PAPER

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Anti-inflammatory activity of a water-soluble polysaccharide from the roots of purple sweet potato

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In this study, a water-soluble polysaccharide was isolated from purple sweet potato roots. The *in vitro* and *in vivo* anti-inflammatory effects of the polysaccharide were evaluated by lipopolysaccharide (LPS)-induced inflammatory RAW264.7 macrophages and mice, respectively. The *in vitro* anti-inflammatory assay showed that the polysaccharide could effectively inhibit the overproduction of nitric oxide and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) while increasing the secretion of anti-inflammatory cytokine (IL-10). The *in vivo* anti-inflammatory assay revealed that mice administered with the polysaccharide showed higher IL-10, SOD, and T-AOC levels but lower TNF-α, IL-1β, IL-6 and MDA levels as compared to the LPS-treated model. Meanwhile, mice administered with the polysaccharide showed increased abundance of *Lachnospiraceae*, *Lactobacillales* and *Parabacteroides* but decreased amounts of *Psychrobacter* and *Staphylococcus* as compared to the LPS model group. Moreover, mice administered with polysaccharide showed enhanced production of short chain fatty acids by gut microbiota in the lipopolysaccharide-induced inflammatory mice. Our results suggested that the water-soluble polysaccharide from purple sweet potato roots could be utilized as a novel anti-inflammatory agent.

1. Introduction

Macrophages can fight off inflammation and infection, playing an essential role in both innate and adaptive immune responses.[1] Macrophages are generally categorized into two main phenotypes: classically activated (M1) macrophages and alternatively activated (M2) macrophages.[2] Macrophages can be stimulated by interferons (IFN) or lipopolysaccharides (LPS), resulting in the secretion of pro- or anti-inflammatory cytokines. M1 macrophages exhibit pro-inflammatory activity by producing tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and nitric oxide (NO), while M2 macrophages exhibit anti-inflammatory activity *via* signature molecules of IL-10. An imbalance in M1–M2 polarization can lead to the development of multifarious acute or chronic inflammatory diseases, such as arthritis and asthma.[3,4] However, natural polysaccharides can induce RAW264.7 macrophages to express IL-10 and change macrophages into M2 phenotypes to maintain body health.[5]

Therefore, macrophages are often used as cell models to evaluate inflammatory responses.

As a complex glycolipid, LPS is the main ingredient of the cytomembrane in Gram-negative bacteria. LPS can trigger inflammatory responses in various eukaryotic species ranging from insects to humans by initiating signal pathways and promoting target proteins.[6,7] LPS can motivate a range of intercellular signaling pathways associated with inflammation, such as nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Stimulation of these pathways facilitate the expression of genes associated with inflammatory response factors.[8,9] TNF-α is initially released during the inflammatory processes, then accelerates the migration of neutrophils to damaged areas resulting in excessive reactive oxygen species (ROS) production.[10] However, excessive accumulation of ROS can lead to the development of inflammation-related diseases. At the same time, IL-1β and IL-6 can accelerate the release of inflammatory mediators by motivating accessorial inflammatory cells. Taken together, strengthening the antioxidant capacity against ROS and inhibiting the expression of pro-inflammatory cytokines are both critical for the treatment of LPS-induced inflammation.[11,12]

The gastrointestinal tract of mammals inhabit a dynamic and complex microbial community comprising the intestinal flora.[13] The relationship of reciprocal symbiosis between microflora and the host is key to maintain the homeostatic balance of intestinal microbiota.[14] Intestinal dysregulation is

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related to the pathogenesis of some inflammatory diseases or infections.[15] As reported, plant-originated polysaccharides can not only improve intestinal microbiota composition but also regulate intestinal immunity.[16] Purple sweet potato, belonging to the family of *Convolvulaceae*, is one type of sweet potato. Tang *et al.* found that three kinds of crude polysaccharides extracted from purple sweet potato possessed immunoregulation activities by reducing opportunistic pathogens and proliferating beneficial bacteria.[17] Moreover, intestinal microbiota can generate short chain fatty acids (SCFAs) by metabolizing polysaccharides.[18] The impact of gut microbiota on the host has been revealed to be mediated in part by SCFAs. Existing studies have documented that SCFAs can suppress histone deacetylases (HDACs) or induce G protein-coupled receptors (GPRs).[19,20]

