

Histologic inflammation in mice with DSS-induced colitis included erosion, crypt shortening or disappearance, edema, and neutrophilic infiltrates in the mucosa and lamina propria. Mice in the control groups showed increased infiltration of inflammatory cells within the epithelium (primarily neutrophils), disappearance of glandular epithelium, moderate to severe erosion of lamina propria mucosa, and even ulcerative damage. Mice treated with *B. bifidum* tended to show less severe effects as evidenced by lower inflammatory cell infiltration and inconsecutive erosion of epithelium (**Figure 3C-3F**).

Overview of Sequencing Analysis

Stool samples were collected from each mouse at different time points and then pooled for the mice from the same group and same time points before DNA extraction. In total, 32 DNA samples were sequenced using a MiSeq instrument. After controlling for the quality of the raw sequences, we obtained 534 506 and 1 493 777 high-quality 16S rRNA gene sequences for the treated and the pretreated groups, respectively, with respective

averages of 66 813 and 62 241 sequences per sample. Using a 97% identity threshold, the sequences were clustered into 3685 and 6176 OTUs, respectively, with respective averages of 461 and 527 OTUs per sample.

The treated and pretreated groups shared 8 predominant bacterial phyla: Bacteroidetes, Firmicutes, Proteobacteria, Deferribacteres, Tenericutes, Cyanobacteria, Verrucomicrobia, and Actinobacteria. Additional predominant bacterial phyla in the treated group included Saccharibacteria and Deinococcus-Thermus, and in the pretreated group were Chloroflexi and Synergistetes. The dominant bacterial phyla in both groups were Bacteroidetes, Firmicutes, and Proteobacteria, which accounted for over 90% of the observed phyla (**Figures 4A, 5A**). At the genus level, the 2 groups shared 6 predominant bacterial genera: *Bacteroides*, *Mucispirillum*, *Lachnospiraceae_NK4A136_group*, *Helicobacter*, *Escherichia-Shigella*, *Lactobacillus*, and *Alistipes*. Additional bacterial genera that characterized the treated group were *Mycoplasma*, *Ruminiclostridium*, and *Oscillibacter*, and in the pretreated group were *Alloprevotella*, *Desulfovibrio*, and *Parabacteroides*.

