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Weizmannia coagulans spores alleviates DSS-induced ulcerative colitis model by modulation of gut flora, metabolites and suppressing TLR4/MyD88/NF-κB pathway

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ABSTRACT

Ulcerative colitis (UC) is a chronic intestinal inflammatory disease characterized by a complex pathogenesis. *Weizmannia coagulans* has emerged as a potential probiotic for treating intestinal disorders. This study aimed to assess the therapeutic impact of *W. coagulans* BC99 on mice with DSS-induced UC and to elucidate its underlying mechanism of action. Our findings revealed that BC99 administration ameliorated symptoms associated with DSS-induced UC mice, as evidenced by reduced disease activity indexes, reversal of weight loss, and normalization of colon length. Furthermore, BC99 treatment also protected the integrity of the intestinal barrier through maintaining the antioxidant activity and the expression of tight junction proteins (ZO-1 and occludin), and regulating the inflammatory cytokines in DSS-induced UC mice. Additionally, BC99 supplementation enhanced the production of short-chain fatty acids (SCFAs) through the proliferation of SCFA-producing bacteria, including *Bidobacterium*, *Blautia* and *Faecallbaculum*. Notably, the NF-κB signaling pathway was found to be closely related to BC99 treatment in DSS-induced UC mice. The positive protein expression and the mRNA expression of TLR4, MyD88 and p65 in colon tissue were all detected in BC99-treated groups, which indicating that BC99 could alleviate UC symptoms by inhibiting TLR4/MyD88/NF-κB signaling pathway. Metabolomics further confirms the previous results. Collectively, these findings provide basic support for the *W. coagulans* as a functional food additive or a promising therapeutic agent for the effective management of UC.

1. Introduction

Ulcerative colitis (UC) is one of the most common forms of inflammatory bowel disease which is characterized by recurrent ulcerative and erosive inflammation occurred in the colon, accompanied by abdominal pain, diarrhea, bowel distension and vomiting[1-2]. The incidence of UC has been increasing around the world in past few decades with a heavy burden on public health. The studies have shown that this disease is mainly caused by many factors, including genetic susceptibility, immune regulation disorder, intestinal microbiota imbalance, environmental triggering or microbial exposure, but the specific etiology of UC is not completely clear[3]. Typically, most patients with UC require lifelong treatment with mesalazine, 5-aminosalicylic acid, and cortisol. Due to the side effects of headache, nausea, osteoporosis and hypertension caused by traditional drugs[4-5], the probiotics is thought to be an effective way to alleviate the symptoms of UC[6-7].

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In recent years, researchers have recognized that gut microbiota plays an important role in intestinal inflammation, and probiotics have been shown to have anti-inflammatory, anti-oxidant, and regulatory effects on gut microbiota[8]. Previous studies have shown that the probiotics, such as *Lactobacillus plantarum*, *Pediococcus acidilacti* and *Lactobacillus johansonii*, have a positive effect on relieving the symptoms of UC[9-11]. Shang et al.[12] found that *Bifidobacterium bifidum* H3-R2 could protect the integrity of colon and alleviate the onset of colitis by inhibiting inflammatory signals, enhancing the expression of tight junction protein, preventing the destruction of intestinal epithelial cell barrier and maintaining intestinal microecological homeostasis. However, due to the acidic environment of the gastrointestinal tract and the action of digestive enzymes, the activity of most probiotics in the digestive tract fluctuates greatly, leading to unstable clinical treatment[13]. Spore-forming probiotics have good stress resistance, which making them have good processing performance and adaptability, thus ensuring a stable intervention effect on diseases. At present, there are mainly strains of spore probiotics used for the treatment of UC, including *Weizmania coagulans*, *Clostridium butyricum*, *Paraclostridium bifermentans* and others[14-17]. Zheng et al.[18] proved that probiotic spores could regulate gut microbiota by increasing the abundance of short-chain fatty acid (SCFA)-producing bacteria and the overall abundance of microbiota. In addition, it is generally believed that probiotics ingested from external sources are difficult to colonize in the intestine for a long time. Therefore, there is a relative lack of research on the mechanisms of probiotics to alleviate the symptoms of UC.

Previous studies have shown that *Weizmannia coagulans* has a significant effect on alleviating ulcerative colitis[16-17]. *W. coagulans* SANK 70258 could inhibit harmful bacteria in patients with UC, and improve the intestinal microenvironment by increasing the content of butyric acid. However, its mechanism of action has not yet been fully elucidated. In this study, BC99 remarkably relieved symptoms associated with dextran sulfate sodium (DSS)-induced UC mice, including reduced the colitis severity, protected the integrity of the intestinal barrier, enriched SCFA-producing bacteria. In addition, this study also identified one of the signaling pathways (the NF-κB signal pathway) through which BC99 alleviates colitis. These results may provide the evidence for the further developed into a suitable functional food additive or promising therapeutic agent of *W. coagulans*.

2. Materials and methods

2.1 Materials

W. coagulans BC99, was purchased from Wecare Probiotics Co., Ltd. (Suzhou, China). The strain was mainly isolated from the feces of healthy infants, and the number of spores was high, and the spore rate was as high as 90%. Its 16S RNA gene sequence was 99% similar to that of different *W. coagulans* strains registered in NCBI database Genbank (Accession CP092692). The bacterial culture was revived and subsequently fermented at 40 ℃ for 96 h in a fermenter (5 L). Following fermentation, the culture was spray-dried at 160 ℃ to yield the probiotic powder.

All mice in this study were conducted in accordance with the Regulations of the People's Republic of China on the Administration of Laboratory Animals, and the experiment was approved by the Institutional Animal Ethics Committee (SYXK-(Yu) 2021-0020).

2.2 Animal experiment design

The male KM mice (12 weeks of age, weighing (30 ± 2) g) were acclimatized for 1 week in a controlled environment with a 12-h light/dark cycle, at a temperature of (22 ± 2) ℃ and humidity of (50 ± 10)%. Then the mice were randomly divided into 5 groups (10 mice per group): control group, DSS (MW 36 000–50 000; Yeasen Biotechnology Co., Ltd., Shanghai, China) group, DSS + low concentration (LC) group, DSS + medium concentration (MC) group and DSS + high concentration (HC) group. Among them, LC, MC and HC corresponded to 3×10^6, 3×10^7 and 3×10^8 CFU/(mL·day) doses of BC99, respectively, and the control group and DSS group were treated with 0.85% normal saline. After 14 days, mice in all groups were given 5% (*m*/*V*) DSS, except for the control group, which was given distilled water (Table 1). During modeling, 2 mice died in DSS group. According to the previous studies[19], the body weight, fecal consistency, and blood in feces of the mice were scored to determine the disease activity index (DAI) per day for all mice, and calculation method as follows:

$$\text{DAI score} = \frac{\text{Weight score} + \text{Fecal condition score} + \text{Hemorrhagic stool score}}{3}$$

Table 1
Disease activity index scoring for DSS-induced colitis mice.

Score	Weight loss ratio (%)	Fecal consistency	Stool bleeding
0	0	Normal	Occult blood test-negative
1	1–5	Normal	Slight
2	6–10	Soft	Blood traces in stool
3	11–15	Soft	Severe bleeding in stool
4	16–20	Diarrhea	Gross rectal bleeding

2.3 Histopathological assessment

After 3 weeks of intervention, all mice were fasted for 12 h and sacrificed, and the samples of blood, spleen, colon, kidney, liver, intestine, and cecum were collected for analysis.

Post-euthanasia, a 0.5 cm segment of the colon tissue was harvested, rinsed, and fixed in 4% paraformaldehyde. The tissues were then dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histological examination. Pathological alterations, including epithelial cell lesions, crypt damage, and inflammatory infiltration in the colonic tissue, were evaluated using a light microscope (E100, Nikon Co., Japan).

2.4 Immunohistochemistry (IHC) staining

IHC analysis of zonula occludens (ZO)-1 and occludin in colon tissue referred to the previous study[20]. The colon tissues were excised, weighed and fixed in 4% paraformaldehyde solution. The samples were embedded in paraffin, sectioned, dewaxed and hydrated. Following antigen retrieval, the endogenous peroxidase was blocked with 3% hydrogen peroxide, and non-specific antigens were blocked with serum at 37 ℃ for 30 min. After washed with PBS, the sections were incubated with anti-ZO-1 antibody overnight at 4 ℃. Secondary antibody was added after three rinses with PBS and incubated for 50 min at room temperature. Then, the sections were stained with

4',6-diamidino-2-phenylindole for 5 min. After being washed, Sections were observed by a fluorescent microscope (ECLIPSE C1, Nikon Corporation, Japan), and analyzed using ImageJ software.

The expression of Toll-like receptor 4 (TLR4), MyD88, nuclear cytokines kappa B (NF-κB) p65 and inhibitor of NF-κB (IκB) was analyzed by IHC. The antigens of TLR4, MyD88, p65 and IκB were diluted with blocking solution at a ratio of 1:100. The IHC analysis was similar to the method mentioned above, while the sections were instead of diaminobenzidine. The sections were sealed after hematoxylin counterstaining and ethanol dehydration, and the results were analyzed using microscope.

2.5 Western blot analysis

Total proteins from colon tissues were extracted and determined using a BCA protein assay. The 25 μg of total protein were separated by 12% SDS-PAGE and transferred to a PVDF membrane. The membrane was blocked with 5% BSA for 1 h at 25 °C and then incubated with the indicated primary antibodies (ZO-1, occludin, TLR4, MyD88, IκB and p65) at 4 °C overnight. The membrane were incubated with HRP-goat anti rabbit (Jackson Lmmuno Research, USA) at 25 °C for 1 h. After that, the protein bands were detected with the western blot substrate and images were obtained using the GE Image Quant Las4000mini (GE Healthcare, Sweden). Image software was used to calculate the relative expression of target protein bands.

2.6 RNA extraction and quantitative real-time PCR

Total RNA was extracted from the colon using a commercial kit (SteadyPure Quick RNA Extraction Kit, China) following the manufacturer's protocols, and measured by Multiskan (Multiskan FC, Thermo Fisher Scientific, America). The RNA was further reverse transcribed into cDNA (Evo M-MLV RT Master Mix, China). The SYBR Green PCR Master Mix kit (Ecoray Bioengineering Co., Ltd., China) was applied using quantitative real-time polymerase chain reaction (qRT-PCR, CFX96 Touch, Bio-Rad Laboratories Inc., USA). The amplification condition: a pre-amplification stage at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The expression of genes related to colitis was counted based on the *β*-actin expression. All primers of these genes are shown in Table S1.

2.7 Determination of oxidative stress in colon tissue

Colon tissues (0.1 g) were homogenized in 0.9 mL normal saline in an ice bath. The homogenate was centrifuged at 3 000 r/min for 10 min at 4 ℃. The contents of total superoxide dismutase (T-SOD), GSH and malondialdehyde (MDA) of supernatant were determined according to the instructions of enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.8 Determination of serum inflammatory cytokines

Blood samples were centrifuged at 4 000 r/min for 15 min at 4 ℃ to obtained serum. The concentrations of IL-10, TNF-α and IL-1β in serum were quantified using ELISA kit (Shanghai COIBO Biotechnology Co., Ltd., China), following the manufacturer's instruction.

2.9 Data analysis

SCFAs from each feces sample were determined by gas chromatography-mass spectrometry (GC-MS, TSQ9000, Thermo Fisher Scientific, America). The samples (0.1 g) were dissolved in 500 μL saturated sodium chloride, then acidified by adding 10% H_2SO_4 and 500 μL anhydrous ether. After centrifugation at 12 000 r/min at 4 °C for 20 min, the supernatant through a 0.22 mm sterile membrane, and then subjected for SCFAs analysis (HP-5 column; injection temperature 250 ℃; column temperature 40 ℃). The standard curves of acetic acid, propionic acid and butyric acid were prepared by external standard method, and the samples were quantified according to the peak area[21].

2.10 16S rRNA sequencing analysis

Total DNA was extracted from the microbial community of each cecal contents according to the instructions of the E.Z.N.A.® soil DNA extraction kit (OMEGA Engineering, USA). The integrity of genomic DNA was checked by 1% agarose gel electrophoresis, and the concentration and purity of DNA were determined. Two DNA samples from the same group were mixed in equal amounts. The mixed DNA were sequenced for 16S rRNA using the Illumina MiSeq platform, targeting the V3–V4 region (the universal primers: 338F, 5′-ACTCCTACGGGAGGCAGCAG-3′; 806R, 5′-GGACTACHVGGGTWTCTAAT-3′). All subsequent data analysis was performed on the Majorbio Cloud platform (https://cloud.majorbio.com).

2.11 Metabolomics analysis

The samples (50 mg) were mixed with methanol and collected the supernatant. After filtered by 0.22 μm filter prior, all metabolites were identified by mass spectrometry and compared with Metlin (https://metlin.scripps.edu/) and HMDB (http://www.hmdb.ca/) databases to obtain the annotation information of metabolites in the database. The annotation information of metabolites in the database was statistically analyzed. Combined with *t*-test, the differential metabolites were screened under the conditions of VIP > 1 and $P < 0.05$. The differential metabolite volcano map was analyzed by MetPA software. Metabonomic analysis was completed on the Shengxin cloud platform of Shanghai Meiji Biomedical Technology Co., Ltd. (https://cloud.majorbio.com).

2.12 Statistical analysis

All statistical analyses were performed using SPSS Statistics 27.0 (IBM, Chicago, IL, USA), Graphpad Prism 5.0 (Graphpad Software, Inc., USA) and MedPeer platform (https://www.medpeer.cn/). Results were expressed as mean ± standard deviation. Multiple groups were analyzed for statistical differences using one-way analysis of variance (ANOVA) with Tukey's *post hoc* test or Kruskal-Wallis test, and $P < 0.05$ was considered significant.

