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A ropy exopolysaccharide producing strain *Bifidobacterium longum* subsp. *longum* YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation†

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B. longum has been reported to exert an alleviative effect on colitis, but the results also suggested significant differences among strains. Here in this study, we compared the effect of *B. longum* subsp. longum strains with different properties in EPS production on DSS-induced colitis. To investigate the alleviative effect of a ropy-exopolysaccharide (EPS) producing strain, *Bifidobacterium longum* subsp. *longum* YS108R, on experimental colitis, C57BL/6J mice (male, 6–8 weeks old) were randomly assigned to six groups (n = 8): normal control, DSS colitis and four DSS colitis groups orally administered with three *B. longum* subsp. *longum* strains (YS108R, C11A10B and HAN4-25) and *B. animalis* subsp. *lactis* BB12, respectively, in which YS108R produced ropy-EPS, C11A10B produced non-ropy-EPS, HAN4-25 did not produce EPS and BB12 was set as a positive control. Ropy-EPS producing strain YS108R could alleviate the symptoms and remit inflammation induced by DSS, in which YS108R could decrease the pro-inflammatory cytokine IL-6 and IL-17A levels after DSS challenge (from 102 ± 45.22 to 37.95 ± 20.33 pg mL^{-1} and from 22.14 ± 5.43 to 12.58 ± 2.74, $p < 0.05$), but another non-ropy-EPS producing strain C11A10B did not decrease the levels of these pro-inflammatory cytokines. Furthermore, YS108R could maintain the expression levels of genes related to the mucosal barrier, but strain HAN4-25, a non-EPS producer, was not able to maintain the expression levels of these genes after DSS challenge. Analysis of gut microbiota showed that DSS treatment significantly increased the relative abundance of *Enterobacteriaceae* and *Peptostreptococcaceae* (0.2623 ± 0.162 and 0.0512 ± 0.0361) and decreased the relative abundance of *S24-7* (0.042 ± 0.0326); however, YS108R administration could decrease the relative abundance of *Enterobacteriaceae* and *Peptostreptococcaceae* to 0.0848 ± 0.0399 and 0.0032 ± 0.0047 and increase the relative abundance of *S24-7* to 0.2625 ± 0.0566 ($p < 0.05$). The results showed that *B. longum* subsp. *longum* YS108R could alleviate DSS-induced colitis by modulating the inflammation related cytokines, maintenance of the normal mucosal barrier and reverting the change of microbiota.

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1. Introduction

Inflammatory bowel disease (IBD) is a kind of chronic intestinal inflammation, and the epidemiological patterns suggest a worldwide prevalence of IBD in the coming years.[1] Pharmaceuticals used to treat IBD include immunosuppressive drugs, antibiotics and some biological agents, in which 5-aminosalicylic acid (5-ASA) and its derivatives are the mainstay of therapy for the induction and maintenance of remission in patients with UC; however, adverse events may appear during the treatment period, such as diarrhea, abdominal pain, headache and nasopharyngitis.[2–4] Although the aetiology and pathogenesis of IBD are not completely understood, it is

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becoming increasingly clear that the genetic susceptibility factors and environmental factors both play important roles in the pathogenesis of IBD, in which environmental factors are primary.[5,6] The correlation between IBD and certain environmental factors, including diet, antibiotic use, germ exposure and social status, is supported by epidemiological, clinical and experimental evidence. Some of the factors, such as diet and antibiotic use, might result in the composition changes of intestinal microbiota.[7,8] Interaction disorder between intestinal microbes and the mucosal immune system has been confirmed as a critical defect resulting in chronic intestinal inflammation.[9]

Bifidobacteria are one of the most common bacteria ingested as probiotics, in food and food supplements, which may interact with the host and exert beneficial modulatory effects on the immune system.[10] Among different species in *Bifidobacterium*, *B. longum* is the most abundant bifidobacterial member in healthy breast-fed infants and adults.[11,12] Randomized controlled clinical trials and animal research studies indicated that *B. longum* could reduce the clinical appearance of chronic mucosal inflammation in ulcerative colitis (UC) patients and prevent the development of DSS-induced experimental colitis.[13–15] However, a study performed by Srutkova *et al.* suggested that *B. longum* exhibited strain-specific abilities to reduce the clinical symptoms and inflammatory level in DSS-induced colitis.[16]

The exopolysaccharide (EPS), attached to and surrounding the bacteria, is an important macromolecule that mediates the interaction between gut microorganisms and the host.[17] The production of EPS exhibits wide difference at the strain level and is associated with the tolerance ability of the bacteria to the gastrointestinal environment (especially cell-surface-bound EPS).[18] The EPS produced by *B. breve* UCC2003 induced a weak immune response in the host after oral administration, which ensured that the bacteria survived against the host immune system, but its isogenic mutants with no surface EPS induced a strong immune response and had a decreased persistence in the host gut.[19] EPS produced by different strains may have variable structures and exhibit different effects on the immune response of the host. For instance, some high molecular weight EPS producing strains may suppress immune response while some low molecular weight EPS producing strains may stimulate immune response.[20]

In our previous research, the EPS production of a number of *B. longum* subsp. *longum* strains was quantified, and there was a wide range of EPS production among these strains.[18] The aim of this study was to compare the effect of *B. longum* subsp. *longum* strains with different properties in EPS production on DSS-induced colitis.

2. Materials and methods

2.1. Bacterial strains

B. longum subsp. *longum* YS108R, C11-A10B and HAN4-25 were isolated from human fecal samples previously,[18,21] and C11A10B and HAN4-25 were deposited at the Collection Center of Food Microbiology, Jiangnan University, and YS108R was deposited at the Guangdong culture collection center, with a collection number of GDMCC 60310. Phylogenetic analysis based on genomic sequences of these strains is shown in Fig. S1.† In particular, strain YS08R (producing ropy EPS) was a spontaneous mutant strain from C11A10B (producing non-ropy EPS) during consecutive cultures, and genome sequencing revealed that there is a single nucleic acid change in a gene related to the synthesis of the exopolysaccharide;[21] strain HAN4-25 did not generate EPS; *B. animalis* subsp. *lactis* BB12 is chosen as a positive control which is a widely used commercial probiotic strain and has been reported to have a potential inflammatory modulation effect and alleviate ulcerative colitis.

2.2. Animal experimental designs

The animal experimental protocol in this study was approved by the Animal Ethics Committee of Jiangnan University, China (Permission No. SYXK 2012-0002). C57BL/6J mice (male, 6–8 weeks old, 18–20 g) were obtained from Shanghai Laboratory Animal Center (Shanghai, China) and housed in the Animal Experiment Center of Jiangnan University. The animals were kept under standard conditions (22 °C ± 2 °C, 55% ± 5% humidity and 12 h light–dark cycle) with a standard pelleted diet and sterilized water. Bifidobacteria were cultured in MRS broth daily, collected by centrifugation and washed twice with normal saline, and then the collected cell pellets were resuspended and adjusted to 5×10^9 CFU mL^{-1} with normal saline, which would be used for oral gavage to mice.

