

## Exopolysaccharides from *Lactobacillus plantarum* YW11 improve immune response and ameliorate inflammatory bowel disease symptoms

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Exopolysaccharides (EPSs) possess many bioactivities such as immune regulation, antioxidant, anti-tumor and modulation of intestinal microbial balance but their direct effect on inflammatory bowel disease (IBD) response has not been studied. The purpose of this study was to evaluate the anti-inflammatory effect of EPS produced by *L. plantarum* YW11 administered at different dosages in IBD mouse model induced with 5% dextran sulphate sodium (DSS). The DSS-induced colitis, accompanied by body weight loss, reduction of colon coefficient and histological colon injury was considerably ameliorated in mice fed the EPS (10 mg/kg). The middle dose of the EPS (25 mg/kg) could effectively recover the intestinal microbial diversity and increase the abundance of *Roseburia*, *Ruminococcus* and *Blautia* with increased content of butyric acid. Moreover, EPS also reduced the production of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-12 and IL-18) and enhanced the anti-inflammatory cytokine IL-10. This study showed that EPS might help in modulation of gut microbiota and improve the immunity of the host to reduce the risk of IBD symptoms.

**Key words:** Exopolysaccharide (EPS); *Lactobacillus plantarum*; Inflammatory bowel disease; Gut microbiota; Immunity

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**Abbreviations:** CD, Crohn's Disease; DSS, Dextran Sulphate Sodium; EPSs, Exopolysaccharides; GC-MS, Gas Chromatography and Mass Spectrometry; IBD, Inflammatory Bowel Disease; LAB, Lactic Acid Bacteria; RDA, Redundancy Analysis; SCFAs, Short Chain Fatty Acids; SIM, Single Ion Monitor; UC, Ulcerative Colitis

### INTRODUCTION

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a common chronic intestinal disease caused by a diversity of complex factors such as genetic, environmental, intestinal, and immune ones (Kong *et al.*, 2008; Dudik *et al.*, 2018). Intestinal flora and mucosal immune disorders play an important role in the pathogenesis of IBD (Dasgupta *et al.*, 2013; Bringiotti *et al.*, 2014). Hansen found that the diversity of intestinal flora of IBD patients was reduced significantly (Hansen *et al.*, 2010) and the number of bacterial genes in feces of the patients was lower by 25% when compared with those of healthy people (Qin *et al.*,

2010). It was shown that the intestinal microbiota balance in IBD patients was broken with reduced number of beneficial *bifidobacterium* and increased sulfate reducing bacteria, as well as decreased formation of butyrate in gut and increased intestinal permeability, allowing bacterial endotoxin pro-inflammatory substances to enter the intestinal mucosa lamina propria to induce immune inflammatory reaction. Sokol and others (Sokol *et al.*, 2008) reported that the number of anti-inflammatory *Clostridium Difficile* in the intestinal mucosa of patients with CD also decreased significantly. In the treatment of IBD, probiotic *Lactobacillus casei* Lbs2 was shown to induce differentiation of regulatory T cells (Tregs) to secrete higher level of IL-10 that exerted therapeutic effect on IBD and then IL-10 inhibited the releasing of the pro-inflammatory factors such as IL-1, IL-6, IL-12 and TNF (Smith *et al.*, 2013; Thakur *et al.*, 2016). Regulation of the intestinal flora to maintain its balance and enhancement of intestinal mucosal immunity were considered vital for treatment of IBD, e.g. by using probiotics or prebiotic preparations (Guarner *et al.*, 2007; Sun *et al.*, 2018).

Prebiotics are generally a kind of indigestible dietary supplements which are useful to stimulate the growth and activity of bacteria, especially they can inhibit the growth of intestinal pathogens, thus conferring beneficial health effect on the host (Gibson *et al.*, 2004). Exopolysaccharides (EPSs) are produced by lactic acid bacteria (LAB), biologically serving as a natural protective barrier to safeguard the bacteria from the adverse environment (Ramchandran *et al.*, 2009), EPSs were reported to possess prebiotic function with various physiological properties, such as anti-oxidation, anti-tumor, and cholesterol lowering (Vuyst *et al.*, 2001). Das and others (Das *et al.*, 2014) proved that the EPS produced by *L. plantarum* DM5 had bifidogenic effect. Peptidoglycan, polysaccharide fragments and glycoproteins from *Bifidobacterium adolescentis* SPM0212 had anti-tumor effect (Arun *et al.*, 2014). The EPS produced by *B. lactis* Bb12 promoted proliferation of T lymphocytes (Amrouche *et al.*, 2006). The EPS synthesized by *L. paracasei* KB28 promoted secretion of immune factors IL-6, IL-12 and TNF- $\alpha$  (Amrouche *et al.*, 2006). By using the acetic acid induced colitis mouse model, *L. delbrueckii* subsp. *bulgaricus* B3 producing higher amount of EPS was found to significantly reduce inflammation in mice when compared to *L. delbrueckii* subsp. *bulgaricus* A13 with low EPS production and the former also showed to have higher antioxidant activity and metal chelating ability than latter. Currently, the EPS produced by *L. plantarum* YW11 was reported to relieve oxidative stress of the galactose-induced aging mice by regulating the gastrointestinal microbiota balance (Jian *et al.*, 2017), but the direct evi-

dence on the curative effect of the EPSs produced by LAB on IBD has not been reported.