3. Results

3.1 BC99 ameliorated the pathological symptoms of DSS-induced colitis in mice

The DSS-induced model was used to evaluate the therapeutic effect and survival rate of *B. coagulans* on UC in mice (Figs. 1A and S1). Compared with DSS group, the DSS + MC group and DSS + HC group alleviated the weight loss of colitis mice ($P < 0.05$), and increased the weight of mice by 9.15% and 8.59%. Compared with the DSS group, DSS resulted in high DAI score and severe body weight loss (Figs. 1B and E). Specifically, the DAI score treated with BD99 in DSS + HC and DSS + MC groups (3×10^7, 3×10^8 CFU/mL) decreased significantly, and the DAI score in DSS + LC group decreased slightly, which indicated that BC99 administration reversed the increase in DAI induced by DSS in a dose-dependent manner (Fig. 1B). BC99 could alleviate diarrhea and hematochezia in mice caused by DSS. Furthermore, colon shortening observed in the DSS group were significantly ameliorated in BC99-treated groups (Figs. 1C and D), underscoring BC99's beneficial effects in alleviating DSS-induced UC.

3.2 BC99 preserved intestinal barrier integrity in DSS-induced mice

To further evaluate the effect of BC99 treatment on pathological changes in the colon tissue of mice, H&E staining was employed. In the control group, colonic epithelial tissue and crypts appeared intact with no signs of inflammatory cell infiltration (Fig. 2A). However, some significant pathological changes were found in the DSS group, such as mucosal ulceration, inflammatory cell infiltration and disappearance of crypt structures (Fig. 2A). Compared with DSS group, the damage of colonic tissues in the mice supplementation with BC99 were recovered as shown in Fig. 2A. The colonic mucosal ulcer, inflammatory cell infiltration and crypt injury in DSS + HC group were mild, and the histopathological score of the model group decreased by 46.43% ($P < 0.05$).

The mucosa damage could increase the intestinal permeability, causing a variety of inflammatory responses and affecting the intestinal mechanical barrier. The integrity of the epithelial barrier is mainly maintained by tight junction protein such as ZO-1 and occludin. To evaluate the intestinal barrier, the tight junction protein (ZO-1 and occludin) were detected using IHC (Figs. 2B-D). Compared with the control group, the ZO-1 and occludin of DSS group decreased significantly, and the groups treated with low dose (3×10^6 CFU/mL) and middle dose (3×10^7 CFU/mL) could not reverse the decreases. However, the ZO-1 and occludin of DSS + HC group increased 15.27% and 21.94% than DSS group. Western blot analysis showed that the relative expression levels of ZO-1 and occludin in the model group decreased sharply. After feeding with BC99, the relative expression of the 2 proteins of colons in mice gradually recovered, and there was no significant difference between the control group and DSS + HC group (Figs. 2E and F). Furthermore, the lipopolysaccharide (LPS) concentration in serum of DSS group increased, while treated with high dose BC99 effectively suppressed this change (Fig. 2G). These results indicated that BC99 could alleviate the colonic tissue damage in DSS-induced mice, protect intestinal epithelial cells, and maintain the integrity of intestinal mucosa to some extent.

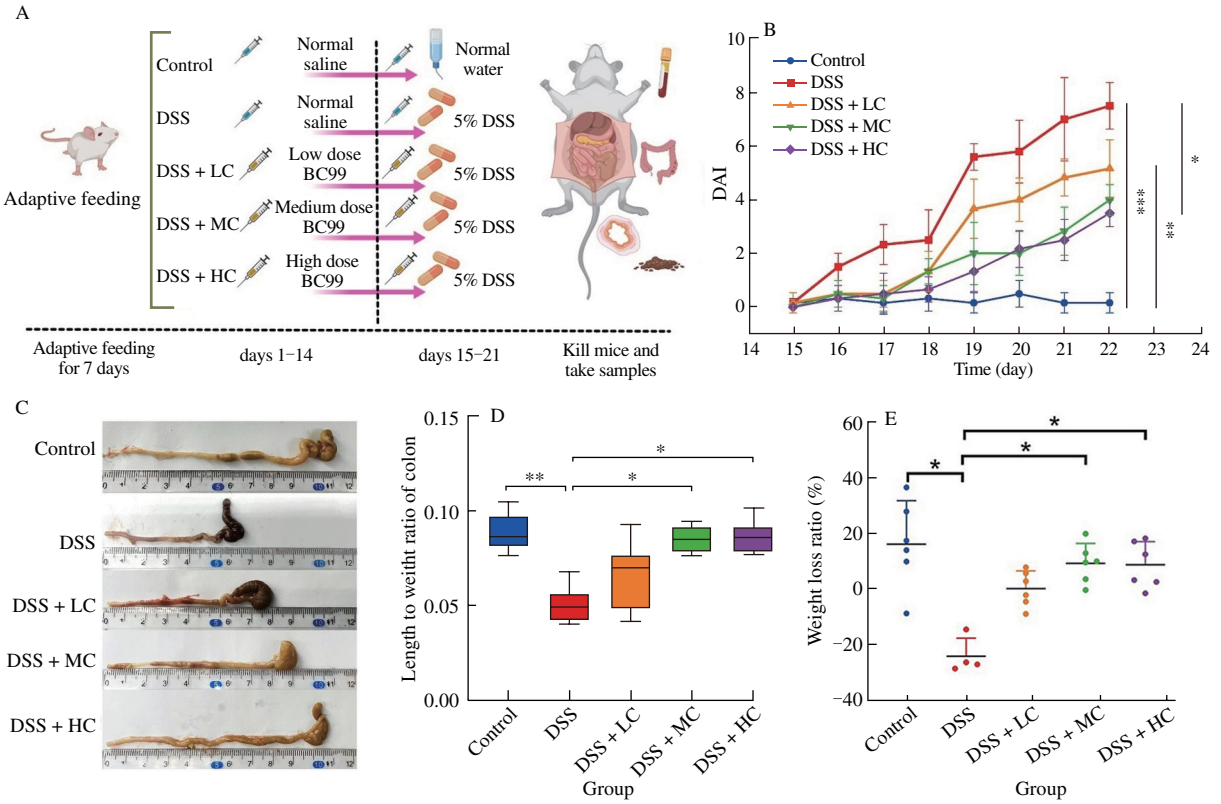


Fig. 1 BC99 alleviated DSS-induced colitis. (A) Animal experiment design; (B) DAI; (C) Macroscopic pictures of colon; (D) Length to weight ratio of colon; (E) Weight loss ratio. Data are presented as the mean ± SD ($n = 6$ per group). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

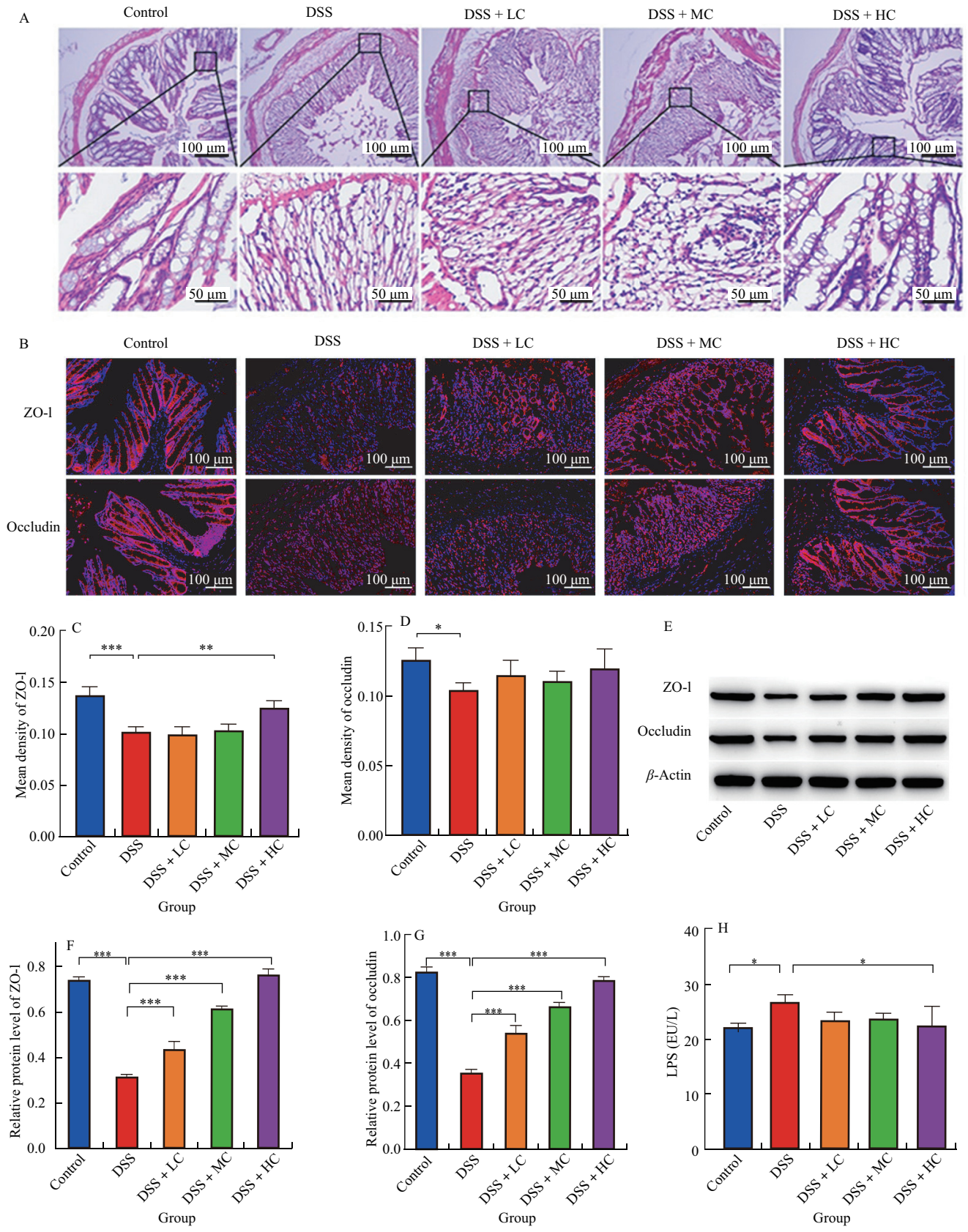


Fig. 2 (A) Representative histological observation of hematoxylin & eosin-stained mouse colon (100× and 400×); (B) IF images of intestinal mechanical barrier (occludin and ZO-1) (400×); (C, D) Mean density of ZO-1 and occludin; (E-G) Expression levels of ZO-1 and occludin; (H) Concentration of LPS in serum; (I) GSH concentration; (J) T-SOD concentration; (K) MDA concentration. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

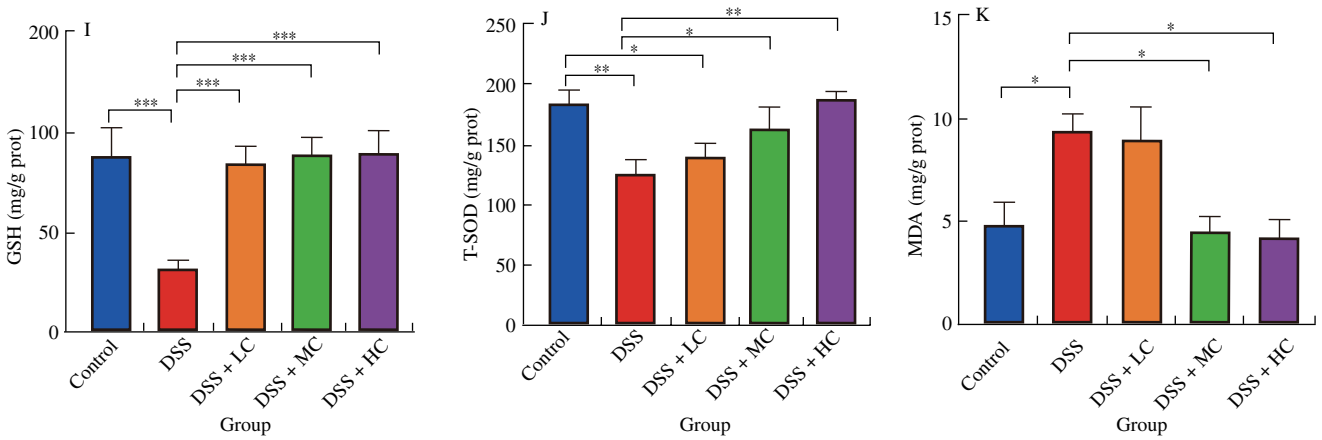


Fig. 2 (*Continued*)

3.3 BC99 mitigated oxidative stress in DSS-induced mice

DSS-induced UC usually causes a large amount of reactive oxygen species in colon, resulting in an imbalance of the redox system in mice and inflammatory damage to the colon tissue. GSH is an important free radical scavenger in the body. As shown in Fig. 2I, the concentration of GSH in DSS group was significantly decreased than that in Control group, which means the dysfunction of non-enzymatic antioxidant system in mice. After treated with BC99, the GSH concentration in DSS + LC, DSS + MC and DSS + HC groups increased significantly than DSS group. The GSH concentration increased by 181.89 % in DSS + HC group ($P < 0.001$). T-SOD, a key antioxidant enzyme, was found to be reduced in the DSS group but significantly elevated in the DSS + HC group (Fig. 2J, 48.73%, $P < 0.01$). MDA, a byproduct of lipid peroxidation, decreased 53.35% and 55.41% significantly in the DSS + MC and DSS + HC groups compared to the DSS group (Fig. 2K, $P < 0.01$), indicating reduced oxidative tissue damage. These results indicated that BC99 could effectively relieve oxidative stress of intestine in mice.