The experimental procedure is described in Fig. 1. After one-week adaptation, forty-eight mice were randomly divided into six groups with 8 mice per group: the control group and DSS treatment group received 200 μL day^{-1} of saline orally; four other groups were subjected to oral gavage of 200 μL day^{-1} of corresponding bifidobacteria resuspension with 10^9 CFU until the end of the experiment (YS108R, C11-A10, HAN4-25 and BB12 for each group). After two weeks of saline or bifidobacteria treatment, colitis was induced in the DSS treatment group and bifidobacteria administered groups by adding DSS in their drinking water (2.5%, w/v, 36–50 kDa, MP Biomedicals, CA) for one week (DSS solution was replaced every two days).[22] Meanwhile, the control group drank sterilized water throughout the experiment period. At the end of the experiment, animals were anesthetized and the blood samples were collected by removing the eyeballs and the serum was obtained by centrifugation at 3000 *g* for 20 min and stored at −80 °C. The colon of each mouse was collected. A 5 mm length of the colon sample 1 cm away from the anus was cut off and fixed in 4% paraformaldehyde fixation solution, and the other part of the colon was stored at −80 °C until analysis. During the DSS induction, the body weight was measured and the feces of each animal were collected daily. The DAI of each mouse was calculated daily according to the method described before and with some modification, and the scoring standard is shown in Table 1, the DAI was calculated as the total score divided by three.[23]

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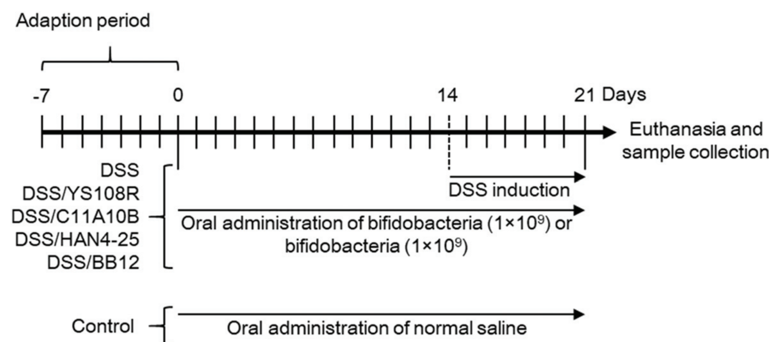

Fig. 1 Procedure of the animal experiment. Control and DSS treatment groups were orally administered with saline and bifidobacteria administered groups were orally administered with the corresponding bifidobacteria strains (YS108R, C11-A10B, HAN4-25 and BB12), respectively, throughout the experiment period; from the 15th day to the end of the experiment, the DSS treatment group and bifidobacteria administered groups were induced with DSS (adding 2.5% DSS in drinking water).

Table 1 Scoring standard of the disease activity index (DAI)

Score	Body weight loss (%)	Stool consistency	Blood
0	<1	Normal	Negative hemoccult
1	1–5	Soft but still formed	Positive hemoccult
2	5–10	Soft and unformed	Visible blood in stool
3	>10	Diarrhea	Rectal bleeding

Table 2 Primers used in real-time PCR

Target gene	Sequence (5′-3′)	Ref.
β-Actin	F: CTTCTAGGCGGACTGTTACTGAG R: TTGCTCCAACCAACTGCTGTC	28
IL-1β	F: CAACCAACAAGTGATATTCTCCATG R: GATCCACACTCTCCAGCTGCA	29
IL-6	F: GGAGCCCACCAAGAACGATAG R: GTGAAGTAGGGAAGGCCGTG	30
IL-10	F: GGTTGCCAAGCCTTATCGGA R: ACCTGCTCCACTGCCTTGCT	31
IL-17A	F: GCTCCAGAAGGCCCTCAGA R: AGCTTTCCCTCCGCATTGA	32
TNF-α	F: GAAAAGCAAGCAGCCAACCA R: TCTTCTGCCAGTTCCACGTC	31
MCP-1	F: CTTCTGGGCCTGCTGTTCA R: CCAGCCTACTCATTGGGATCA	33
ZO-1	F: GCTGCCTCGAACCTCTACTC R: TTGCTCATAACTTCGCGGGT	28
Occludin	F: TCACTTTTCCTGCGGTGACT R: GGGAACGTGGCCGATATAATG	28
Claudin-1	F: ATGCAAAGATGTTTTGCCACAG R: TACAAATTCCCATTGCAGCCC	28
MUC2	F: ATGCCCACCTCCTCAAAGAC R: GTAGTTTCCGTTGGAACAGTGAA	34

2.3. Determination of myeloperoxidase (MPO) activity in colonic tissue samples

Activity of MPO in colonic tissue was detected using a Myeloperoxidase Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Colonic tissues were weighed and washed with 10 mmol L^{-1} phosphate buffered saline (PBS, pH 7.0) and homogenized in 9 volumes of PBS, and the homogenate was used to determine MPO activity according to the manufacturer's manual.

2.4. Determination of cytokine levels in serum samples

IL-1β, IL-6, IL-10, IL-17A, MCP-1 and TNF-α in the serum were determined using a MILLIPLEX MAP Kit Mouse Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore Corporation, Billerica, MA, USA) following the instructions from the manufacturer and performed with a Luminex MAGPIX System (Luminex, Austin, TX).

2.5. Determination of cytokine and tight junction protein expression in colon tissue by real-time PCR

To isolate total RNA in colon tissue, about 50 mg colon was ground with liquid nitrogen and RNA was extracted using the TRIzol™ reagent (Life Technologies Corporation, Carlsbad, CA) as described previously.[24] The purity and concentration of the isolated RNA were measured using a NanoPhotometer N60 Tough spectrophotometer (Implen GmbH, München, Germany). Then, cDNA was obtained by reverse transcribing 1 μg RNA using a Random Hexamer primer and reagents in a RevertAid RT Kit (Thermo Scientific, Carlsbad, CA). 10 ng cDNA, 500 nM specific primers (Table 2), 10 μL SYBR™ Green PCR Master Mix (Applied Biosystems, Carlsbad, CA) and RNase-Free water were mixed to a final volume of 20 μL. Then the amplification was performed with a CFX96 Touch™ Real-Time PCR System (Bio-Rad Laboratories, Hercules, CA). The values of the β-actin gene was used to normalize the values of other genes and the values of each gene belonging to the control group were set as in ref. 25 and 26. The expression level of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method.[27]

2.6. Histological analysis of colon

After fixing in paraformaldehyde solution for 24 h, colon samples were dehydrated with graded alcohol solutions and then embedded in paraffin wax. Ultrathin slices (5 μm) were obtained by cutting the paraffin-embedded colon samples and then stained with Hematoxylin and Eosin (H&E). The stained

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slices were finally covered with coverslips using neutral balsam as adhesive. A pathologic slice scanner was used to record the photomicrographs. Histological damage scores were assessed using a published system.[23] Alcian blue with post-staining with Nuclear Fast Red (All from Vector, Burlingame, CA) was used to perform the observation of mucin in the colon.[16]

2.7. Analysis of fecal microbiota by high-throughput sequencing

Total DNA in mice fecal samples was extracted using a FastDNA Spin Kit for Feces (MP Biomedicals, Santa Ana, CA) according to the manual. The V3–V4 region of 16S rRNA gene was amplified with a universal bacterial primer pair: 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). A TAG barcode of 7 bases was linked to the 5′ end of 341F to identify different samples. 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). Generation of libraries, sequencing on Illumina MiSeq and data processing were performed according to the work by Mao *et al.*[35] Particularly, sequences with a lower quality score (<30) and a short length (<200 bp) and sequences containing ambiguous bases and showing mismatches in primers were removed. Random rarefaction of each library was performed according to the sample with the lowest number of sequences, and then β-diversity calculations were performed within QIIME. Sets of trimmed sequences with more than 97% identity were defined as an Operational Taxonomic Unit (OTU). A Ribosomal Database Project (RDP) Naive Bayes classifier was used to assign the OTUs to phylotypes.

