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Dose-response efficacy and mechanisms of orally administered CLA-producing *Bifidobacterium breve* CCFM683 on DSS-induced colitis in mice

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ABSTRACT

This study designed to explore the dose-effect relationship of CCFM683 in relieving colitis and investigate the mechanisms involved. Specifically, the concentration of mucin2, goblet cells and tight junction proteins were significantly up-regulated by 10^{10} and 10^{9} cfu/day CCFM683. Moreover, interleukin (IL)-1β and IL-6 were significantly down-regulated by 10^{10} and 10^{9} cfu/day CCFM683. Furthermore, gut microbiota in mice treated with 10^{10} and 10^{9} cfu/day CCFM683 were rebalanced via improving the unbalanced interaction, regulating the diversity, increasing *Bifidobacterium* and decreasing *Bacteroides* and *Sutterella*. Moreover, the colonic conjugated linoleic acid (CLA) concentration was significantly positive correlated with the effectiveness of the strain in relieving colitis. The gavage dose of CCFM683 should be more than $10^{8.65}$ cfu/day in mice for improving colitis according to dose-effect curve. In conclusion, CCFM683 supplementation alleviated colitis in a dose-dependent manner by improving intestinal epithelial barriers, protecting the intestinal mucus layer, restoring gut microbiota, and down-regulating the inflammatory cytokines.

1. Introduction

One of the main characteristics of inflammatory bowel disease (IBD) is intestinal mucosal inflammation with frequent recurrences and severe clinical forms (Molodecky et al., 2012; Sairenji, Collins, & Evans, 2017; Hanauer, 2006). Prolonged use of conventional pharmacological treatments, such as 5-aminosalicylic acid, budenisonide and hydrocortisone, may cause other complications including osteoporosis, hypertension and diabetes, thus compromising treatment success (Goyal, Rana, Ahlawat, Bijjem, & Kumar, 2014; Biondo-Simões, Mandelli, Pereira, & Faturi, 2003; Kaser, 2009). New therapeutic options for IBD are being explored including the noteworthy use of monoclonal anti-tumor necrosis factor-α (TNF-α) antibodies, prebiotics and probiotic microorganisms that can modulate the gut microbiota and the host immune response (Osman et al., 2006; Howarth, 2009; Pascal et al., 2008; Curro, Ianiro, Pecere, Bibbo, & Cammarota, 2017).

Lactobacillus casei DN-114 001 (6×10^{8} cfu/day) improved the integrity and permeability of intestinal mucosa by preserving ZO-1 expression, which can ameliorate colitis, in a mouse model of IBD (Zakostelska et al., 2011). A cocktail of lactic acid bacteria (LAB) designated G17 (1×10^{8} cfu/day) improved colitis in mice by reducing the concentration of TNF-α and IL-6 in dextran sulphate sodium (DSS)-induced colitis (Kim et al., 2017). *L. fermentum* HY01 (10^{9} cfu/kg·bw) effectively reduced the pro-inflammatory factors IL-12, IFN-γ, TNF-β and IL-6 in mice resulting in colitis relief (Chen et al., 2017). Moreover, *L. brevis* K65 (10^{9} cfu/day) improved ulcerative colitis (UC) symptoms in mice by reducing the levels of TNF-α, IL-6 and IL-1β (Liu et al., 2016). Others LAB, such as *L. fermentum* CECT5716 (5×10^{8} cfu/day), *L. brevis* KY21 (10^{9} cfu/day), *Bifidobacterium longum* HY8004 (2×10^{10} cfu/day), *B. bifidum* 17 (10^{9} cfu/day) and *L. paracasei* (10^{9} cfu/day) also improved colitis by reducing these pro-inflammatory cytokines (Rodrigueznogales et al., 2015; Kim, Koh, Ahn, Oh, & Kim, 2015; Lee

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et al., 2010; Philippe, Heupel, Blumsperisen, & Riedel, 2011; Oliveira et al., 2011; Liu, Su, Ong, Cheng, & Tsai, 2011; Jadhav, Shandilya, & Kansal, 2012). Thus, the above results suggest that the effect of LAB on colitis might be species-specific and even strain specific. The onset of remission was found to be related to the oral dose of the strain. These results suggest that different kinds of LAB have different remission effects, and the effective dose to alleviate colitis is within the range of 10^8-10^{10} cfu/day.

The probiotic definition stresses the administration of an 'adequate amount' to get the health benefit. However, the size of this 'adequate' dose is not specified (Hill et al., 2014). The health endpoint, delivery vehicles, specific probiotics used, and route of administration all have important effects on the effective dose of probiotics. In our previous study, treatment with CLA-producing *B. breve* CCFM683 (10^9 cfu/day) significantly decreased the expression of TNF-α, IL-1β and IL-6, and up-regulated PPAR-gamma (γ) in mRNA levels, as well as improved the histological damages (Yang et al., 2018). However, the maximum and minimum effective doses for relieving colitis by CCFM683 are not clear. Also, the relationship between the amount of CLA produced in the intestinal tract of mice and colitis alleviation is unknown. The mechanism of alleviating colitis involved needs further investigation. This limits further human and clinical trials.

Against this background, this study aimed to explore the dose–effect relationship of relieving colitis by CCFM683 and thus determine the optimal dose of orally administered CCFM683 required. The results will elucidate the relationships between oral dose of CCFM683, CLA content in the colon, and colitis remission. Moreover, it may help provide guidance and foundation for human colitis experiments.

2. Materials and methods

2.1. B. breve strains culture conditions

B. breve CCFM683, isolated from feces of a healthy breast-fed neonate in The Ninth People's Hospital of Wuxi, Jiangsu, China, and was deposited in the Culture Collection of Food Microorganisms (CCFM) of Jiangnan University (Wuxi, China). CCFM683 were sub-cultured three times before use as described previously (Yang et al., 2018). Bacteria used in animal experiment was cultured as described previously (Wang et al., 2018). Fresh *B. breve* CCFM683 suspension was prepared daily by centrifuging at 3000*g* for 10 min at 4 °C and diluted to a suspension with 5×10^6, 5×10^7, 5×10^8, 5×10^9 and 5×10^{10} cfu/mL by using 0.9% sterile saline before administration.

2.2. Animals and experimental design

C57BL/6J mice (male, 72, 8 weeks old, 22–24 g) were purchased from Institute of Model Zoology, Nanjing University (Nanjing, Jiangsu, China) and adapted for 7 days before experiments. All the animals were maintained in wire cages under standard conditions of constant temperature (20 °C ± 2 °C), humidity (50% ± 5%) and photoperiod (12 h/12 h light/dark period) in the barrier facility of Animal Center of Jiangnan University. Mice were divided into 9 groups (four mice in each cage) and fed with standard chow and sterile water. The nutrient compositions and fatty acid profiles of the standard chow were listed in Table S1 and Table S2, which met the nutrient requirements of mice. CLA and precursors are not observed in the crude fat diet.

The entire experiment lasted for 14 days. The colitis mouse model was induced as previous description and the experimental design was presented in Table 1. The experimental protocols were as follow: C57BL/6J male mice were distributed into 9 groups (n = 8) randomly: control, DSS, mesalazine, CLA, 10^6 cfu/day CCFM683, 10^7 cfu/day CCFM683, 10^8 cfu/day CCFM683, 10^9 cfu/day CCFM683, 10^{10} cfu/day CCFM683. All the mice were freely drunk with sterilized water from day 1 to day 7. Colitis was induced by adding 2.5% (w/v) DSS (36,000–50,000, MP Biomedicals, LLC, Irvine, CA, USA) in the drinking water at 8–14 days for all the mice except those mice in control group. All the mice were orally gavaged with different composition (0.2 mL) once daily from day 1 to 14. Each mice in mesalazine group was gavaged with 10 mg/mL mesalazine (Etiasa pharmaceutical Co., Ltd., Saint-Cloud, Paris, France) which was dissolved in 0.9% saline; CLA (50:50 mixture of t10, c12-CLA and c9, t11-CLA isomers, purity: > 99%, Nu-Chek-Prep, Elysian, MN, USA) was administered at a dose of 100 mg/mL; the mice in control group and DSS group were only gavaged with 0.9% saline; A dose of 5×10^6, 5×10^7, 5×10^8, 5×10^9, 5×10^{10} cfu/mL of *B. breve* CCFM683 were orally administered to the mice in these five groups once daily, respectively.

The animal trial were approved by the Ethics Committee of Experimental Animals of Jiangnan University, China (qualified number: JN.No20180615c1400930[111]) and complied with the Directive of 2010/63/European Community.

2.3. Assessment of colitis

The body weight of each mice was measured daily, and the percentage of weight change relative to the initial weight itself prior to DSS exposure on day 0 was calculated. Then, each mice was moved into a clean and empty cage; fecal samples were collected in individual sterile Eppendorf tubes to observe the morphology and to measure the occult blood using an Occult Blood Kit (Nanjing Jiancheng Co., Ltd., Nanjing, China). Daily assessments of weight loss, stool consistency and fecal blood, as indicators of disease activity index (DAI), were measured (Table 2) (Chen et al., 2019; Murthy, Cooper, Shim, Shah, & Sedergran, 1993).

The mice were sacrificed on the 14th day, after 12 h fasting. Blood samples were collected from the orbital plexus of mice and centrifuged at 3000*g* for 30 min to collect the serum. After sacrificing, the colons were removed, and the entire length of each colon was measured. The distal colon samples (0.5 cm) were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (Yang et al., 2018). To identify the occurrence of colitis, the stained tissues were analyzed using a panoramic viewer (3DHISTECH, Ltd., Budapest, Hungary). The valuation system of the pathological score is referred to the Table S3 (Dieleman et al., 1998).

2.4. Biochemical assays

The colon tissues were weighed and homogenized in RIPA lysis buffer (100 mg tissue per milliliter) (Beyotime Biotechnology, Shanghai, China) on ice using the MM 400 Mixer Mill homogenizer (Retsch, GmbH, Haan, Germany). The homogenate was centrifuged at 15,000*g* at 4 °C for 10 min and the supernatant was collected and stored at −80 °C prior to analysis.

The colon tissue supernatant were used to measure the inflammatory enzymatic activity including myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2), which were assessed with commercially available ELISA kits (Nanjing SenBeiJia Biotechnology Co., Ltd., Nanjing, Jiangsu, China). BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China) was used to measure the protein concentration of the colon tissue supernatant.

2.5. Colon epithelial barrier

Alcian blue with post-staining by Nuclear Fast Red (All from Vector, Burlingame, CA) was used to perform the observation of mucin in colon (Steedman, 1950). Periodic acid-schiff (PAS) stain was used to investigate the number of goblet cells (Wu et al., 2018). The concentrations of tight junction (TJ) and adherens junction (AJ) proteins (occludin, ZO-1, claudin-3, α-catenin1, β-catenin and E-Cadherin1) of colon tissue supernatant were measured by commercially available ELISA kits (Nanjing SenBeiJia Biotechnology Co., Ltd., Nanjing, Jiangsu, China).

Table 1
Animal model experimental design.

Group	Daily gavage treatment (0.2 mL)	1–7 day	8–14 day
Control	Saline (0.9%)	Free drinking sterilized water	Free drinking sterilized water
DSS	Saline (0.9%)	Free drinking sterilized water	Free drinking DSS water (2.5%)
Mesalazine	10 mg/mL mesalazine	Free drinking sterilized water	Free drinking DSS water (2.5%)
CLA	100 mg/mL CLA	Free drinking sterilized water	Free drinking DSS water (2.5%)
10^6 cfu/day	5×10^6 cfu/mL CCFM683	Free drinking sterilized water	Free drinking DSS water (2.5%)
10^7 cfu/day	5×10^7 cfu/mL CCFM683	Free drinking sterilized water	Free drinking DSS water (2.5%)
10^8cfu/day	5×10^8 cfu/mL CCFM683	Free drinking sterilized water	Free drinking DSS water (2.5%)
10^9 cfu/day	5×10^9 cfu/mL CCFM683	Free drinking sterilized water	Free drinking DSS water (2.5%)
10^{10} cfu/day	5×10^{10} cfu/mL CCFM683	Free drinking sterilized water	Free drinking DSS water (2.5%)

Table 2
Calculated disease activity index (DAI) score.[a]

Score	Weight loss (%)	Stool consistency[b]	Blood in feces
0	None	Normal	Negative (no bleeding)
1	1.0–5.0	Loose stools	Negative
2	5.0–10.0	Loose stools	Hemoccult positive (slight)
3	10.0–15.0	Diarrhea (slight)	Hemoccult positive
4	Over 15.0	Diarrhea (Watery diarrhea)	Gross bleeding

[a]Disease activity index is a summation of the three parameters weight loss, stool consistency, and hematochezia.
[b]Normal stool: well-shaped stool; loose stool: unformed pasty stool that does not stick to the anus; diarrhea: liquid stool that is attached to the anus.

2.6. The level of cytokines in colon tissue

The concentration of cytokines (IL-10, TNF-α, IL-6, IL-1β and IL-17) in colon tissue supernatant were measured by commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA). BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China) was used to measure the protein concentration of the colon tissue supernatant.

2.7. Fatty acid analysis

Fatty acids in liver, blood and colon were extracted and methylated as previous described (Bligh, & Dyer, 1959; Wang et al., 2016). Pentadecanoic acid (Sigma-Aldrich, St. Louis, MO) and heneicosanoic acid (Sigma-Aldrich, St. Louis, MO) were used as internal standard. FAMEs were recovered with hexane and analyzed using a gas chromatograph (GC2010 plus, Shimadzu, Kyoto, Japan) fitted with a QP2010 ultra mass spectrometer (Shimadzu, Kyoto, Japan) using an Rtx-WAX column (30 m × 0.25 mm i.d. with 0.25 μm thickness) (Restek Corporation, Bellefonte, PA). The type of gas chromatograph, mass spectrometer, column and the temperature programming were as previously described (Yang et al., 2017).