3.4 BC99 enhanced immune regulatory in DSS-induced mice

The pro-inflammatory cytokines (TNF-α, IL-1β) and the anti-inflammatory cytokines (IL-10), play an important role in immune regulatory in UC, were determined in this study. As shown in Fig. 3, the TNF-α and IL-1β concentration in DSS group increased and IT-10 decreased significantly compared with control group, indicating that inflammatory response in mice was aggravated by DSS induction. The TNF-α and IL-10 concentration in DSS + HC group were significantly reduced 24.87% and 46.81%, showed an effectively inhibition in the pro-inflammatory cytokines (Figs. 3E and G). However, the IL-1β concentration was no significant between DSS group and BC99-treated groups (Fig. 3F). Overall, BC99 appears to mitigate DSS-induced inflammation by suppressing pro-inflammatory cytokines and enhancing anti-inflammatory factors.

The signaling pathway that contribute to UC may be activated by pro-inflammatory cytokines (TNF-α, IL-1β) released from immune cells[22].

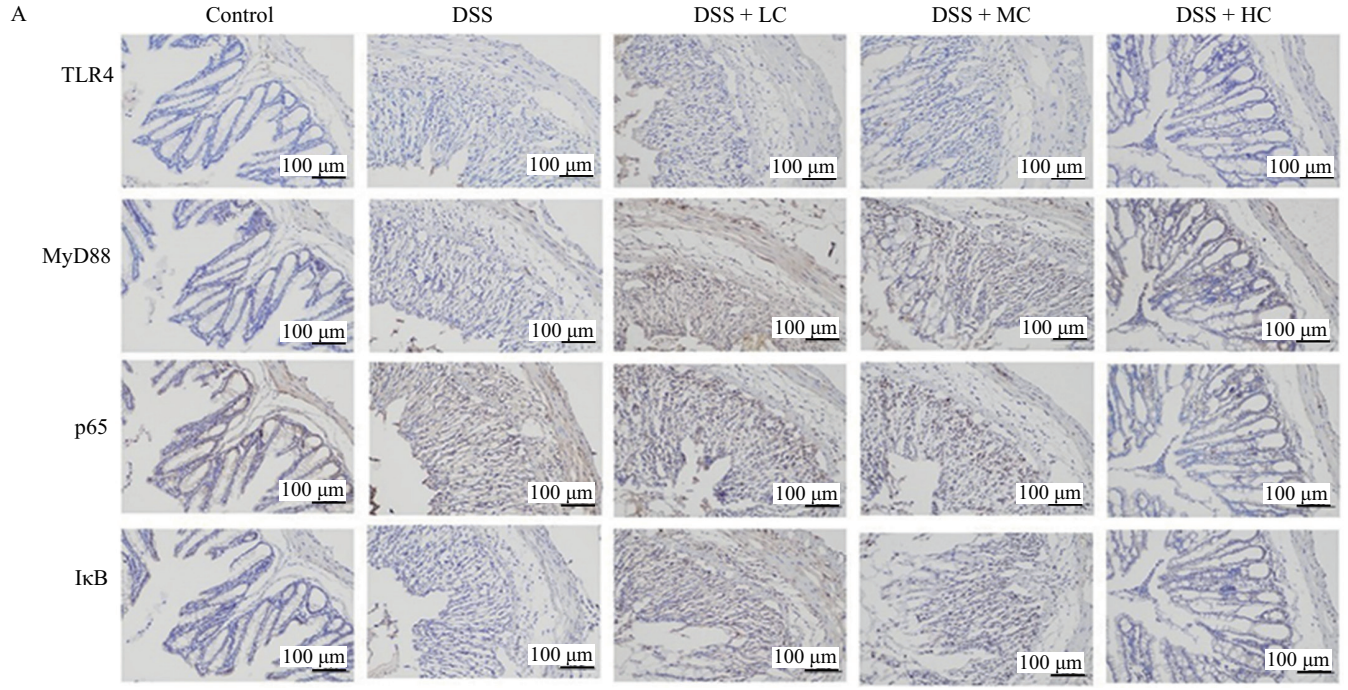


Fig. 3 BC99 regulated inflammatory response and TLR4/NF-κB signaling. (A) IHC images of TLR4, MYD88, p65 and IκB (200×); (B) Mean density of TLR4, MYD88, p65 and IκB; (C, D, H) Gene and protein expression of TLR4, MYD88, p65 and IκB; Concentration of (E) TNF-α, (F) IL-1β, and (G) IL-10. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

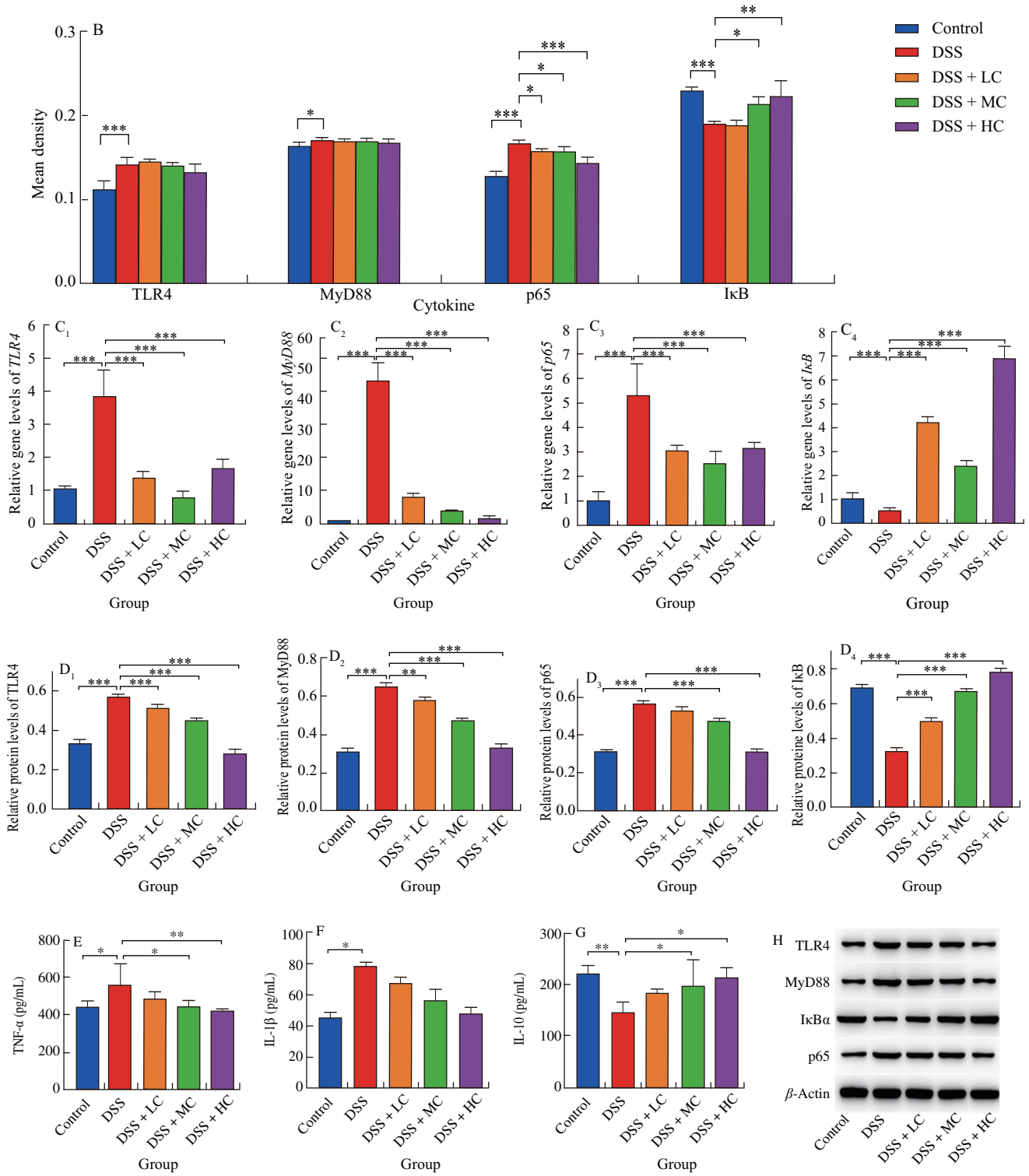


Fig. 3 (*Continued*)

Therefore, we investigated the effect of BC99 on inflammatory response signaling pathways in mice. As shown in Figs. 3E and F, the TNF-α and IL-1β concentration increased significantly in DSS group, but decreased after BC99 treated. Similarly, LPS concentration, which can specifically activate TLR4 and trigger an inflammatory response, was regulated as shown in Fig. 2H[23]. Immunohistochemistry revealed that TLR4, MyD88, and p65 expressions significantly increased in DSS-induced UC mice, with IκB downregulation. However, these expressions were restored to varying extents in the DSS + MC and DSS + HC groups (Figs. 3A and B). Compared to the DSS-induced group, the relative protein expression and relative gene expression of TLR4, MyD88 and p65 in BC99-treatment groups decreased, and there was no significant difference between the DSS-HC group and the control group (Figs. 3C, D and H), which suggesting that BC99 may inhibit the TLR4/NF-κB signaling pathway, thereby reducing pro-inflammatory cytokine secretion.

3.5 BC99 regulated gut flora in DSS-induced mice

16S rRNA was used to analyze evaluated the changes of the gut flora community in cecal contents after treated with BC99 in this study. The model stress < 0.1 and $P < 0.001$ through NMDS analysis, indicating that the model was reliable. Moreover, NEMD analysis also showed a separation in the gut flora structure among control group, DSS group and BC99-treated groups (Fig. 4A). Compared with control group, the Simpson index of DSS group increased, while BC99-treated groups decreased significantly than DSS group (Fig. 4B). The results showed that gut flora diversity of mice in DSS group was significantly reduced, while the gut flora diversity of mice in DSS + MC and DSS + HC groups was similarly to that of healthy group.

At the phylum level, Firmicutes and Bacteroidetes were absolutely dominant in all samples, accounting for 80%–90% of the total samples. The Wilcoxon rank sum test was used to explore the distribution of bacteria at different taxonomic levels in the 5 groups. The gut flora community at phylum level was shown in Fig. 4C. The dominant bacterial community in each group were Firmicutes, Bacteroidota, Actinobacteriota, Proteobacteria, Desulfobacterota. Compared with healthy control group, there was a significant decrease of Firmicutes and Actinobacteriota in the cecal contents in DSS group, the content of Bacteroidota increased. After treated with BC99, the Bacteroidota was remarkably decreased, while Firmicutes and Actinobacteria increased than DSS group (Fig. 4C).

Furthermore, at the genus level, the intestinal microbiota in all groups were mainly *Romboutsia*, Muribaculaceae, *Allobaculum*, and *Dubosiella* (Fig. 4D). Specifically, compared to the control group, the abundance of the main pathogenic bacteria (*Clostridium-sensu-stricto*-1, *Ruminococcus-gauvreauii*-group, *Turicibacter*, and *Prevotella*) causing UC, markedly increased in DSS group (Fig. 4F). The abundance of these bacteria decreased in BC99-treated groups, indicating that BC99 could effectively inhibit the growth of pathogenic bacteria and maintain healthy intestinal microenvironment. In addition, the abundance of four potential probiotic bacteria (*Bifidobacterium*, *Blautia*, *Lachnoclostridium*, *Butyricimonas* and *Faecallbaculum*) increased in the DSS + HC group (Fig. 4F). The differentially abundant bacterial taxa in DSS-induced mice response to BC99 treatment were further identified by LEfSe analysis. The opportunistic pathogens (*Allobaculum* and *Clostridium-sensu-stricto*-1) were enriched in DSS group, while the potential beneficial microorganisms (*Faecallbaculum*, *Blautia* and *Bidobacterium*), which produced SCFAs, enriched in DSS + HC group (Figs. 4E and G). Moreover, further analysis through thermal map of the top 50 species in genus abundance showed significant differences between Control and DSS groups, and the intestinal microbiota of DSS + MC and DSS + HC groups was similar to that of the control group. These results provide the evidence for the regulation of BC99 to the gut flora community in DSS-induced mice.