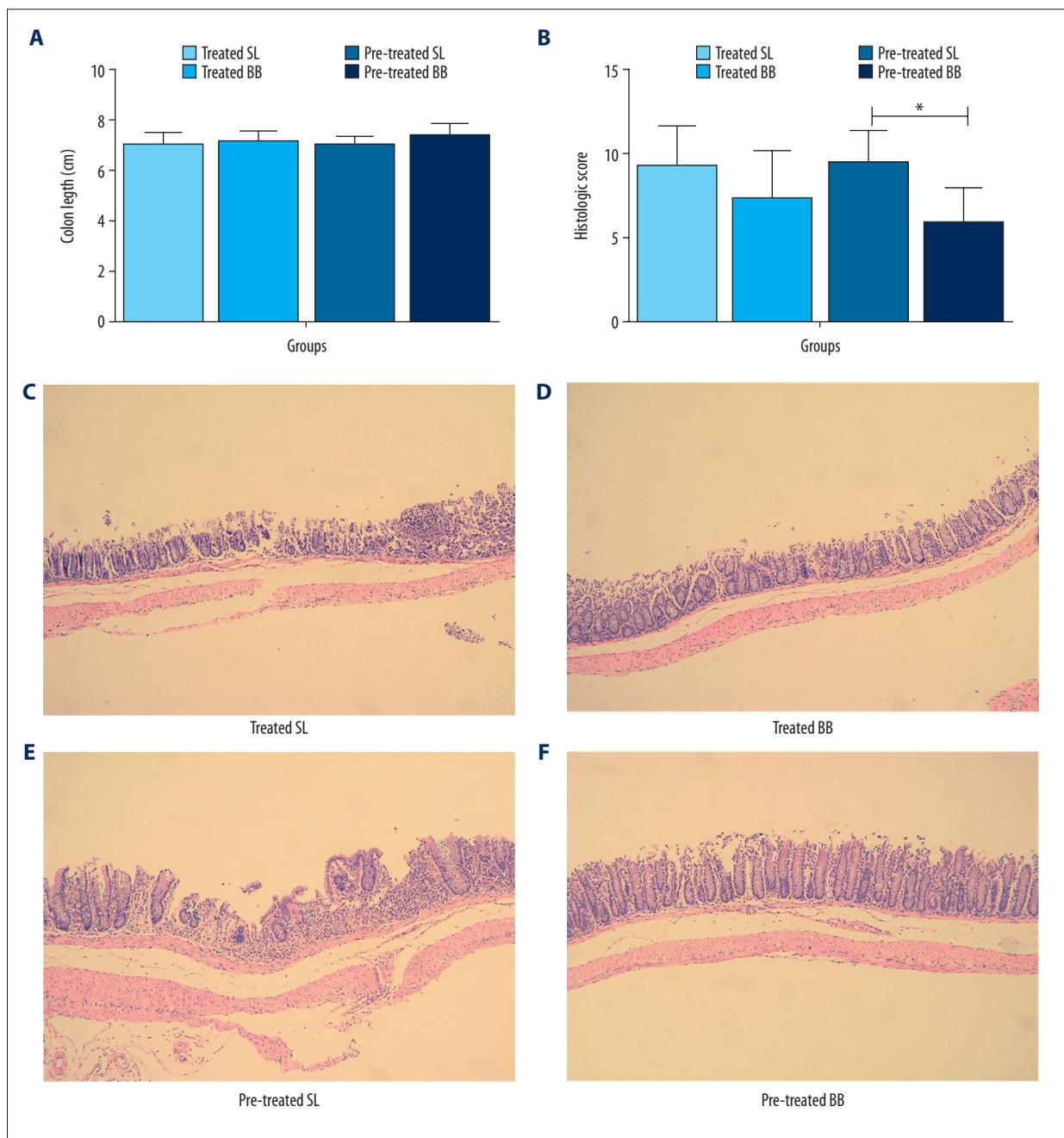


Figure 3. Colon length and histopathology analysis for effect of *Bifidobacterium bifidum* colonization on dextran sulfate sodium-induced colon damage. **(A)** Average colon length was shorter in the control groups than those in the treated groups, but the difference was not significant. **(B)** Histologic scores were significantly lower in the mice pretreated with *B. bifidum* for 5 weeks (* $P < 0.05$). **(C–F)** Inflammation was more serious in the control groups than in the *B. bifidum*-treated and -pretreated groups. The control groups exhibited inflammatory cell infiltration, moderate to severe erosion of the lamina propria mucosa, and even ulcerative damage (hematoxylin and eosin staining, magnification $\times 100$).

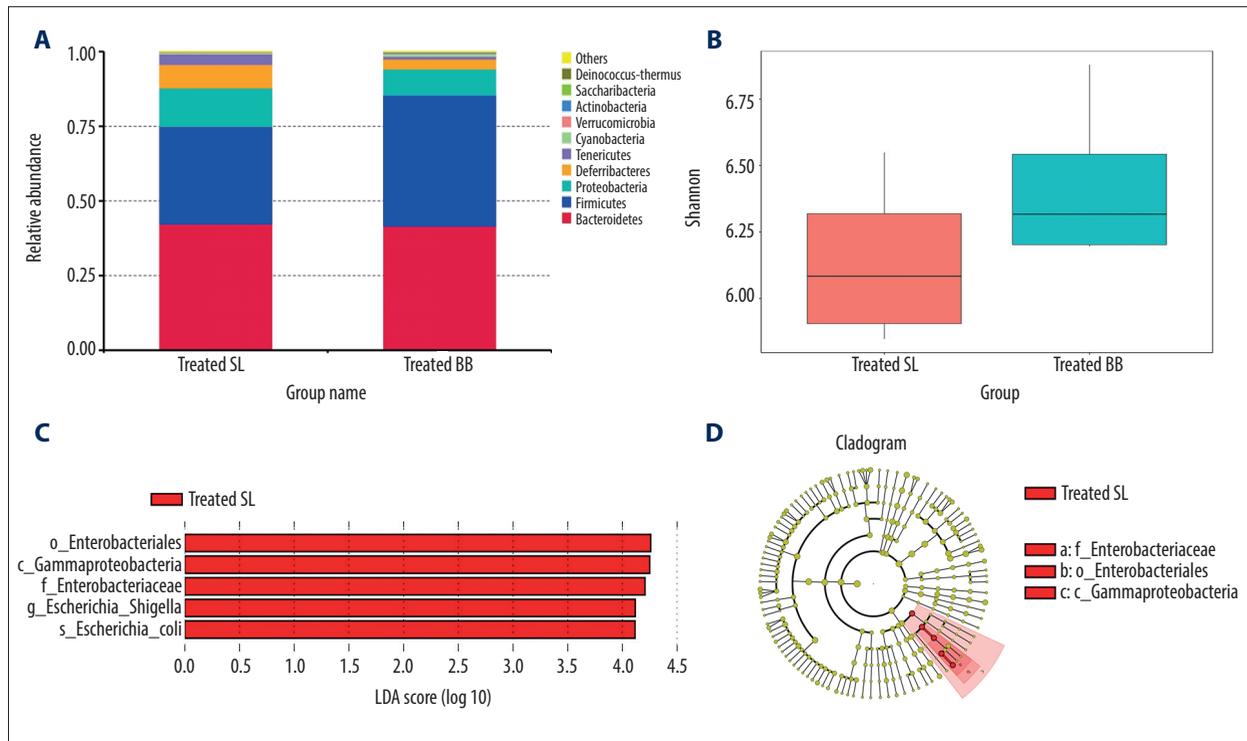


Figure 4. Changes in the microbial communities in the treated group. (A) The top 10 most frequently observed bacterial phyla in the communities. Sequences that could not be classified into any known group were labeled “other.” (B) The Shannon diversity index of the *Bifidobacterium bifidum*-treated group was greater than that of the control group. (C) Different features in the control and *B. bifidum*-treated groups were identified using linear discriminate analysis effect size, with a local-density approximation value of 4.0. (D) Differences are represented by the color of the most abundant class.