For the treatment of IBD, probiotics, such as lactobacilli and bifidobacteria, were extensively studied for their regulating effects on gut microbiota, and there was a certain causal relationship between intestinal flora and IBD (Dong *et al.*, 2019). The EPS-producing probiotic strains were shown to alleviate the gut oxidative damage in experimental colitis (Sengul *et al.*, 2011b). Although EPSs produced by LAB were reported with different bioactivities, there was no direct evidence on their curative effects on IBD. Previously in our lab, we found that the EPS produced by *L. plantarum* YW11 possessed the function of regulating intestinal flora to relieve the oxidative stress of a mouse model (Jian *et al.*, 2017). In this study, the EPS produced by YW11 was further shown to be effective to alleviate the IBD related symptoms by recovering the microbial diversity and improving the immune response of the IBD mice.

After the experimental mice were modeled by DSS, they gradually lost body weight when compared to the healthy mice without DSS treatment, probably due to reduced diet and water intake of the model mice as reported earlier (Morhardt *et al.*, 2019). Administration of the EPS produced by *L. plantarum* YW11 increased the weight gain compared to the negative group, and improved the symptoms of the colon inflammation, indicating that the EPS had a certain effect on the recovery of gastroenteritis in mice. Similarly, Kim showed that the *Portulaca oleracea* extracts probably containing its secreted polysaccharides ameliorated IBD *in vivo*, and the weight of the mice treated with the *Portulaca oleracea* extracts increased (Kim *et al.*, 2018). It was also observed that the ratio of colon weight to body weight significantly increased in the DSS groups, accompanying with different degrees of hyperemia, edema, and thickening of the inflammatory response in the colon, and the corresponding HE scores were higher as reported previously (Mueller *et al.*, 2009). In the preventive approach, the animals treated with the EPS from *L. plantarum* YW11 showed less edema and neutrophil infiltration, as well as a restoration of the mucosa, submucosa and muscular layers. Comparatively, the EPS-producing *B. adolescentis* IF1-03 was also reported to reduce the area of ulceration and thickening of the intestinal wall compared to the DSS-colitis model mice (Yu *et al.*, 2019), but whether this EPS played the roles or not was not confirmed.

In this study, the effect of the EPS produced by *L. plantarum* YW11 on the dextran sulfate sodium (DSS) induced IBD mice colitis model was studied, focusing on the regulatory effect of the EPS on the gut microbiota composition and the immune response. The EPS was previously characterized with a molecular mass of  $1.1 \times 10^5$  Da, with the monosaccharide composition of glucose and galactose in a molar ratio of 2.71:1 (Wang *et al.*, 2018). The present study will provide further understanding of the prebiotic function and mechanism of the EPSs produced by *L. plantarum* in the treatment of intestinal inflammatory diseases.

The EPS produced by *L. plantarum* YW11 exerted its curative effect on IBD by simulating expression of the anti-inflammatory cytokine IL-10, and decreasing levels of the pro inflammatory TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-12 and IL-18 in the colon tissue of the DSS mice. IL-10 could increase expression of anti-inflammatory proteins such as soluble TNF- $\alpha$  receptor and matrix metalloproteinase tissue inhibitors, which protected the

intestinal epithelial barrier (Staples *et al.*, 2000). IL-1 $\beta$  could promote the macrophages and monocytes to secrete TNF- $\alpha$  and IL-6 in an autocrine and paracrine mode, leading to inflammation that damaged intestinal epithelial barrier function and caused further deterioration of IBD (Ishiguro *et al.*, 1999). Synergistic effect of IL-12 and IL-18 promoted secretion of large amounts of IFN- $\gamma$  by NK and Th1/Th2 cells and played a pro-inflammatory role (Nakanishi *et al.*, 2003). Previously the bacterial  $\beta$ -(1,3)-glucan was found to exert its anti-inflammatory effect by promoting gene expression of IL-10 in DSS-induced colitis mice (Lee *et al.*, 2014). Other polysaccharides inhibited expression of the pro inflammatory factors such as IL-1 $\beta$ , IL-6, TNF- $\alpha$  in IBD tissues (Pereira *et al.*, 2016). Lactulose had immunomodulatory effect on TNBS-induced colitis rats by decreasing the content of TNF- $\alpha$ , thus reducing epithelial cell injury and intestinal mucosa ulcer (Yan *et al.*, 2015). *Portulaca oleracea* inhibited IBD by controlling the levels of the inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-1 $\beta$  in mice with DSS-induced IBD (Kim *et al.*, 2018).