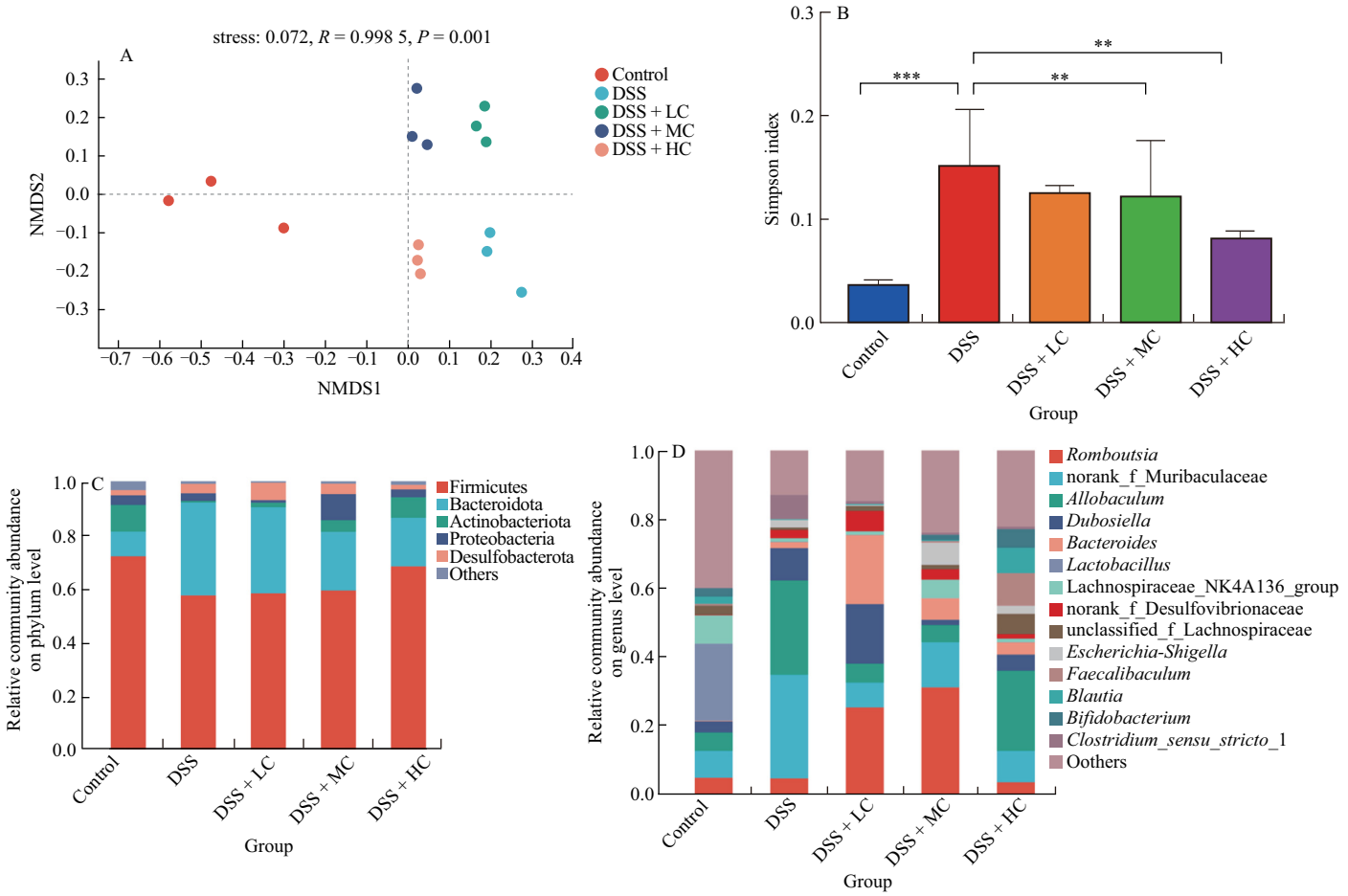
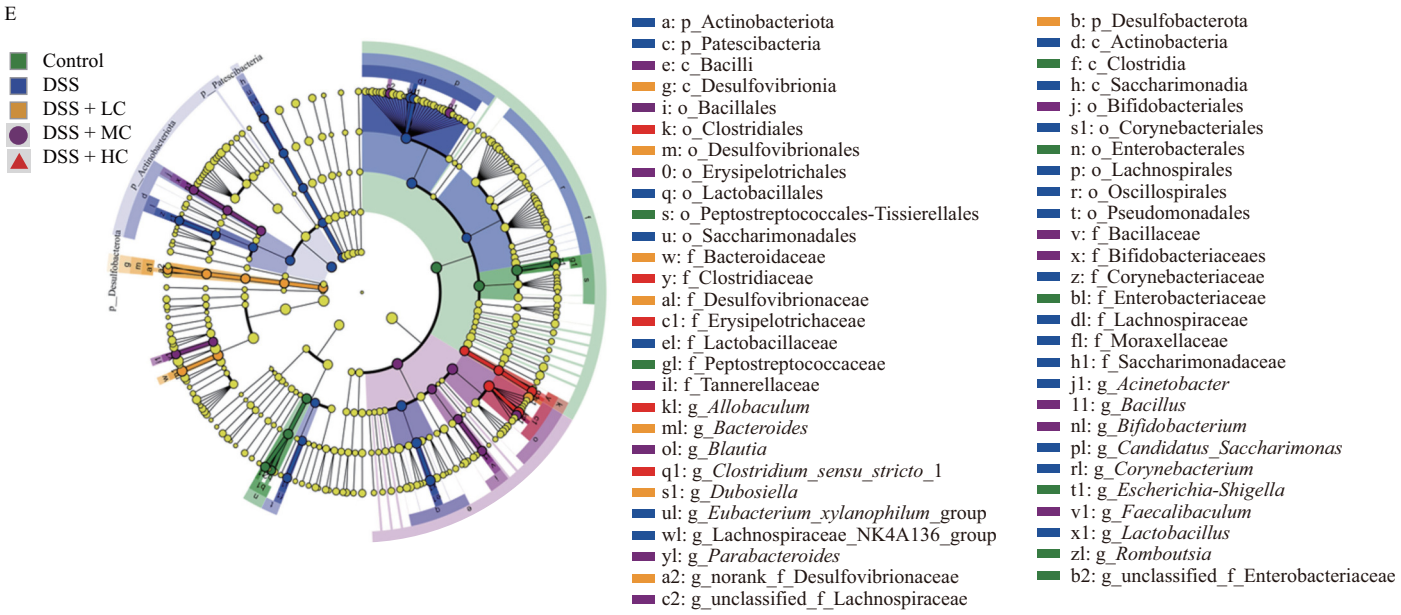


Fig. 4 (A) Gut flora of cecal contents. NMDS on OUT level; (B) Simpson index; (C) Relative community abundance on phylum level; (D) Relative community abundance on genus level; (E) Cladogram; (F) Heatmap of the differential microorganism at the genus among all groups; (G) Distribution histogram based on LDA (LDA score > 4.0).

E



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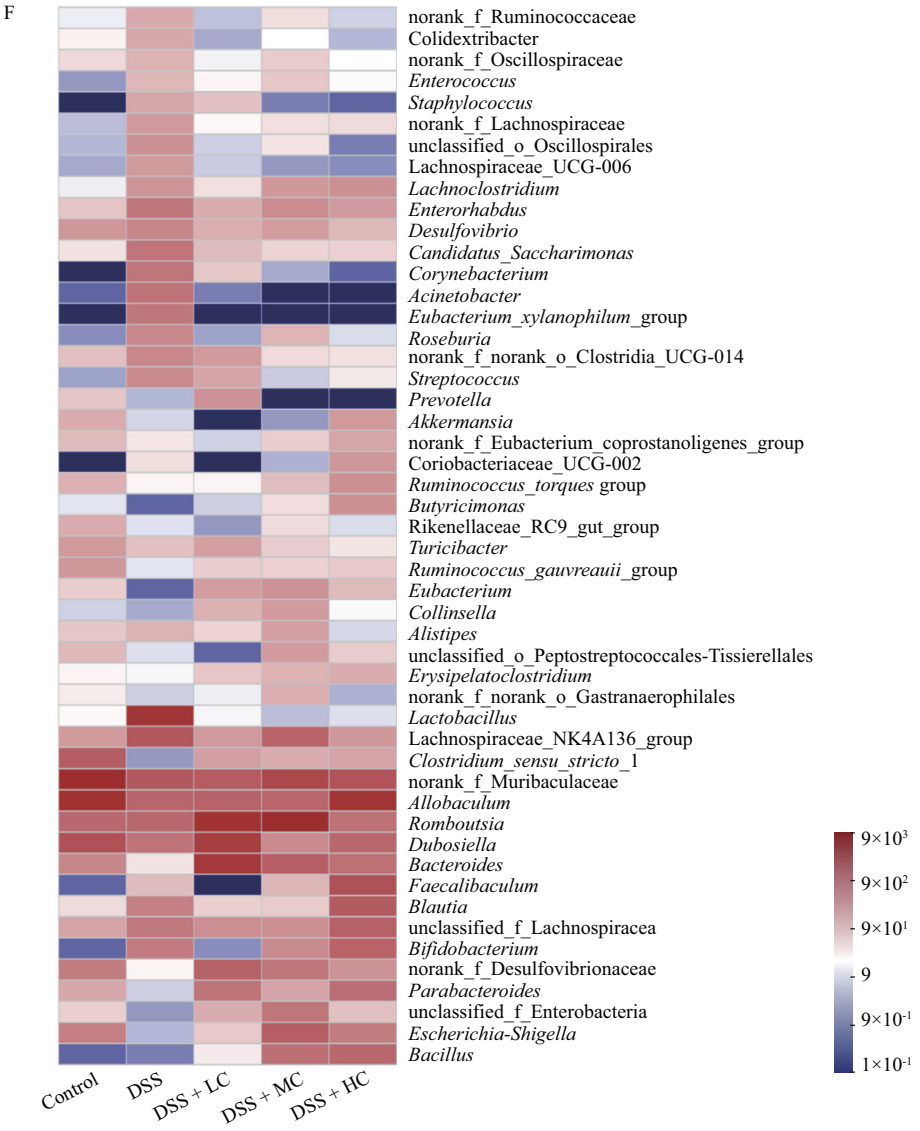

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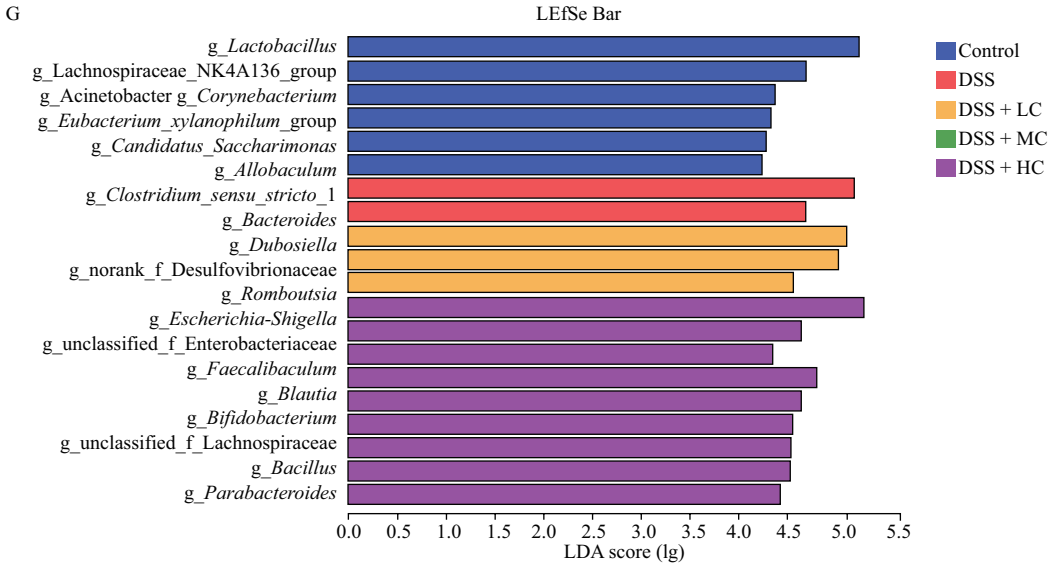


Fig. 4 (*Continued*)

The correlation between gut flora, inflammatory factors was analyzed, and the key strains to alleviate the inflammatory response of BC99-treated DSS-induced mice were studied. At the genus level, the *Dubosiella*, *Clostridium_sensu_stricto*_1, *Ruminococcus_gauvreauii*_ group, *Prevotella* were positively correlated with pro-inflammatory cytokines in DSS-induced mice. In addition, *Faecalibaculum*, *Blautia*, *Bifidobacterium*, *Bacillus*, *Enterorhabdus*, *Candidatus_Saccharimonas*, *Lachnoclostridium*, *Eubacterium_ xylanophilum*_group, *Streptococcus*, *Roseburia*, Coriobacteriaceae_UCG-002, and *Enterococcus* exhibited the remission effect of inflammatory response. Moreover, these strains showed a significant negative correlation with pro-inflammatory cytokines, but extremely positive correlation with anti-inflammatory cytokines, and length to weight ratio of colon (Fig. 5A).

At the species level, the strains such as *Delftia_tsuruhatensis* and *Citrobacter_rodentium*_ICC168 played a driving role in promoting the inflammatory response. While *Bifidobacterium_animalis*, *Phascolarctobacterium_faecium*, *Burkholderiales_bacterium*_YL45, *Bacillus_coagulans*, *Streptococcus_hyointestinalis*, *Enterococcus_ faecium* and other strains played an important role in alleviating the disease species in UC mice (Fig. 5B). In addition, there were some unclassified or uncultured strains at the genus and species levels, and they were extremely associated with inflammatory responses, which may be the focuses in the future.

3.6 Relationship between the levels of SCFAs and microbial signatures or the expression of inflammatory cytokines in DSS-induced mice treated with BC99

SCFAs are one of the main metabolites produced by gut flora, and play an important role in maintaining intestinal balance and relieving UC. Compared with the Control group, the content of acetic acid, propionic acid, and butyric acid in DSS group was significantly decreased ($P < 0.01$), and the contents of acetic acid, propionic acid and butyric acid in BC99-treated groups increased significantly in a stepwise manner with the intake dose of BC99 (Fig. 5C). These results indicated that BC99 may alleviate UC by increasing the content of SCFAs.

Furthermore, the relationship between SCFAs and gut flora was investigated by Pearson correlation analysis. *Faecalibaculum*, *Blautia*, *Bifidobacterium*, *Bacillus*, *Enterohabdus*, *Lachnoclostridium*, *Eubacterium_xylanophilum*_group, *Roseburia* were positively correlation with acetic acid, propionic acid and butyric acid. However, the *Clostridium_sensu_stricto*_1 and *Turicibacter* increased significantly in DSS group, which showed a negative correlation with SCFAs. The microbiota was strongly correlated with SCFAs content (Fig. 5D). The microbiota is closely related to the content of SCFAs in the intestine. The results showed that treatment with BC99 could colonize more bacteria in the intestine and produced more SCFAs in mice.

Additionally, the correlation between SCFAs and TLR4/NF-κB signaling pathway-related proteins was investigated. Acetic acid, propionic acid, and butyric acid showed positive correlations with IL-10 and IκB, but negative correlations with TNF-α, IL-1β, TLR4, MyD88, and p65 (Fig. 5E). These results suggested that SCFAs exerted anti-inflammatory effects by inhibiting the activation of TLR4/NF-κB signaling pathway and regulating the expression of inflammatory cytokines.