Distinctive Fecal Microbial Communities Associated with the *B. bifidum* ATCC 29521-treated Group

In the treated group, mice with acute DSS-induced colitis received *B. bifidum* supplements for only 2 weeks. Although no significant difference was observed in the diversity of the gut microbiota, the number of unique OTUs and the Chao 1 and Shannon indexes of mice treated with *B. bifidum* were higher than those observed in the control group (Figure 4B).

Of the top 10 most predominant phyla, the relative abundance of Firmicutes was more enriched in the *B. bifidum*-treated group than that in the control group, whereas Proteobacteria, Deferribacteres, and Tenericutes were less enriched. The abundances of Actinobacteria and Bifidobacteriaceae were also significantly higher in the *B. bifidum*-treated group than those in the control group, with the abundance of Actinobacteria increasing from 0.067% to 0.29% and the abundance of Bifidobacteriaceae increasing from 0.042% to 0.28%, respectively. At the genus level, the abundance of *Bifidobacterium* was too low to identify any differences. We performed LefSe to determine the differential abundances of the microbial communities between the 2 groups. Mice that received saline exhibited an increased enrichment of Enterobacteriales,

Enterobacteriaceae, and *Escherichia-Shigella*. This showed that abundances of these pathogens, which belong to phylum Proteobacteria, were significantly inhibited in mice that received *B. bifidum* supplements (Figure 4C, 4D).

Effects on Gut Microbiota in Mice of *B. bifidum* ATCC 29521-pretreated Group

Mice in the pretreated group received *B. bifidum* ATCC 29521 for 5 weeks to evaluate its preventative and treatment effects. The gut microbial community is considered a key indicator of the overall health of the host and is influenced by the health of the host [25]. In a comparison of 3 time points in the *B. bifidum*-pretreated group, 472 OTUs were observed to be shared. The most unique OTUs were identified at the time point at which the mice had been administered *B. bifidum* for 3 weeks, before the DSS treatment (preA BB, day 21), followed by the time point at which the mice were sacrificed (preT BB, day 35) and the baseline, at which time mice were untreated (preB BB, day 0). In a horizontal comparison, more unique OTUs were identified in the *B. bifidum* group than those in the saline group at the time the mice were sacrificed. In a longitudinal comparison, the gut microbiota was more diverse in mice after 3 weeks of treatment with *B. bifidum* than in mice