2.8. DNA extraction, PCR amplification, sequencing and bioinformatics analysis

The FastDNA Spin Kit (MP Biomedicals, LLC, Irvine, CA, USA) was used to extract the genomic DNA of fecal samples. The V3-V4 region of 16S rDNA was then PCR-amplified using primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′), which was carried out with the Premix TaqTM (Takara, Dalian, China) following the manuals. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) and quantified with a Qubit™ 4 Fluorometer (Life Technologies Corporation, Carlsbad, CA). Libraries were prepared using a TruSeq DNA LT Sample Preparation Kit (Illumina, Santiago, CA, USA) and sequenced at Illumina MiSeq PE300 platform using a MiSeq Reagent Kit (Illumina, Santiago, CA, USA). After sequencing, the 16S rDNA sequence data were analyzed using the QIIME pipeline (Caporaso et al., 2010).

The β-diversity of microbial communities was reflected by principal coordinates analysis (PCoA) of the weighted UniFrac distance online (https://www.microbiomeanalyst.ca/) (Chong, Liu, Zhou, & Xia, 2020). Network diagram of samples and OTUs were performed by Cytoscape 3.6.0 (Yang et al., 2016). Co-occurrence network of bacterial-interaction patterns were constructed using the random matrix theory (RMT)-based network approach in molecular ecological network analyses (MENA) (http://ieg4.rccc.ou.edu/MENA/) and Cytoscape 3.6.0 (Deng et al., 2012). The role of OTU in the network was characterized by its within-module connectivity (Zi) and amongmodule connectivity (Pi) (Guimera, & Amaral, 2005). Network hubs (z-score > 2.5; c-score > 0.6) were defined as OTUs that were highly connected both in general and within a module; module hubs (z-score > 2.5; c-score < 0.6) were OTUs that are highly connected only within a module; OTUs that link modules are known as connectors (z-score < 2.5; c-score > 0.6), while peripherals (z-score < 2.5; c-score < 0.6) have few links to other species (Fan et al., 2018).

Linear Discriminant Analysis Effect Size (LEfSe) were performed online (http://huttenhower.sph.harvard.edu/galaxy) (Segata et al., 2011). Correlation analysis of colonic CLA concentration, significant taxa, colitis indices, TJ proteins, antioxidant enzymes and cytokines were performed by R 3.5.0 and Gephi 0.9.2 (Barberan, Bates, Casamayor, & Fierer, 2014). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to infer metagenomes based on 16S data and predicted by Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Monk et al., 2017).

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 and SPSS 22.0. Significant difference was evaluated using one-way analysis of variance (ANOVA) followed by a Tukey test for multiple comparisons.

3. Results

3.1. B. breve CCFM683 improved colitis symptoms

The effect of five doses of *B. breve* CCFM683 (10^6 to 10^{10} cfu/day) on DSS induced colitis was investigated. Different effects were observed for mice treated with 10^{10} cfu/day CCFM683, 10^9 cfu/day CCFM683, CLA and mesalazine. Indeed, these specific treatments showed different extents of alleviation in weight loss (Fig. 1A), while 10^8 cfu/day, 10^7 cfu/day and 10^6 cfu/day CCFM683 did not show these effects.

The DAI of DSS treated mice reached 11.38 ± 0.37 on the 14th day, while mesalazine and CLA treatments led to an extreme decrease in the DAI of 41.78% and 27.50%, respectively (Fig. 1B). The most effective dose, 10^{10} cfu/day CCFM683, led to a decrease in the DAI of 70.34%. In the other groups, compared to the DSS group, the DAI was reduced to 3.75 ± 0.53 (10^9 cfu/day CCFM683), 9.50 ± 0.57 (10^8 cfu/day CCFM683), 10.00 ± 0.63 (10^7 cfu/day CCFM683) and 10.75 ± 0.53 (10^6 cfu/day CCFM683), with decreases of 67.05%,

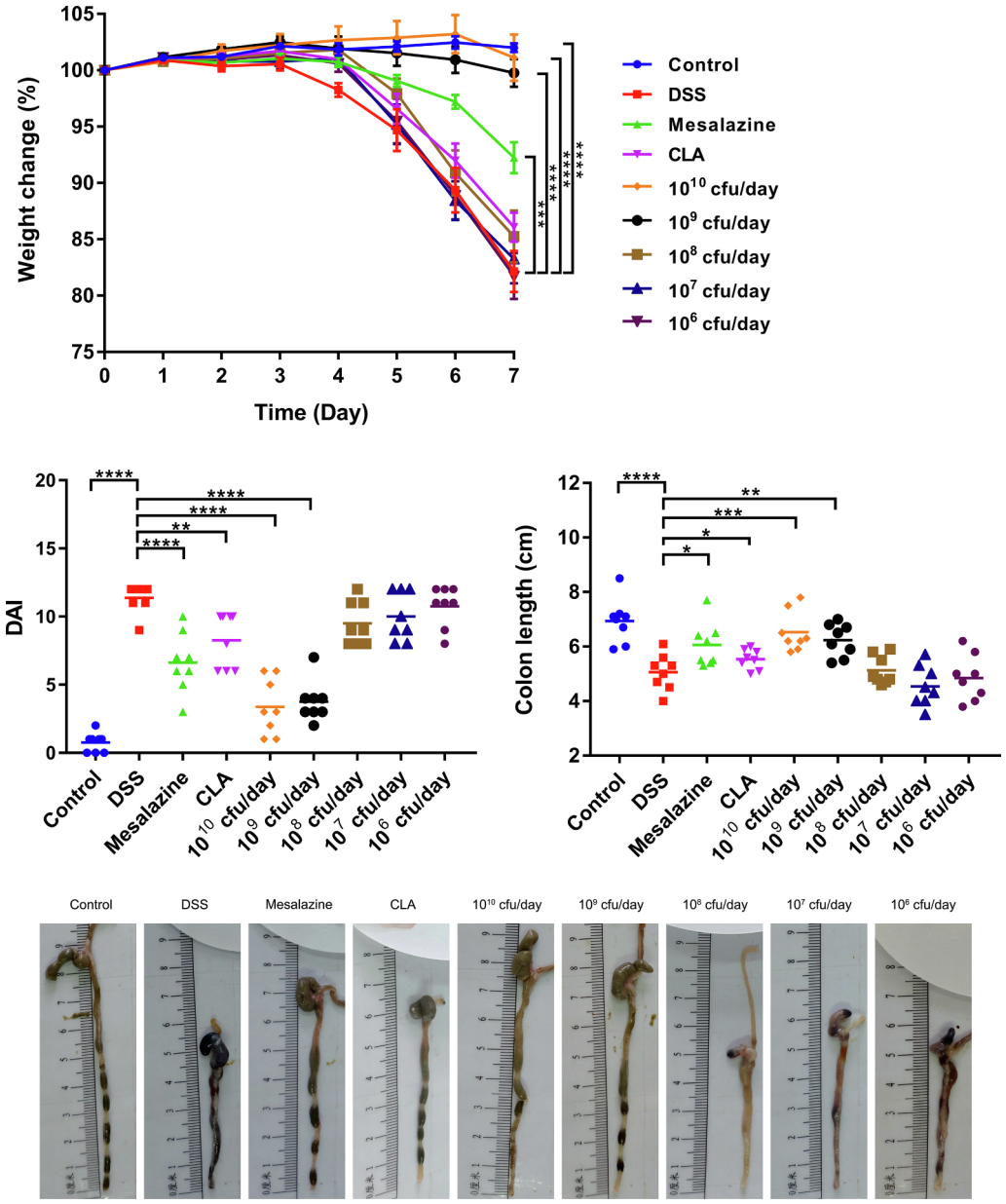

Fig. 1. Effect of different doses of *B. breve* CCFM683 on the symptoms of colitis. (A) Body weight, (B) DAI, (C) Colon length, (D) Macroscopic pictures of colons. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$. $n = 8$ mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

16.52%, 12.13% and 5.53%, respectively. Unlike the other doses, 10^8 cfu/day CCFM683, 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 showed insignificant decreases of DAI compared to the DSS group.

The colon length of mice in the control group (normal, non-diseased mice) was 6.94 ± 0.28 cm (Fig. 1C). The colon of the mice in control group was reddish with granular stool (Fig. 1D). In comparison, the colon of DSS treated mice was swollen, dark red, and bleeding, which was only 5.06 ± 0.23 cm. Moreover, the colon length of mesalazine, CLA, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treated mice increased by 19.75%, 9.38%, 28.87% and 23.12% compared with DSS treated mice, respectively (Fig. 1C). However, 10^8 cfu/day CCFM683, 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 did not significantly prevent colon shortening.

3.2. B. breve CCFM683 recovered the damage in colonic tissue and regulated inflammatory enzymes associated with colitis

Histopathological injury was evaluated by H&E staining. The normal mice showed an intact colonic mucosa, tidy colonic villi, healthy crypt structure, and abundant goblet cells without inflammatory cell, while the intestinal mucosa, crypt, epithelium and inflammatory cell in DSS treated mice were edematous, letheral, damaged and abundant, respectively (Fig. 2A).

The injury score of colon tissue in DSS treated mice (12.63 ± 0.32) was 5.93 times as much as that in normal mice (2.13 ± 0.29) (Fig. 2B). CLA, 10^{10} cfu/day CCFM683, 10^9 cfu/day CCFM683 and mesalazine treatments significantly reduced the tissue damage. Among those groups, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments were most effective for protecting the colon: the crypts were intact and goblet cells were no significant disappearance. However, the colons in 10^8 cfu/day CCFM683, 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 treated mice showed severe oedema and dramatic reduction in crypt lengths, goblet cells and villi.

To evaluate the influences of different doses of CCFM683 on inflammatory enzymes, iNOS, COX-2 and MPO activities in the colon were measured. Mesalazine, CLA, 10^{10} cfu/day CCFM683, 10^9 cfu/day

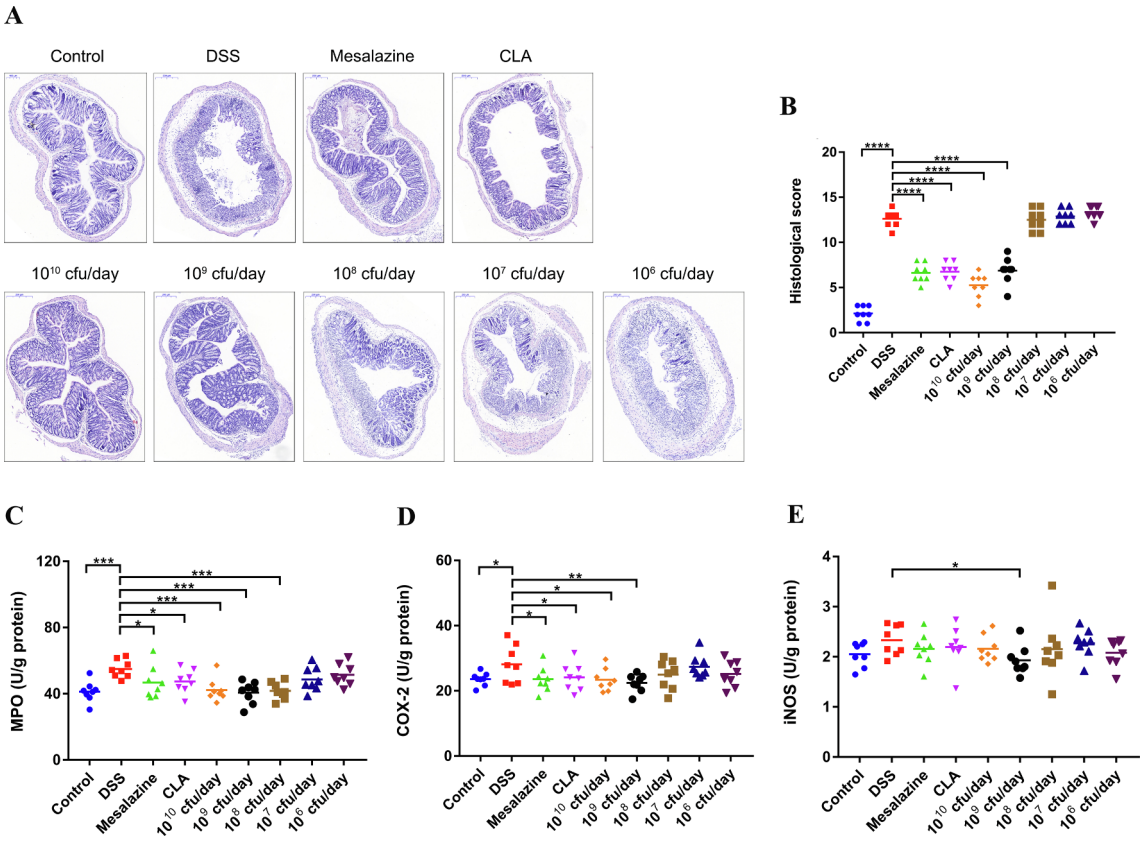

Fig. 2. Effect of different doses of *B. breve* CCFM683 on the histological injury and colonic enzyme activities. (A) Histological examination, Scale bars, 200 μm, (B) Colonic histological injury score, (C) MPO, (D) COX-2, (E) iNOS. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$. n = 8 mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

CCFM683 and 10^8 cfu/day CCFM683 treatments significantly decreased the MPO activity compared with DSS treatment (Fig. 2C). However, the MPO activity of 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 treated mice were similar with the DSS treated mice. Moreover, DSS treatment showed highest COX-2 activity, while those in mesalazine, CLA, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments were significantly decreased by 16.22%, 14.12%, 16.82% and 20.21%, respectively ($p < 0.05$) (Fig. 2D). Compared with DSS treatment, the iNOS activity among all the groups, except for 10^9 cfu/day CCFM683, showed no significant differences (Fig. 2E).