3.7 BC99 regulates the metabolite in DSS-induced UC mice

The untargeted metabonomics were used to explore the intervention of BC99 on DSS-induced UC mice. The principle component analysis (PCA) showed divergences in Fig. 6A. There are significant differences in intestinal contents between healthy and diseased mice. As shown in Fig. 6B, compared with the control group, there were 19 up-regulated and 45 down-regulated metabolites were traced in DSS group. However, compared with DSS group, there were 41 up-regulated and 19 down-regulated metabolites in DSS + HC group (VIP > 1 and $P < 0.05$; Fig. 6B, Table S2).

Cluster analysis based on Euclidean distance was used to study the relationship of metabolites between DSS-induced and BC99-treated groups. The methylnoradrenalin, succinic acid, and cholic acid-related bile acid metabolites were significantly down-regulated in DSS group, and pantothenic acid, glutaconic acid and other metabolites were significantly up-regulated in DSS group and DSS + HC group.

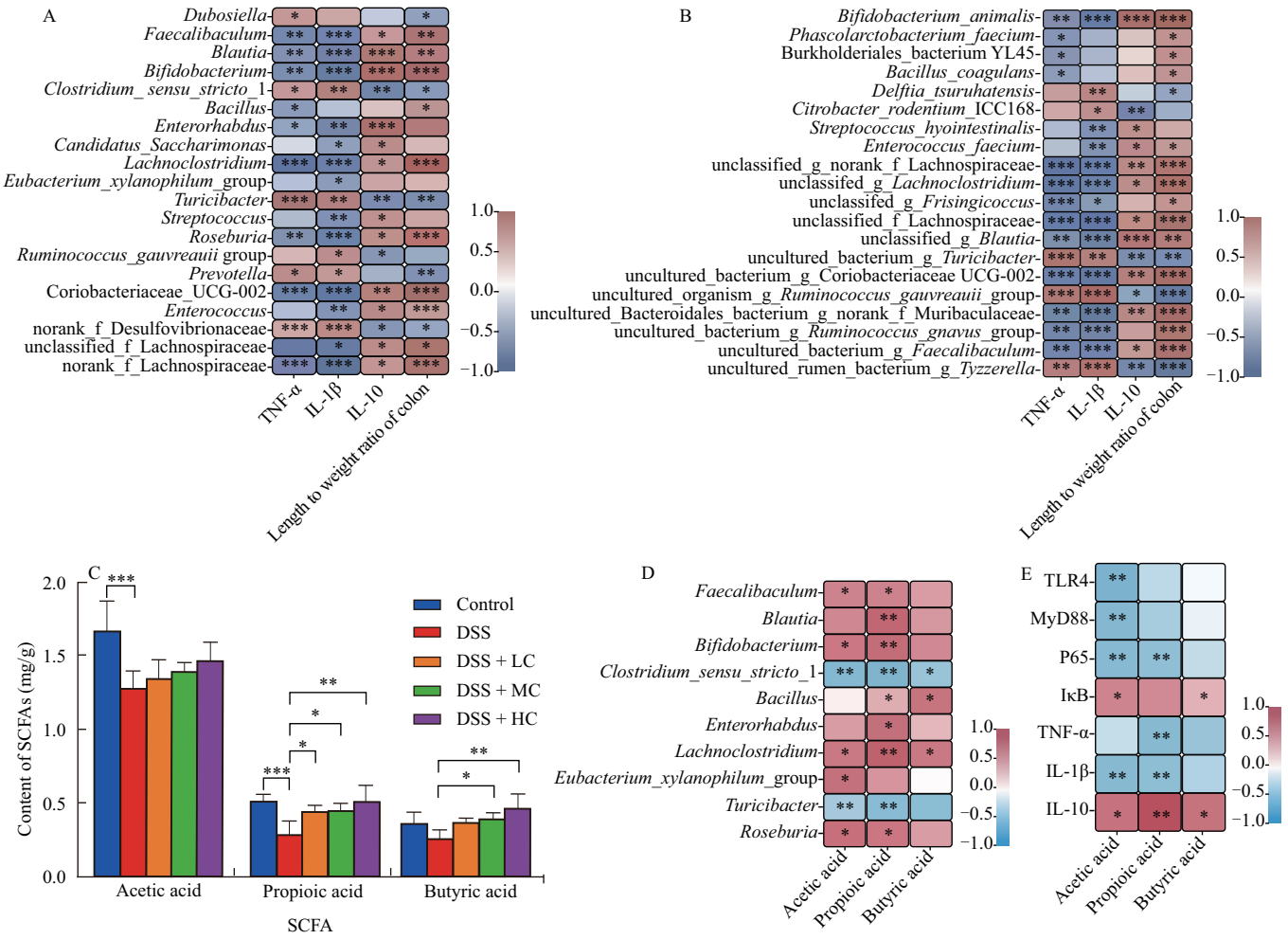


Fig. 5 (A) Heatmap of genus level correlations between gut flora and colonic length to weight ratio, TNF-α, IL-1β, IL-10; (B) Heat map of species level of gut flora to Colonic length to weight ratio, TNF-α, IL-1β, IL-10. (C) SCFA content determined by gas chromatography-mass spectrometry. (D) Spearman's correlation heat map of bacterial species and SCFAs. (E) Correlation heat map of SCFAs and TLR4, MyD88, p65, IκB, TNF-α, IL-1β and IL-10. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

However, the metabolism in BC99-treated mice was basically the same as that of the control group (Fig. 6C). Moreover, the metabolism pathways of tryptophan, glycine, serine, threonine and bile acid were enriched in the Fig. 6E.

The correlation between gut flora and metabolites was showed in Figs. 6F and G. There was a positive correlation between various probiotics such as *Bifidobacterium*, *Enterobacterium*, *Bacillus*, and some metabolites classified as organic acids and derivatives, as well as nucleosides, nucleotides and their analogues (Fig. 6F). Similar results were also observed at species levels (Fig. 6G). The 7-dehydrocholesterol and 24-dihydroxyvitamin D3 are precursors in the synthesis of VD3[24]. Compared with DSS group, the samples in BC99-treated group showed an upregulation of 7-dehydrocholesterol and 24-dihydroxyvitamin D3 (Table S2), as well as an upregulation of bile acid metabolites (Fig. 6C). The biosynthesis pathway of primary bile acids is also enriched in Fig. 6E.

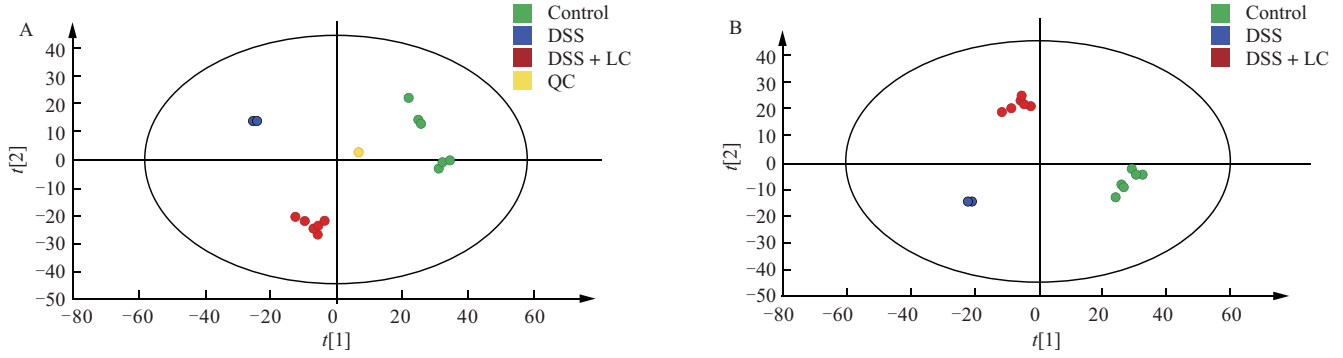
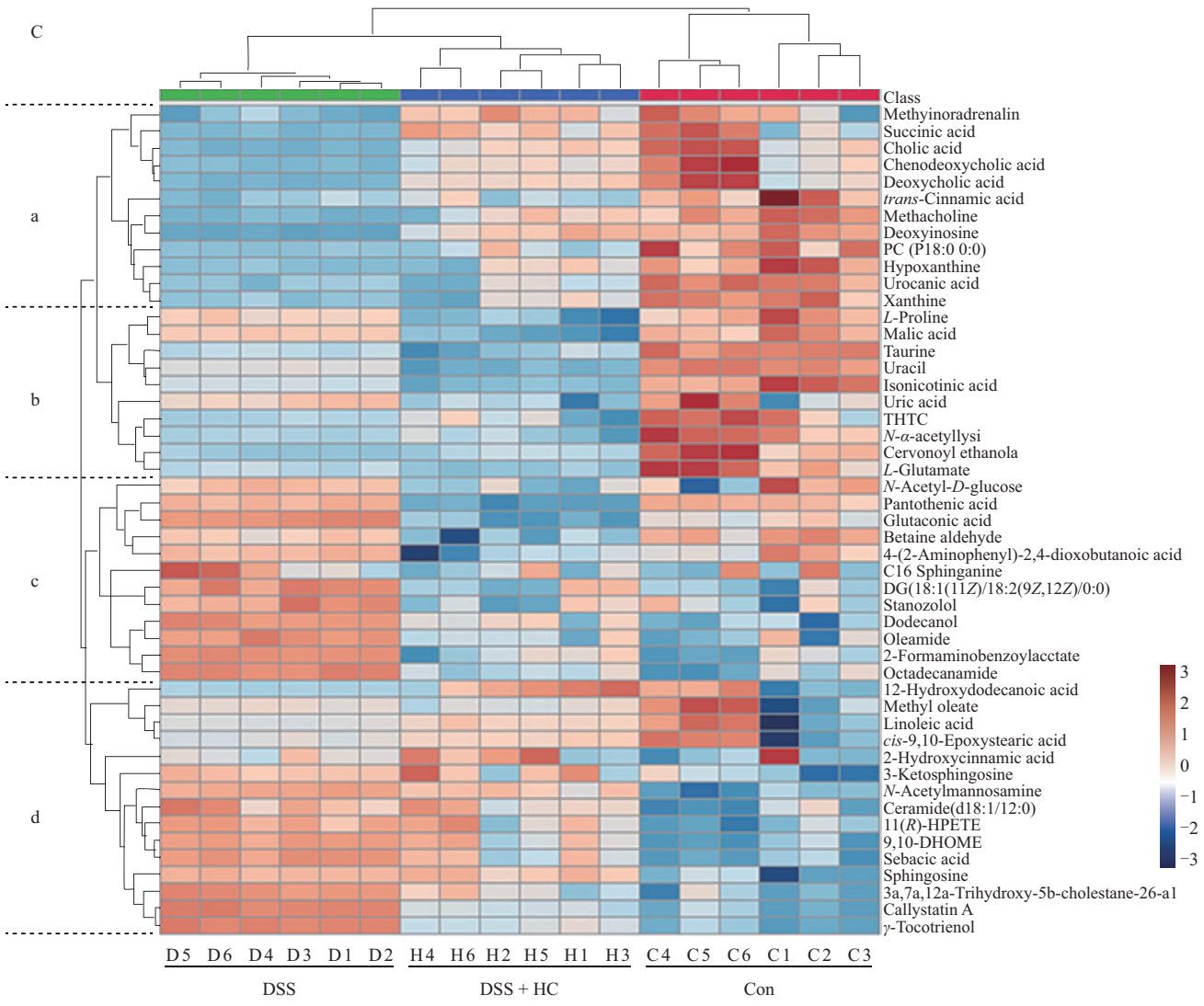


Fig. 6 Metabolomic analysis of cecal contents. (A) The PCA cluster diagram; (B) PLS-DA analysis; (C) Heat map of metabolites in mouse caecum contents. Metabolic pathway construction based on MetPA database, (D) Control *vs*. DSS group; (E) DSS group *vs*. DSS + HC group. Heatmap of correlation analysis between differential metabolites and intestinal microflora, (F) genus level; (G) species level.