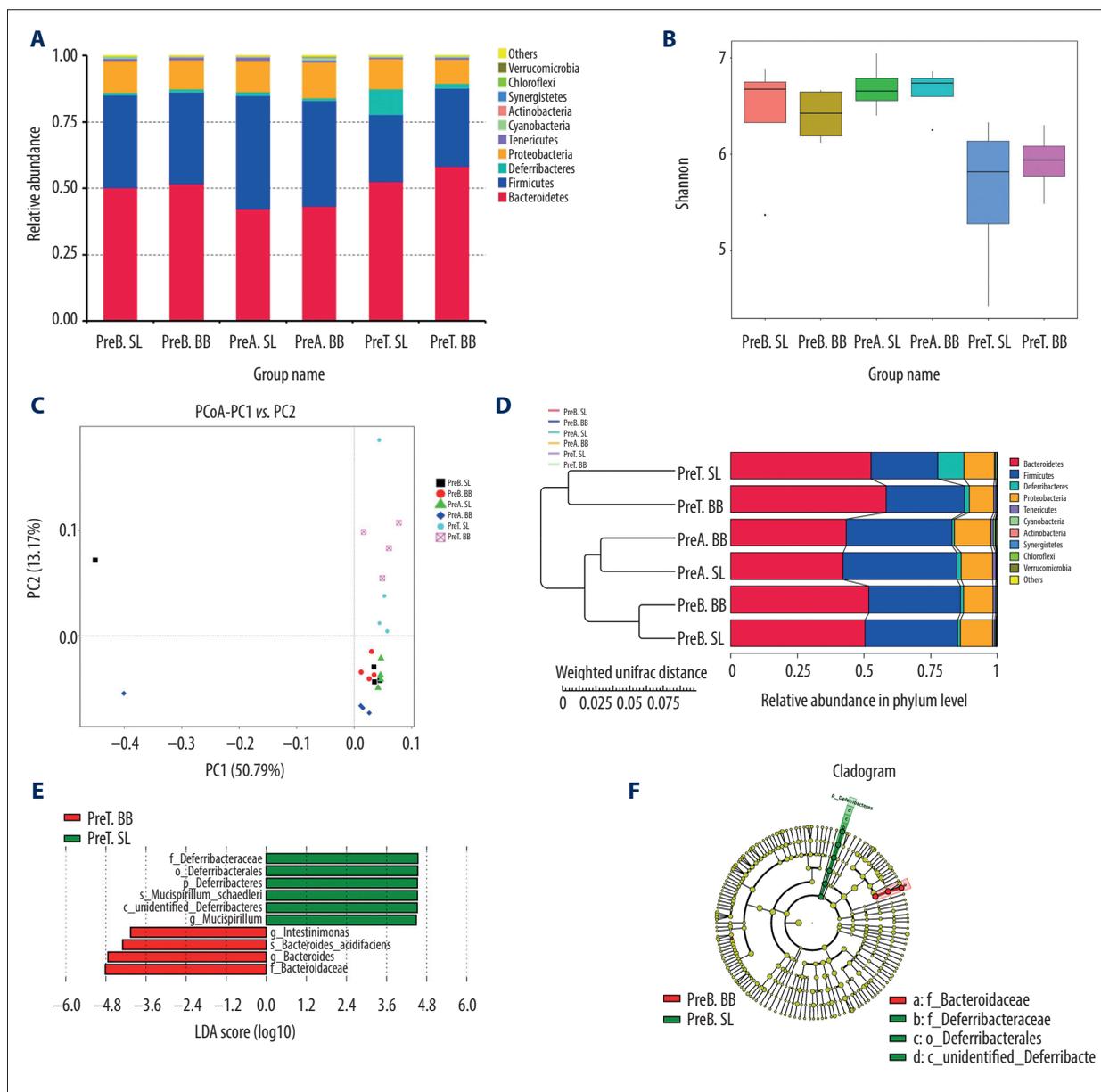


Figure 5. Changes in the microbial communities in the pretreated group. **(A)** The top 10 most frequently observed bacterial phyla in the communities. **(B)** The Shannon diversity index was higher in mice that received *Bifidobacterium bifidum* supplements for 3 weeks than in mice assessed on the day of sacrifice. The principal coordinate analysis **(C)** and unweighted pair group method with arithmetic mean tree **(D)** showed that the microbiota was initially clustered by different time points and treatments. Different features in the control and *B. bifidum*-pretreated groups on the day of sacrifice were identified using linear discriminate analysis effect size, with a local-density approximation value of 4.0 **(E, F)**.

at the time of sacrifice. The observed species matrix, which was simplified to the number of unique OTUs, showed an obvious increase in the preA BB group. The α diversity in the community was estimated using the Chao 1 and Shannon indexes, which were all significantly higher in mice that received *B. bifidum* supplements for 3 weeks than those in mice assessed on day of sacrifice **(Figure 5B)**.

According to PCoA, the gut microbial community shifted between the *B. bifidum*-pretreated and saline-pretreated groups and varied among the different time points **(Figure 5C)**. When the mice were sacrificed, mice that received the *B. bifidum* treatment were separated from mice that received the saline treatments. In particular, the preA BB group was distinctly separated from mice that received *B. bifidum* treatment at other time points. Meanwhile, the microbial communities of the

untreated mice and the mice treated with 3 weeks of saline supplementation were clustered together. A structural comparison of the gut microbiota among different groups was also assessed on the basis of samples that clustered in a pairwise similarity matrix with hierarchical clustering according to their OTUs. Notably, groups initially clustered by different time points and the distance between the preA BB and the preT BB groups was shorter than distance between preA SL and preT SL groups (Figure 5D). Therefore, the structure of the gut microbiota was shaped by different treatments and time points.

We also observed longitudinal changes in the *B. bifidum*-pretreated group. Compared with baseline conditions, mice pretreated with *B. bifidum* ATCC 29521 supplements for 3 weeks exhibited an enrichment of Cyanobacteria and Fusobacteria. On the day of sacrifice, the relative abundances of Firmicutes, Actinobacteria, Saccharibacteria, and Fusobacteria were significantly decreased, whereas the abundances of Bacteroidetes and Verrucomicrobia were increased.

Furthermore, we used LEfSe to identify differentially abundant features in *B. bifidum*-pretreated and saline-pretreated groups at time of sacrifice. *Mucispirillum*, which belongs to the family Deferribacteraceae, was enriched in the saline-pretreated group at the time of sacrifice, whereas *Intestinimonas* and *Bacteroides* were enriched in the *B. bifidum*-pretreated group. When we compared the *B. bifidum*-pretreated group with the saline-pretreated group after 3 weeks of *B. bifidum* supplementation, the former group was enriched for Cyanobacteria, Actinobacteria, and Fusobacteria, and the latter group was enriched for Deferribacteres. Regardless of whether the mice received 3 weeks or 5 weeks of *B. bifidum* supplementation before or after DSS treatment, the abundance of *Bifidobacterium* was not significantly different between the *B. bifidum*-pretreated and saline-pretreated groups (Figure 5E, 5F).

Discussion

The present study determined that treatment with *B. bifidum* ATCC 29521 could alleviate the severity of acute colitis. Treatment with *B. bifidum* ATCC 29521 shifted composition of the gut microbiome in a DSS-induced acute colitis model. Mice pretreated with *B. bifidum* for 21 days exhibited an increase in α diversity of the gut microbiome. The structure of the gut microbiota was shaped by different treatments of *B. bifidum*. The LEfSe illustrated that abundance of the genus *Escherichia-Shigella* was reduced in the *B. bifidum*-treated group, suggesting that pathogens were inhibited by *B. bifidum* treatment. The genera *Intestinimonas* and *Bacteroides* were associated with the *B. bifidum*-pretreated group.