3.3. Dose-effect curve and relationship between CCFM683 dose and colitis alleviation

In order to analyze the relationship between CCFM683 dose in gavage and pathological indexes of colitis, dose–response curves were generated between CCFM683 dose and colon length, histological score, MPO, and DAI. CCFM683 dose versus colon length, histological score, MPO and DAI revealed S-shaped dose–response curves (Fig. 3). The EC_{50} value of the dose–response curves between CCFM683 dose and colon length was $10^{8.42}$ cfu/day. However, the EC_{50} value of the dose–response curves between the CCFM683 dose and histological score, MPO or DAI were $10^{8.65}$ cfu/day, $10^{7.21}$ cfu/day and $10^{8.41}$ cfu/day, respectively. Thus, the gavage dose of CCFM683 is significantly related to its colitis relieving effects. The gavage dose of CCFM683 should be more than $10^{8.65}$ cfu/day to relieve colitis.

3.4. The effect of B. breve CCFM683 on colonic mucous layer and mucosal barrier

The goblet cell numbers and mucin2 (MUC2) concentration were measured to evaluate the mucous layer. The results from alcian blue staining and periodic acid-schiff staining indicated that the mucous layer and goblet cells in the DSS treated mice were destroyed (Fig. 4A and B). Mesalazine, CLA, 10^{10} cfu/day CCFM683, and 10^9 cfu/day CCFM683 treatments significantly protected against the destruction of the mucosal layer and the reduction of goblet cells.

MUC2 of DSS treated mice was significantly decreased, whereas 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments maintained its content at a normal level (Fig. 4C). Moreover, the content of MUC2 in CLA and mesalazine treated mice significantly increased compared with that in DSS treated mice, while MUC2 in the 10^8 cfu/day CCFM683, 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 groups were similar to that of DSS group. Remarkably, DSS treated mice suffered a loss of mucus-producing goblet cells. However, 10^{10} cfu/day CCFM683, 10^9 cfu/day CCFM683 and 10^8 cfu/day CCFM683 treatments significantly increased the numbers of goblet cells ($p < 0.05$) (Fig. 4D).

To evaluate the effect of *B. breve* on the mucosal barrier, the concentrations of TJ proteins (claudin-3, ZO-1, occludin) and AJ proteins (α-catenin1, β-catenin, E-Cadherin1) were measured. CLA, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments up-regulated the concentrations of ZO-1 by 26.62%, 22.07% and 20.00%, respectively, compared with DSS treatment (Fig. 5D). In addition, DSS treatment decreased the concentration of α-catenin1, claudin-3, β-catenin, occludin and ZO-1 compared with the control (Fig. 5). On the contrary, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments significantly increased the concentrations of β-catenin, ZO-1 and occludin compared with DSS group ($p < 0.05$). The concentrations of occludin and β-catenin were up-regulated to certain levels in 10^8 cfu/day CCFM683 and 10^7 cfu/day CCFM683 groups, but were not significantly different to DSS mice (Fig. 5B and E). Indeed, α-catenin1 and E-

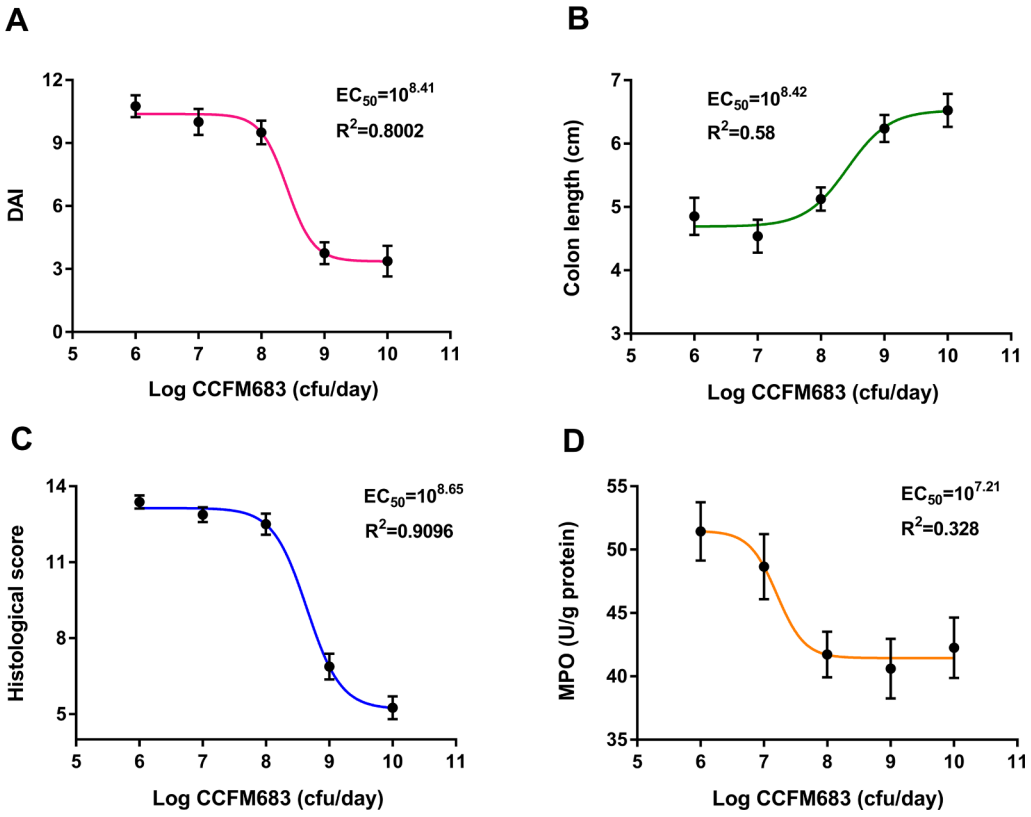

Fig. 3. Dose-effect curve between CCFM683 dose and inflammatory index of colitis. (A) DAI, (B) Colon length, (C) Histological score, (A) MPO. n = 8 mice per group.

Cadherin1 were also up-regulated in 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treated mice, but were not significantly different to that in DSS treated mice (Fig. 5A and C).

3.5. B. breve CCFM683 regulated the inflammatory cytokines

DSS treatment increased the concentration of TNF-α, IL-6 and IL-1β, while 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments decreased them (Fig. 6A, 6B and 6C). 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments significantly decreased the concentrations of IL-6 and IL-1β, while 10^{8} cfu/day CCFM683, 10^{7} cfu/day CCFM683 and 10^{6} cfu/day CCFM683 treatments showed no significant reduction compared with DSS treatment (Fig. 6B and C). Moreover, the anti-inflammatory cytokine, IL-10, increased to 16.76% and 17.45%, in mesalazine and 10^{10} cfu/day CCFM683 treated mice, respectively, compared with DSS treatment (52.04 ± 2.64 pg/mg protein) (Fig. 6E). However, mice administrated with all the five doses of CCFM683 showed no significant differences in IL-17 compared with DSS treatment (Fig. 6D).

3.6. B. breve CCFM683 influenced the concentration of CLA in the liver, blood and colon

To explore the influence of orally administered doses of CCFM683 on CLA concentrations in mice, the fatty acid contents in colon, liver and blood were analyzed. The colonic CLA concentration in CLA, 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treated mice were 671%, 650% and 531% as much as that in DSS treated mice, respectively ($p < 0.0001$). Moreover, the colonic CLA concentration in 10^{8} cfu/day CCFM683, 10^{7} cfu/day CCFM683 and 10^{6} cfu/day CCFM683 treated mice showed insignificance compared with DSS treated mice (Fig. 7A). However, none of the five doses of CCFM683 treatments increased CLA concentrations in liver or blood compared with DSS treated mice (Fig. 7B and C).

The colonic CLA concentration negatively correlated with DAI and tissue histological scores ($p < 0.0001$) (Fig. 7E and F). Moreover, colonic CLA concentration showed highly positive correlation with colon length ($p < 0.0001$), and negatively correlated with MPO ($p < 0.001$) (Fig. 7D and G). The results showed that the content of CLA produced by *B. breve* in the colon displayed significantly and positive correlations with the relief effect of colitis. Furthermore, the gavage concentration of CCFM683 showed an extremely positive correlation with the content of colonic CLA (Fig. 7H).

3.7. B. breve CCFM683 regulated gut microbiota

Shannon and Chao1 indexes were used to evaluate alpha diversity of gut microbiota. Both Shannon indexes and Chao1 indexes of 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treated mice were significantly higher than that of DSS treated mice (Fig. 8A and B). The gut microbiota between DSS treated mice and normal mice were significantly different base on β-diversity, while administration of 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 improved the shift in gut microbiota ruined by DSS challenge (Fig. 8C).

To analyze the effects of different doses of *B. breve* CCFM683 on intestinal microbiota, the gut microbiota composition was investigated. The dominant phyla in control mice were Firmicutes (40.71%), Bacteroidetes (30.19%), Actinobacteria (16.53%) and Verrucomicrobia (6.45%) (Fig. 9A). However, relative abundances of Proteobacteria and Deferribacteres in DSS treated mice increased to 31.12% and 3.72% while the relative abundances of Actinobacteria, Verrucomicrobia and Firmicutes decreased to 1.50%, 2.14% and 22.78%, respectively (Fig. 9A). However, 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments increased the relative abundances of Actinobacteria, Firmicutes and Verrucomicrobia as well as significantly decreased the relative abundance of Proteobacteria, compared with DSS treatment.

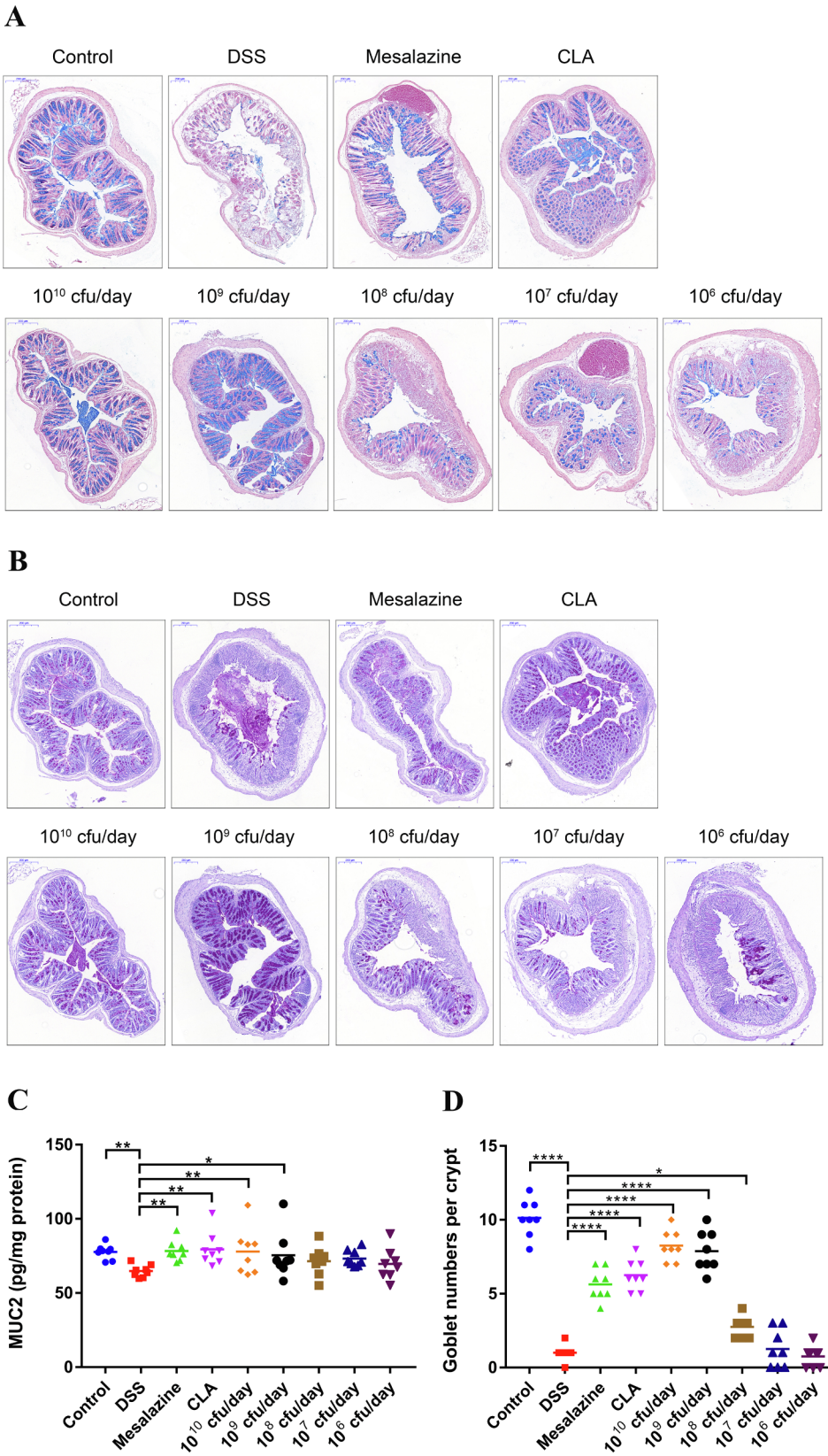

Fig. 4. Effects of different doses of *B. breve* CCFM683 on the mucous layer. (A) Alcin blue staining, Scale bars, 200 μm (B) Histological sections of the colon (stained with PAS), Scale bars, 200 μm, (C) Concentration of MUC2, (D) The number of goblet cells. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. n = 8 mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