In our previous work, a water-soluble polysaccharide (named WPSPP-1) was purified from purple sweet potato.[21] Structural characterization showed WPSPP-1 was mainly constituted by 1,4-linked-α-Glc$p$, 1,6-linked-α-Glc$p$, 1,4,6-linked-α-Glc$p$ and 1,2-linked-α-Man$p$, and the branch terminated with β-Glc$p$. Its molecular weight was determined by high performance gel permeation chromatography (HPGPC) as 1.03×10^4 Da. Moreover, WPSPP-1 was proved to ameliorate mice colitis inflammation induced by dextran sulfate sodium (DSS).[21] Apart from chemical-induced colitis, toll-like receptor 4 (TLR4) ligand LPS can also contribute to the onset of intestinal and systemic inflammation. In this study, we further evaluated the *in vitro* and *in vivo* anti-inflammatory effect of WPSPP-1 on LPS-induced inflammatory RAW264.7 macrophages and mice, respectively. Moreover, the effects of WPSPP-1 on gut microbiota and SCFA production in LPS-induced inflammatory mice were also determined.

2. Materials and methods

2.1. Materials and reagents

Purple sweet potato roots were provided by the Sweet Potato Research Institute, Chinese Academy of Agricultural Sciences (Jiangsu, China). Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) were obtained from Gibco/Invitrogen Co. (Carlsbad, CA). LPS was purchased from Sigma Chemicals Company (St. Louis, USA). Enzyme-linked immunosorbent assay (ELISA) kits used for the determination of TNF-α, IL-1β, IL-6 and IL-10 were from Nanjing SenBeiJia Biological Technology Co., Ltd (Nanjing, China). The commercial kits used for assaying the activities of superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) level were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). All other reagents and chemicals were analytical grade.

2.2. Extraction and purification of WPSPP-1

WPSPP-1 was extracted based on our previously established method.[21] Briefly, lyophilized purple sweet potato roots was powdered, degreased with 95% alcohol at 80 °C and then leached at hot water (70 °C) for 2 h. The obtained crude extract

was firstly decolorized with AB-8 macroporous resin, and then fractionated on a DEAE-52 ion-exchange column and Sephadex G-100 gel column. The resultant fraction was collected, concentrated and lyophilized to obtain a purified water-soluble polysaccharide, namely WPSPP-1.

2.3. Measurement of the cytotoxicity of WPSPP-1

RAW264.7 cells (CBP No. 74098) were obtained from Shanghai Institute of Cell Biology and Biochemistry of Chinese Academy of Sciences (Shanghai, China). The cells were maintained in DMEM along with penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}) and 10% (v/v) FBS, and were cultured at 37 °C in an atmosphere with 5% CO_2.[22] The cytotoxicity of WPSPP-1 was determined by the MTT method.[23] In short, RAW264.7 macrophages were cultured with a density of 1×10^5 cells per well in 96-well plates and incubated at 37 °C overnight. Then, the cells were pre-treated with various concentrations (25, 50, 100, 200, 400, 800, 1000, 2000, and 4000 μg mL^{-1}) of WPSPP-1 for 24 h. Afterwards, 10 μL of 5 mg mL^{-1} MTT solution was supplemented to each well of 96-well plates and the cells were incubated for 4 h. Subsequently, the formed formazan crystals were completely dissolved by addition of 100 μL dimethyl sulfoxide. Finally, the absorbance of 96-well plates was recorded at 570 nm by a microplate spectrophotometer.

2.4. *In vitro* anti-inflammatory activity of WPSPP-1

Briefly, RAW264.7 cells with a density of 1×10^5 cells per well were cultured in 96-well plates at 37 °C for 24 h. Then, the cells were pre-treated with WPSPP-1 at different concentrations (100, 200, 400 and 800 μg mL^{-1}) and 1 μg mL^{-1} of LPS, and the cells were then incubated at 37 °C for another 24 h. The supernatant was collected and the levels of inflammatory mediator (NO) and cytokines (TNF-α, IL-1β, IL-6 and IL-10) were determined according to the instructions of commercial ELISA kits.[12,24]

2.5. *In vivo* anti-inflammatory activity of WPSPP-1

2.5.1. Animal experiments design. The *in vivo* anti-inflammatory activity of WPSPP-1 was evaluated by a previously reported method.[25] ICR mice were purchased from the Comparative Medical Center of Yangzhou University (Yangzhou, China). All mice were housed under controlled environmental conditions at 25 °C and a 12 h light–darkness cycle. Water and standard chow were freely available for mice. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Yangzhou University and approved by the Animal Ethics Committee of Yangzhou University (permit no. SYXK2016-0019).