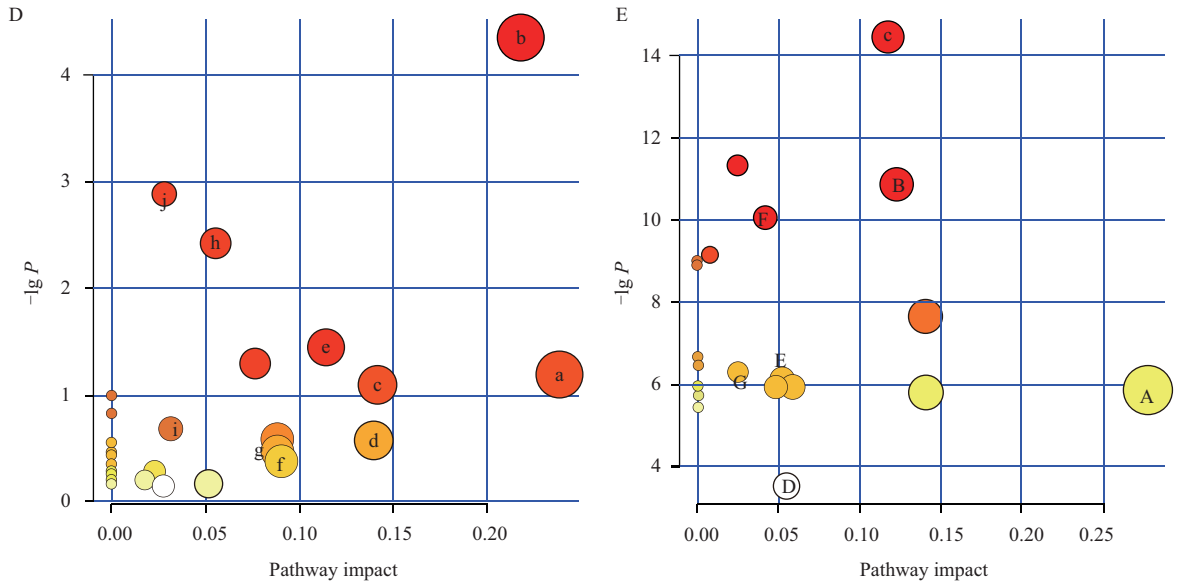


Fig. 6 (*Continued*)

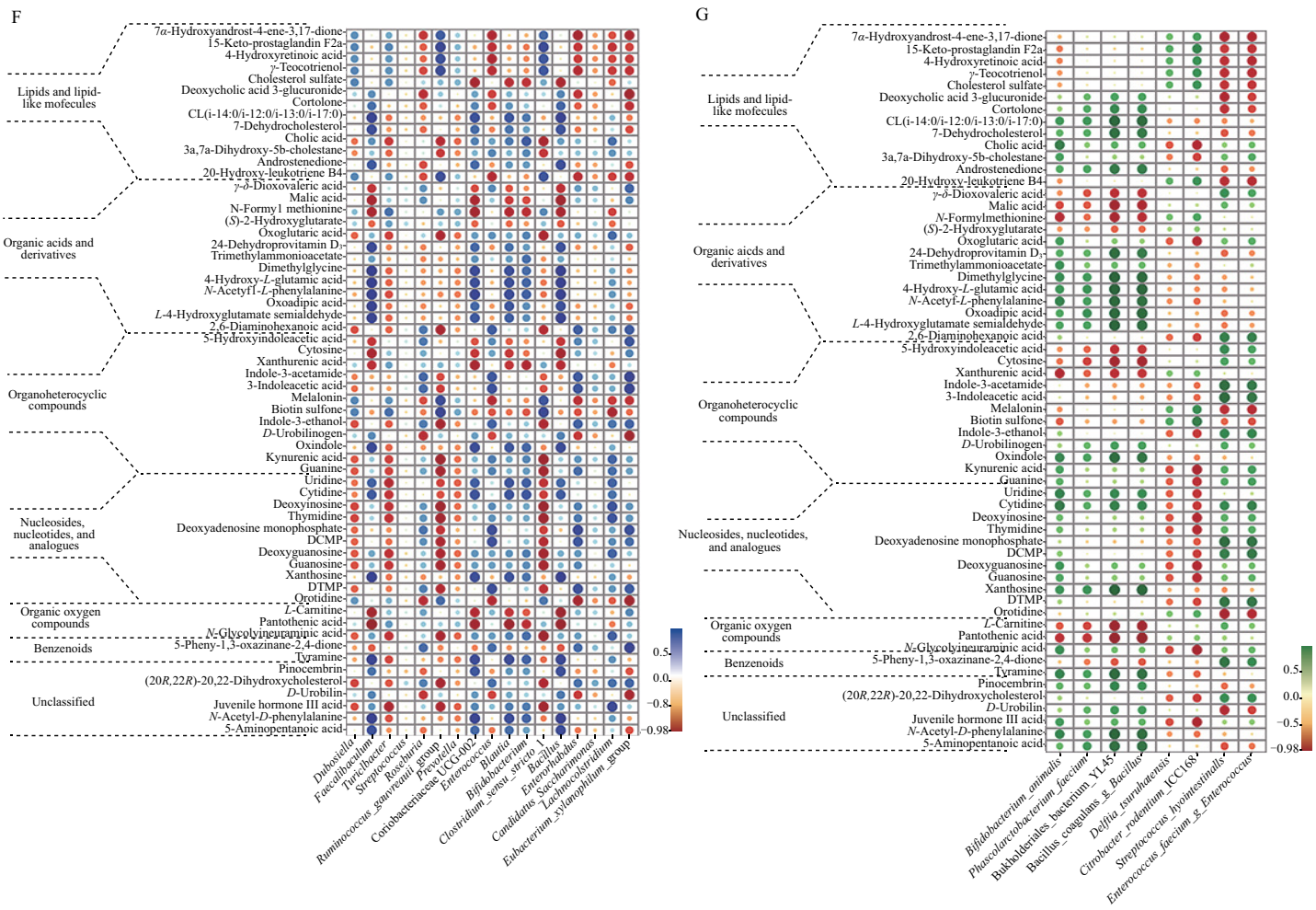


Fig. 6 (*Continued*)

4. Discussion

UC is a chronic, recurrent intestinal inflammatory disease, often linked to disrupted intestinal barriers, gut flora imbalances, and inflammatory cytokine dysregulation[25]. The DSS-induced mouse model, exhibiting symptoms like weight loss, diarrhea, and colon shortening, effectively mimics these UC characteristics. This study highlights the therapeutic potential of BC99 in UC treatment. BC99, known for its high acid resistance and stability, significantly alleviated DSS-induced UC symptoms in mice. These findings reinforce the role of probiotics, particularly spore-forming strains like BC99, in managing complex pathologies of UC. Our research contributes to the growing evidence supporting probiotic use in UC, positioning BC99 as a promising candidate for future therapeutic strategies against this chronic inflammatory condition.

Numerous clinical studies have shown that the intestinal mechanical barrier can effectively prevent harmful substances, such as bacteria and endotoxin, from entering the bloodstream through the intestinal mucosa[26]. The main features of intestinal mucosal mechanical barrier, including the enlargement of intercellular space, the loss of crypt structures and goblet cells, and the increase of inflammatory infiltration of the lamina propria, are closely related to the occurrence and development of UC[27-28]. Previous studies have found that intestinal epithelial cells, mucin layer, and tight junction proteins played the important roles in maintaining the integrity of colon tissue[25,29-30]. In the present study, BC99 treatment restored normal epithelial structure of the intestinal mucosa and enhanced the expression of occludin and ZO-1 in colonic tissues, which is consistent with previous study[31]. Additionally, the LPS concentration in serum of DSS-induced UC mice treated with BC99 decreased significantly. The results indicated that BC99 treatment had a protective effect to restore the integrity of intestinal mechanical barrier and intestinal permeability in DSS-induced UC mice.

The oxidative damage is an inescapable factor on intestine. As an important free radical scavenger in body, GSH reduce cell damage in tissues, and helpful to activated and differentiated of immune cells, thereby enhancing immunity[32]. As one of the important antioxidant enzymes, T-SOD can decompose the reactive oxygen species produced by the body into oxygen and hydrogen peroxide, thereby inhibiting lipid peroxidation in colon tissues[33]. Nevertheless, MDA, a product of lipid peroxidation, can reflect the degree of lipid peroxidation in the tissues and indirectly reflect the damage degree of histiocytes[34]. The results showed that GSH and T-SOD were significantly increased, and MDA is significantly decreased after BC99 treatment, which indicates that BC99 could effectively improve the oxidative stress level of the colon of the UC mice, thereby reducing the injury of the intestinal mucosa.

Inflammatory cytokines play a crucial role in the pathogenesis of UC. Previous studies have shown that overexpression of TNF-α may aggravate intestinal mucosal injury, and IL-1β promote the expression

of myosin light chain kinase, thereby increasing intestinal wall permeability[35-36]. Besides, IL-10 alleviate UC by protecting intestinal barrier[37-38]. In this study, we found that BC99 significantly down-regulated the expression of pro-inflammatory cytokines (TNF-α and IL-1β), while up-regulated the anti-inflammatory cytokines (IL-10). All these data suggest that BC99 could improve immune regulatory in DSS-induced UC mice. The adverse factors may induce oxidative stress reactions, which produces oxygen-containing derivatives that further activate inflammation and amplify the inflammatory response[39-40].

NF-κB has been proved to be one of the key pathways to activate inflammation[41-42]. Toll-like receptors (TLRs), as one of the main types of pattern recognition receptor families, mainly induce inflammation responses and establish adaptive immunity. Among TLRs receptors, pathogen associated molecular patterns recognized by TLR4 are activated, and MyD88 is recruited to induce IκB activation and degradation, release and exposure of NF-κB core localization sequence to promote NF-κB p50 and NF-κB p65 translocates from cytoplasm to nucleus and binds to DNA, inducing the release of a large amount of inflammatory cytokines in colon tissue and exacerbating inflammation in UC patients[43-45]. Therefore, TLR4/MyD88/NF-κB signaling pathway is an important target for drug therapy or nutritional intervention in UC patients. The results in this study showed that the relative protein expressions and gene expressions of TLR4, MyD88 and p65 were highly up-regulated in DSS-induced UC mice, and IκB down-regulated. In contrast, the expressions of p65 and IκB was showed an opposite trend after the intervention of middle and high dose of BC99. Relevant studies have shown that lipopolysaccharide could significantly up-regulate the expression of inflammatory pathway proteins TLR4, MyD88, NF-κB, IL-1β, IL-6 and TNF-α in the cecum of rat, but *W. coagulans* TL3 could significantly reverse this phenomenon, which could protect the cecum of rat from lipopolysaccharide-induced inflammatory injury by inhibiting the activation of NF-κB and the expression of downstream pro-inflammatory cytokines[46]. The relative expression of *TLR4*, *MyD88* and *NF-κB* are consistent with those reported by Wang et al.[46]. Therefore, it was speculated that BC99 may inhibit the regulates the release of pro-inflammatory cytokines and anti-inflammatory cytokines by regulating the TLR4/NF-κB signal pathway in DSS-induced UC mice.

The gut flora and pathogens, as important component of the micro-ecological environment in intestine, may lead to certain diseases in humans due to their changes of species and functions. In this study, the diversity of gut flora was improved in BC99 treatment group, indicating the effective regulation of the microbial community, while the relative abundances of *Allobaculum*, *Clostridium_sensu_stricto*_1, *Ruminococcus_gauvreauii*_group, *Turicibacter*, and *Prevotel* were decreased in BC99-treated groups than DSS group[47-48]. In addition, the ratio of Firmicutes/Bacteroidetes decreased in the DSS group, but increased after BC99 supplementation. This result was consistent with many previous animal and clinical studies. In addition, the abundance of *Actinomycetes* increased after ingestion of BC99, which may be due to a significant increase in the abundance of *Bididobacterium*. The therapeutic effect of *Bididobacterium* on UC also demonstrated in several studies[49-50]. *B. coagulans* could consume free oxygen in the intestine, providing a better anaerobic environment for the growth of *Bididobacterium*[51].

In recent years, researchers have focused on the key strains associated with the inflammatory responses and alleviate UC symptoms by ingesting anti-inflammatory strains. Tyler noted that *Allobaculum* and its associated taxa were important drivers of human IBD and potential therapeutic targets for the treatment of inflammatory diseases[52]. *Clostridium_sensu_stricto*_1 belongs to *Clostridium* cluster I and has been found to have potential pathogenicity[53]. *Ruminococcus_gauvreauii*_group is considered to be the most changed microbiota in IBD, which usually increases significantly during the active stage of disease. Moreover, *Ruminococcus_gauvreauii*_group can produce a polysaccharide, such as TNF-α, which induces the secretion of inflammatory cytokines by dendritic cells. Furthermore, *Turicibacter* is often found to be increased in patients with IBD, and *Prevotella* was found to inhibit the secretion of the anti-inflammatory cytokines IL-18 and increase intestinal permeability[54]. The DSS-induced UC mice have marked changes in the species abundances of intestinal probiotics and pathogens, leading to aberrant exacerbations of inflammatory responses[55]. The probiotics associated with anti-inflammatory activity increased in the BC99 treated mice in this study. At the genus level, BC99 treatment markedly reduced the abundance of *Faecalibaculum*, *Blautia*, *Bifidobacterium*, *Bacillus*, *Enterorhabdus*, *Candidatus*, *Lachnoclostridium*, *Eubacterium*, *Streptococcus*, *Roseburia*, Coriobacteriaceae, *Enterococcus*, which were significantly and positively correlated with anti-inflammatory cytokines, and the similar results were also observed at species level. *B. coagulans* spores are highly resistant to gastric acid and bile, and can be colonized and produce lactic acid in the intestine, thereby reducing intestinal pH and inhibiting pathogenic bacteria[56]. In addition, *B. coagulans* can also secrete amino acids, vitamins, proteases, amylases and other metabolites to promote the growth of beneficial bacteria in intestine, and cooperate with them to alleviate intestinal inflammation.