Short-chain fatty acids (SCFAs) produced by gut microbial fermentation with polysaccharides were considered to be beneficial in maintaining a relatively low pH value of colon and preventing the growth of potentially harmful bacteria. The SCFAs produced by probiotic *Clostridium difficile* could induce differentiation of primary CD4+ T cells into regulatory T cells (Treg), which stimulated secretion of IL-10 by macrophages, thereby reducing the inflammatory response (Bhat *et al.*, 2019). In this study, the contents of SCFAs were different in the 6 experimental groups with butyric acid to be the highest, possibly because most of the butyric acid producing colonies existed in the intestinal flora. The experimental statistics of the butyric acid producing colonies found that *Roseburia* increased by 0.39%, *Ruminococcus* increased by 0.08% and *Blautia* increased by 0.56%. Butyrate could be the main energy source for colonic epithelial cells, and it inhibited the immune inflammatory response and the downstream pro-inflammatory IL-12, and up-regulated the production of anti-inflammatory cytokine IL-10 (Backhed *et al.*, 2007). This might also be a mechanism of alleviating the inflammatory response of IBD by the EPS from *L. plantarum* YW11.

Previous studies showed that decrease of intestinal flora diversity was closely related to the generation of IBD inflammation (Gevers *et al.*, 2014). Complex carbohydrates could increase levels of beneficial bacteria bifidobacterial and regulate intestinal microenvironment, stimulating macrophages and lymphocytes in the gut, and fighting inflammatory diseases (Gevers *et al.*, 2014). In this study, treatment of the IBD mice with the EPS from *L. plantarum* YW11 increased the richness of intestinal flora, indicating that the EPS could alleviate symptoms of IBD inflammation by increasing the diversity of intestinal flora. As indicated by correlation analysis between intestinal flora and cytokines, the relative abundance of beneficial *Lactobacillus* and *Bacteroidales-S24-7* was significantly positively correlated with IL-10 anti-inflammatory cytokines. The relative abundance of *Lachnospiraceae*, *Faecalibaculum*, *Fissicatena*, *Escherichia*, *Shigella* and *Streptococcus* was positively correlated with TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-12 and IL-18 pro-inflammatory cytokines. The results of this study regarding the relationship among the SCFAs, cytokines and intestinal flora suggested that the

mechanism of the effective treatment with the LAB EPSs for IBD was gaining new understanding.

## MATERIAL AND METHODS

**Strains and culture condition.** *L. plantarum* YW11 isolated from Tibetan kefir maintained in the culture bank of Dairy Laboratory in Beijing Technology and Business University of China at  $-80^{\circ}\text{C}$  was activated by consecutive transfer for three times in MRS medium (Beijing Aoboxing Co Ltd) at  $37^{\circ}\text{C}$  for 18 hr. Skim milk medium made by reconstituting 10% skim milk powder (Fonterra, Auckland, New Zealand) in deionized water was used for growth of the strain for preparing the EPS sample used in this study (Wang *et al.*, 2018).

**Preparation of EPS.** The EPS produced by *L. plantarum* YW11 was isolated and purified by the following method as described (Wang *et al.*, 2015). Briefly, the skim milk medium (1 L) was inoculated with *L. plantarum* YW11 at 3% (v/v). After growth at  $37^{\circ}\text{C}$  for 18 hr, the culture was heated in a boiling water bath for 15 min to inactivate possible polysaccharide-degrading enzymes. Then trichloroacetic acid (TCA) at 80% (w/v) was added to the final concentration of 4% (w/v). The mixture was stirred at room temperature for 2 hr, and then centrifuged at 10000 rpm at  $4^{\circ}\text{C}$  for 45 min. The supernatant was added with two volumes of cold ethanol ( $4^{\circ}\text{C}$ ), kept at  $4^{\circ}\text{C}$  for 12 hr, and centrifuged at 10000 rpm at  $4^{\circ}\text{C}$  for 30 min. The precipitate was dissolved with distilled water, dialyzed for 48 hr with a molecular cutoff of 8000–14000 Da, and then lyophilized to obtain the crude EPS. Purification of the EPS sample (20 mg/mL distilled  $\text{H}_2\text{O}$ ) was performed with a diethylaminoethyl cellulose column (GE Healthcare, Pittsburgh, PA), and subsequently with a Sepharose CL-6B column (GE Healthcare, Pittsburgh, PA). Peak fractions containing the EPS were collected and lyophilized to obtain the purified EPS sample, which was stored at  $-20^{\circ}\text{C}$  for further use.