To estimate the effect of WPSPP-1 on the LPS-induced inflammatory mice model, forty-eight mice were randomly sorted into four groups (twelve mice per group), including a normal control group treated with 0.9% (w/v) of saline solution, The LPS model group was treated with 2 mg kg^{-1} of LPS, one sample treatment group was treated with 400 mg kg^{-1} of WPSPP-1 and 0.9% (w/v) of saline solution, and another sample treatment group was treated with 400 mg kg^{-1} of WPSPP-1 and 2 mg kg^{-1} of LPS. Mice in the two LPS

treatment groups were intraperitoneally injected with LPS for three days, while mice in the other two groups were intra-gastrically administered with 0.9% (w/v) of saline solution. Mice feces in the four treatment groups were separately collected and stored at $-80\ ^{\circ}$C. After consecutive gavage for 28 days, mice were sacrificed and their blood, thymus, spleen and liver were quickly collected.

2.5.2. Measurement of immune organ index. The thymus and spleen indexes were calculated according to eqn (1):

Thymus or spleen index = thymus or spleen weight (mg)/body weight (g) (1)

2.5.3. Measurement of inflammatory cytokines. Blood samples were centrifuged (10 000g, 4 °C) to afford serum. The contents of TNF-α, IL-1β, IL-6 and IL-10 in the serum were measured with ELISA kits based on instructions.

2.5.4. Measurement of hepatic antioxidant and lipid levels. The activities of SOD, T-AOC and MDA in hepatic tissues were assayed according to the manufacturer's instructions (Jiancheng Institute of Biotechnology, Nanjing, China).

2.6. SCFAs analysis

The concentrations of SCFAs including acetate, propionate and butyrate in the feces of mice were measured according to a previous report with some modifications.[20] Briefly, fecal samples were pre-treated by deionized water and 10% sulphuric acid, then SCFAs were extracted with diethyl ether. SCFAs in the supernatant were analyzed by a Thermo Trace DSQ II GC-MS System (Thermo Fisher Scientific, USA) with a DB-Wax capillary column (30 m × 0.25 mm × 0.25 μm). The initial column temperature was set at 100 °C for 1 min, then raised to 120 °C at 5 °C min^{-1}, and finally reached 260 °C at 2 °C min^{-1}. The flow rate of carrier gas (helium) was 1.3 mL min^{-1}. For mass spectra, the ionization energy and electron multiplier voltage was 70 eV and 500 V, respectively. Quantitative analysis was conducted according to the peak areas of internal compound (caprylic acid methyl ester).

2.7. 16S rRNA gene sequencing analysis

Total microbial DNA from mice feces was prepared with the TIANamp Stool DNA Extraction kit. PCR was adopted for amplifying the V3–V4 regions of the bacterial 16S rRNA with a forward primer and a reverse primer. A paired-end method was introduced to identify operational taxonomic units (OTUs) after the library was quantified. Sequencing analysis was performed by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

2.8. Statistical analysis

All data were presented as mean ± standard deviation (SD). Statistical significance was estimated by one-way analysis of variance (ANOVA), which was followed by Duncan test using SPSS software (Version 19.0). Differences were considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Extraction and purification of WPSPP-1

Water-soluble polysaccharides were extracted from freeze-dried purple sweet potato at 70 °C for 2 h. The resultant crude polysaccharides were purified by a DEAE-52 ion exchange column and Sephadex G-100 gel column to obtain a purified polysaccharide, named WPSPP-1. GC-MS, 1D NMR, and 2D NMR were employed to investigate the structure of WPSPP-1. Our previous study showed that WPSPP-1 was composed of glucose and mannose in the molar ratio of 20.44 : 1.00 (Fig. 1).[21]

3.2. Cytotoxicity of WPSPP-1

In this study, the cytotoxic effect of WPSPP-1 on normal RAW264.7 macrophages was determined by the MTT assay. The cell viability of RAW264.7 macrophages treated with WPSPP-1 is shown in Fig. 2A. At the concentration of 25–800 μg mL^{-1}, WPSPP-1 was non-toxic to RAW264.7 macrophages. However, the cell viability of RAW264.7 macrophages significantly increased when the concentration of WPSPP-1 increased from 800 to 4000 μg mL^{-1} ($p < 0.05$).