Whether there is a sufficiently preventive and therapeutic approach for IBD that does not allow recurrence is currently under investigation. Conventional therapies, including 5-aminosalicylic acid, corticosteroids, and thiopurines [26], and new therapies, such as infliximab and adalimumab, have been shown to reduce inflammation and relieve symptoms, but many patients become refractory to the treatments during the course of their disease and are unable to bear the economic burden. Commensal enteric bacteria in patients with IBD are characterized by increased numbers of opportunistic pathogens, such as *E. coli*, and decreased numbers of beneficial bacteria, such as *Lactobacillus* and *Bifidobacteria*, in the colon [27]. Probiotics may inhibit inflammation or activate innate immunity and represent treatment strategies that may be used to restore the host gut microbiota [28,29]. Moreover, the administration of probiotics has been shown to restore the microbiota composition and improve the symptoms of patients with IBD [30]. In particular, the clinical efficacy of the administration of VSL#3 has been shown in adult and pediatric patients with active UC [31-33]. The abundances of potentially beneficial *Bifidobacterium* species are reduced in adult and pediatric patients with IBD [12,34,35].

In the present study, we evaluated the anti-inflammatory activity of *B. bifidum* ATCC 29521 in the prevention and treatment of clinical symptoms in a mouse model of DSS-induced colitis. We observed that daily administration of *B. bifidum* ATCC 29521 significantly reduced the severity of DSS-induced acute colitis. The evaluation of colonic specimens from colitic mice treated with *B. bifidum* revealed significant decreases in body weight loss and CAS when compared with the colitic control group, indicating that *B. bifidum* protected the barrier integrity of the colon. The beneficial effect was also observed in assessments of macroscopic and histologic inflammation, as a significant reduction in inflammatory cell infiltration and epithelial damage was observed, particularly in the *B. bifidum*-pretreated group.

Most current probiotics are sold as food or nutritional supplements to improve individuals' health. The development of probiotics is rarely based on the goal of preventing or treating a specific disease. Because of their limited purity and viability, the curative efficacy of commercial probiotics is difficult to determine [36]. Compared with other probiotics, *Bifidobacterium* might be developed as a "second-generation probiotic," which are selected for studies of their mechanistic advantages based on established mechanisms of prevention or treatment of a specific disease [37,38]. In our study, colonization with *B. bifidum* ATCC 29521 did not cause any clinical adverse reactions, such as body weight loss, diarrhea, or hemafecia, indicating the safety of *B. bifidum* ATCC 29521 administration in mice. Of note, *Bifidobacterium* can also be integrated as part of a complex diet, depending on its ability to be carried by the food

matrix. Such a complex diet might be used in susceptible individuals to reduce the risk of IBD development.

Bifidobacterium bifidum has been shown to increase the abundance of total anaerobic bacteria and the level of *Bifidobacteria* and possesses antagonistic activity against enteropathogens by producing antimicrobial substances [39-41]. In another study, *B. bifidum* ATCC 29521 was shown to be useful in enhancing the microbial diversity of the healthy mouse intestine especially by increasing the populations of *Lactobacillus intestinalis* and *Lactobacillus crispatus* and inhibiting colonization by pathogenic bacteria [20]. Decreased diversity of bacterial species has been observed in both fecal and mucosal samples from human patients with UC and CD as well as in fecal samples from rats and mice with DSS-induced colitis [42,43]. Our study showed that the number of unique OTUs and the Shannon index in mice treated with *B. bifidum* were higher than the values for the control-treated group. Similarly, the most unique OTUs were identified after 3 weeks of *B. bifidum* administration, and significant differences in bacterial diversity were observed in the preA BB group. However, no significant differences in diversity were observed between the saline-treated and *B. bifidum*-treated groups at the same time point, further demonstrating that colonization by *B. bifidum* ATCC 29521 modifies bacterial diversity to different extents. Compared with healthy mice, it was harder for *B. bifidum* ATCC 29521 to significantly rebuild the microbial diversity in mice with DSS-induced colitis because of the disturbed microecological balance.