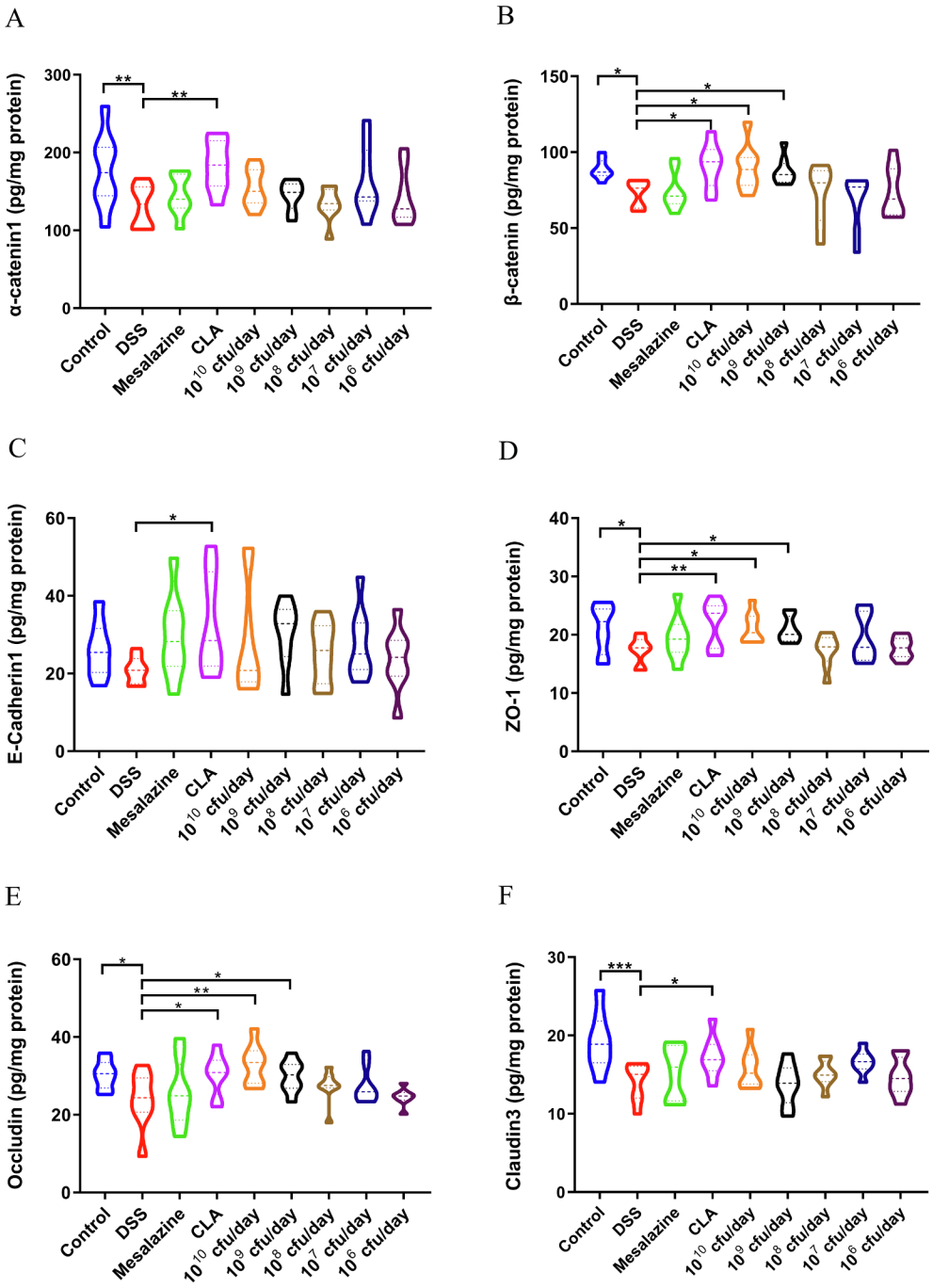

Fig. 5. Effects of different doses of *B. breve* CCFM683 on AJ and TJ proteins. (A) α-catenin1, (B) β-catenin, (C) E-Cadherin1, (D) ZO-1, (E) Occludin, (F) Claudin-3. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$. n = 8 mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

The biomarkers and dominant microorganisms were authenticated by the histogram of LDA scores (Fig. 9B and C). Dominant communities of twelve and eight taxa were found in 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treated mice, respectively (Fig. 9B and C). Among them, Proteobacteria and *Bacteroides* were dominant in DSS group; Firmicutes and *Allobaculum* was the dominant bacteria in the 10^{10} cfu/day CCFM683 group; while Turicibacteraceae and *Turicibacter* were the dominant microbes in the 10^9 cfu/day CCFM683 (Fig. 9C). The abundance of *Lactobacillus*, *Allobaculum* and *Bifidobacterium* in DSS treated mice significantly decreased, but the abundance of *Sutterella*, *Bacteroides* and *Mucispirillum* significantly increased compared with normal mice (Fig. 9D). However, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treated mice showed an increased abundance of *Bifidobacterium,* a significant increase of *Allobaculum* and *Akkermansia* ($p < 0.05$) as well as a significant decrease of *Sutterella*, *Bacteroides* and *Mucispirillum* compared with DSS treated mice ($p < 0.01$).

Furthermore, the topological role of each OTU in microbial networks consisting of all samples was determined via RMT-based network analysis. There were a greater number of negatively correlated edges in 10^{10} cfu/day CCFM683 (27.35%) and 10^9 cfu/day CCFM683 groups (20.00%), compared with DSS group (4.95%) and control group (13.04%) (Fig. 9E and Table S6). The negative links of total network in 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 groups were significantly more than those in either DSS or control group. 10^{10} cfu/day CCFM683 group showed higher module hubs (3.92%) than that in 10^9 cfu/day CCFM683 (0%), DSS (1.96%) and control groups (0%) (Fig. S1,

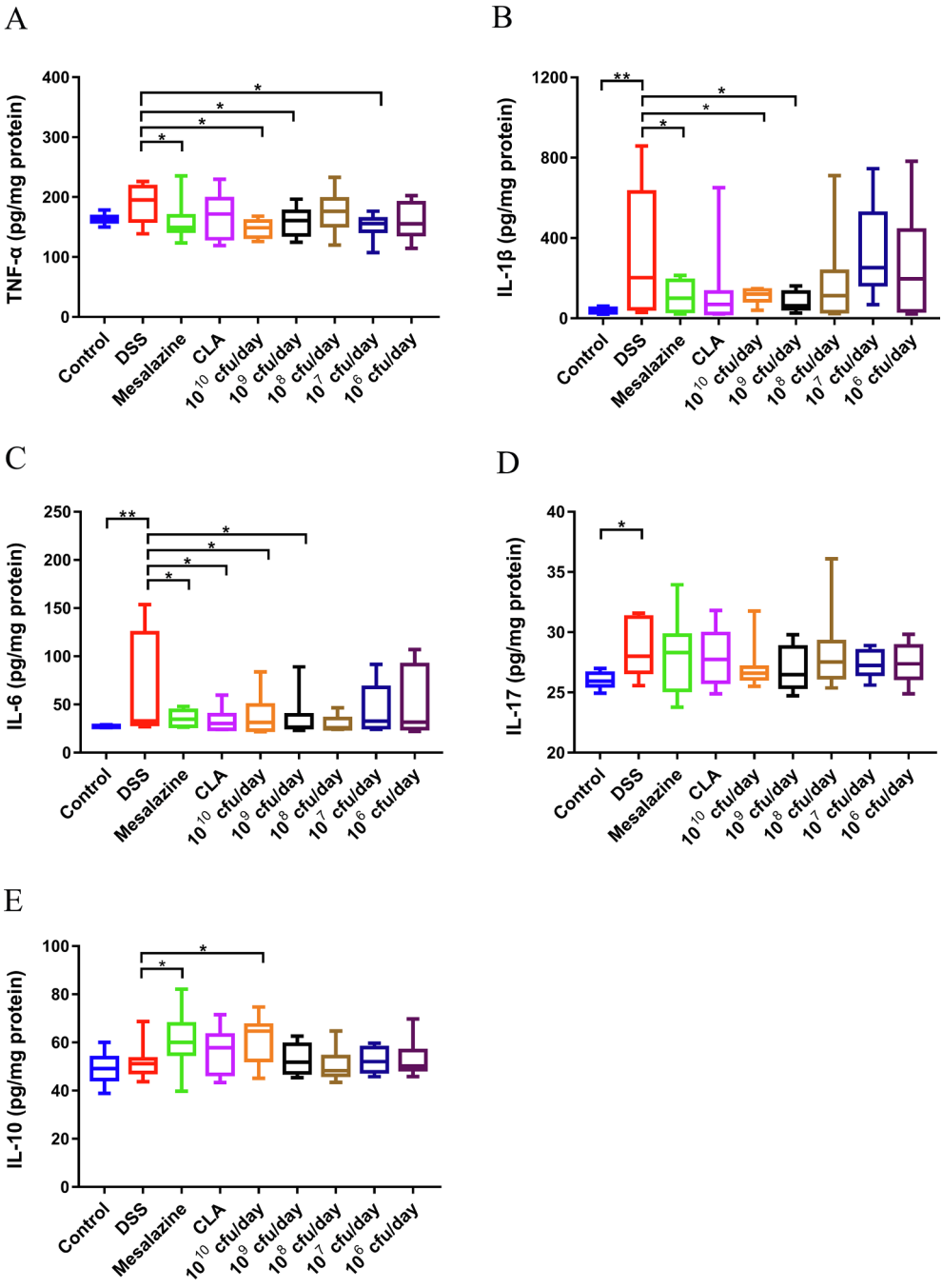

Fig. 6. Effects of different doses of *B. breve* CCFM683 on the cytokines in colon. TNF-α (A), IL-1β (B), IL-6 (C), IL-17 (D) and IL-10 (E) concentration in colonic tissue. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. $n = 8$ mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

TableS5 and TableS7). Moreover, the connectors in 10^9 cfu/day CCFM683 were much higher than those in all the other groups.

The core microbes were evaluated by combining the degree, Pi and Zi values (Fig. 9E, Fig. S1, Table S4, Table S5, Table S6 and Table S7). Epulopiscium (OTU111) represented the core microbes in the DSS group (Fig. 9E, Fig. S1 and Table S7), in which Epulopiscium correlated positively with *Oscillospira* (OTU120), *Dorea* (OTU110) and Clostridiales (OTU92). However, Anaerotruncus (OTU121), *Odoribacter* (OTU39) and *Mucispirillum* (OTU60) were the core microbes in normal mice. In 10^{10} cfu/day CCFM683 treatment, Lactobacillaceae (OTU82), *Streptococcus* (OTU87) and *Bifidobacterium* (OTU19) were the core microbes (Fig. 9E). And Lactobacillaceae positively correlated with *Dehalobacterium* (OTU103) while negatively correlated with *Blautia* (OTU108), Enterobacteriaceae (OTU220), Peptostreptococcaceae (OTU116), Bacteroidales (OTU26) and *Trabulsiella* (OTU229). Furthermore, *Streptococcus* (OTU87), *Turicibacter* (OTU88), Desulfovibrionaceae (OTU210) were the core microbes in 10^9 cfu/day CCFM683 treated mice, in which *Streptococcus* positively correlated with Peptococcacea (OTU115), *Pseudomonas* (OTU243) and *Anaeroplasma* (OTU252) (Fig. 9E, Fig. S1 and Table S7). Thus, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments changed the core gut microbiota and their interactions

Moreover, the correlations among differential microorganisms, colonic CLA concentration and inflammation markers were analyzed. It is well known that histological score, DAI and colon length were the most important indicators for colitis, and showed the bigger weightiness in the network analysis (Fig. 9F). The concentration of colonic CLA positively correlated with ZO-1 and colon length, in contrast, negatively

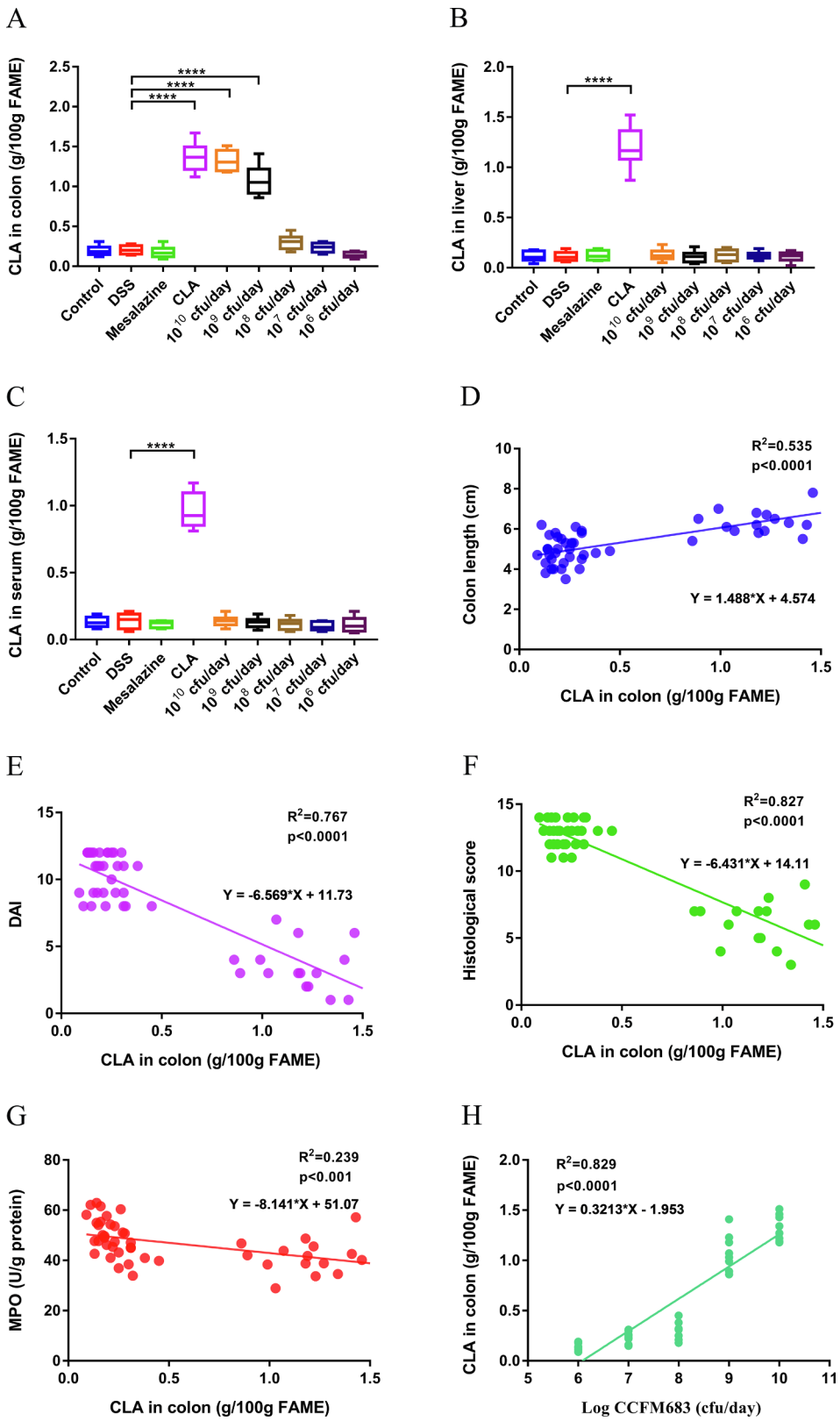

Fig. 7. CLA concentration in the colonic contents, blood and liver, and the interdependent quantitative relationships between the colonic CLA concentration and inflammatory markers. Colonic contents (A), liver tissue (B) and blood (C). The interdependent quantitative relationships between the colonic CLA concentration and (D) colon length, (E) DAI, (F) histological scores, (G) MPO and (H) doses of CCFM683. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. n = 8 mice per group. One-way ANOVA followed by Tukey multiple-comparison test.