3.3. *In vitro* anti-inflammatory activity of WPSPP-1

3.3.1. NO production in LPS-stimulated RAW264.7 macrophages. In LPS-induced macrophages, NO production is considered a representative signal of ordinary inflammatory responses. As shown in Fig. 2B, 1.0 μg mL^{-1} of LPS significantly stimulated the release of NO in RAW264.7 cells. WPSPP-1 reduced the NO level in a dose-dependent manner. In a previous study, Wang *et al.* found that 150 μg mL^{-1} of neutral polysaccharide from North American ginseng could also suppress LPS-stimulated NO overproduction.[26]

3.3.2. Cytokines secretion in LPS-stimulated RAW264.7 macrophages. Cytokines secreted by RAW264.7 cells can regulate immunity.[27] TNF-α plays a pro-inflammatory role in the immune system. IL-1β is concerned with fever during the

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(1→4)-α-Glc*p*-(1→4)-α-Glc*p*(1→2)-α-Man*p* (1→]$_{n}$

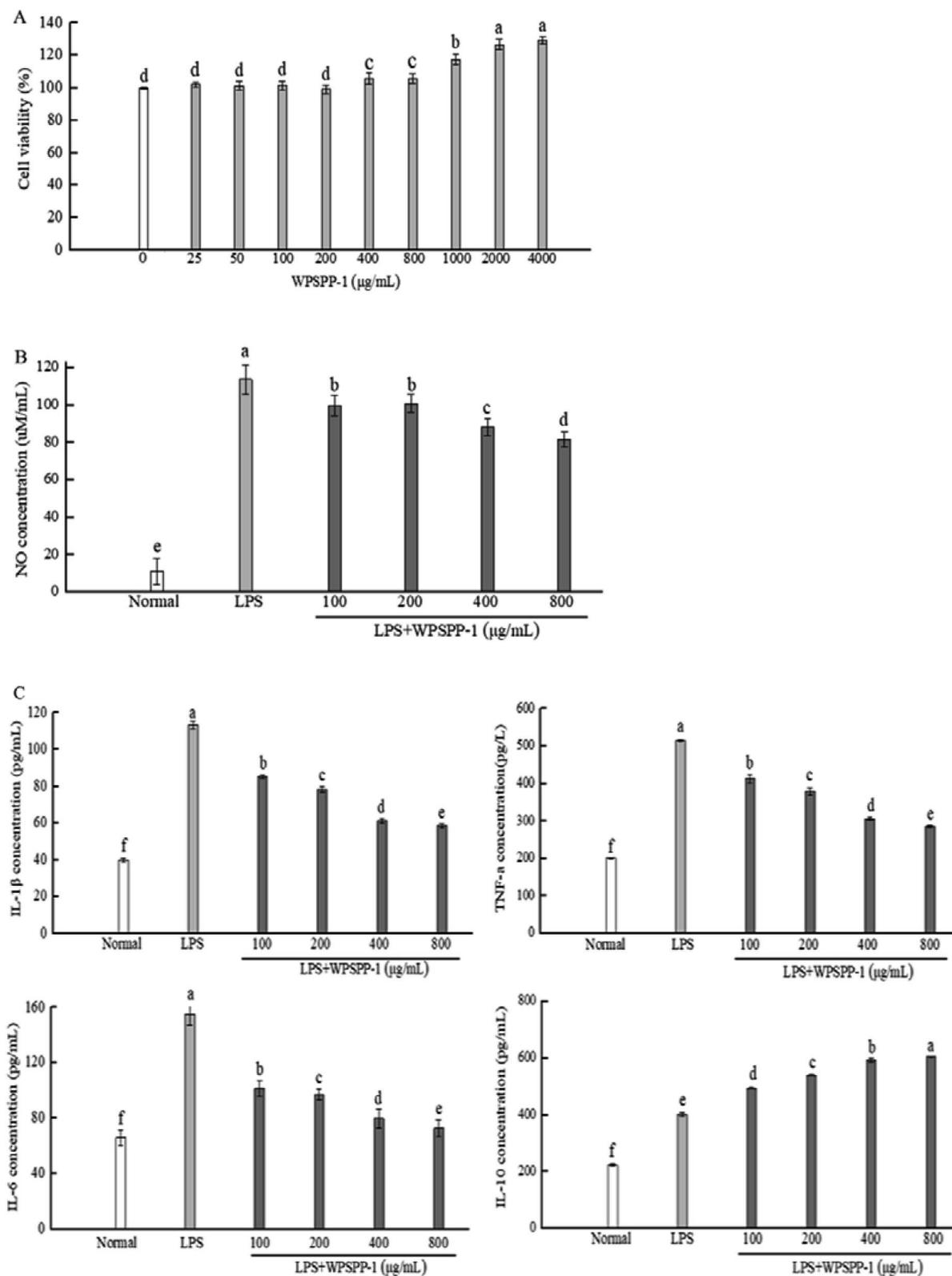**Fig. 1** Structure of WPSPP-1 from purple sweet potato with a molecular weight of 103 kDa.