Celiberto et al [44] observed an effect of probiotic beverage consumption (*Enterococcus faecium* CRL 183 and *Bifidobacterium longum* ATCC 15707) in rats with daily oral gavage for 30 days and observed that no significant changes occurred in the population of *Bifidobacterium* spp or in potentially pathogenic bacteria such as Enterobacteria. In addition, Ekmekci et al [45] observed that the abundance of *Bifidobacterium* spp was unchanged after VSL#3 treatment by conventional colonization. Our results are consistent with these reports, showing that even though the relative abundance of Actinobacteria and Bifidobacteriaceae increased in the treated BB group, the relative abundance of *B. bifidum* did not significantly increase in the test groups. These findings further confirmed the role of commensal bacterial colonization of the host and physiological colonization resistance in the prevention of stable pathogenic colonization. Bedani et al [46] and Cavallini et al [47] reported that when rats consumed a fermented soy product without the addition of *B. longum*, an increase in the population of *Bifidobacterium* spp was observed, indicating that probiotic microorganisms have an advantage in regulating the fecal microbiota. We further observed that *B. bifidum* changed the composition of the gut microbiota. Specifically, compared with mice in the *B. bifidum*-treated group, mice in the saline-treated group exhibited a significant enrichment of *Escherichia-Shigella*,

which belongs to the family Enterobacteriaceae. Most IBD studies have revealed that the levels of Proteobacteria species, particularly Enterobacteriaceae species, are increased in both fecal and mucosal samples [35,48-50]. Enterobacteriaceae, particularly *E. coli*, has been reported to induce or aggravate the chronic inflammation observed in patients with IBD. In human and mouse cells, *E. coli* has also been shown to enhance epithelial adherence and invasion and to increase the survival rates of macrophages by inhibiting NF- κ B signaling [51,52]. This bacterium can also modulate the levels of microRNAs (miRNAs) in intestinal epithelial cells to reduce autophagy [53]. As shown in our study, *B. bifidum* effectively suppresses colonization of Enterobacteriaceae in mice with DSS-induced colitis. This result is consistent with previous studies showing that *Bifidobacterium* elicited a beneficial effect on antagonistic antioxidant activity and exerted a barrier effect against pathogens by producing antimicrobial substances [20,39,41]. In the present study, in the pretreated group, control mice exhibited an enrichment of *Mucispirillum* and *Deferribacteraceae*, which belong to the family *Deferribacteres*. In other studies, an increase in the levels of *Deferribacteres* was observed in piglets with carrageenan gum-induced colitis and in signal-transducing activator of transcription (STAT1) and wild-type mice with DSS-induced colitis [54,55]. Certain strains of *Mucispirillum* have also been observed to be physically associated with the secreted mucus layer [56]. In the current study, mice treated with *B. bifidum* supplements exhibited an enrichment of *Bacteroides*, the levels of which were decreased in studies that focused on more accurate measures of the mucosal microbiota in a different population of patients with IBD [35,37]. Certain Bacteroidetes species, particularly *Bacteroides fragilis*, exert protective functions in IBD models. These species alleviate intestinal inflammation by regulating regulatory T cells, balancing helper T1 and helper T2 immunoreactions, maintaining the mucosal barrier, and promoting bacteriologic functions that mediate mucosal homeostasis [58-60]. However, a previous study [61] also determined that *Bifidobacterium bifidum* ATCC 29521 demonstrated a probiotic role via an anti-inflammatory function and through regulating miRNA-associated TJP and NF- κ B modulation, which is not consistent with the mechanism involved in this study. Our findings mainly focused on effects of *B. bifidum* ATCC 29521 on gut microbiota of mice with DSS-induced colitis.

A few limitations should be stated to improve further investigations. First, the association between severity of acute colitis and the gut microbiome has not been analyzed. Second, the mechanism for *B. bifidum* ATCC 29521 inhibition of acute inflammation of the DSS-induced colitis model has not been fully clarified (such as the targeting molecules or associated signaling pathways). Third, the balance of gut microbiomes in a DSS-induced colitis animal model treated with *B. bifidum* ATCC 29521 has not been examined.

Conclusions

In the present mouse model of acute colitis, 16S rRNA gene sequencing showed that pretreatment with *B. bifidum* ATCC 29521 reduced intestinal inflammation and altered the gut microbiota to favor the genera *Intestinimonas* and *Bacteroides*. *Bifidobacterium bifidum* ATCC 29521 might be a promising therapeutic probiotic that suppresses colonization of pathogens clinically; however, clinical experiments need to be conducted in future studies.

References:

1. Kelsen JR, Russo P, Sullivan KE. Early onset inflammatory bowel disease. *Immunol Allergy Clin North Am*, 2019;39:63-79
2. Jeong DY, Kim S, Son MJ, et al. Induction and maintenance treatment of inflammatory bowel disease. A comprehensive review. *Autoimmun Rev*, 2019;18:439-54
3. Mangiola F, Ianiro G, Franceschi F, et al. Gut microbiota in autism and mood disorders. *World J Gastroenterol*, 2016;22:361-68
4. Shi N, Li N, Duan X, et al. Interaction between the gut microbiome and mucosal immune system. *Mil Med Res*, 2017;4:14
5. Xie Y, Guo Q, Li S, et al. Anti-inflammatory properties of *Bifidobacterium longum* expressing human manganese superoxide dismutase using the TNBS-induced rat model of colitis. *J Microbiol Biotechnol*, 2017;8:4014
6. Satish Kumar CS, Kondal Reddy K, Boobalan G, et al. Immunomodulatory effects of *Bifidobacterium bifidum* 231 on trinitrobenzenesulfonic acid-induced ulcerative colitis in rats. *Res Vet Sci*, 2017;110:40-46
7. Palumbo VD, Romeo M, Marino Gammazza A, et al. The long-term effects of probiotics in the therapy of ulcerative colitis: A clinical study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 2016;160:372-77
8. Guandalini S, Sansotta N. Probiotics in the treatment of inflammatory bowel disease. *Adv Exp Med Biol*, 2019;1125:101-7
9. Goncalves P, Araujo JR, Di Santo JP. A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. *Inflamm Bowel Dis*, 2018;24:558-72
10. Neu J. Multiomics-based strategies for taming intestinal inflammation in the neonate. *Curr Opin Clin Nutr Metab Care*, 2019;22:217-22
11. Howarth GS. Inflammatory bowel disease, a dysregulated host-microbiota interaction: Are probiotics a new therapeutic option? *J Gastroenterol Hepatol*, 2008;23:1777-79
12. Khan I, Ullah N, Zha L, et al. Alteration of gut microbiota in inflammatory bowel disease. Cause or consequence? IBD treatment targeting the gut microbiome. *Pathogens*, 2019;8:126
13. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*, 2008;134:577-94
14. Aggeletopoulou I, Konstantakis C, Assimakopoulos SF, et al. The role of the gut microbiota in the treatment of inflammatory bowel diseases. *Microb Pathog*, 2019;137:1103774
15. Veerappan GR, Betteridge J, Young PE. Probiotics for the treatment of inflammatory bowel disease. *Curr Gastroenterol Rep*, 2012;14:324-33
16. Wirtz S, Popp V, Kindermann M, et al. Chemically induced mouse models of acute and chronic intestinal inflammation. *Nat Protoc*, 2017;12:1295-309
17. He J, Zhang F, Jian Z, et al. Stachyose modulates gut microbiota and alleviates dextran sulfate sodium-induced acute colitis in mice. *Saudi J Gastroenterol*, 2020;26:153-59
18. Zheng B, van Bergenhenegouwen J, Overbeek S, et al. *Bifidobacterium breve* attenuates murine dextran sodium sulfate-induced colitis and increases regulatory T cell responses. *PLoS One*, 2014;9:e95441
19. Zheng B, van Bergenhenegouwen J, van de Kant HJ, et al. Specific probiotic dietary supplementation leads to different effects during remission and relapse in murine chronic colitis. *Benef Microbes*, 2016;7:205-13
20. Wang BG, Xu HB, Xu F, et al. Efficacy of oral *Bifidobacterium bifidum* ATCC 29521 on microflora and antioxidant in mice. *Can J Microbiol*, 2016;62:249-62
21. Lau SK, Woo PC, Li NK, et al. Globicatella bacteraemia identified by 16S ribosomal RNA gene sequencing. *J Clin Pathol*, 2016;59:303-7
22. Christensen B, Gibson PR, Micic D, et al. Safety and efficacy of combination treatment with calcineurin inhibitors and vedolizumab in patients with refractory inflammatory bowel disease. *Clin Gastroenterol Hepatol*, 2019;17(3):486-93
23. Rachmilewitz D, Karmeli F, Takabayashi K, et al. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. *Gastroenterology*, 2002;122:1428-41
24. Wang YN, Meng XC, Dong YF, et al. Effects of microbiota in mice with acute colitis based on 16S rRNA gene sequencing. *Chin Med J (Engl)*, 2019;132:1833-42
25. Mithieux G. Gut microbiota and host metabolism: What relationship. *Neuroendocrinology*, 2018;106:352-56
26. Hindryckx P, Novak G. Can we move directly from 5-ASA to a biologic agent in ulcerative colitis? *Best Pract Res Clin Gastroenterol*, 2018;32-33:9-15
27. Specia S, Dubuquoy L. Chronic bowel inflammation and inflammatory joint disease: Pathophysiology. *Joint Bone Spine*, 2017;84:417-20
28. Yousefi B, Eslami M, Ghasemian A, et al. Probiotics importance and their immunomodulatory properties. *J Cell Physiol*, 2019;234:8008-18
29. Kanauchi O, Andoh A, AbuBakar S, et al. Probiotics and paraprobiotics in viral infection: Clinical application and effects on the innate and acquired immune systems. *Curr Pharm Des*, 2018;24:710-17
30. Nishida A, Inoue R, Inatomi O, et al. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol*, 2018;11:1-10
31. Sood A, Midha V, Makharia GK, et al. The probiotic preparation VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol*, 2009;7:1202-209,1209.e1
32. Tursi A, Brandimarte G, Papa A. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: A double-blind, randomized, placebo-controlled study. *Am J Gastroenterol*, 2010;105:2218-27
33. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol*, 2009;104:437-43
34. Fan H, Du J, Liu X, et al. Effects of pentasa-combined probiotics on the microflora structure and prognosis of patients with inflammatory bowel disease. *Turk J Gastroenterol*, 2019;30:680-85
35. Gevers D, Kugathasan S, Denson LA. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe*, 2014;15:382-92
36. Marcobal A, Underwood MA, Mills DA. Rapid determination of the bacterial composition of commercial probiotic products by terminal restriction fragment length polymorphism analysis. *J Pediatr Gastroenterol Nutr*, 2008;46:608-11
37. Strisciuglio C, Miele E, Giugliano FP, et al. *Bifidobacteria* enhance antigen sampling and processing by dendritic cells in pediatric inflammatory bowel disease. *Inflamm Bowel Dis*, 2015;21:1491-98
38. Underwood MA. Intestinal dysbiosis: Novel mechanisms by which gut microbes trigger and prevent disease. *Prev Med*, 2014;65:133-37
39. Ramare F, Nicoli J, Dabard J, et al. Trypsin-dependent production of an antibacterial substance by a human *Peptostreptococcus* strain in gnotobiotic rats and in vitro. *Appl Environ Microbiol*, 1993;59:2876-83