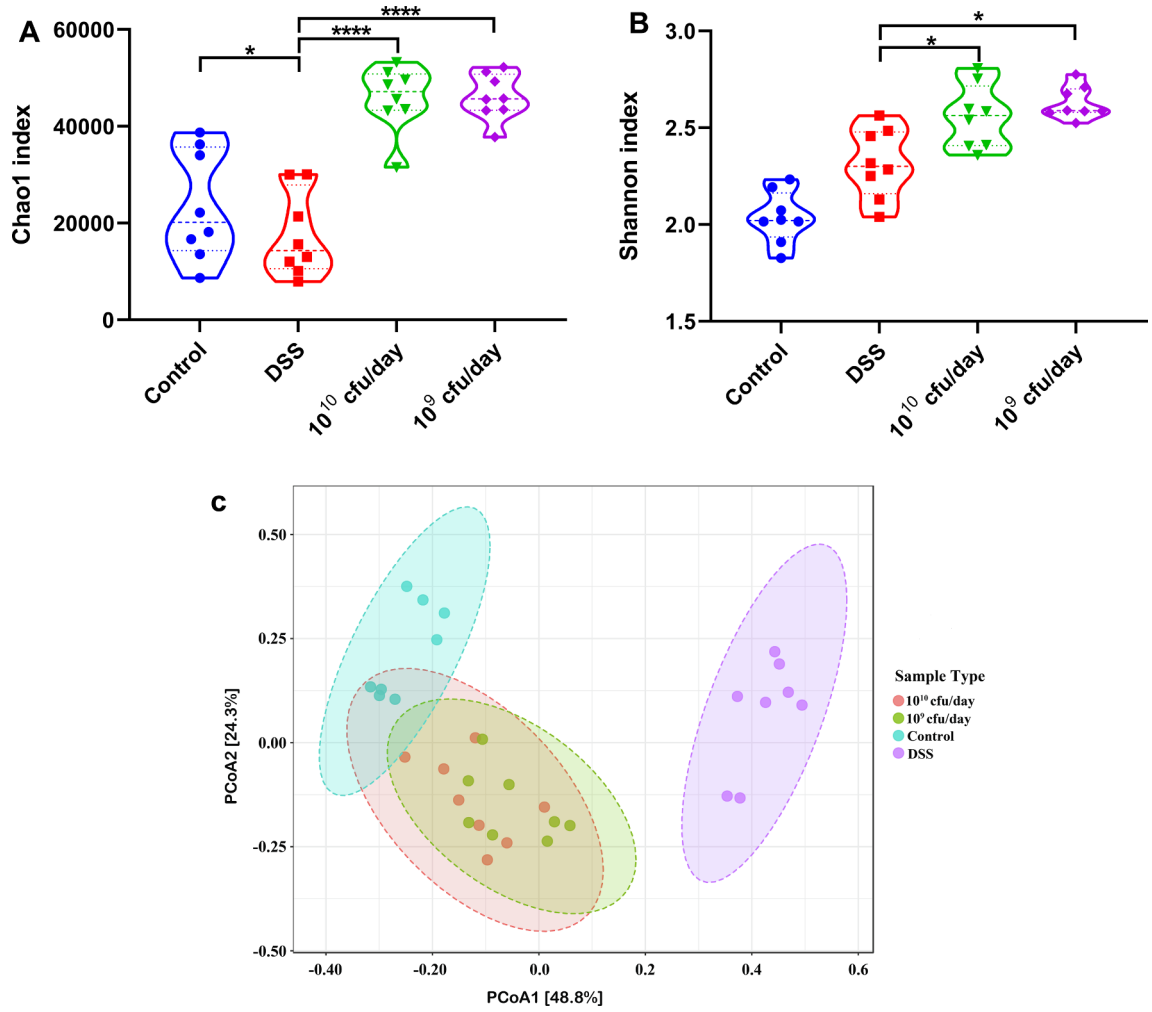

Fig. 8. Effect of *B. breve* CCFM683 *on* the overall structure of gut microbiota. Alpha diversity indicated by Chao1 index (A) and Shannon index (B). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. One-way ANOVA followed by Tukey multiple-comparison test. (C) PCoA, with extended functionality for labeling groups, with normal probability ellipsoids for different groups.

correlated with MPO, DAI and histological scores. Interestingly, colonic CLA concentration showed a big weightiness in the network analysis, which indicated that CLA production may be a factor for relieving colitis in CCFM683 treated mice. The relative abundance of *Bacteroides* was positive correlation with DAI and histological scores; however, negatively correlated with MUC2 and TJ proteins (β-catenin and occludin), indicating that *Bacteroides* has a negative effect on colitis. Moreover, *Sutterella* showed positive correlations with DAI, histological scores and TNF-α, while negatively correlated with colon length, TJ proteins (β-catenin, ZO-1 and occludin). However, *Akkermansia* only negatively correlated with IL-17. Furthermore, *Bifidobacterium* showed positive correlation with colon length and negative correlations with histological score and DAI (Fig. 9F). Notably, there was no significant correlation between the differential microorganisms (*Turicibacter* and *Odoribacter*) and AJ proteins and inflammation markers (colon length, DAI, MPO and histological score).

PICRUSt was performed to predict KEGG categories represented in mice after different dose of CCFM683 treatment. It showed 14 differentially abundant KEGG pathways in control and DSS groups (11 increased and 3 decreased, respectively), including those microbial genes related to amino acid metabolism, metabolism of cofactors and vitamins as well as carbohydrate metabolism (Fig. 10A). Moreover, compared with DSS group, 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments recovered the abundance of metabolic pathways, increasing lysine biosynthesis, tyrosine metabolism, glycolysis/gluconeogenesis, vitamin B6 metabolism, starch and sucrose metabolism, and D-glutamine and D-glutamate metabolism (Fig. 10B).

4. Discussion

Many animal studies have shown that LAB can regulate colitis and this ability was found to depend on the oral dose of the bacteria (Rodrigueznogales et al., 2015; Kim, Koh, Ahn, Oh, & Kim, 2015; Lee et al., 2010; Philippe, Heupel, Blumsperisen, & Riedel, 2011; Oliveira et al., 2011; Liu, Su, Ong, Cheng, & Tsai, 2011). In the current study, five doses of CLA-producing CCFM683 were used to compare their effects on relieving colitis. Administration of 10^{10} cfu/day and 10^{9} cfu/day CCFM683 relieved the symptoms (DAI and colon shortening) of colitis and maintained the normal anatomical structure of colonic tissue; however, 10^{8} cfu/day CCFM683, 10^{7} cfu/day CCFM683 and 10^{6} cfu/day CCFM683 did not elicit these effects (Fig. 1 and Fig. 2). Furthermore, the MPO (inflammatory activity) and COX-2 activity (inhibited the expression of inflammatory mediators NO) in mice treated with 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 were significantly lower than those in DSS treated mice (Fig. 2) (Chassaing, Aitken, Malleshappa, & Vijaykumar, 2014; Zwolinskawcislo et al., 2011). Thus, *B. breve* CCFM683 treatments ameliorated the symptoms of colitis in a dose-dependent manner.

The mucous layer is an important component of colonic epithelial cells, which protects against harmful antigenic microbiota and luminal

antigens (Peterson, & Artis, 2014; Kim, & Ho, 2010). Specifically, MUC2 is produced by goblet cells and is the key component of mucus (Hansson, 2012). In the current study, only 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments increased the number of goblet cells and the concentration of MUC2, but other doses did not elicit those effects (Fig. 4). Another CLA-producing strain *L. plantarum* ZS2058 (10^{9}

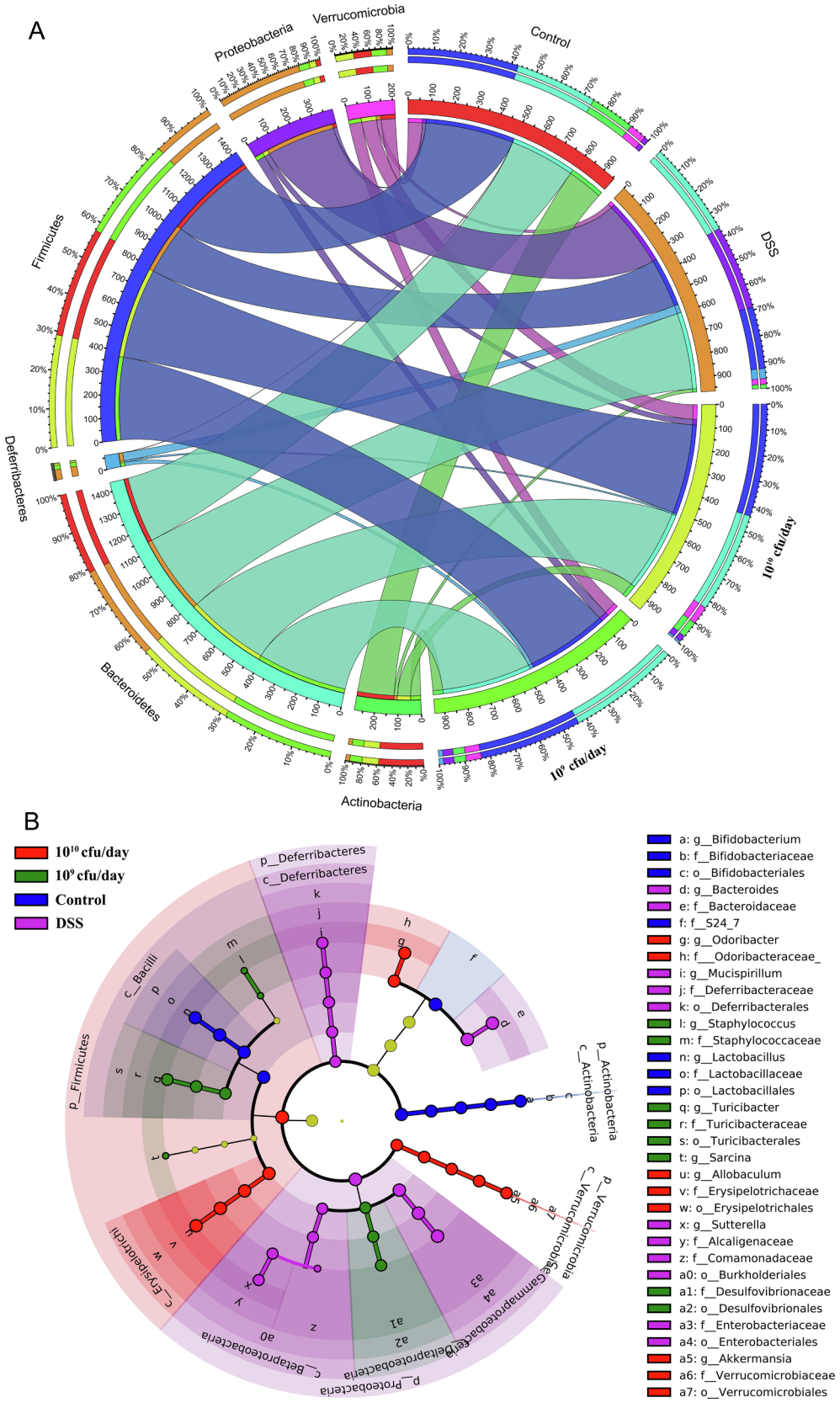

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Fig. 9. Effects of *B. breve* CCFM683 on dominant microorganisms. (A) Microbial distribution at the phylum level. (B) Cladogram. (C) Distribution histogram based on LDA, with a log LDA score above 3.0. Significant taxa were labeled and annotated with tags in the right panel. (D) Relative abundance of differential microorganism. P value represents discrepancy compared with the DSS treated mice. One-way ANOVA followed by Tukey multiple-comparison test. (E) The co-occurrence network structure of gut bacteria. Only significant correlations (p-value < 0.05, $|r^2| > 0.6$) are displayed with an edge. The edge colors indicated positive (red) or negative (green) correlations, which depended on Spearman's correlation coefficient. The node size represents the degree. (F) Correlation analysis of the concentration of colonic CLA, significant taxa, colitis indices, TJ and AJ protein and cytokine in colon. Only significant correlations (p-value < 0.05, $|r^2| > 0.5$) are displayed with an edge. The edge colors indicate positive (red) or negative (green) correlations, which depended on Spearman's correlation coefficient. The node size represents the weight.