2.8. Statistical analysis

Statistical analysis of the data was conducted with the GraphPad Prism 7.0 software. A Shapiro–Wilk normality test was performed to verify the normality of distribution of the values. Tukey's test of one-way analysis of variance (ANOVA) was performed to determine the significant differences among groups. The results were expressed as the mean value ± standard deviation (SD). Significant differences were defined as $p < 0.05$.

3. Results

3.1. Ropy EPS producing *B. longum* subsp. *longum* strain YS108R could alleviate the colonic damage induced by DSS

In the present study, the effect of different bifidobacterial strains on DSS induced colitis was investigated, including three *B. longum* subsp. *longum* strains with different properties in EPS production and a widely used commercial strain *B. animalis subsp. lactis* BB12. DAI, which is a composite score comprising weight loss, stool consistency and bleeding, continued to increase from the second day (day 15) of DSS induction in the DSS treatment group and bifidobacteria administered groups, and the DAI values were kept at 0 in the control group (Table 3). The maximum DAI values were obtained at the end of the experiment, in which only the group administered with YS108R showed a significantly lower DAI value ($p < 0.05$) than that of the DSS treatment group. The BB12 fed group also showed a lower DAI value than the DSS treatment group, but the difference was not significant. The DAI values of C11A10B and HAN4-25 fed groups were close to that of DSS group and higher than that of YS108R fed group (Table 3). Colon shortening could serve as a macroscopic marker of colonic injury, and the colon lengths of DSS induced groups were shorter than those of the control group, but the colon length of mice supplemented with YS108R was similar to that of the control group without any significant difference (Table 4 and Fig. 2). During the DSS treatment, the activity of MPO increased; however, in YS108R and BB12 administered groups, the activities of MPO were significantly lower than those in the DSS treatment group (Table 4).

3.2. Histological analysis of colon

DSS exposure could result in histological damage to colonic tissues. In order to evaluate the protective function of different bifidobacteria strains, the histological scores of colonic tissues were assessed based on the crypt architecture, degree of inflammatory cell infiltration, muscle thickening, goblet cell depletion and crypt abscess, and the histological score was the sum of each individual score of the five indicators mentioned above. In the control group (Fig. 3A), the crypts in the mucosal

Table 3 Changes of the disease active index (DAI) during the experimental period

experimental period (Days)	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
14[a]	0(0,0)	0(0,0)	0(0,0)	0(0,0)	0(0,0)	0(0,0)
15[a]	0(0,0)	0.17(0,0.33)	0(0,0.33)	0(0,0.33)	0.17 (0,0.33)	0(0,0.33)
16[a]	$0(0,0)^{a}$	$0.67(0.33,0.83)^{c}$	$0.17(0,0.67)^{ab}$	$0.33(0.33,0.83)^{bc}$	$0.33(0,1)^{bc}$	$0.33(0,0.33)^{ab}$
17	0.00 ± 0.00^{a}	0.81 ± 0.14^{d}	0.38 ± 0.28^{ab}	0.73 ± 0.24^{cd}	0.63 ± 0.22^{bcd}	0.50 ± 0.18^{bc}
18	0.00 ± 0.00^{a}	1.02 ± 0.19^{c}	0.48 ± 0.3^{b}	0.94 ± 0.28^{c}	0.82 ± 0.20^{c}	0.70 ± 0.14^{bc}
19	0.00 ± 0.00^{a}	1.42 ± 0.4^{c}	0.77 ± 0.23^{b}	1.25 ± 0.32^{c}	1.19 ± 0.28^{bc}	1.00 ± 0.25^{bc}
20	0.00 ± 0.00^{a}	1.73 ± 0.36^{c}	1.15 ± 0.33^{b}	1.58 ± 0.35^{bc}	1.59 ± 0.56^{bc}	1.30 ± 0.36^{bc}
21	0.00 ± 0.00^{a}	2.12 ± 0.43^{c}	1.38 ± 0.37^{b}	1.91 ± 0.35^{bc}	2.09 ± 0.40^{c}	1.62 ± 0.29^{bc}

[a] Representing the data that are not in accordance with normal distribution and are shown as the median (minimum, maximum); other data are in accordance with normal distribution and data are shown as mean ± SD. Different letters in each row indicate significant difference ($P < 0.05$) among groups.

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Table 4 Effects of different bifidobacteria strains on the colonic damage induced by DSS

	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
Colon length	7.67 ± 0.36[a]	5.24 ± 0.57[c]	7.01 ± 0.50[ab]	5.85 ± 0.92[c]	5.52 ± 0.59[c]	6.11 ± 0.74[bc]
MPO	0.08 ± 0.02[a]	0.39 ± 0.09[c]	0.22 ± 0.06[ab]	0.37 ± 0.16[c]	0.31 ± 0.05[bc]	0.21 ± 0.10[ab]
Histological score	0.50 ± 1.00[a]	11.88 ± 2.23[c]	7.00 ± 2.39[b]	10.88 ± 2.59[c]	11.25 ± 2.38[c]	9.13 ± 3.14[bc]

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference (P < 0.05) among groups.

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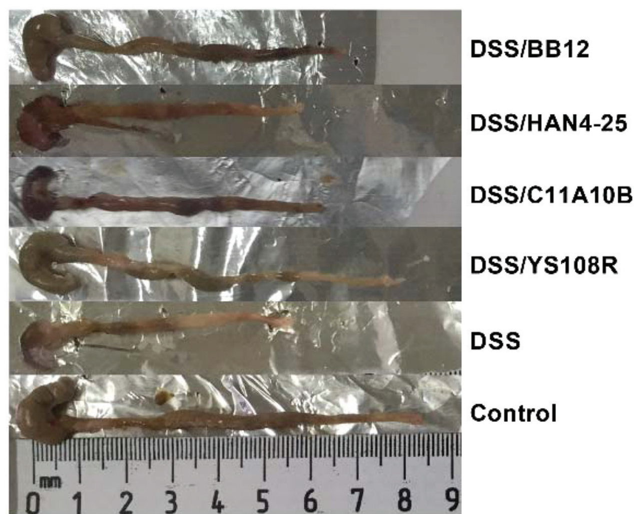

Fig. 2 Macroscopic pictures of colons.

layer presented regular structures, and the thicknesses of mucosal and muscular layers were normal, and there were also no inflammatory cell infiltration and goblet cell depletion. However, in the DSS treatment group (Fig. 3A), the crypt structure was irregular or even disappeared, and it was accompanied by severe inflammatory cell infiltration and goblet cell disappearance, and partial thickening of submucosa was observed. In contrast, in the mice with YS108R administration, the crypt structures were entire and almost similar to those in the control group, and some distortion could be found; additionally, the inflammatory cell infiltration was more moderate or absent. In C11A10B and HAN4-25 fed mice, the structures of crypts were mostly distorted or had disappeared, and the severity of inflammatory cell infiltration was similar to that in the DSS treatment group. Mice fed with BB12 had a visible structure of the crypt, but there were inflammatory cells in lamina propria (Fig. 3A). In control and YS108R administered groups, the cavities of crypts were filled with mucin produced by goblet cells (stained with alcian blue), but in the DSS treatment group, mucin was remarkably decreased, as well as in three other *Bifidobacterium* strain administered groups (Fig. 3B). Taking these factors into consideration, the histological scores of each group were assessed (shown in Table 4). Correspondingly, the histological scores of all the DSS induced colitis groups were significantly higher than those of the control; however, YS108R supplementation could reduce the colonic injury compared with other groups.