Conflict of Interest

None.

40. Akram W, Garud N, Joshi R. Role of inulin as prebiotics on inflammatory bowel disease. *Drug Discov Ther*, 2019;13:1-8
41. Lievin V, Peiffer I, Hudault S, et al. *Bifidobacterium* strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut*, 2000;47:646-52
42. Wills ES, Jonkers DM, Savelkoul PH, et al. Fecal microbial composition of ulcerative colitis and Crohn's disease patients in remission and subsequent exacerbation. *PLoS One*, 2014;9:e90981
43. Samanta AK, Torok VA, Percy NJ, et al. Microbial fingerprinting detects unique bacterial communities in the faecal microbiota of rats with experimentally induced colitis. *J Microbiol*, 2012;50:218-25
44. Celiberto LS, Bedani R, Dejana NN. Effect of a probiotic beverage consumption (*Enterococcus faecium* CRL 183 and *Bifidobacterium longum* ATCC 15707) in rats with chemically induced colitis. *PLoS One*, 2017;12:e0175935
45. Ekmekciu I, von Klitzing E, Fiebiger U, et al. The probiotic compound VSL#3 modulates mucosal, peripheral, and systemic immunity following murine broad-spectrum antibiotic treatment. *Front Cell Infect Microbiol*, 2017;7:167
46. Bedani R, Pauly-Silveira ND, Roselino MN, et al. Effect of fermented soy product on the fecal microbiota of rats fed on a beef-based animal diet. *J Sci Food Agric*, 2010;90:233-38
47. Cavallini DC, Suzuki JY, Abdalla DS, et al. Influence of a probiotic soy product on fecal microbiota and its association with cardiovascular risk factors in an animal model. *Lipids Health Dis*, 2011;10:126
48. Mirsepasi-Lauridsen HC, Vallance BA, Krogfelt KA, et al. *Escherichia coli* pathobionts associated with inflammatory bowel disease. *Clin Microbiol Rev*, 2019;32:e00060-18
49. Papa E, Docktor M, Smillie C, et al. Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. *PLoS One*, 2012;7:e39242
50. Goyal A, Yeh A, Bush BR, et al. Safety, clinical response, and microbiome findings following fecal microbiota transplant in children with inflammatory bowel disease. *Inflamm Bowel Dis*, 2018;24:410-12
51. Litvak Y, Sharon S, Hyams M, et al. Epithelial cells detect functional type III secretion system of enteropathogenic *Escherichia coli* through a novel NF- κ B signaling pathway. *PLoS Pathog*, 2017;13:e10006472
52. Rahman K, Sasaki M, Nusrat A, et al. Crohn's disease-associated *Escherichia coli* survive in macrophages by suppressing NF κ B signaling. *Inflamm Bowel Dis*, 2014;20:1419-25
53. Nguyen HT, Dalmaso G, Muller S, et al. Crohn's disease-associated adherent invasive *Escherichia coli* modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. *Gastroenterology*, 2014;146:508-19
54. Munyaka PM, Sepehri S, Ghia JE, et al. Carrageenan gum and adherent invasive *Escherichia coli* in a piglet model of inflammatory bowel disease: Impact on intestinal mucosa-associated microbiota. *Front Microbiol*, 2016;7:462
55. Berry D, Schwab C, Milinovich G. Phylotype-level 16S rRNA analysis reveals new bacterial indicators of health state in acute murine colitis. *ISME J*, 2012;6:2091-106
56. Herp S, Brugiroux S, Garzetti D, et al. *Mucispirillum schaedleri* antagonizes *Salmonella* virulence to protect mice against colitis. *Cell Host Microbe*, 2019;25:681-94
57. Frank DN, St Amand AL, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA*, 2007;104:13780-85
58. Park JS, Choi JW, Jhun J, et al. *Lactobacillus acidophilus* improves intestinal inflammation in an acute colitis mouse model by regulation of Th17 and Treg cell balance and fibrosis development. *J Med Food*, 2018;21:215-24
59. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA*, 2010;107:12204-9
60. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, 2008;453:620-25
61. Din AU, Hassan A, Zhu Y, et al. Inhibitory effect of *Bifidobacterium bifidum* ATCC 29521 on colitis and its mechanism. *J Nutr Biochem*, 2020;79:108353