**Animal experiments.** Male ICR mice weighing 18–22 g were used for the experiments. Mice were housed in sawdust-lined plastic cages at a constant temperature ( $22\text{--}24^{\circ}\text{C}$ ) and maintained under a 12 hr light/dark cycle (lights on at 6:00 a.m.) with free access to laboratory chow and water. The animals were maintained under the conditions and used for the experiment after 7 days of acclimation. Groups of 6–8 animals were used in the experiments. The animal experiments conducted strictly according to the Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation.

Mice ( $n=48$ ) were randomized into the following six groups: the blank (B) group consisted of healthy animals; the negative (N) group was administered with 5% DSS (M.W. 36000–50000, MP Biomedicals, LLC, France) to induce bowel inflammation and then treated with 0.9% normal saline at a dose of 20 mL/kg (body weight) per day; the positive (P) group was administered with 5% DSS to induce bowel inflammation and then treated with prednisolone acetate injection (Hua-Yi-Hu-Bei Pharmaceutical Co., Ltd, China) at a dose of 5.0 mg/kg per day; the YW11-L group was administered with 5% DSS to induce bowel inflammation and then treated with the EPS at a low dose of 10.0 mg/kg per day; the YW11-M group was administered with 5% DSS to induce bowel inflammation and then treated with the EPS at a medium dose of 25.0 mg/kg per day; the YW11-H group was administered with 5% DSS to induce bowel

inflammation and then treated with the EPS at a high dose of 50.0 mg/kg per day.

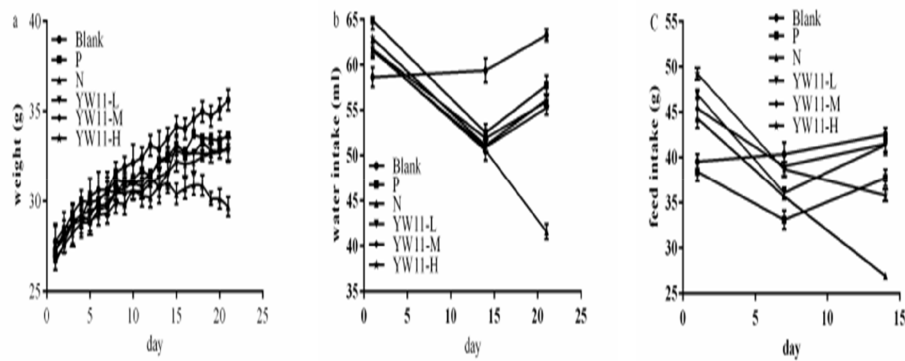
The animals received DSS solution on days 1–14 to induce bowel inflammation, and in the following week (days 15–21) the animals in different groups received the different treatments as described above. On day 22, all mice were sacrificed and the colon without caecum was immediately removed, measured and weighed. The colon coefficient was calculated as the weight of colon divided by the weight of mouse (Paquola *et al.*, 2019). Then the fecal pellets were removed. Clinical parameters including diarrhea were observed daily, and animal body weight was assessed.

**Histology evaluation.** The colon was fixed using 4% paraformaldehyde and embedded in paraffin. Five-micrometer-thick sections were sliced from the paraffin block and stained with hematoxylin and eosin (H&E). Histological scoring was performed on H&E-stained colon tissue sections; the degree of involvement was graded on a scale of 1–4 (1, 0–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%), and the extent of injury on a scale of 0–4 (0, negative; 1, mucosal; 2, submucosa; 3, muscularis mucosal; 4, serosa regions). Two scores per mouse were added for the total score of the individual animal. The average scores for each group were calculated (Horino *et al.*, 2008).

**Cytokine analysis.** Local levels of IL-1 $\beta$ , IL-18, IL-6, IL-12, TNF- $\alpha$ , IFN- $\gamma$ , IL-10 were assessed according to the instructions of the ELISA kit (Hua-Mei-Wu-Han Pharmaceutical Co., Ltd-China). Sample preparation: Precooled cracking fluid was added to colonic tissue, grinded, and centrifuged (12000 rpm,  $4^{\circ}\text{C}$ , 10 min). Then the protein concentration of the supernatant was determined.

**Assay procedure:** 100  $\mu\text{L}$  of sample was added to each well and incubated for 2 hr at  $37^{\circ}\text{C}$ . The liquid in each well was removed without washing. 100  $\mu\text{L}$  of biotin-antibody (1X) was then added to each well, incubated for 1 hr at  $37^{\circ}\text{C}$ , aspirated and washed 3 times. 100  $\mu\text{L}$  of HRP-avidin (1X) was added to each well, incubated for 1 hr at  $37^{\circ}\text{C}$ , aspirated and washed 5 times. 90  $\mu\text{L}$  of TMB substrate was added to each well and incubated for 15–30 minutes at  $37^{\circ}\text{C}$  under dark conditions. 50  $\mu\text{L}$  of Stop Solution was added to each well, and optical density was determined at 450 nm within 5 minutes.