SCFAs, the indirect nutrients produced by the gut flora, play an important role in modulating immune activity and enhancing epithelial the barrier function in the gut. Acetic acid, propionic acid and butyric acid, important components of SCFAs, related to the occurrence of UC[57]. For example, previous studies proved that the relieved intestinal barrier injury and inflammation by regulating the cascade response of microbiota-regulated SCFAs-TLR4/MyD88/NF-κB cascade response in Acrylamide-induced rats[58]. In our study, acetic acid, propionic acid and butyric acid decreased significantly in DSS group, and increased gradually after BC99 treatment with different doses. This conclusion is further supported by the previous study[55]. The enrichment of acetic acid, propionic acid and butyric acid in the DSS + HC group in the determination of SCFAs, which confirmed the increase of SCFAs-producing bacteria in the intestine. At the same time, we observed that *Faecalibaculum*, *Blautia*, *Bifidobacterium*, *Bacillus*, *Enterorhabdus* and *Lachnoclostridium* had a strong positive correlation with the increase of acetic acid, propionic acid and butyric acid, while *Clostridium* and *Turicibacter* had a strong negative correlation with SCFAs in DSS group. Supplementation with BC99 increased the abundance of SCFAs-producing probiotics such as *Faecallbaculum*, *Blutia* and *Bididobacterium*. *Faecallbaculum* and *Blutia* were found to be significantly reduced in patients with colon cancer, and they maintained intestinal epithelial barrier function through production of SCFAs, regulate glucose and lipid metabolism, improve intestinal balance of nature, and inhibit cancer cells and intestinal inflammatory responses[59-60]. These studies provide the evidence that BC99 could regulate the gut flora of UC mice to produce more SCFAs. SCFAs are also involved in the

development of regulatory T cells (Tregs), affecting the activity of macrophages and the secretion of anti-inflammatory cytokines[61]. Feng et al.[62] found that SCFAs could inhibit the activation of NF-κB by preventing the translocation of p65 into the nucleus. SCFAs in the gut are consistent with a conserved signaling receptor signature of TLR4, and thus SCFAs can inhibit the inflammatory response triggered by activation of TLR4[63-64]. In addition, Tian et al.[65] also found that SCFAs mixture could improve the symptoms of DSS-induced by colitis by improving colon inflammation, and inhibiting the expression of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-7. Therefore, the increase of SCFAs content after probiotic intervention is expected to alleviate colitis. Probiotics could regulate the release of immune cytokines IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α and IFN-γ by inhibiting TLR4/NF-κB signaling pathway and PI3K/AKT/NF-κB signaling pathway[66]. These data suggested that BC99 could regulate the inflammatory response of mice by promoting the production of SCFAs by beneficial bacteria, thus protecting the intestinal barrier. Moreover, the results of metabolomics showed that bile acid metabolism, purine metabolism and pyrimidine metabolism were involved in the process of BC99 regulating UC induced by DSS. Bile acid metabolism plays an important role in intestinal barrier function and inflammatory homeostasis, once bile acid metabolism is disordered, it will cause intestinal barrier dysfunction and mucosal inflammation[67]. The results showed that the level of bile acid metabolites was up-regulated after BC99 intervention. Uridine, cytidine, xanthine and other metabolites related to pyrimidine and purine metabolism also changed, which indirectly proved that BC99 could alleviate intestinal barrier dysfunction.

There was positive correlation between gut flora and metabolites at genus level and species level. This indicates that pathogenic bacteria may cause disturbances in bile acid metabolism, purine metabolism, and pyrimidine metabolism, thereby promoting the occurrence of inflammatory reactions and exacerbating UC symptoms in mice. Previous studies have shown that the bile acids play important roles in various important physiological activities, such as immune inflammation and metabolism through binding with vitamin D receptor (VDR)[68], which relieving intestinal barrier function by inhibiting NF-κB signal pathway[69-70]. Nucleotides, including pyrimidine and purine, are precursors for the synthesis of DNA and RNA, and are involved in almost all biochemical activities in the body. Xanthine is a metabolite produced during purine metabolism. This study found that uridine, cytidine and xanthosine were correlated with the probiotics, indicating that BC99 could enhance pyrimidine and purine metabolism, thereby enhancing immune system and better resisting inflammatory responses.

5. Conclusion

In conclusion, the present study revealed that BC99 has a significant protective effect on the morphology of pathological tissue, the physiological indexes of serum and colon, and the related indexes of intestinal barrier in DSS-induced UC mice. The potential mechanism of BC99 in alleviating UC symptoms is shown in Fig. 7. These results provide new insights into the further investigation for mitigating symptoms in human UC.

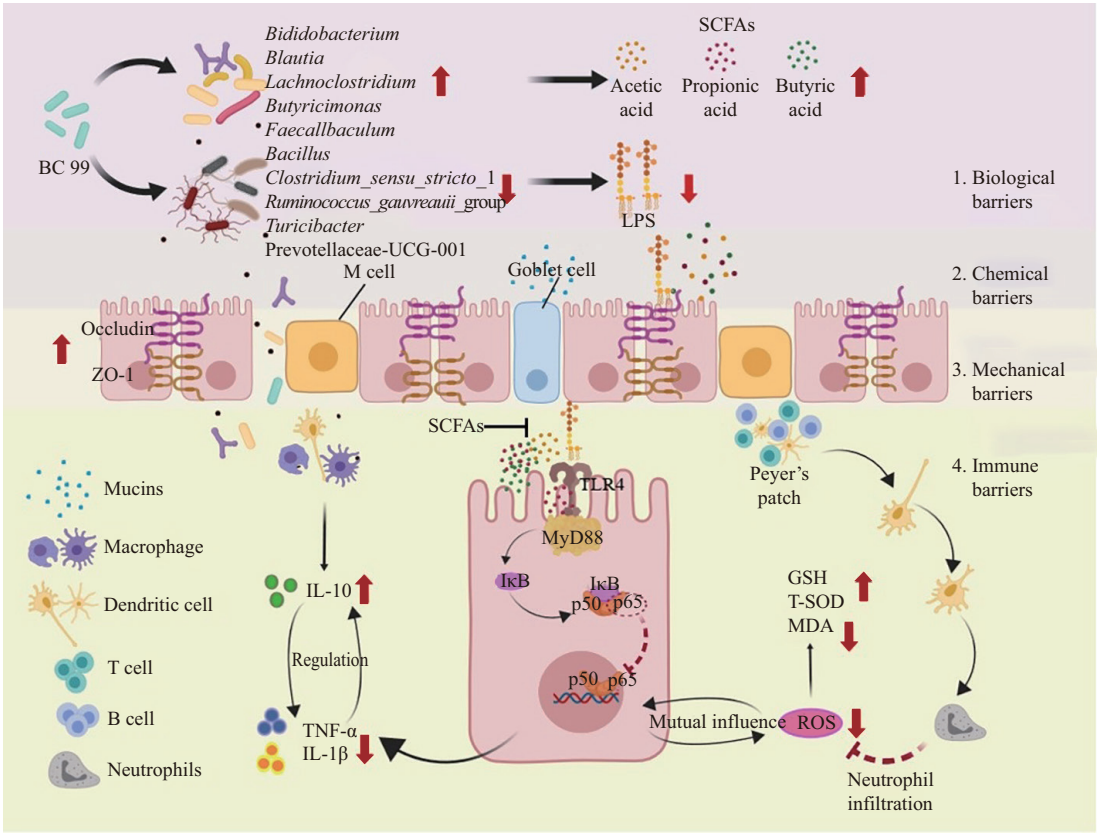


Fig. 7 Potential mechanism of action of BC99 in relieving DSS-induced UC. BC99 intervention increased the abundance of SCFAs producing bacteria, resulting in more SCFAs being produced. SCFAs affect TLR4/NF-κB signal pathway inhibits inflammatory response. At the same time, the abundance of gram-negative pathogenic bacteria that produce LPS decreases, making LPS less secreted. The decrease in LPS causes a small number of TLR4 receptors to be activated, thereby inhibiting LPS/TLR4/NF-κB signal pathway, reducing the secretion of pro-inflammatory cytokines.

Availability of data and material

The 16S rRNA gene sequence of mice fecal has been submitted to NCBI genome database under SRP486629.

Competing interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250351.

References

[1] R.V. Bryant, D.C. Burger, J. Delo, et al., Beyond endoscopic mucosal healing in UC: histological remission better predicts corticosteroid use and hospitalisation over 6 years of follow-up, Gut 65(3) (2016) 408-414. https://doi.org/10.1136/gutjnl-2015-309598.

[2] L. Agüero, D. Zaldivar-Silva, L Peña, et al., Alginate microparticles as oral colon drug delivery device: a review, Carbohydr. Polym. 168 (2017) 32-43. https://doi.org/10.1016/j.carbpol.2017.03.033.

[3] S. Tie, Y. Chen, M. Tan, An evaluation of animal models for using bioactive compounds in the treatment of inflammatory bowel disease, Food Front. 5(2) (2024) 1-20. https://doi.org/10.1002/fft2.360.

[4] R. Opheim, M.L. Hoivik, I.C. Solberg, et al., Complementary and alternative medicine in patients with inflammatory bowel disease: the results of a population-based inception cohort study (IBSEN), J. Crohns Colitis 6(3) (2012) 345-353. https://doi.org/ 10.1016/j.crohns.2011.09.007.

[5] T. Sairenji, K.L. Collins, D.V. Evans, An update on inflammatory bowel disease, Primary Care 44 (2017) 673. https://doi.org/10.1016/j.pop.2017.07.010.

[6] B.P. Blackwood, C.Y. Yuan, D.R. Wood, et al., Probiotic *Lactobacillus* species strengthen intestinal barrier function and tight junction integrity in experimental necrotizing enterocolitis, J. Probiotics Health 5(1) (2017) 159. https://doi.org/10.4172/2329-8901.1000159.

[7] C. You, J. Xing, J. Sun, et al., Anti-inflammatory and anti-oxidant impacts of lentinan combined with probiotics in ulcerative colitis, Mol. Biotechnol. 66(10) (2024) 2778-2791. https://doi.org/10.1007/s12033-023-00878-w.

[8] M.S. Geier, R.N. Butler, G.S. Howarth, Inflammatory bowel disease: current insights into pathogenesis and new therapeutic options; probiotics, prebiotics and synbiotics, Int. J. Food Microbiol. 115(1) (2007) 1-11. https://doi.org/10.1016/j.ijfoodmicro.2006.10.006.

[9] H. Hao, X. Zhang, L. Tong, et al., Effect of extracellular vesicles derived from *Lactobacillus plantarum* Q7 on gut microbiota and ucerative colitis in mice, Front. Immunol. 12 (2021) 777147. https://doi.org/10.3389/fimmu.2021.777147.

[10] K.C.D.O. Vieira, A.B.B. Da Silva, S.A. Felicio, et al., Orange juice containing *Pediococcus acidilactici* CE51 modulates the intestinal microbiota and reduces induced inflammation in a murine model of colitis, Sci. Rep. 13(1) (2023) 18513. https://doi.org/10.1038/s41598-023-45819-4.

[11] Z. Wu, J. He, Z. Zhang, et al., Propionic acid driven by the *Lactobacillus johnsonii* culture supernatant alleviates colitis by inhibiting M1 macrophage polarization by modulating the MAPK pathway in mice, J. Agric. Food Chem. 71(41) (2023) 14951-14966. https://doi.org/10.1021/acs.jafc.3c00278.

[12] J. Shang, S. Yang, Z. Tang, et al., *Bifidobacterium bifidum* H3-R2 and its molecular communication within the context of ulcerative colitis. J. Agric. Food Chem. 70(37) (2022) 11678-11688. https://doi.org/10.1021/acs.jafc.2c02909.

[13] K. Enck, S. Banks, H. Yadav, et al., Development of a novel oral delivery vehicle for probiotics, Curr. Pharm. Des. 26 (2020) 3134-3140. https://doi.org/10.2174/1381612826666200210111925.

[14] W. Pu, H. Zhang, T. Zhang, et al., Inhibitory effects of *Clostridium butyricum* culture and supernatant on inflammatory colorectal cancer in mice, Front. Immunol. 14 (2023) 1004756. https://doi.org/10.3389/fimmu.2023.1004756.

[15] R. Kutsuna, T. Miyoshi-Akiyama, K. Mori, et al., Description of *Paraclostridium bifermentans* subsp. muricolitidis subsp. nov., emended description of *Paraclostridium bifermentans* (Sasi Jyothsna et al., 2016), and creation of *Paraclostridium bifermentans* subsp. *bifermentans* subsp. nov, Microbiol. Immunol. 63(1) (2019) 1-10. https://doi.org/10.1111/1348-0421.12663.

[16] Z. Liu, Z. Jiang, Z. Zhang, et al., *Bacillus coagulans* in combination with chitooligosaccharides regulates gut microbiota and ameliorates the DSS-induced colitis in mice, Microbiol. Spectrum. 10(4) (2022) e0064122. https://doi.org/10.1128/spectrum.00641-22.

[17] K. Sasaki, D. Sasaki, J. Inoue, et al., *Bacillus coagulans* SANK 70258 suppresses Enterobacteriaceae in the microbiota of ulcerative colitis in vitro and enhances butyrogenesis in healthy microbiota, Appl. Microbiol. Biotechnol. 104(9) (2020) 3859-3867. https://doi.org/10.1007/s00253-020-10506-1.

[18] D. Zheng, R. Li, J. An, et al., Prebiotics-encapsulated probiotic spores regulate gut microbiota and suppress colon cancer, Adv. Mater. 32(45) (2020) 2004529. https://doi.org/10.1002/adma.202004529.

[19] H.S. Cooper, S.N. Murthy, R.S. Shah, et al., Clinicopathologic study of dextran sulfate sodium experimental murine colitis, Lab. Invest. 69(2) (1993) 238-249.

[20] Y. Zou, L. Ma, Y. Zhao, et al., Inhibition of Rho kinase protects against colitis in mice by attenuating intestinal epithelial barrier dysfunction *via* MLC and the NF-B pathway, Int. J. Mol. Med. 41(1) (2018) 430-438. https://doi.org/10.3892/ijmm.2017.3197.

[21] B.L. Eberhart, A.S. Wilson, S.J.D. O'keefe, et al., A simplified method for the quantitation of short-chain fatty acids in human stool, Anal. Biochem. 612 (2021) 114016. https://doi.org/10.1016/j.ab.2020.114016.

[22] Z. Shen, C. Zhu, Y. Quan, et al., Relationship between intestinal microbiota and ulcerative colitis: mechanisms and clinical application of probiotics and fecal microbiota transplantation, World J. Gastroenterol. 24(1) (2018) 5-14. https://doi.org/10.3748/wjg.v24.i1.5.

[23] G.M. De Waal, W.J. S.De Villiers, T. Forgan, et al., Colorectal cancer is associated with increased circulating lipopolysaccharide, inflammation and hypercoagulability, Sci. Rep. 10(1) (2020) 8777. https://doi.org/10.1038/s41598-020-65324-2.