3.3. Cytokine levels in serum samples and expression levels of cytokine genes in colon tissue

In order to understand the effects of administration of different bifidobacterial strains on immune parameters, the concentrations of several cytokines in the serum (Table 5) and the expression levels of their structural genes (Table 6) were measured. DSS treatment induced the increase of pro-inflammatory (TNF-α, IL-1β and IL-6) and chemokine MCP-1 in the DSS treatment group, both in the serum and the gene expression in colitis tissue; the serum concentration of IL-17A of the DSS treatment group was significantly higher than that in the control group; however the difference at the mRNA level was not significant. Notably, IL-6 was the most significant pro-inflammatory cytokine that increased by 56 fold in gene expression and by 12 fold in the serum. Administration of the bifidobacterial strains could decrease the concentrations of TNF-α and IL-1β in the serum and their expression in the colon. Compared with the DSS treatment group, YS108R and BB12 could significantly decrease the IL-6 concentration in the serum and its colonic expression level. In YS108R and HAN4-25 administration groups, the concentrations of IL-17A in the serum were significantly lower than that in the DSS treatment group; however, no significant difference was observed in the expression level in the colon. The concentrations of MCP-1 in bifidobacterial administered groups were lower than that in the DSS treatment group, but its gene expression levels were similar to those in the DSS treatment group, except for the C11A10B administered group which had an even higher expression level of MCP-1 than the DSS treatment group. Administration of YS108R and C11A10B could increase the expression level of IL-10, but only the YS108R administered group had a higher concentration of IL-10 in the serum. Overall, YS108R exerted the most significant inflammatory modulation effect, followed by HAN4-25 and BB12, and the profiles of cytokines in C11A10B treatment mice were similar to those of colitis mice, which was indicated by the higher IL-6 and IL-17A levels.

3.4. Expression levels of genes related to tight junction proteins (TJP) and mucin in colon tissue

In order to evaluate the influence of bifidobacteria on the mucosal barrier, expression levels of genes related to TJP (ZO-1, occludin and claudin-1) and mucin2 (MUC2) were

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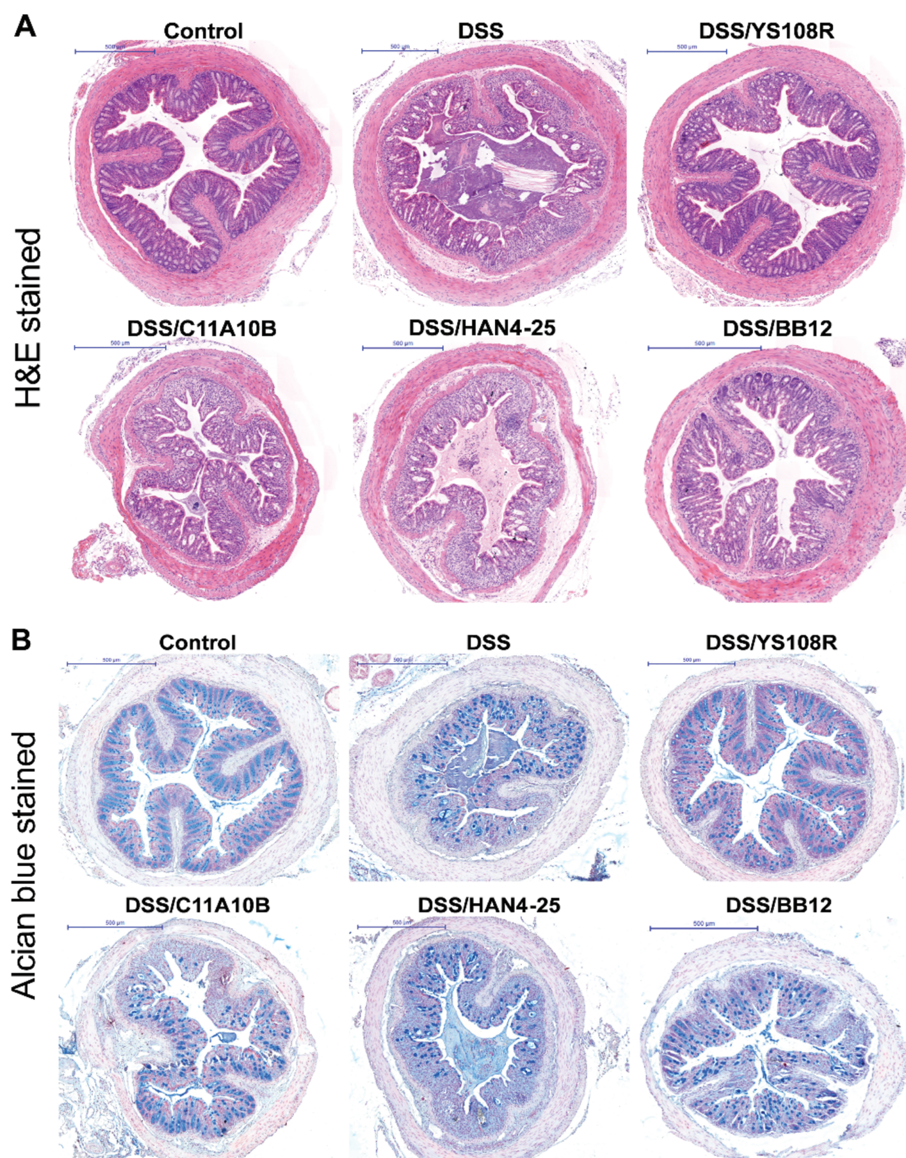

Fig. 3 Impact of different bifidobacteria strains on the histological evaluation of DSS induced colitis. (A) H&E stained sections of the colon. (B) Alcian blue stained sections of the colon.

Table 5 Effects of different bifidobacteria strains on the concentrations of cytokines in the serum (pg mL^{-1})

Cytokines	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
TNF-α	3.29 ± 0.92^{a}	8.81 ± 5.06^{b}	4.47 ± 0.58^{a}	4.70 ± 0.71^{a}	5.10 ± 0.83^{a}	6.14 ± 1.01^{ab}
IL-1β	401.16 ± 79.29^{a}	1023.03 ± 255.10^{b}	509.45 ± 314.01^{a}	459.35 ± 116.39^{a}	562.76 ± 149.22^{a}	522.65 ± 348.96^{a}
IL-6	6.66 ± 3.88^{a}	102.03 ± 45.22^{c}	37.95 ± 20.33^{ab}	69.40 ± 27.16^{bc}	29.83 ± 13.28^{ab}	40.23 ± 23.79^{ab}
IL-10	3.23 ± 0.73^{a}	6.03 ± 1.70^{a}	9.72 ± 2.80^{b}	5.95 ± 0.69^{a}	5.98 ± 0.99^{a}	6.48 ± 1.64^{a}
IL-17A	3.15 ± 2.16^{a}	22.14 ± 5.43^{c}	12.58 ± 2.74^{ab}	20.89 ± 5.48^{bc}	13.24 ± 7.69^{ab}	16.54 ± 7.33^{bc}
MCP-1	9.40 ± 4.18^{a}	59.17 ± 30.81^{b}	18.88 ± 8.62^{a}	13.00 ± 3.95^{a}	13.73 ± 8.15^{a}	28.72 ± 18.56^{a}