**Determination of short chain fatty acids (SCFAs) in feces.** The SCFAs in feces were determined using a method described by (Shixiang *et al.*, 2018). Briefly, feces were collected from individual mouse. Fecal samples (50 mg) were added to 2 ml water, acidified with sulfuric acid (10%) to adjust the pH to 2–3, and resuspended by shaking for 2 min. Then, 1 ml diethyl ether was added; 10 min later, the sample was centrifuged at 1800 rpm for 10 min to remove the solid material. Supernatants were retained, 2-methyl-3-heptanone solution in ether was added as internal standard, and the solution was filtered through a 0.45  $\mu\text{m}$  microporous membrane. Samples were analyzed by GC-MS within 24 h. 1  $\mu\text{L}$  of sample was injected into GC-MS, which was equipped with a DB-Wax column. Helium was the carrier gas at a flow rate of 0.8 mL/min. The injection temperature was  $180^{\circ}\text{C}$  and the GC temperature program was as follows: beginning at  $60^{\circ}\text{C}$ , increasing to  $160^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$ , then holding at  $160^{\circ}\text{C}$  for 6 min. The ion source temperature was  $200^{\circ}\text{C}$ . Concentration of SCFAs was analyzed using Single Ion Monitor (SIM) scan mode, calculated using the internal standard method and expressed in  $\mu\text{g}/\text{g}$  sample.



**Figure 1.** (a) shows changes of body weights of the mice during the 3 weeks of experimental period. (b) and (c) show the changes of water and feed intake of the mice, respectively, during the experimental period

**16S RNA analysis.** Feces from three mice were randomly selected from each group. 16S rRNA gene analysis was used to examine the diversity of fecal microbiota in the subjects. Using the NEXT flex™ 16S V4 Amplicon-Seq Kit (Bioo Scientific, USA), primers were used to generate amplicons spanning the variable regions 3-4 (V3-V4) of the 16S rRNA gene that were subsequently sequenced on an Illumina MiSeq (Illumina, USA). Data were processed using QIIME software (v1.9.0).

The partial least square-discriminant analysis (PLS-DA) plot was generated based on the UniFrac distance matrix and was used to test the similarity among individual subject groupings of intestinal bacteria. A redundancy analysis (RDA) was completed with CANOCO (Microcomputer Power, Ithaca, NY) according to the software manual (Braak *et al.*, 2002). Briefly, species and environmental data were input into CANOCO software. In the method-selection inspection, the lengths of gradient (1.42) in the detrended correspondence analysis was lower than 3.0, suggesting that the method of RDA was more suitable for data analysis than for canonical correspondence analysis. The significance of RDA analysis was used to analyze the relationship between environmental factors and species.

**Statistical Analysis.** All data were expressed as mean  $\pm$  standard deviation (S.D.). Statistically significant differences were determined by one-way ANOVA analysis followed by Tukey's tests in SPSS 13.0 software (SPSS, Inc., USA).

## RESULTS AND DISCUSSION

### Change of physical state of mice

Figure 1a shows changes of body weights of the mice during the 3 weeks of experimental period. All the mice in the 5 groups treated with DSS (N, P, YW11-L, YW11-M, YW11-H) showed obviously slower increase in the body weight than the normal healthy mice without DSS treatment (group B). Starting from day 15, treatment with different doses of EPS (groups YW11-L, YW11-M, YW11-H) sustained the growth of the mice, but the body weights of these mice were still lower than those of the untreated mice (group B) till the last day of the experimental period, while the mice without EPS treatment (group N) showed substantial decrease of body weight. Figure 1b and Fig. 1c show the changes of water and feed intake of the mice, respectively, during the experimental period. During the first 2 weeks, treatment

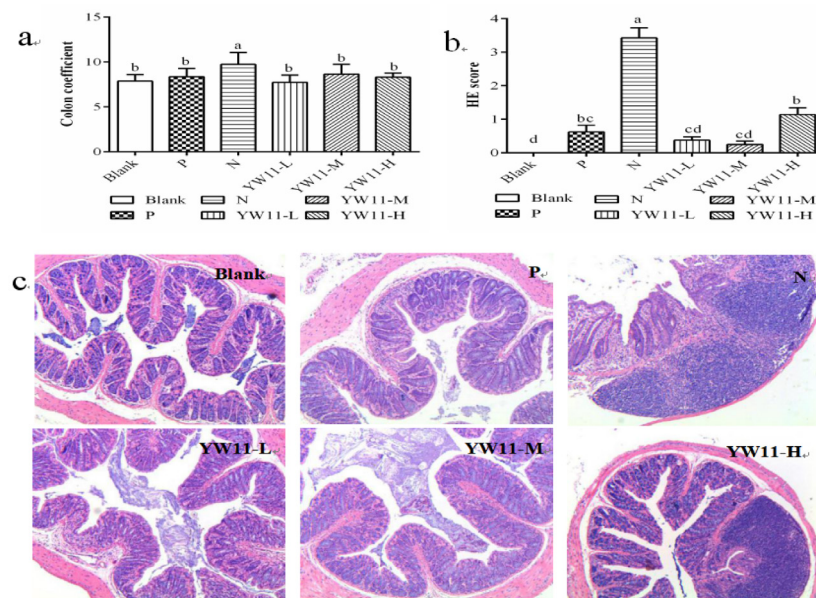
of the mice with DSS significantly affected the water and feed intake, but this was obviously improved when the mice were treated with different doses of EPS starting from day 15, compared to the mice without EPS treatment (group N) that continued to decrease in the water and diet intake till the end of the experiment period.