[24] D. Tiosano, V. Gepstein, Vitamin D action: lessons learned from hereditary 1,25-dihydroxyvitamin-*D*-resistant rickets patients, Curr. Opin. Endocrinol. 19(6) (2012) 452-459. https://doi.org/10.1097/MED.0b013e32835a3415.

[25] J. Wang, C. Chen, Y. Ren, et al., Metformin alleviates intestinal epithelial barrier damage by inhibiting endoplasmic reticulum stress-induced cell apoptosis in colitis cell model, Journal of Zhejiang University Medical Sciences 50(5) (2021) 627-632. https://doi.org/10.3724/zdxbyxb-2021-0242.

[26] A. Ruffatti, A. Calligaro, C.S. Lacognata, et al., Insights into the pathogenesis of catastrophic antiphospholipid syndrome. A case report of relapsing catastrophic antiphospholipid syndrome and review of the literature on ischemic colitis, Clin. Rheumatol. 39(4) (2020) 1347-1355. https://doi.org/10.1007/s10067-019-04888-5.

[27] B. Pawlowska, B. M. Sobieszczanska, Intestinal epithelial barrier: the target for pathogenic *Escherichia coli*, Adv. Clin. Exp. Med. 26(9) (2017) 1437-1445. https://doi.org/10.17219/acem/64883.

[28] S. Xie, B. Liu, H. Ye, et al., *Dendrobium huoshanense* polysaccharide regionally regulates intestinal mucosal barrier function and intestinal microbiota in mice, Carbohydr. Polym. 206 (2019) 149-162. https://doi.org/10.1016/j.carbpol.2018.11.002.

[29] X. Wang, X. Xie, Y. Li, et al., Quercetin ameliorates ulcerative colitis by activating aryl hydrocarbon receptor to improve intestinal barrier integrity, Phytother. Res. 38(1) (2024) 253-264. https://doi.org/10.1002/ptr.8027.

[30] Y. Xiao, H. Lian, X.S. Zhong, et al., Matrix metalloproteinase 7 contributes to intestinal barrier dysfunction by degrading tight junction protein Claudin-7, Front. Immunol. 13 (2022) 1020902. https://doi.org/10.3389/fimmu.2022.1020902.

[31] D. Srutkova, M. Schwarzer, T. Hudcovic, et al., *Bifidobacterium longum* CCM 7952 promotes epithelial barrier function and prevents acute DSS-induced colitis in strictly strain-specific manner, PLoS One 10(7) (2015) e0134050. https://doi.org/ 10.1371/journal. pone.0134050.

[32] A.K. Al-Asmari, R. Abbasmanthiri, A. M. Al-Elewi, et al., Camel milk beneficial effects on treating gentamicin induced alterations in rats, J. Toxicol. 2014 (2014) 917608-917608. https://doi.org/10.1155/2014/917608.

[33] K. Arunachalam, A.S. Damazo, A. Macho, et al., *Piper umbellatum* L. (Piperaceae): phytochemical profiles of the hydroethanolic leaf extract and intestinal anti-inflammatory mechanisms on 2,4,6 trinitrobenzene sulfonic acid induced ulcerative colitis in rats, J. Ethnopharmacol. 254 (2020) 112707. https://doi.org/10.1016/j.jep.2020.112707.

[34] G. Yildiz, Y. Yildiz, P. A. Ulutas, et al., Resveratrol pretreatment ameliorates TNBS colitis in rats, recent patents on endocrine, Recent Pat. Endocr. Metab. Immune Drug Discovery. 9(2) (2015) 134-140. https://doi.org/ 10.2174/1872214809666150806105737.

[35] T. Horiuchi, H. Mitoma, S.I. Harashima, et al., Transmembrane TNF-α: structure, function and interaction with anti-TNF agents, Rheumatology 49 (2010) 1215-1228. https://doi.org/10.1093/rheumatology/keq031.

[36] R. Al-Sadi, D. Ye, K. Dokladny, et al., Mechanism of IL-1β-induced increase in intestinal epithelial tight junction permeability, J. Immunol. 180 (2008) 5653-5661. https://doi.org/10.4049/jimmunol.180.8.5653.

[37] M. Hillman, B. Westrom, K. Aalaei, et al., Skim milk powder with high content of Maillard reaction products affect weight gain, organ development and intestinal inflammation in early life in rats, Food Chem. Toxicol. 125 (2019) 78-84. https://doi.org/10.1016/j.fct.2018.12.015.

[38] M. Chalubinski, K. Wojdan, P. Gorzelak, et al., The effect of oxidized cholesterol on barrier functions and *IL-10* mRNA expression in human intestinal epithelium co-cultured with dendritic cells in the transwell system, Food Chem. Toxicol. 69 (2014) 289-293. https://doi.org/10.1016/j.fct.2014.04.005.

[39] F. Carini, M. Mazzola, F. Rappa, et al., Colorectal carcinogenesis: role of oxidative stress and antioxidants, Anticancer Res. 37 (2017) 4759-4766. https://doi.org/10.21873/anticanres.11882.

[40] Y. Ji, Y. Yang, S. Sun, et al., Insights into diet-associated oxidative pathomechanisms in inflammatory bowel disease and protective effects of functional amino acids, Nutr. Rev. 81(1) (2022) 95-113. https://doi.org/10.1093/nutrit/nuac039.

[41] C. Monaco, E. Andreakos, S. Kiriakidis, et al., Canonical pathway of nuclear factor kappa B activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis, PNAS 101(15) (2004) 5634-5639. https://doi.org/10.1073/pnas.0401060101.

[42] G.B. Atkins, D.I. Simon, Interplay between NF-κB and Kruppel-like factors in vascular inflammation and atherosclerosis: location, location, location, J. Am. Heart Assoc. 2(3) (2013) e000290. https://doi.org/10.1161/jaha.113.000290.

[43] M.J. Szczepanski, M. Czystowska, M. Szajnik, et al., Triggering of toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack, Cancer Res. 69(7) (2009) 3105-3113. https://doi.org/10.1158/0008-5472.Can-08-3838.

[44] A. Huppertsberg, L. Kaps, Z. Zhong, et al., Squaric ester-based, pH-degradable nanogels: modular nanocarriers for safe, systemic administration of toll-like receptor 7/8 agonistic immune modulators, J. Am. Chem. Soc. 143(26) (2021) 9872-9883. https://doi.org/10.1021/jacs.1c03772.

[45] X. Zhang, H. Wang, Y. Wang, et al., Nalmefene non-enantioselectively targets myeloid differentiation protein 2 and inhibits toll-like receptor 4 signaling: wet-lab techniques and in silico simulations, Phys. Chem. Chem. Phys. 23(21) (2021) 12260-12269. https://doi.org/10.1039/d1cp00237f.

[46] Y. Wang, J. Lin, Z. Cheng, et al., *Bacillus coagulans* tl3 inhibits lps-induced caecum damage in rat by regulating the tlr4/myd88/Nf-κb and Nrf2 signal pathways and modulating intestinal microflora. Oxid. Med. Cell Longev. 2022 (2022) 5463290. https://doi.org/10.1155/2022/5463290.

[47] H. Wu, Q. Rao, G. Ma, et al., Effect of triptolide on dextran sodium sulfate-induced ulcerative colitis and gut microbiota in mice, Front. Pharmacol. 10 (2020) 1652. https://doi.org/10.3389/fphar.2020.00319.

[48] X. Bian, W. Wu, L. Yang, et al., Administration of *Akkermansia muciniphila* ameliorates dextran sulfate sodium-induced ulcerative colitis in mice, Front. Microbiol. 10 (2019) 2259. https://doi.org/10.3389/fmicb.2019.02259.

[49] Y. Chen, B. Yang, C. Stanton, et al., *Bifidobacterium pseudocatenulatum* ameliorates DSS-induced colitis by maintaining intestinal mechanical barrier, blocking proinflammatory cytokines, inhibiting TLR4/NF-κB signaling, and altering gut microbiota, J. Agric. Food Chem. 69(5) (2021) 1496-1512. https://doi.org/10.1021/acs.jafc.0c06329.

[50] D. Philippe, E. Heupel, S. Blum-Sperisen, et al., Treatment with *Bifidobacterium bifidum* 17 partially protects mice from Th1-driven inflammation in a chemically induced model of colitis, Int. J. Food Microbiol. 149(1) (2011) 45-49. https://doi.org/10.1016/j.ijfoodmicro.2010.12.020.

[51] D.S. Kalman, H.I. Schwartz, P. Alvarez, et al., A prospective, randomized, double-blind, placebo-controlled parallel-group dual site trial to evaluate the effects of a *Bacillus coagulans*-based product on functional intestinal gas symptoms, BMC Gastroenterol. 9 (2009) 85. https://doi.org/10.1186/1471-230x-9-85.

[52] T. A. Rice, A. A. Bielecka, M. T. Nguyen, et al., Interspecies commensal interactions have nonlinear impacts on host immunity, Cell Host Microbe 30(7) (2022) 988-1002. https://doi.org/10.1016/j.chom.2022.05.004.

[53] L.R. Lopetuso, F. Scaldaferri, V. Petito, et al., Commensal Clostridia: leading players in the maintenance of gut homeostasis, Gut Pathog. 5(1) (2013) 23. https://doi.org/10.1186/1757-4749-5-23.

[54] S. Yu, I. Balasubramanian, D. Laubitz, et al., Paneth cell-derived lysozyme defines the composition of mucolytic microbiota and the inflammatory tone of the intestine, Immunity 53(2) (2020) 398-416. https://doi.org/10.1016/j.immuni.2020.07.010.

[55] M. Lleal, G. Sarrabayrouse, J. Willamil, et al., A single faecal microbiota transplantation modulates the microbiome and improves clinical manifestations in a rat model of colitis, Ebiomedicine 48 (2019) 630-641. https://doi.org/10.1016/j.ebiom.2019.10.002.

[56] E.P. Nyangale, S. Farmer, D. Keller, et al., Effects of prebiotics on the fecal microbiota of elderly volunteers after dietary supplementation of *Bacillus coagulans* GBI-30, 6086, Anaerobe 34 (2015) 187. https://doi.org/10.1016/j.anaerobe.2014.11.005.

[57] D. Parada Venegas, M. K. De La Fuente, G. Landskron, et al., Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases, Front. Immunol. 10 (2019) 277. https://doi.org/10.3389/fimmu.2019.01486.

[58] Y. Yuan, L. Li, B. Nan, et al., Allicin ameliorates intestinal barrier damage via microbiota-regulated short-chain fatty acids-TLR4/MyD88/NF-κB cascade response in acrylamide-induced rats, J. Agric. Food Chem. 69(43) (2021) 12837-12852. https://doi.org/10.1021/acs.jafc.1c05014.

[59] X. Liu, B. Mao, J. Gu, et al., *Blautia*-a new functional genus with potential probiotic properties? Gut Microbes 13 (2021) 1875796. https://doi.org/10.1080/19490976.2021.1875796.

[60] Z. Wu, S. Huang, T. Li, et al., Gut microbiota from green tea polyphenol-dosed mice improves intestinal epithelial homeostasis and ameliorates experimental colitis, Microbiome 9(1) (2021) 184. https://doi.org/10.1186/s40168-021-01115-9.

[61] M. Sun, W. Wu, Z. Liu, et al., Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases, J. Gastroenterol. 52(1) (2017) 1-8. https://doi.org/10.1007/s00535-016-1242-9.

[62] Y. Feng, Y. Wang, P. Wang, et al., Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy, Cell. Physiol. Biochem. 49(1) (2018) 190-205. https://doi.org/10.1159/000492853.

[63] M.Y. Lin, M.R. De Zoete, J.P.M. Van Putten, et al., Redirection of epithelial immune responses by short-chain fatty acids through inhibition of histone deacetylases, Front. Immunol. 6 (2015) 160390. https://doi.org/10.3389/fimmu.2015.00554.

[64] Q.H. Sam, H. Ling, W.S. Yew, et al., The divergent immunomodulatory effects of short chain fatty acids and medium chain fatty acids, Int. J. Mol. Sci. 22(12) (2021) 6453. https://doi.org/10.3390/ijms22126453.

[65] Y. Tian, Q. Xu, L. Sun, et al., Short-chain fatty acids administration is protective in colitis-associated colorectal cancer development. J. Nutr. Biochem. 57 (2018) 103-109. https://doi.org/10.1016/j.jnutbio.2018.03.007.

[66] K. Halloran, M.A. Underwood, Probiotic mechanisms of action, Early Hum. Dev. 135 (2019) 58-65. https://doi.org/10.1016/j.earlhumdev.2019.05.010.

[67] X. Liu, Y. Zhang, W. Li, et al., Fucoidan ameliorated dextran sulfate sodium-induced ulcerative colitis by modulating gut microbiota and bile acid metabolism. J. Agric. Food Chem. 70(47) (2022) 14864-14876. https://doi.org/10.1021/acs.jafc.2c06417.

[68] T. Jiao, Y. Ma, X. Guo, et al., Bile acid and receptors: biology and drug discovery for nonalcoholic fatty liver disease, Acta Pharmacol. Sin. 43(5) (2022) 1103-1119. https://doi.org/10.1038/s41401-022-00880-z.

[69] J. Du, Y. Chen, Y. Shi, et al., 1,25-Dihydroxyvitamin D protects intestinal epithelial barrier by regulating the myosin light chain kinase signaling pathway, Inflamm. Bowel Dis. 21(11) (2015) 2495-2506. https://doi.org/10.1097/mib.0000000000000526.

[70] W. Liu, Y. Chen, M.A. Golan, et al., Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis, J. Clin. Invest. 123(9) (2013) 3983-3996. https://doi.org/10.1172/jci65842.