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference ($P < 0.05$) among groups.

measured by RT-PCR (Table 7). The expression of all the TJ and MUC2 genes significantly decreased in the DSS treatment group. Animals administered with YS108R could maintain the expression of the ZO-1, Claudin-1 and MUC2 genes at levels similar to or even higher than the control group. The C11A10B administered group had higher expression levels of claudin-1

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Table 6 Effects of different bifidobacteria strains on the relative expression of cytokines in colonic tissue (fold change of mRNA)

Cytokines	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
TNF-α	1.07 ± 0.47^{a}	9.85 ± 3.00^{c}	1.73 ± 0.85^{ab}	4.68 ± 1.55^{b}	2.02 ± 0.92^{ab}	2.25 ± 0.89^{ab}
IL-1β	1.06 ± 0.42^{a}	26.09 ± 11.18^{b}	3.37 ± 2.99^{a}	5.16 ± 3.45^{a}	13.74 ± 8.56^{ab}	5.53 ± 3.48^{a}
IL-6	1.21 ± 0.86^{a}	56.73 ± 17.86^{c}	7.68 ± 4.74^{ab}	31.28 ± 15.83^{bc}	37.93 ± 14.49^{c}	10.55 ± 6.61^{ab}
IL-10	1.04 ± 0.31^{a}	0.57 ± 0.06^{a}	2.73 ± 1.67^{ab}	4.71 ± 2.06^{b}	1.18 ± 0.82^{a}	0.7 ± 0.44^{a}
IL-17A	1.07 ± 0.38^{a}	8.34 ± 4.28^{a}	3.85 ± 2.23^{a}	21.68 ± 7^{b}	5.15 ± 3.14^{a}	5.45 ± 3.51^{a}
MCP-1	1.01 ± 0.29^{a}	4.64 ± 0.92^{b}	3.49 ± 1.28^{ab}	7.88 ± 1.81^{c}	4.59 ± 1.04^{b}	3.78 ± 1.05^{b}

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference (P < 0.05) among groups.

Table 7 Effects of different bifidobacteria strains on the relative expression of tight junction protein and mucin

	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
ZO-1	1.02 ± 0.11^{a}	0.64 ± 0.07^{bc}	1.18 ± 0.13 ^{a}	0.91 ± 0.14 ^{ac}	0.47 ± 0.17 ^{bd}	0.33 ± 0.15 ^{d}
Occludin	1.03 ± 0.19^{a}	0.25 ± 0.12^{c}	0.59 ± 0.04^{b}	0.46 ± 0.11^{bc}	0.26 ± 0.13^{c}	0.32 ± 0.11^{bc}
Claudin-1	1.01 ± 0.11^{ab}	0.29 ± 0.08^{c}	1.53 ± 0.37^{b}	0.83 ± 0.42^{ad}	0.36 ± 0.09^{cd}	0.17 ± 0.09^{c}
MUC2	1.01 ± 0.18^{a}	0.45 ± 0.23^{b}	1.65 ± 0.21^{c}	1.13 ± 0.26^{ac}	0.59 ± 0.23^{ab}	0.44 ± 0.32^{b}

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference (P < 0.05) among groups.

and MUC2 genes than the DSS treatment group, but still lower than the YS108R administered group. In HAN4-25 and BB12 administered groups, the expression levels of TJP and mucin2 genes were similar to those of the DSS treatment group.

3.5. Modulation of intestinal microbiota

To analyze the modulatory effects of different bifidobacteria on gut microbiota of DSS induced mice, the V3–V4 region of the 16S rRNA gene was sequenced with Illumina Miseq. A total of 1 468 687 high-quality 16S rRNA gene sequences was obtained from all samples, and the average sequence number was 40 796 for each sample, which is suitable for analysis. At the phylum level, 11 phyla were identified in all of the samples, and the relative abundance of 7 phyla was more than 0.1%, which comprised more than 98% of all the reads (Table 8). In the control group, the dominant phyla were *Bacteroidetes* (44.12%), *Firmicutes* (35.14%), *Verrucomicrobia* (15.68%) and *Proteobacteria* (2.08%). However, DSS treatment dramatically shifted the bacterial composition at the phylum level, and the relative abundance of *Proteobacteria* increased to 28.78% and the relative abundance of *Bacteroidetes* and *Verrucomicrobia* decreased to 15.97% and 1.68% (Table 8). Even though the relative abundance of *Proteobacteria* in YS108R, C11A10B and HAN4-25 administered groups was higher than that in the control group, it was significantly decreased compared with the DSS group. However, administration of BB12 did not decrease the abundance of *Proteobacteria* after DSS challenge. All bifidobacterial strains used in this study could significantly increase the relative abundance of *Bacteroidetes* after DSS challenge, in which three *B. longum* strains could make it recover to the level similar to that of the control group. Specifically, our results showed that the oral administration of YS108R could maintain the relative abundance of *Verrucomicrobia* after DSS challenge. Principal coordinates analysis (PCoA) of weighted UniFrac distances showed that the gut microbiota of DSS treatment mice was significantly different from that of the mice in the control group

Table 8 Composition of the gut microbiota at the phylum level

Phylum	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
Actinobacteria	0.0167 ± 0.0047^{ab}	0.0099 ± 0.01^{bc}	0.0047 ± 0.0014^{c}	0.0055 ± 0.0025^{c}	0.0052 ± 0.0037^{c}	0.0288 ± 0.0196^{d}
Bacteroidetes	0.4412 ± 0.0444^{a}	0.1597 ± 0.0405^{c}	0.3917 ± 0.0602^{a}	0.44 ± 0.0779^{a}	0.4229 ± 0.044^{a}	0.3315 ± 0.0086^{b}
Deferribacteres	0.0002 ± 0.0001^{a}	0.0413 ± 0.0373^{c}	0.0079 ± 0.0059^{ab}	0.0212 ± 0.0115^{abc}	0.0313 ± 0.0251^{bc}	0.0194 ± 0.0152^{abc}
Firmicutes	0.3514 ± 0.0439^{a}	0.4662 ± 0.1341^{b}	0.3441 ± 0.0723^{a}	0.3239 ± 0.059^{a}	0.2898 ± 0.1222^{a}	0.2576 ± 0.0516^{a}
Proteobacteria	0.0208 ± 0.0155^{a}	0.2878 ± 0.1449^{c}	0.0969 ± 0.0325^{ab}	0.1287 ± 0.0782^{b}	0.17 ± 0.1066^{b}	0.3105 ± 0.0398^{c}
Tenericutes	0.001 ± 0.0008^{a}	0.0001 ± 0.0001^{a}	0.0029 ± 0.0042^{ab}	0.0079 ± 0.0113^{b}	0.002 ± 0.0026^{ab}	0.0008 ± 0.0005^{a}
Verrucomicrobia	0.1568 ± 0.0513^{a}	0.0168 ± 0.0127^{c}	0.1328 ± 0.0428^{a}	0.0566 ± 0.0351^{bc}	0.0608 ± 0.0343^{b}	0.0366 ± 0.0191 ^{bc}

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference (P < 0.05) among groups.