### Colon coefficient and histological analysis

A major hallmark of DSS-induced colitis is leukocyte infiltration into the colon wall resulting in massive tissue damage (Yan *et al.*, 2009). As shown in Fig. 2a, mice in group N treated only with DSS had the highest colon coefficient, and also the highest histological HE score (Fig. 2b). This was confirmed by the histopathological assessment of H&E stained tissue sections of the mice in group N that revealed a massive destruction of the epithelial lining, a shortening or absence of crypts, loss of goblet cells, and excessive leukocyte infiltrates in the mucosa and submucosa (Fig. 2c). Although there was no significant difference in the values of colon coefficient among the other five groups, treatment of the mice with the EPS showed different extent of protective effect on the colonic tissue. As shown in Fig. 2c, the healthy mice (group B) showed obvious crypt and intact epithelium with no inflammation, and treatment with different doses of the EPS improved the shape of colon tissue in different degrees. This was in line with the result of histological HE scoring where mice in groups YW11-L and YW11-M gave significantly lower histological scores than groups N, P and YW11-H, but higher than group B (Fig. 2b).

### Change of cytokine levels in colon tissue of mice

Interleukin (IL)-10 is an anti-inflammatory cytokine that plays an important role in intestinal homeostasis, while other cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-12 and IL-18 are pro inflammatory with well-established pro inflammatory functions in IBD in humans and animal models (Amaral *et al.*, 2016). Treatment of the mice with DSS (group N) significantly ( $P < 0.05$ ) decreased the level of the anti-inflammatory IL-10 in the intestinal tissue, with almost 0.33-fold lower level of the cytokine than those in the other five groups (Fig. 3a). In contrast, the levels of the pro inflammatory TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-12 and IL-18 increased significantly ( $P < 0.05$ ) in the mice treated with DSS (group N) with 1–6-fold increase compared to those of the healthy mice (group B) (Fig. 3b, 3c, 3d, 3e, 3f, 3g). However, treatment of the mice with different doses of the EPS



**Figure 2.** Effect of the EPS produced by *L. plantarum* YW11 on the colon tissue of the IBD mice.

(a) Colon coefficient. (b) Histological score. (c) Representative H&E-stained colons. Abcd: different letters indicating significant differences at the level  $P < 0.05$ .

significantly ( $P < 0.05$ ) decreased the levels of these six proinflammatory cytokines to different extents, with medium or low dose EPS being more effective than the high dose EPS.

#### Fecal SCFA concentration of mice

The SCFAs and their concentrations in feces ( $\mu\text{g/g}$ ) of mice were determined as shown in Table 1. Comparing with the healthy mice (group B), treatment of the mice with DSS (group N) significantly ( $P < 0.05$ ) decreased the total SCFA concentration, with decreased concentration of formic acid, propanoic acid, isobutyric acid, butyric acid and valeric acid except for acetic acid and isovalic acid. Administration of the EPS at low and medium doses recovered the total SCFA content in feces, especially butyric acid that had the highest concentration among all the SCFAs determined. However, treatment with high-dose EPS decreased the total SCFA concentration in feces with major decrease of butyric acid.

#### Gut Microbiota Change in mice treated with EPS

A total of 713465 sequences were obtained after pyrosequencing, and unique sequences were classified and grouped into 402 operational taxonomic units (OTUs)

based on 97% nucleotide sequence identity, and the average length was 443.19. The rarefaction curves (Fig. 4) for all mice reached a plateau, indicating that the bacterial diversity in these communities was mostly covered. The partial least square-discriminant analysis (PLS-DA) of UniFrac distances based on the relative abundance of OTUs revealed the difference in intestinal microbiota at the genus level between the fecal samples from each group (Fig. 5a). The 3 symbols representing the microbial communities from each of the fecal samples from group N mice were well separated from those of the other five groups, with the distance between the symbols representing the degree of difference of the microbiota. Therefore, the overall microbiota was different in the fecal samples from group N compared with the other five groups, whereas there was no significant difference between these five groups (B, P, YW11-L, YW11-M and YW11-H). This was further confirmed by microbial community heat map that showed considerable difference in the microbiota composition at genus level between the fecal samples from group N and those from the other 5 groups (Fig. 5b).