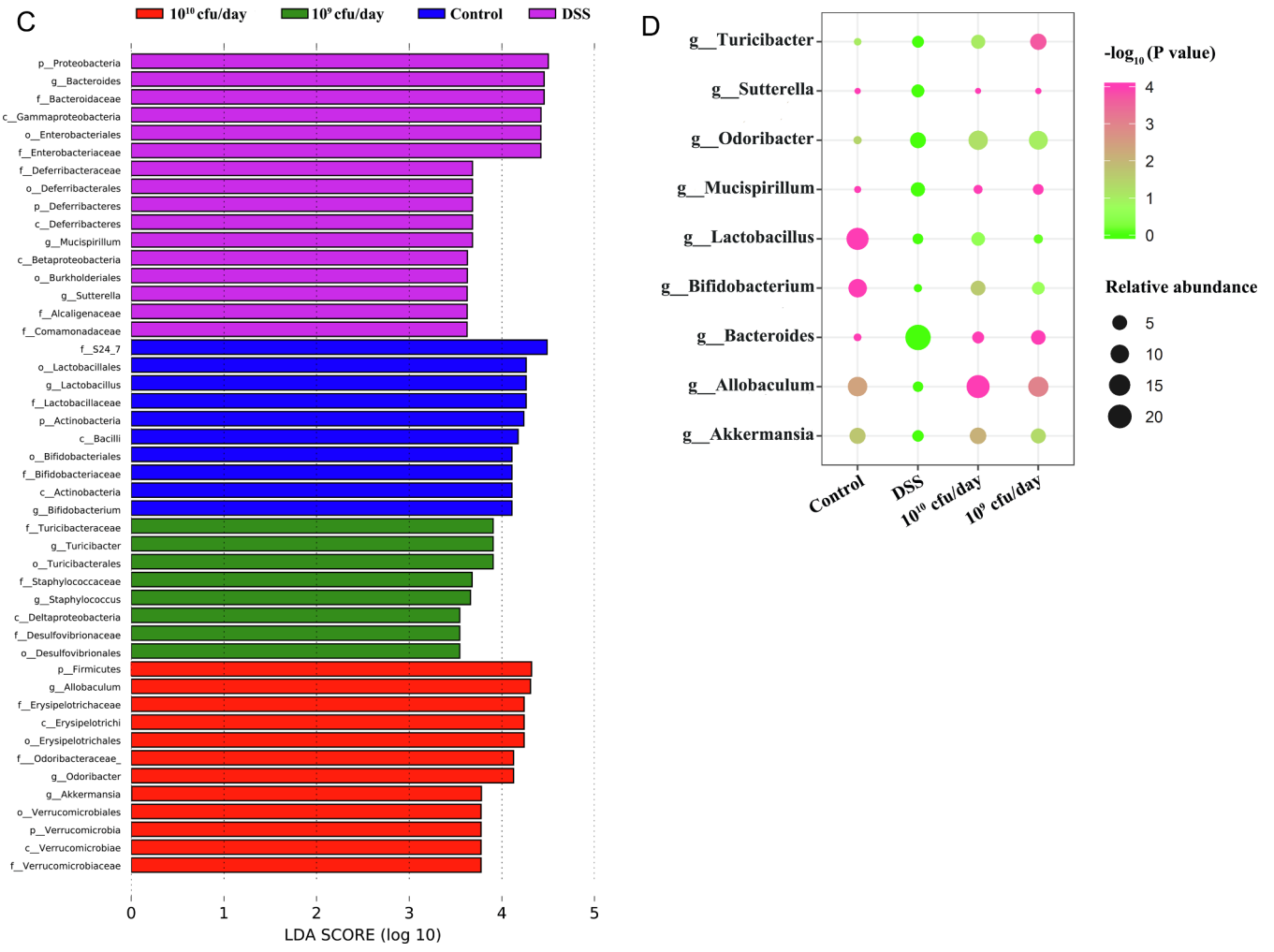

Fig. 9. (*continued*)

cfu/day) has also been reported to up-regulate mRNA expression of MUC2 (Wang et al., 2016). These results are in line with that of UC patients, in which mucosal inflammation is associated with the concentration of MUC2 (Van Klinken, Der Wal, Einerhand, Buller, & Dekker, 1999; Theodoratou et al., 2014). Thus, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 were able to maintain the intestinal mucus layer via promoting the goblet cells to secrete MUC2.

Furthermore, the integrity of the epithelial monolayer is critical for IBD (Schmitz et al., 1999). Epithelial barrier integrity is controlled by AJs (control epithelial permeability) and TJs (stable cell–cell adhesion) (Shen, Weber, Raleigh, Yu, & Turner, 2011; Ivanov, & Naydenov, 2013; Ivanov, Parkos, & Nusrat, 2010). Treatment with 10^9 cfu/day *L. plantarum* ZS2058 increased the expression ZO-1 and claudin-3 (Wang et al., 2016). However, due to the limitations of qPCR these results need further confirmation at protein level. In the current study, 10^{10} cfu/day CCFM683 and 10^9 cfu/day CCFM683 treatments showed higher contents of AJ proteins (β-catenin) and TJ proteins (occludin, ZO-1) compared with DSS treatment group ($p < 0.05$) (Fig. 5). Similar results were found in *L. casei* DN-114 001 (6×10^8 cfu/day), which has been shown to improve the integrity and permeability of intestinal mucosa by preserving ZO-1 expression (Zakostelska et al., 2011). In Srutkova's reseach, *B. longum* CCM 7952 (2×10^8 cfu/day) treatment preserved expression of occludin and ZO-1 and reduced clinical symptoms (Srutkova et al., 2015). However, 10^8 cfu/day CCFM683, 10^7 cfu/day CCFM683 and 10^6 cfu/day CCFM683 treatments did not significantly increase the concentration of ZO-1, occludin, β-catenin and MUC2 compared with DSS treatment (Fig. 4 and Fig. 5). Therefore, *B. breve* CCFM683 regulated TJ and AJ proteins in a dose-dependent manner.

Intestinal immune response disorder is an important characteristic of IBD (Hegazy, & Elbedewy, 2010). In mice with colitis, *Lactococcus lactis* NCDO 2118 (5×10^9 cfu/day) increased TGF-β and IL-10 production, which improved colitis symptoms (Luerce et al., 2014). Moreover, *L. plantarum* Lp91 (10^9 cfu/day) evoked significant down regulation of TNF-α in a colitis mouse model, while IL-10 and IL-4 were

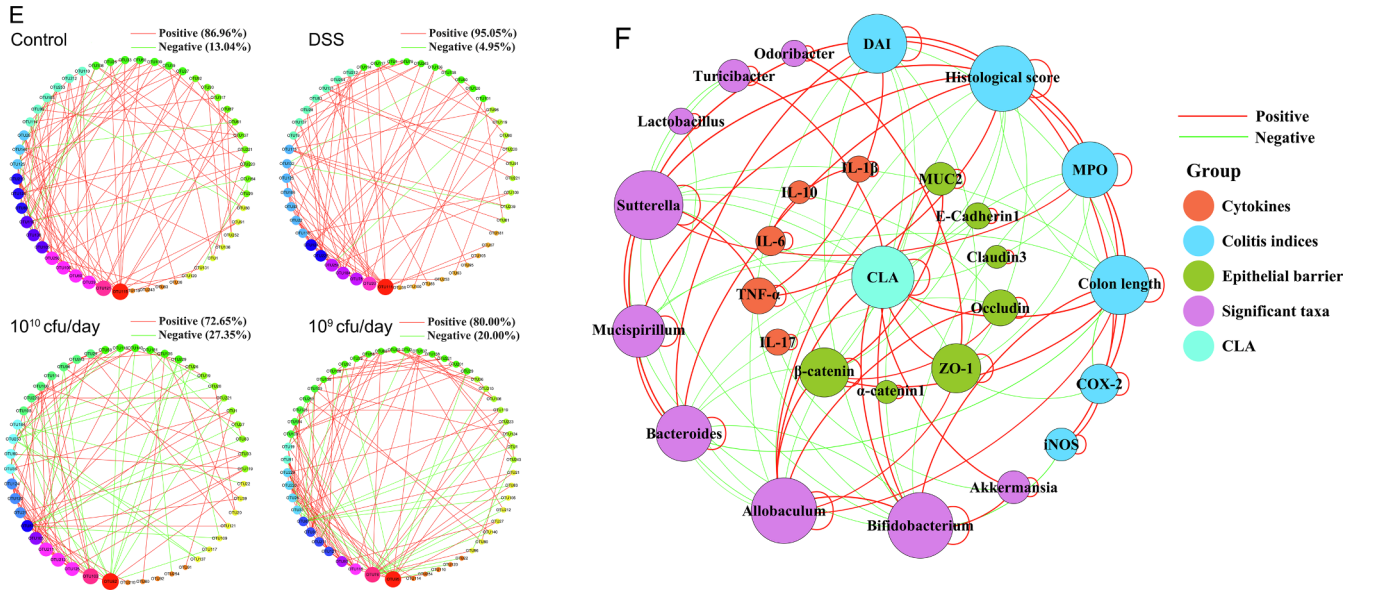

Fig. 9. (*continued*)

significantly up regulated (Duary, Bhausaheb, Batish, & Grover, 2012). In the present study, 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 significantly decreased the concentration of IL-1β and IL-6. However, there was no such effect in 10^{8} cfu/day, 10^{7} cfu/day and 10^{6} cfu/day CCFM683 treated mice (Fig. 6). TNF-α has been reported to lead to mucosal inflammation and intestinal barrier damage (Horiuchi, Mitoma, Harashima, Tsukamoto, & Shimoda, 2010). Moreover, IL-1β and IL-6 resulted in immune disorders and amplified the inflammation (Lee et al., 2010). Thus, regulating inflammatory cytokines was an important way for *B. breve* CCFM683 to relieve colitis.

Gut microbiota plays an important role in IBD (Jin et al., 2017). The diversity of gut microbiota in IBD patients are much lower than those in healthy individuals (Yeom, Kim, Kim, & Kim, 2016). After 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments, microbial diversity improved and the microbiota profiles changed (Fig. 8A, B and C). The relative abundances of Proteobacteria and Deferribacteres in DSS treated mice were 18.09 and 45.93 times as high as the control group, while these bacteria significantly decreased in CCFM683 administered groups (Fig. 9A). Furthermore, Verrucomicrobia, was associated with MUC2 (Fujio-Vejar et al., 2017), which increased following treatment with 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683. Compared with DSS treatment, 10^{9} cfu/day *B. longum* YS108R treatment also increased the abundance of Verrucomicrobia (Clemente, Manasson, & Scher, 2018), which was consistent with the current results. At the genus level, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treated mice increased the abundance of *Akkermansia* and *Bifidobacterium* and decreased the abundance of *Sutterella*, *Bacteroides* and *Mucispirillum* (Fig. 9C and 9D). Notably, *Akkermansia* only negatively correlated with IL-17, while *Bifidobacterium* showed positive correlation with colon length and negative correlations with histological score and DAI (Fig. 9F). Many *Bifidobacterium* can regulate colitis via improving the tight junctions and regulating inflammation factors to sustain the mucosal barrier (Srutkova et al., 2015; Yan, Yang, Zhao, Zhao, & Chen, 2019; Chae et al., 2018). Furthermore, some *Bacteroides* have been reported to degrade the mucous layer and break down the intestinal barriers (Ramakrishna, 2013; Ma et al., 2018). In the present study, *Bacteroides* negatively correlated with MUC2 and TJ proteins (β-catenin and occludin) and positively correlated with DAI as well as histological scores (Fig. 9F) In a previous study, *Bacteroides* has been proved to negatively correlate with TJ proteins (claudin-1, occludin, ZO-1) and positively correlate with pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) (Yan, Yang, Zhao, Zhao, & Chen, 2019), which is consistent with the current results. Interestingly, *Sutterella* showed extremely significantly positive correlations with DAI, histological scores and TNF-α, while negatively correlated with colon length, TJ proteins (β-catenin, ZO-1 and occludin) (Fig. 9F). In a clinical study, *Sutterella* is enriched in the feces of UC patients (Paramsothy et al., 2019), and is widely found in autistic children with gastrointestinal dysfunction (Williams, Hornig, Parekh, & Lipkin, 2012). Moreover, *Sutterella* is responsible for low levels of IgA antibodies, which are presented in large quantities in the lining of gut and have an important protective effect on the gut (Moon et al., 2015). 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments increased the beneficial bacteria (*Bifidobacterium*) and decreased harmful bacteria (*Sutterella* and *Bacteroides*), which was an important factor in colitis alleviation.

Moreover, the interaction pattern of gut microbiota in IBD patients has been reported to be disorganized (Peng et al., 2019), and the co-occurrence network is unbalanced. In our study, an unbalanced network was found in the DSS treated mice, the core microbes of which was *Epulopiscium*. The bacteria correlated positively with *Oscillospira*, *Dorea*, Clostridiales (Fig. 9E, Fig. S1 and Table S7). Indeed, *Oscillospira, Epulopiscium, Dorea,* and Clostridiales have been confirmed to aggravate colitis (Pascal et al., 2017; Clemente, Manasson, & Scher, 2018). However, 10^{10} cfu/day CCFM683 and 10^{9} cfu/day CCFM683 treatments improved the unbalanced microbiota profiles. In 10^{10} cfu/day CCFM683 treatment group, Lactobacillaceae, *Streptococcus* and *Bifidobacterium* were the core microbes (Fig. 9E, Fig. S1 and Table S7). Interestingly, Lactobacillaceae, *Streptococcus* and *Bifidobacterium* have been associated with improved colitis (Pascal et al., 2017; Clemente, Manasson, & Scher, 2018; Lamas et al., 2016). Furthermore, *Streptococcus*, *Turicibacter* and Desulfovibrionaceae were the core microbes in 10^{9} cfu/day CCFM683 treated mice, which were different from DSS treated mice. Co-occurrence networks had fewer negative correlations in DSS group when compared with that of either 10^{10} cfu/day or 10^{9} cfu/day CCFM683, which can be interpreted as reduced inter-species competition in 10^{10} cfu/day and 10^{9} cfu/day CCFM683 groups (Fig. 9E and Fig. S1). Thus, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments improved the unbalanced microbiota and regulate the core microbiota, which was another factor for improving colitis.