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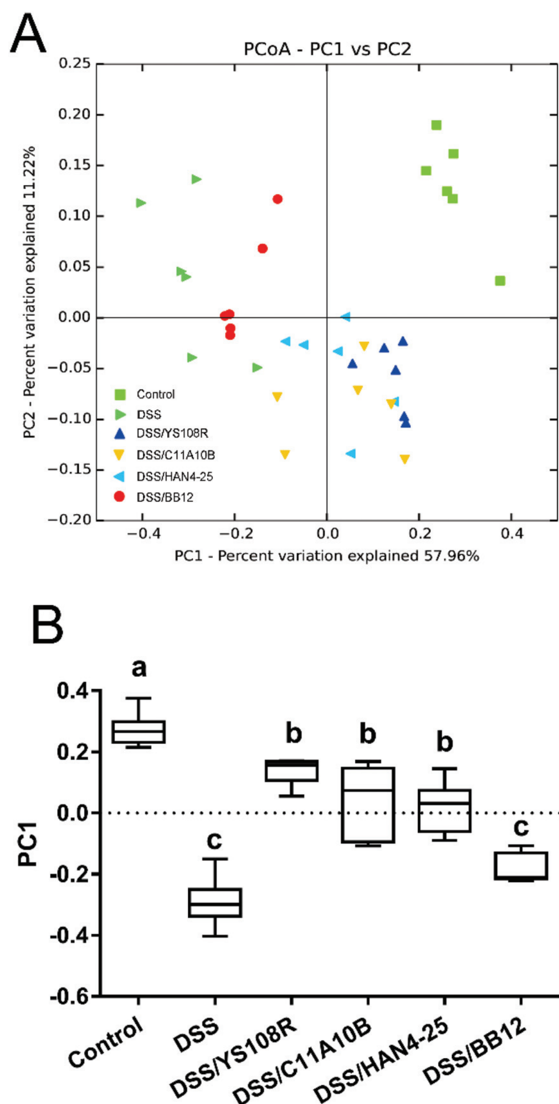

Fig. 4 Effect of different bifidobacteria strains on the gut microbiota of DSS induced mice. (A) Principal coordinates analysis (PCoA) of weighted UniFrac distances; and (B) PC1 values. Each bar presents the range from the minimum value to the maximum value, and bars that do not share a common letter on the top are significantly different according to multiple comparisons using Tukey's test ($p < 0.05$).

(Fig. 4A), and administration of *B. longum* strains could remit the shift of gut microbiota induced by DSS treatment, in which PC1 explained 57.96% of the difference (Fig. 4B). At the genus level, 167 genera were identified in the combined sample set, in which 14 genera had a relative abundance of more than 1% and comprised 90% of all the reads. The relative abundance of 3 genera was decreased by DSS treatment, including the genera of unclassified *S24-7*, *Akkermansia* and *Lactobacillus*, whose relative abundance values were 42.95%, 15.68% and 15.97%, respectively, and their relative abundance decreased to 4.20%, 1.68% and 3.43% after DSS challenge. In addition, the relative abundance of 7 genera increased after DSS induction, and they consist of the genera of unclassified *Enterobacteriaceae*, *Bacteroides*, *Turicibacter*, *Mucispirillum*, and *Oscillospira*, and the genera of unclassified *Peptostreptococcaceae* and *Enterococcus* (Table 9). Administration of *B. longum* subsp. *longum* strains could increase the relative abundance of unclassified *S24-7* and decrease the relative abundance of unclassified *Peptostreptococcaceae* and unclassified *Enterobacteriaceae* compared with the DSS group, even though the effect of HAN4-25 on *Enterobacteriaceae* was not significant (Table 9). Particularly, in the YS108R administration group, the relative abundance of *Enterobacteriaceae* was decreased from 26.44% to 8.54% which was similar to that in the control group. Spearman correlation analysis (Table 10) showed that the relative abundance of *S24-7* and *Akkermansia* was negatively correlated with pro-inflammatory cytokines, but they were positively correlated with the expression levels of TJ proteins and MUC2; in contrast, the relative abundance of *Enterobacteriaceae*, *Peptostreptococcaceae* and *Turicibacter* was positively correlated with pro-inflammatory cytokines and the former two were negatively correlated with the expression levels of TJ proteins and MUC2. In addition, the relative abundance of *Lachnospiraceae* was positively correlated with the expression levels of TJ proteins and MUC2.

4. Discussion

Administration of ropy EPS producing strain YS108R could relieve the symptoms of colitis such as the DAI and colon shortening; and histological analysis showed that YS108R could maintain the normal anatomical structure of colonic tissue, including reducing the extents of neutrophil infiltration and relatively entire crypts; however, another EPS producing strain C11A10B could not exert such an influence. Furthermore, the MPO activity of mice administered with YS108R was significantly lower than that of mice administered with C11A10B. MPO is an enzyme that exists in granulocytes such as neutrophils, which is associated with inflammatory activity, and the activity of MPO in colonic tissue correlates positively with the severity of neutrophil infiltration.[36] These results suggested that ropy EPS may be related to the alleviative effect of YS108R on DSS induced colitis.

DSS induced colitis is presumed to be the result of damage of the epithelial monolayer which allows the dissemination of pro-inflammatory intestinal environmental antigens (*e.g.*, intestinal bacteria) into underlying tissue and then leads to dysregulation of the intestinal immune response.[36,37] The levels of most of the pro-inflammatory cytokines and chemokine MCP-1 involved in this study increased in the serum and their gene expression levels in the colonic tissue of the DSS treatment group, in which the change of IL-6 was most significant. IL-6 has been repeatedly reported to be strongly expressed in the development of IBD, and there are other researchers suggesting that IL-6-receptor blockade could be a novel therapeutic target for IBD.[38,39] Our results showed that YS108R, HAN4-25 and BB12 administered mice had signifi-

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Table 9 Composition of the gut microbiota at the genus level