Differences in gut microbial communities before and after EPS administration were also evaluated by  $\alpha$ -diversity analysis, consisting of richness estimates

**Table 1.** Content of short-chain fatty acids in feces of mice

Content ( $\mu\text{g/g}$ )	Blank	N	P	YW11-L	YW11-M	YW11-H
acetic acid	32.40 $\pm$ 8.66 <sup>b</sup>	33.14 $\pm$ 2.03 <sup>b</sup>	40.72 $\pm$ 6.06 <sup>b</sup>	68.95 $\pm$ 9.60 <sup>a</sup>	22.90 $\pm$ 8.30 <sup>b</sup>	65.52 $\pm$ 9.31 <sup>a</sup>
formic acid	67.36 $\pm$ 9.97 <sup>ab</sup>	60.80 $\pm$ 11.67 <sup>ab</sup>	65.87 $\pm$ 6.23 <sup>a</sup>	34.20 $\pm$ 3.36 <sup>b</sup>	38.77 $\pm$ 12.16 <sup>ab</sup>	51.05 $\pm$ 19.67 <sup>ab</sup>
propanoic acid	60.10 $\pm$ 6.13 <sup>cd</sup>	53.69 $\pm$ 7.00 <sup>d</sup>	97.69 $\pm$ 7.50 <sup>a</sup>	92.86 $\pm$ 8.25 <sup>ab</sup>	82.06 $\pm$ 8.66 <sup>bc</sup>	62.70 $\pm$ 7.81 <sup>cd</sup>
isobutyric acid	12.88 $\pm$ 2.86 <sup>a</sup>	7.73 $\pm$ 2.55 <sup>a</sup>	11.46 $\pm$ 3.43 <sup>a</sup>	18.43 $\pm$ 5.67 <sup>a</sup>	19.34 $\pm$ 11.55 <sup>a</sup>	16.37 $\pm$ 8.93 <sup>a</sup>
butyric acid	201.29 $\pm$ 12.47 <sup>bc</sup>	169.46 $\pm$ 8.49 <sup>c</sup>	221.97 $\pm$ 6.71 <sup>b</sup>	313.62 $\pm$ 14.61 <sup>a</sup>	264.06 $\pm$ 17.71 <sup>a</sup>	124.61 $\pm$ 18.46 <sup>d</sup>
isovalic acid	28.82 $\pm$ 7.71 <sup>bc</sup>	42.51 $\pm$ 7.76 <sup>b</sup>	64.88 $\pm$ 4.56 <sup>a</sup>	20.56 $\pm$ 3.23 <sup>c</sup>	29.16 $\pm$ 7.35 <sup>bc</sup>	28.16 $\pm$ 6.45 <sup>bc</sup>
valeric acid	25.62 $\pm$ 9.04 <sup>a</sup>	14.79 $\pm$ 6.38 <sup>ab</sup>	24.83 $\pm$ 5.67 <sup>a</sup>	5.82 $\pm$ 1.60 <sup>b</sup>	5.70 $\pm$ 0.31 <sup>b</sup>	10.64 $\pm$ 3.32 <sup>b</sup>
Total SCFAs	428.47 $\pm$ 8.12 <sup>c</sup>	382.13 $\pm$ 6.55 <sup>d</sup>	527.42 $\pm$ 5.74 <sup>b</sup>	554.45 $\pm$ 6.62 <sup>a</sup>	461.99 $\pm$ 9.36 <sup>b</sup>	359.06 $\pm$ 10.56 <sup>d</sup>

abcd stands for different letters indicating significant differences at the level  $P < 0.05$ .