Meanwhile, significantly different functional profiles among different groups were predicted by PICRUSt to highlight the importance of gut microbiota in those metabolic pathways. In the present study, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments increased three carbohydrate metabolism (glyoxylate and dicarboxylate metabolism, glycolysis/Gluconeogenesis, starch and sucrose metabolism) (Fig. 10A and

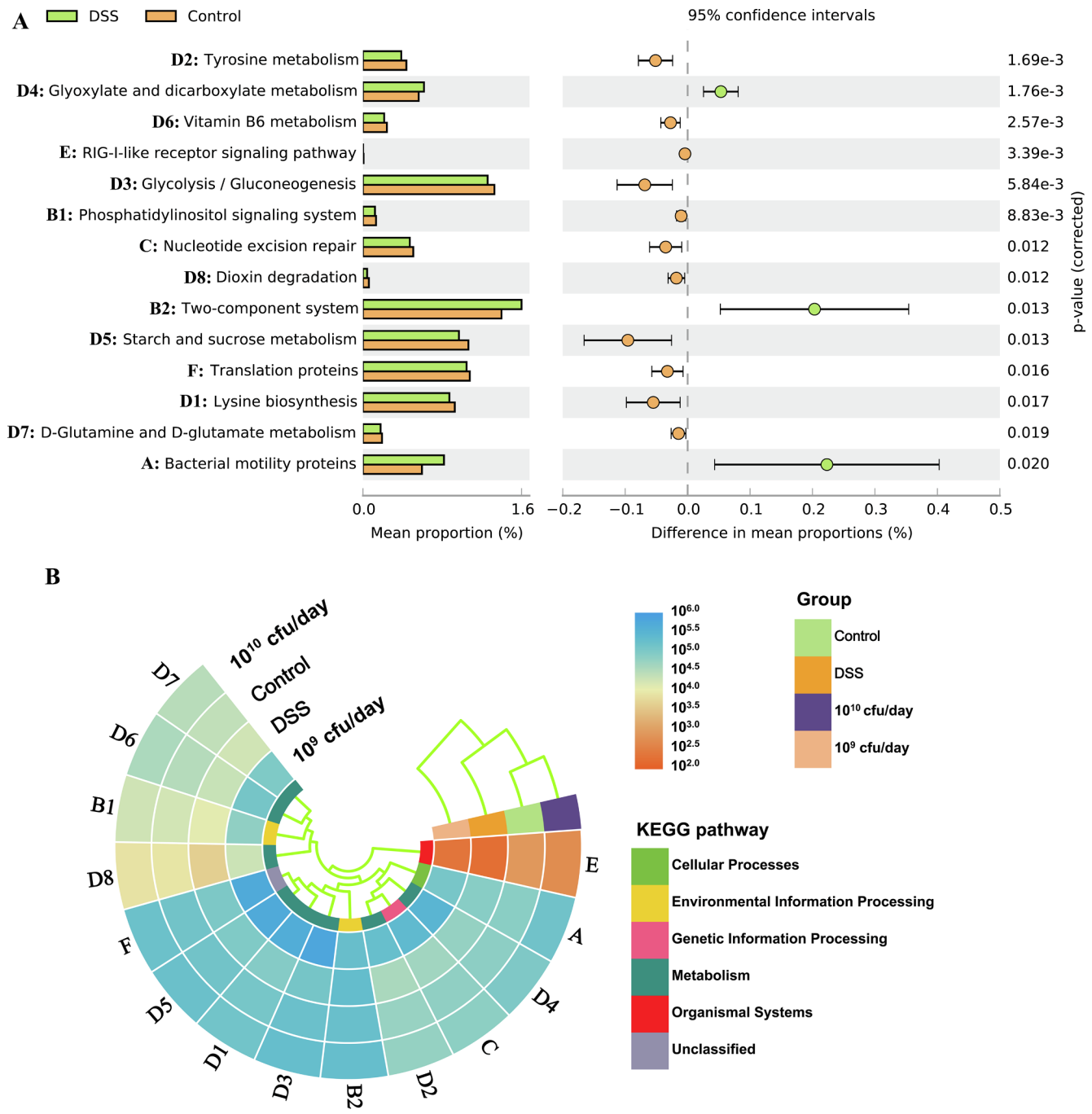

Fig. 10. Prediction of functional content from 16S rDNA surveys by PICRUSt. (A) Differential KEGG pathways between control and DSS groups using two-sided Welch's *t* test at p-value $<$ 0.05. (B) Heat map of the differential pathways among all four groups.

B), which can produce short chain fatty acids (SCFAs), acting as nutrients for colonocytes and other intestinal epithelial cells (Koropatkin, Cameron, & Martens, 2012). Amino acids not only support the growth and survival of bacteria in the gastrointestinal tract, but also regulate the balance of energy and protein in the organism (Neis, Dejong, & Rensen, 2015). 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments increased the tyrosine metabolism, whose metabolites may have better anti-inflammatory effects (Xu, Fang, Li, Yang, & Chen, 2019). Moreover, lysine biosynthesis was increased in 10^{10} cfu/day and 10^{9} cfu/day CCFM683 groups (Fig. 10A and 10B), which had been demonstrated with anti-inflammatory benefits (Dong et al., 2018). Vitamin B6 is involved in various metabolisms in human, and its metabolism is implicated in the adaptive response to a wide array of adverse conditions, such as nutrient deprivation, hypoxia (Galluzzi et al., 2012). In the present study, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments increased vitamin B6 metabolism, which may contribute to colon cells resisting the harsh intestinal environment induced by DSS modeling. Thus, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments may regulate amino acid anabolism, carbohydrate metabolism and vitamin metabolism, and produce fatty acids and amino acids to improve epithelial cell barrier and intestinal inflammation.

Oral administration of different doses of CCFM683 resulted in different CLA levels in the colon. Interestingly, CLA contents in the colon positively correlated with colitis alleviation (Fig. 7). Thus, CLA production may be one of the factors for relieving colitis in *B. breve* CCFM683. Moreover, the role of CLA needs to be further confirmed by

gene deletion approach in the future.

Some probiotic products were used to improve UC patients, however different probiotics showed different efficacious doses (Groeger et al., 2013; Oliva et al., 2012; Wildt, Nordgaard, Hansen, Brockmann, & Rumessen, 2011). 10^{10} cfu/day viable *B. infantis* 35,264 significantly decreased the TNF-α and IL-6 of UC patients (Groeger et al., 2013). In a clinical study involving children with UC, *L. reuteri* ATCC 55730 (10^{10} cfu/day) significantly increased IL-10, whereas IL-8, TNF-α and IL-1β significantly decreased (Oliva et al., 2012). Moreover, UC patients treated with *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 (2.5×10^{11} cfu/day) maintained the remission (Wildt, Nordgaard, Hansen, Brockmann, & Rumessen, 2011). In the present study, 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments significantly relieved colitis in mice, and the gavage dose of CCFM683 is significantly related to the relieving effects. Through analysing dose–effect curve, the gavage dose of CCFM683 should be more than $10^{8.65}$ cfu/day to relieve colitis in mice (Fig. 3). The conversion of probiotics doses between mice and human was not clear at present, therefore, clinical trials should be conducted to investigate the efficacy of CCFM683 in UC patients in the future.

5. Conclusions

In summary, at five doses, only 10^{10} cfu/day and 10^{9} cfu/day CCFM683 treatments significantly relieved colitis. The gavage dose of CCFM683 significantly related to its relieving effects. The gavage dose of CCFM683 should be more than $10^{8.65}$ cfu/day in mice according to the dose–effect curve. The multiple mechanisms of alleviating DSS-induced colitis mainly included decreasing the pro-inflammatory cytokines, maintaining the concentration of intestinal TJ proteins and AJ proteins, protecting the intestinal mucus layer via promoting the goblet cells to secrete MUC2, improving the unbalanced microbiota and regulating the core microbiota, increasing beneficial bacteria and inhibiting harmful bacteria. These results aid our understanding of the mechanisms by which CCFM683 alleviates colitis and regulates other immune-related diseases, which is conducive to guiding the research of clinical trials and the development of probiotics products.

CRediT authorship contribution statement

Yang Chen: Investigation, Formal analysis, Writing - original draft. **Yan Jin:** Data curation. **Catherine Stanton:** Supervision, Writing - review & editing. **R. Paul Ross:** Supervision, Writing - review & editing. **Zhi Wang:** Resources, Validation. **Jianxin Zhao:** Resources, Validation. **Hao Zhang:** Conceptualization, Supervision. **Bo Yang:** Writing - review & editing. **Wei Chen:** Funding acquisition, Supervision.

Declaration of Competing Interest

The 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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104245.

References

Barberan, A., Bates, S. T., Casamayor, E. O., & Fierer, N. (2014). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal, 6*, 343–351.

Biondo-Simões, M. L. P., Mandelli, K. K., Pereira, M. S. C., & Faturi, J. L. (2003). Opções terapêuticas para as doenças inflamatórias intestinais: revisão. *Revista Brasileira de Coloproctologia, 23*(3), 172–182.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Biochemistry and Cell Biology, 37*(8), 911–917.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335–336.

Chae, J. M., Heo, W., Cho, H. T., Lee, D. H., Kim, J. H., Rhee, M., ... Kim, Y. J. (2018). Effects of orally-administered *Bifidobacterium animalis* subsp. lactis strain BB12 on dextran sodium sulfate-induced colitis in mice. *Journal of Microbiology and Biotechnology, 28*(11), 1800–1805.

Chassaing, B., Aitken, J. D., Malleshappa, M., & Vijaykumar, M. (2014). Dextran sulfate sodium (DSS)-induced colitis in mice. *Current Protocols in Immunology, 104*(1), 15–25.

Chen, Y., Yang, B., Ross, R. P., Jin, Y., Stanton, C., Zhao, J., ... Chen, W. (2019). Orally administered CLA ameliorates DSS-induced colitis in mice via intestinal barrier improvement, oxidative stress reduction, and inflammatory cytokine and gut microbiota modulation. *Journal of Agricultural and Food Chemistry, 67*(48), 13282–13298.

Chen, X., Zhao, X., Wang, H., Yang, Z., Li, J., & Suo, H. (2017). Prevent effects of *Lactobacillus fermentum* HY01 on dextran sulfate sodium-induced colitis in mice. *Nutrients, 9*(6), 545.

Chong, J., Liu, P., Zhou, G., & Xia, J. (2020). Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nature Protocols, 15*(3), 799–821.

Clemente, J. C., Manasson, J., & Scher, J. U. (2018). The role of the gut microbiome in systemic inflammatory disease. *BMJ, 360*, Article j5145.

Curro, D., Ianiro, G., Pecere, S., Bibbo, S., & Cammarota, G. (2017). Probiotics, fibre and herbal medicinal products for functional and inflammatory bowel disorders. *British Journal of Pharmacology, 174*(11), 1426–1449.

Deng, Y., Jiang, Y., Yang, Y., He, Z., Luo, F., & Zhou, J. (2012). Molecular ecological network analyses. *BMC Bioinformatics, 13*(1), 113.

Dieleman, L. A., Palmen, M. J., Akol, H., Bloemena, E., Pena, A. S., Meuwissen, S. G., & Van Rees, E. P. (1998). Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clinical and Experimental Immunology, 114*(3), 385–391.

Dong, W., Zhu, X., Zhou, X., Yang, Y., Yan, X., Sun, L., & Shang, D. (2018). Potential role of a series of lysine-/leucine-rich antimicrobial peptide in inhibiting lipopolysaccharide-induced inflammation. *Biochemical Journal, 475*(22), 3687–3706.

Duary, R. K., Bhausaheb, M. A., Batish, V. K., & Grover, S. (2012). Anti-inflammatory and immunomodulatory efficacy of indigenous probiotic *Lactobacillus plantarum* Lp91 in colitis mouse model. *Molecular Biology Reports, 39*(4), 4765–4775.

Fan, K., Weisenhorn, P., Gilbert, J. A., Shi, Y., Bai, Y., & Chu, H. (2018). Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. *Soil Biology & Biochemistry, 121*, 185–192.

Fujio-Vejar, S., Vasquez, Y., Morales, P., Magne, F., Vera-Wolf, P., Ugalde, J. A., ... Gotteland, M. (2017). The gut microbiota of healthy Chilean subjects feveals a high abundance of the phylum Verrucomicrobia. *Frontiers in Microbiology, 8*, 1–11.

Galluzzi, L., Vitale, I., Senovilla, L., Olaussen, K. A., Pinna, G., Eisenberg, T., ... Kroemer, G. (2012). Prognostic impact of vitamin B6 metabolism in lung cancer. *Cell Reports, 2*(2), 257–269.

Goyal, N., Rana, A., Ahlawat, A., Bijjem, K. R., & Kumar, P. (2014). Animal models of inflammatory bowel disease: A review. *Inflammopharmacology, 22*(4), 219–233.

Groeger, D., Omahony, L., Murphy, E. F., Bourke, J. F., Dinan, T. G., Kiely, B., ... Quigley, E. M. (2013). *Bifidobacterium infantis* 35624 modulates host inflammatory processes beyond the gut. *Gut Microbes, 4*(4), 325–339.

Guimera, R., & Amaral, L. A. (2005). Functional cartography of complex metabolic networks. *Nature, 433*(7028), 895–900.

Hanauer, S. B. (2006). Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. *Inflammatory Bowel Diseases, 12*(5), S3–S9.