Genus	Control	DSS	DSS/YS108R	DSS/C11A10B	DSS/HAN4-25	DSS/BB12
Unclassified *S24-7*	0.4295 ± 0.0414^{a}	0.042 ± 0.0326^{e}	0.2625 ± 0.0566^{b}	0.2354 ± 0.0384^{bc}	0.18 ± 0.0738^{c}	0.1096 ± 0.0715^{d}
Akkermansia	0.1568 ± 0.0513^{a}	0.0168 ± 0.0127^{c}	0.1328 ± 0.0428^{a}	0.0566 ± 0.0351^{bc}	0.0608 ± 0.0343^{b}	0.0366 ± 0.0191^{bc}
Lactobacillus	0.1597 ± 0.0671^{a}	0.0343 ± 0.0387^{b}	0.0063 ± 0.0042^{b}	0.0092 ± 0.0081^{b}	0.0196 ± 0.0106^{b}	0.0297 ± 0.0232^{b}
Unclassified *Clostridiales*	0.1078 ± 0.0231^{ac}	0.0886 ± 0.0475^{ab}	0.1564 ± 0.0555^{c}	0.1523 ± 0.042^{c}	0.1108 ± 0.0703^{ac}	0.0489 ± 0.0166^{b}
Dorea	0.0038 ± 0.0026^{a}	0.006 ± 0.0053^{a}	0.0484 ± 0.0255^{c}	0.0206 ± 0.0138^{ab}	0.0296 ± 0.0197^{bc}	0.0287 ± 0.0307^{bc}
Unclassified *Enterobacteriaceae*	0.0028 ± 0.0013^{a}	0.2623 ± 0.162^{cd}	0.0848 ± 0.0399^{ab}	0.1118 ± 0.08^{b}	0.1643 ± 0.1088^{bc}	0.2897 ± 0.0442^{d}
Bacteroides	0.0081 ± 0.0024^{a}	0.0932 ± 0.0217^{b}	0.1198 ± 0.0446^{bc}	0.191 ± 0.0779^{cd}	0.2305 ± 0.0992^{d}	0.2128 ± 0.0664^{d}
Turicibacter	0.0004 ± 0.0001^{a}	0.0931 ± 0.0217^{c}	0.0387 ± 0.0223^{b}	0.0276 ± 0.0095^{b}	0.0532 ± 0.0502^{b}	0.0342 ± 0.0133^{b}
Unclassified *Lachnospiraceae*	0.0315 ± 0.0083^{ac}	0.049 ± 0.039^{c}	0.0378 ± 0.0087^{c}	0.0327 ± 0.0145^{ac}	0.0087 ± 0.0058^{b}	0.0126 ± 0.0087^{ab}
Mucispirillum	0.0002 ± 0.0001^{a}	0.0413 ± 0.0373^{c}	0.0079 ± 0.0059^{ab}	0.0212 ± 0.0115^{abc}	0.0313 ± 0.0251^{bc}	0.0194 ± 0.0152^{abc}
Oscillospira	0.014 ± 0.0071^{a}	0.0389 ± 0.0293^{b}	0.0127 ± 0.0063^{a}	0.0263 ± 0.0155^{ab}	0.0122 ± 0.011^{a}	0.0123 ± 0.003^{a}
Unclassified *Peptostreptococcaceae*	0.0003 ± 0.0001^{a}	0.0512 ± 0.0361^{b}	0.0032 ± 0.0047^{a}	0.0011 ± 0.0008^{a}	0.004 ± 0.0042^{a}	0.0434 ± 0.02^{b}
Enterococcus	0.0004 ± 0.0003^{a}	0.0445 ± 0.0554^{b}	0.0091 ± 0.0121^{a}	0.0006 ± 0.0006^{a}	0.0136 ± 0.014^{ab}	0.0211 ± 0.0306^{ab}
Unclassified *Ruminococcaceae*	0.0134 ± 0.0053^{ab}	0.0168 ± 0.0153^{a}	0.0072 ± 0.0012^{b}	0.0118 ± 0.0045^{ab}	0.0089 ± 0.0035^{ab}	0.0074 ± 0.0044^{b}

The data are in accordance with normal distribution. Data are shown as mean ± SD (n = 8). Different letters in each row indicate significant difference ($P < 0.05$) among groups.

cantly lower IL-6 concentrations in the serum than the DSS treatment group; however, mice administered with C11A10B had a higher serum IL-6 level that was similar to the DSS treatment group. Additionally, it was noticed that C11A10B induced higher expression of IL-17A and MCP-1, indicating that C11A10B could induce inflammation which was correlated with the severe DAI score and higher activity of MPO. IL-17A is a pro-inflammatory cytokine secreted by Th17 cells and it is able to stimulate the expression of a wide range of inflammatory cytokines and plays an important role in the pathogenesis of colitis.[40–42] The role of IL-17A has been demonstrated by DSS-induced colitis in IL-17A deficient mice and IL-17A blockade treatment; furthermore, MCP-1 and G-CSF were thought to be involved in the inflammation induced by IL-17A.[43,44] An investigation on bindarit, an inhibitor of MCP-1 synthesis, showed that treatment with bindarit could reduce the clinical severity of TNBS-induced colitis.[45] A study associated with *B. longum* subsp. *longum* 35624, which involved production of a cell surface exopolysaccharide, revealed that strain 35624 could prevent the disease symptoms of the colitis model and dampen the pro-inflammatory host response to the strain by reducing Th17 responses.[46] However, our results show that the serum concentrations of IL-17A and MCP-1 were not completely coincident with their expression levels in colonic tissue. The cytokine concentration in the serum represents the inflammation of the whole body. In this study, bacteria treatment firstly modulated the colonic mucosal immune response and affected the expression of several cytokines which would release into the serum. Furthermore, the expression of cytokines could be regulated at the post-transcriptional level with a different treatment; so the change of cytokines in the serum may be inconsistent with the colonic mucosa.[47] IL-10 is an interleukin that could be produced by Treg cells, and it acts as a regulator in immune response and is thought to reduce the severity of inflammation. Hidalgo-Cantabrana *et al.* showed that the oral administration of the ropy EPS producing *B. animalis* subsp. *lactis* strain increased the number of Fxop3 + Treg cells in mesenteric lymphoid nodes, which also reduced the DAI score in DSS induced colitis mice.[48] In the current YS108R treatment group, the serum concentration of IL-10 was significantly higher than those of other groups; therefore reducing the increase of Treg cells and then repressing Th17 responses may be a possible mechanism by which YS108R reduces the inflammatory level in colitis.

In recent years, EPS produced by bifidobacteria has been reported to present immunomodulatory properties.[19] However, the diversity of the compositions of the monosaccharide and the molecular weights and structures make EPS present different immune responses *in vivo* or *in vitro*. For example, some EPS with high molecular weights could suppress the immune response, but some EPS with low molecular weights could stimulate the immune response.[20,48] Size exclusion chromatography analysis of EPS showed that the molecular weight of EPS produced by YS108R was higher than that of C11A10B (Fig. S2†). Hence, it could be surmised that the

Table 10 Correlation between the composition of the gut microbiota and colitis damage

Spearman's rho	TNF-α	IL-1β	IL-6	IL-10	IL-17A	MCP-1	ZO-1	Occludin	Claudin-1	MUC2
Unclassified *S24-7*	−0.702**	−0.547**	−0.623**	−0.348*	−0.473**	−0.471**	0.626**	0.722**	0.624**	0.570**
Akkermansia	−0.723**	−0.405*	−0.608**	−0.239	−0.548**	−0.613**	0.590**	0.725**	0.702**	0.633**
Lactobacillus	−0.185	−0.054	−0.417*	−0.593**	−0.367*	−0.075	−0.150	0.240	−0.308	−0.275
Unclassified *Clostridiales*	−0.284	−0.224	−0.112	0.213	−0.063	−0.375*	0.465**	0.250	0.681**	0.553**
Dorea	0.092	−0.127	0.044	0.369*	0.196	0.002	0.047	−0.131	0.163	0.122
Unclassified *Enterobacteriaceae*	0.787**	0.345*	0.557**	0.361*	0.510**	0.514**	−0.670**	−0.656**	−0.715**	−0.584**
Bacteroides	0.217	0.225	0.324	0.238	0.305	−0.011	−0.449**	−0.526**	−0.289	−0.184
Turicibacter	0.634**	0.610**	0.508**	0.432**	0.436**	0.616**	−0.229	−0.527**	−0.390*	−0.285
Unclassified *Lachnospiraceae*	−0.126	−0.083	0.061	0.168	0.015	0.003	0.595**	0.367*	0.450**	0.439**
Mucispirillum	0.530**	0.495**	0.574**	0.197	0.409*	0.270	−0.412*	−0.674**	−0.495**	−0.310
Oscillospira	0.086	0.144	0.268	0.061	0.170	−0.073	0.094	−0.122	0.070	0.170
Unclassified *Peptostreptococcaceae*	0.719**	0.371*	0.574**	0.425**	0.515**	0.568**	−0.572**	−0.663**	−0.630**	−0.591**
Enterococcus	0.412*	0.277	0.180	0.197	0.292	0.509**	−0.403*	−0.482**	−0.393*	−0.471**
Unclassified *Ruminococcaceae*	−0.117	−0.049	−0.110	−0.061	0.032	−0.156	0.133	0.036	0.112	0.112

* indicates that the correlation is significant. * $P < 0.05$, ** $P < 0.01$, $P < 0.001$.