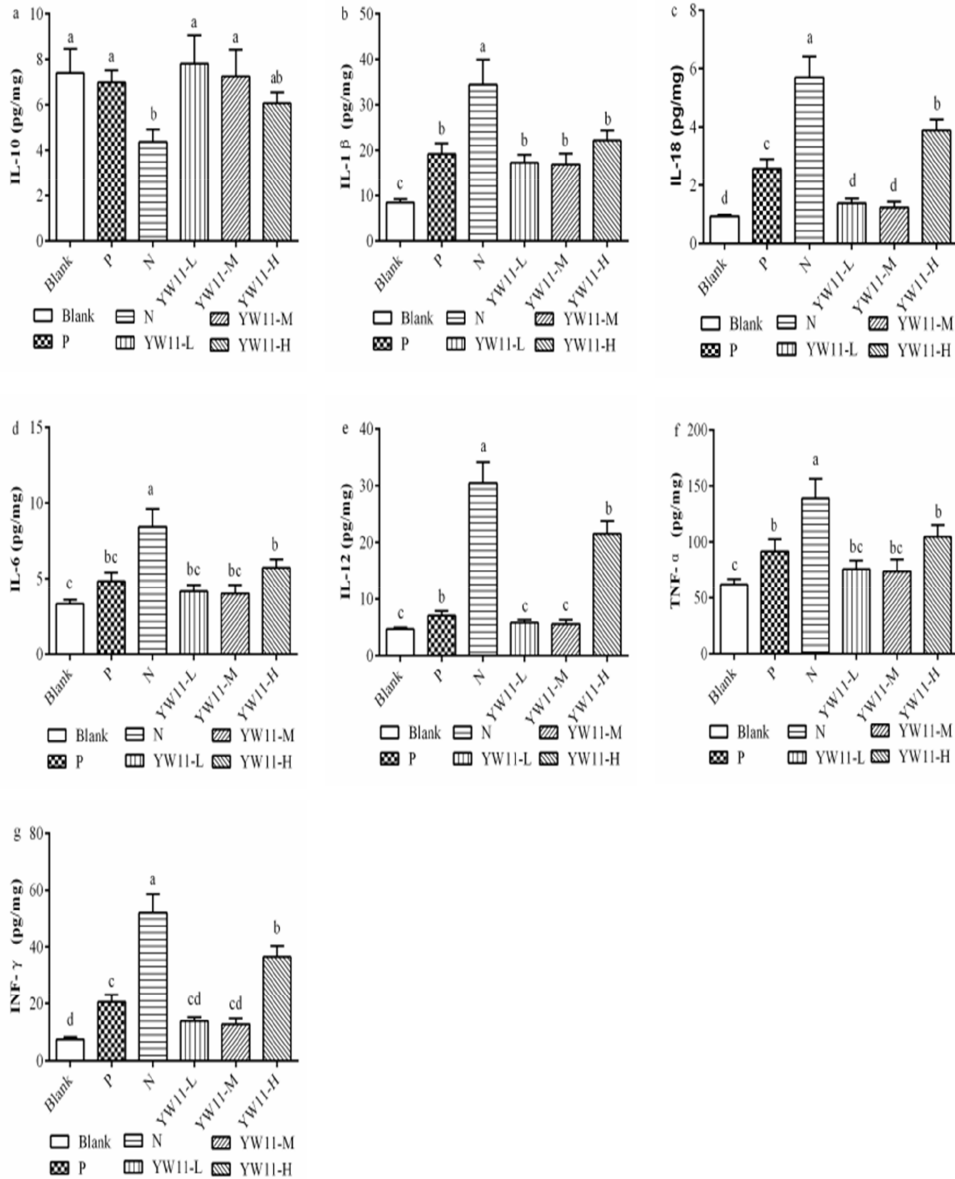


Figure 3. Effect of the EPS produced by *L. plantarum* YW11 on the cytokine levels of anti-inflammatory IL-10 (a), and proinflammatory IL-1β (b), IL-18 (c), IL-6 (d), IL-12 (e), TNF-α (f) and INF-γ (g) in the colon tissue of IBD mice, as determined by ELISAs.

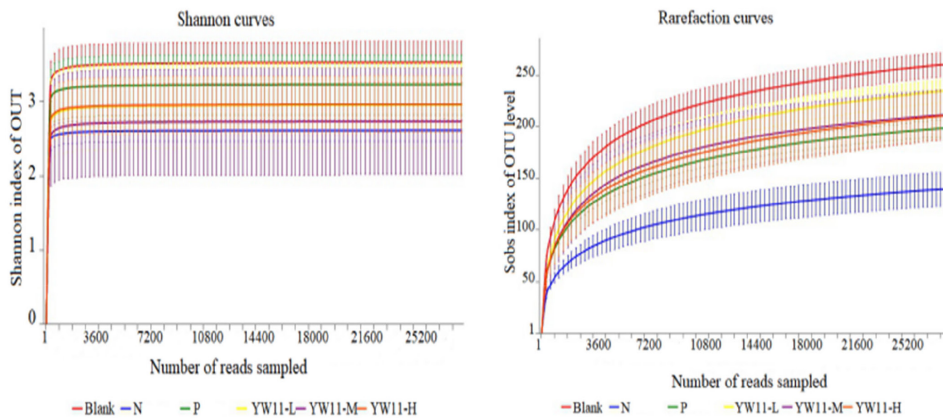


Figure 4. Rarefaction curves for each group calculated at OTU level and at 97% of sequence similarity clustering.



pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-12 and IL-18) that relieved enteritis by modulating the immune response. Our results contributed to further understanding of the functional mechanisms of probiotics and probiotic foods.

#### Conflict of Interest:

The authors declare that they have no conflict of interest

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