Hansson, G. C. (2012). Role of mucus layers in gut infection and inflammation. *Current Opinion in Microbiology, 15*(1), 57–62.

Hegazy, S., & Elbedewy, M. M. (2010). Effect of probiotics on pro-inflammatory cytokines and NF-κB activation in ulcerative colitis. *World Journal of Gastroenterology, 16*(33), 4145–4151.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., & Pot, B. (2014). Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506–514.

Horiuchi, T., Mitoma, H., Harashima, S. I., Tsukamoto, H., & Shimoda, T. (2010). Transmembrane TNF-α: Structure, function and interaction with anti-TNF agents. *Rheumatology, 49*(7), 1215–1228.

Howarth, G. S. (2009). Inflammatory bowel disease, a dysregulated host-microbiota interaction: Are probiotics a new therapeutic option? *Journal of Gastroenterology & Hepatology, 23*(12), 1777–1779.

Ivanov, A. I., Parkos, C. A., & Nusrat, A. (2010). Cytoskeletal regulation of epithelial barrier function during inflammation. *American Journal of Pathology, 177*(2), 512–524.
Ivanov, A. I., & Naydenov, N. G. (2013). Dynamics and regulation of epithelial adherens junctions: Recent discoveries and controversies. *International Review of Cell and Molecular Biology, 303*, 27–99.
Jadhav, S. R., Shandilya, U. K., & Kansal, V. K. (2012). Immunoprotective effect of probiotic dahi containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on dextran sodium sulfate-induced ulcerative colitis in mice. *Probiotics and Antimicrobial Proteins, 4*(1), 21–26.
Jin, S., Zhao, D., Cai, C., Song, D., Shen, J., Xu, A., ... Zheng, Q. (2017). Low-dose penicillin exposure in early life decreases Th17 and the susceptibility to DSS colitis in mice through gut microbiota modification. *Scientific Reports, 7*(1), 43662.
Kaser, A. (2009). Inflammatory bowel disease. *Medical Journal of Australia, 92*(3), 72.
Kim, Y. S., & Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: Recent insights and progress. *Current Gastroenterology Reports, 12*(5), 319–330.
Kim, Y., Koh, J. H., Ahn, Y. J., Oh, S., & Kim, S. H. (2015). The synergic anti-inflammatory impact of *Gleditsia sinensis Lam.* and *Lactobacillus brevis* KY21 on intestinal epithelial cells in a DSS-induced colitis model. *Korean Journal for Food Science of Animal Resources, 35*(5), 604–610.
Kim, M. S., Byun, J. S., Yoon, Y. S., Yum, D. Y., Chung, M. J., & Lee, J. C. (2017). A probiotic combination attenuates experimental colitis through inhibition of innate cytokine production. *Beneficial Microbes, 8*(2), 231–241.
Koropatkin, N. M., Cameron, E. A., & Martens, E. C. (2012). How glycan metabolism shapes the human gut microbiota. *Nature Reviews Microbiology, 10*(5), 323–335.
Lamas, B., Richard, M. L., Leducq, V., Pham, H., Michel, M., Costa, G. D., ... Sokol, H. (2016). CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nature Medicine, 22*(6), 598–605.
Lee, I., Bae, E., Lee, J., Lee, H., Ahn, Y., Huh, C., & Kim, D. (2010). *Bifidobacterium longum* HY8004 attenuates TNBS-induced colitis by inhibiting lipid peroxidation in mice. *Inflammation Research, 59*(5), 359–368.
Liu, Y., Su, Y., Ong, W., Cheng, T., & Tsai, Y. (2011). Oral administration of *Lactobacillus plantarum* K68 ameliorates DSS-induced ulcerative colitis in BALB/c mice via the anti-inflammatory and immunomodulatory activities. *International Immunopharmacology, 11*(12), 2159–2166.
Liu, Y., Ong, W. K., Su, Y., Hsu, C. C., Cheng, T., & Tsai, Y. (2016). Anti-inflammatory effects of *Lactobacillus brevis* K65 on RAW 264.7 cells and in mice with dextran sulphate sodium-induced ulcerative colitis. *Beneficial Microbes, 7*(3), 387–396.
Luerce, T. D., Gomessantos, A. C., Rocha, C. S., Moreira, T. G., Cruz, D. N., Lemos, L., ... Miyoshi, A. (2014). Anti-inflammatory effects of *Lactococcus lactis* NCDO 2118 during the remission period of chemically induced colitis. *Gut Pathogens, 6*(1), 33.
Ma, N., Guo, P., Zhang, J., He, T., Kim, S. W., Zhang, G., & Ma, X. (2018). Nutrients mediate intestinal bacteria-mucosal immune crosstalk. *Frontiers in Immunology, 9*, 5.
Moon, C., Baldridge, M. T., Wallace, M. A., Burnham, C. D., Virgin, H. W., & Stappenbeck, T. S. (2015). Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation. *Nature, 521*(7550), 90–93.
Molodecky, N., Soon, I. S., Rabi, D. M., Ghali, W. A., Ferris, M., Chernoff, G. W., ... Kaplan, G. G. (2012). Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology, 142*(1), 46–54.
Monk, J. M., Lepp, D., Wu, W., Pauls, K. P., Robinson, L. E., & Power, K. A. (2017). Navy and black bean supplementation primes the colonic mucosal microenvironment to improve gut health. *Journal of Nutritional Biochemistry, 49*, 89–100.
Murthy, S. N. S., Cooper, H. S., Shim, H., Shah, R. S., & Sedergran, D. J. (1993). Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. *Digestive Diseases and Ences, 38*(9), 1722–1734.
Neis, E. P., Dejong, C. H., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients, 7*(4), 2930–2946.
Oliveira, M., Bosco, N., Perruisseau, G., Nicolas, J., Seguraroggero, I., Duboux, S., ... Benyacoub, J. (2011). *Lactobacillus paracasei* reduces intestinal inflammation in adoptive transfer mouse model of experimental colitis. *Clinical & Developmental Immunology, 2011*, Article 807483.
Oliva, S., Nardo, G. D., Ferrari, F., Mallardo, S., Rossi, P., Patrizi, G., ... Stronati, L. (2012). Randomised clinical trial: The effectiveness of *Lactobacillus reuteri* ATCC 55730 rectal enema in children with active distal ulcerative colitis. *Alimentary Pharmacology & Therapeutics, 35*(3), 327–334.
Osman, N., Adawi, D., Molin, G., Ahrne, S., Berggren, A., & Jeppsson, B. (2006). *Bifidobacterium infantis* strains with and without a combination of Oligofructose and Inulin (OFI) attenuate inflammation in DSS-induced colitis in rats. *BMC Gastroenterology, 6*(1), 31.
Paramsothy, S., Nielsen, S., Kamm, M. A., Deshpande, N., Faith, J. J., Clemente, J. C., ... Kaakoush, N. O. (2019). Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology, 56*(5), 1440–1454.
Pascal, V., Pozuelo, M., Borruel, N., Casellas, F., Campos, D., Santiago, A., ... Manichanh, C. (2017). A microbial signature for Crohn's disease. *Gut, 66*(5), 813–822.
Pascal, J., Valérie, P., Jean-Luc, B., Idris, G., Teodora, A. A., & Christian, M. (2008). Prevalence of inflammatory bowel disease in the canton of vaud (switzerland): A population-based cohort study. *Journal of Crohns & Colitis, 2*(2), 131–141.
Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews Immunology, 14*(3), 141–153.
Peng, Y., Yan, Y., Wan, P., Chen, D., Ding, Y., Ran, L., ... Cao, Y. (2019). Gut microbiota modulation and anti-inflammatory properties of anthocyanins from the fruits of *Lycium ruthenicum* Murray in dextran sodium sulfate-induced colitis in mice. *Free Radical Biology and Medicine, 136*, 96–108.
Philippe, D., Heupel, E., Blumsperisen, S., & Riedel, C. U. (2011). Treatment with *Bifidobacterium bifidum* 17 partially protects mice from Th1-driven inflammation in a chemically induced model of colitis. *International Journal of Food Microbiology, 149*(1), 45–49.
Ramakrishna, B. S. (2013). Role of the gut microbiota in human nutrition and metabolism. *Journal of Gastroenterology and Hepatology, 28*, 9–17.
Rodrigueznogales, A., Algieri, F., Vezza, T., Garridomesa, N., Olivares, M., Comalada, M., ... Galvez, J. (2015). The viability of *Lactobacillus fermentum* CECT5716 is not essential to exert intestinal anti- inflammatory properties. *Food & Function, 6*(4), 1176–1184.
Sairenji, T., Collins, K. L., & Evans, D. V. (2017). An update on inflammatory bowel disease. *Primary Care, 44*(4), 673–692.
Schmitz, H., Barmeyer, C., Fromm, M., Runkel, N., Foss, H., Bentzel, C. J., ... Schulzke, J. (1999). Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology, 116*(2), 301–309.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology, 12*(6), 1–18.
Shen, L., Weber, C. R., Raleigh, D. R., Yu, D., & Turner (2011). Tight junction pore and leak pathways: A dynamic duo. *Annual Review of Physiology, 73*(1), 283–309.
Srutkova, D., Schwarzer, M., Hudcovic, T., Zakostelska, Z., Drab, V., Spanova, A., ... Schabussova, I. (2015). *Bifidobacterium longum* CCM 7952 promotes epithelial barrier function and prevents acute DSS-induced colitis in strictly strain-specific manner. *PLoS One, 10*, Article e0134050.
Steedman, H. F. (1950). Alcian blue 8GS: A new stain for mucin. *Journal of Cell Science, 91*(16), 477–479.
Theodoratou, E., Campbell, H., Ventham, N. T., Kolarich, D., Pucicbakovic, M., Zoldos, V., ... Lauc, G. (2014). The role of glycosylation in IBD. *Nature Reviews Gastroenterology & Hepatology, 11*(10), 588–600.
Van Klinken, B. J., Der Wal, J. G., Einerhand, A. W., Buller, H. A., & Dekker, J. (1999). Sulphation and secretion of the predominant secretory human colonic mucin MUC2 in ulcerative colitis. *Gut, 44*(3), 387–393.
Wang, J., Chen, H., Yang, B., Gu, Z., Zhang, H., Chen, W., & Chen, Y. Q. (2016). *Lactobacillus plantarum* ZS2058 produces CLA to ameliorate DSS-induced acute colitis in mice. *RSC Advances, 6*(18), 14457–14464.
Wang, G., Xu, Q., Jin, X., Hang, F., Liu, Z., Zhao, J., ... Chen, W. (2018). Effects of lactobacilli with different regulatory behaviours on tight junctions in mice with dextran sodium sulphate-induced colitis. *Journal of Functional Foods, 47*, 107–115.
Williams, B. L., Hornig, M., Parekh, T., & Lipkin, W. I. (2012). Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBIO, 3*(1), e00261–11.
Wildt, S., Nordgaard, I., Hansen, U., Brockmann, E., & Rumessen, J. J. (2011). A randomised double-blind placebo-controlled trial with *lactobacillus acidophilus* la-5 and *Bifidobacterium animalis* subsp. lactis bb-12 for maintenance of remission in ulcerative colitis. *Journal of Crohns & Colitis, 5*(2), 115–121.
Wu, H., Ye, L., Lu, X., Xie, S., Yang, Q., & Yu, Q. (2018). *Lactobacillus acidophilus* alleviated *Salmonella*-induced goblet cells loss and colitis by Notch pathway. *Molecular Nutrition & Food Research, 62*(22), 1800552.
Xu, J. J., Fang, X., Li, C. Y., Yang, L., & Chen, X. Y. (2019). *General and specialized tyrosine metabolism pathways in plants. aBIOTECH, 1*, 97–105.
Yan, S., Yang, B., Zhao, J., Zhao, J., & Chen, W. (2019). A ropy exopolysaccharide producing strain *Bifidobacterium longum* subsp. longum YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation. *Food & Function, 10*(3), 1595–1608.
Yang, T., Weisenhorn, P., Gilbert, J. A., Ni, Y., Sun, R., Shi, Y., & Chu, H. (2016). Carbon constrains fungal endophyte assemblages along the timberline. *Environmental Microbiology, 18*(8), 2455–2469.
Yang, B., Chen, H., Gao, H., Wang, J., Stanton, C., Ross, R. P., ... Chen, W. (2018). *Bifidobacterium breve* CCFM683 could ameliorate DSS-induced colitis in mice primarily via conjugated linoleic acid production and gut microbiota modulation. *Journal of Functional Foods, 49*, 61–72.
Yang, Q., Wang, S., Ji, Y., Chen, H., Zhang, H., Chen, W., ... Chen, Y. Q. (2017). Dietary intake of n-3 PUFAs modifies the absorption, distribution and bioavailability of fatty acids in the mouse gastrointestinal tract. *Lipids in Health and Disease, 16*(1), 10.
Yeom, Y., Kim, B. S., Kim, S. J., & Kim, Y. (2016). Sasa quelpaertensisleaf extract regulates microbial dysbiosis by modulating the composition and diversity of the microbiota in dextran sulfate sodium-induced colitis mice. *BMC Complementary & Alternative Medicine, 16*(1), 481.
Zakostelska, Z., Kverka, M., Klimesova, K., Rossmann, P., Mrazek, J., Kopecny, J., ... Tlaskalovahogenova, H. (2011). Lysate of probiotic *Lactobacillus casei* DN-114 001 ameliorates colitis by strengthening the gut barrier function and changing the gut microenvironment. *PloS One, 6*, Article e27961.
Zwolinskawcislo, M., Brzozowski, T., Ptakbelowska, A., Targosz, A., Urbanczyk, K., Kwiecien, S., & Sliwowski, Z. (2011). Nitric oxide-releasing aspirin but not conventional aspirin improves healing of experimental colitis. *World Journal of Gastroenterology, 17*(36), 4076–4089.