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different effects of YS108R and C11A10B on the DSS induced colitis may be attributed to the different properties of the produced EPS. In other research of ours, the genomes of YS108R and C11A10B (GenBank accession no. SAMN09355369 and SAMN09355370) were analyzed, in which a single nucleic acid change in a gene related to the synthesis of EPS was found and the product of this gene is theoretically responsible for the determination of the chain length of the saccharide polymer.[21] In a research study on *B. animalis* subsp. *lactis*, the author also found a single mutation in an EPS cluster which may be responsible for the ropy phenotype.[49]

IBD is thought to be associated with increased intestinal permeability caused by the decrease of the expression of tight junction proteins in the mucosa with inflammation.[50,51] The epithelium and the mucosal layer between the gut lumen and the underlying tissues form a physical barrier to prevent the potentially toxic and noxious agents from disseminating into the underlying tissues and then inflammation is induced in the colon. The changes of cytokine profiles, in turn, further trigger the decreasing of tight junctions; thus, a vicious cycle of mucosal barrier dysfunction and inflammation is set up.[52,53] Mucins are important substances constructing the colonic mucosal layer, and the mainly expressed mucin in the colonic epithelium of humans, rats and mice is mucin2, which is stored in bulky apical granules of the goblet cells.[54,55] In UC patients, the activity of the mucosal inflammation has been shown to correlate with decreasing mucin2 synthesis and secretion.[56,57] In the current study, the decreased expression level of TJP and mucin2 genes indicated that DSS treatment could damage the mucosal barrier, which was consistent with previous research studies.[58,59] In the YS108R administered group, the expression levels of TJP and mucin2 genes were even higher than those in the control group (except for occludin), indicating that YS108R was able to maintain the mucosal barrier which was consistent with the moderate DAI and histological scores. Wang *et al*. demonstrated that the effects on tight junction recovery by lactobacilli seem to be inter- and intra-species specific, and different strains could maintain the level of specific TJ proteins but not all the TJ proteins.[60,61] Thus, we presumed that *B. longum* or the strains used in this study were not able to recover the level of occludin due to species or strain specificity, but the mechanism remains clear, which could be further investigated.

However, the C11A10B administration group maintained higher expression levels of claudin-1 and mucin2 when compared with the DSS treatment group, inversely related to their histological damage. These results indicated that EPS-producing strains (YS108R and C11A10B) were able to maintain the expression of TJP and MUC2 genes, even though histological analysis showed the damage of colonic mucosa in C11A10B treatment mice. This may because that C11A10B is not able to completely recover the injured mucosal barrier which is caused by multiple factors and can not be fully reflected by the expression level of TJP. Furthermore, the increased inflammatory response in C11A10B treatment mice may affect the expression of TJP and lead to the damage of the mucosal layer.

In normal individuals, there is a symbiotic relationship between the gut microbiota and host. Gut microbiota is closely related to the pathogenesis of IBD, and an abnormal interaction between the gut microorganisms and the mucosal immune system may lead to their deterioration. The result of PCoA in this study indicated that the gut microbiota of DSS treatment mice was dramatically different from that of control mice, and administration of bifidobacteria could remit the shift of the gut microbiota resulting from DSS challenge. Furthermore, the gut microbiota of the mice administered with different *B. longum* subsp. *longum* strains were similar to each other and were separated from that in mice fed with BB12, and the microbiota profile of BB12-administered mice was similar to that of DSS colitis mice. At the phylum level, the relative abundance of *Proteobacteria* in the DSS treatment group was 10 times more than that in the control group. Administration of *B. longum* subsp. *longum* strains could decrease the abundance of *Proteobacteria*, but *B. animalis* was not able to decrease *Proteobacteria*, which suggested a species-

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specific effect of bifidobacteria on the gut microbiota. However, only YS108R could increase the relative abundance of *Verrucomicrobia*, which mainly consists of genus *Akkermansia* in the current work, and is able to use mucin as the carbon and nitrogen source; therefore the increase of *Verrucomicrobia* in YS108R administered mice was considered to be associated with the higher expression of MUC2.[62] At the genus level, the relative abundance of three genera, especially the genera of unclassified *S24-7*, significantly decreased and the relative abundance of 7 genera significantly increased after DSS induction. *S24-7* has been reported to be associated with the remission of experimental colitis, and studies focused on the gut microbiome of IBD patients have demonstrated the expansion of *Proteobacteria*, particularly *Enterobacteriaceae*.[63–65] In a mouse model that closely mimics human diarrheal pathogens, the overgrowth of *Enterobacteriaceae* was hypothesized to be prompted by the intestinal inflammation.[66] Examination of biopsies and stool samples from chronic pouchitis patients indicated that the abundance of *Peptostreptococcaceae* increased in the patients, and the severity of intestinal mucosal ulceration was associated with the increased number of species within *Peptostreptococcaceae*.[67,68] Our results showed that *S24-7* had a negative correlation with pro-inflammatory cytokines, but *Enterobacteriaceae* and *Peptostreptococcaceae* had a positive correlation with pro-inflammatory cytokines, which verified the results reported previously. Noticeably, our results indicated that administration of *B. longum* subsp. *longum* strains could significantly increase the relative abundance of unclassified *S24-7* and decrease the relative abundance of unclassified *Enterobacteriaceae* and *Peptostreptococcaceae* compared with DSS treatment. However, the relative abundance values of these three genera in the BB12 administration group were close to that of the DSS treatment group.

To comprehensively consider these results, *B. longum* YS108R could alleviate the symptoms of DSS-induced colitis by multiple mechanisms including the anti-inflammatory effect (increasing the level of anti-inflammatory cytokine IL-10 and decreasing the level of pro-inflammatory cytokines IL-6 and IL-17A), maintaining the expression of intestinal tight junction proteins and partially recovering the imbalanced gut microbiota after DSS challenge. Furthermore, the different phenotypes and effects of YS108R and C11A10B on colitis indicated that ropy EPS produced by YS108R may be an important substance that makes it useful to alleviate colitis.

DSS-induced colitis is the most commonly used model to mimic inflammation seen in ulcerative colitis. But the severity of colitis induced by DSS is species and strain specific, and the differences in the intestinal microbiota may influence the result of DSS treatment. In addition, because the sample size was somewhat limited, the effect observed in this study may not be reproduced in a clinic trial. Despite its shortcomings, DSS induced colitis has an important use in helping us understand the pathophysiology of intestinal inflammation or in preliminary screening of pharmaceuticals and understanding their mechanisms of action.

5. Conclusion

Oral administration of YS108R could reduce the severity of colitis indicated by the DAI score; however, the non-ropy EPS producing strain C11A10B did not have the ability to alleviate the symptoms of colitis. The results of cytokines in the serum and the expression levels of cytokine genes in colon tissues showed that YS108R could reduce the inflammation. The higher expression levels of TJP and mucin in colonic tissue suggested that YS108R could maintain the integrity of the mucosal barrier. Analysis of gut microbiota showed that YS108R could partially revert the change of the bacterial composition; in particular, this strain could prevent the increase of *Proteobacteria* (particularly *Enterobacteriaceae*), which consist of many of the pathogenic bacteria. Furthermore, the results of gut microbiota analysis suggested that the effect of bifidobacteria on gut microbiota might be species-specific.

Conflicts of interest

All authors declared no conflict of interest.

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