Fig. 2 The cytotoxicity of WPSPP-1 on normal RAW264.7 macrophages (A) and the effect of WPSPP-1 on NO production (B) and IL-1β, IL-6, TNF-α and IL-10 levels (C) in LPS-induced inflammatory RAW264.7 macrophages. Data are expressed as mean ± SD ($n = 3$). Different lowercase alphabet letters are significantly different at the level of $p < 0.05$.

induction of acute phase protein response. IL-6, generated by monocytes and macrophages, can promote the proliferation and differentiation of B cells and T cells. IL-10 is a major anti-inflammatory cytokine with an immunosuppressive effect.[28–30] Compared with the normal control group, the levels of TNF-α, IL-1β, IL-6, and IL-10 were significantly elevated ($p < 0.05$) in the LPS treatment group (Fig. 2C), indicating that LPS successfully induced RAW264.7 cells to release inflammatory cytokines. As compared to the LPS treatment group, the WPSPP-1 treatment group showed dose-dependent decreased TNF-α, IL-1β and IL-6 levels but increased IL-10 level in RAW264.7 cells ($p < 0.05$). Our study showed that WPSPP-1 could inhibit the expression of pro-inflammatory cytokines, while stimulating the expression of anti-inflammatory cytokines in a dose-dependent manner.

Under normal circumstance, T helper (Th) 1 cytokines and Th2 cytokines regulate relative homeostasis. However, various stimulus-mediated disruptions of Th1 and Th2 immune responses may result in the onset and development of several disorders. Severe Th1 immune responses are involved in the initiation of inflammatory bowel disease (IBD), whereas abnormal Th2 immune responses participate in the development of asthma.[31] Plant-derived polysaccharides, such as *Astragalus* polysaccharides, could reduce the secretion of Th1 and modulate the imbalance between Th1 and Th2 in inflammatory disorders.[32] Our results showed that overproduction of Th1 cytokine IL-1β and Th2 cytokine IL-6 was suppressed by WPSPP-1 treatment, suggesting that WPSPP-1 had the potential to regulate the immune response balance between Th1 and Th2 in LPS-stimulated RAW264.7 macrophages. In this study, WPSPP-1 treatment was proven to boost the secretion of anti-inflammatory cytokine IL-10 in LPS-treated macrophages. Previous literature also elucidated that IL-10 possessed the ability to decrease excessive pro-inflammatory cytokines (TNF-α, IL-1β and IL-6).[33] Thus, the improvement of IL-10 secretion contributed to the anti-inflammatory activity of WPSPP-1.

Our results showed that high concentrations of WPSPP-1 (more than 400 μg mL^{-1}) exhibited better anti-inflammatory effects in LPS-stimulated RAW264.7 macrophages. Although there was no significant differences between 400 μg mL^{-1} and 800 μg mL^{-1} of WPSPP-1 on the cell viability of RAW264.7 cells (Fig. 2A), a high dose of sweet potato polysaccharide might decrease the abundance of gut microbiota.[34] So 400 mg kg^{-1} of WPSPP-1 was used in *in vivo* experiments.

3.4. *In vivo* anti-inflammatory effect of WPSPP-1

3.4.1. Immune organ index of LPS-induced inflammatory mice. The effect of WPSPP-1 on the immune organ indices (thymus and spleen indexes) of mice is shown in Fig. 3A. No significant differences were observed in thymus and spleen indices between the normal control group and WPSPP-1 treatment group, revealing that WPSPP-1 had no side-effect on the immune organ index of normal mice. Compared with the normal control group, the thymus index of mice in the LPS treatment group was significantly reduced, but the spleen index in the LPS treatment group was significantly increased ($p <$

0.05), indicating that the LPS-induced inflammation model was successfully established. As compared with the LPS model group, the thymus index increased while the spleen index decreased in the WPSPP-1 + LPS treatment group ($p < 0.05$). These results suggested that WPSPP-1 possessed a positive effect on improving the immune organs of inflammatory mice. Similar effects were also found in inflammatory mice administered with sulfated *Cyclocarya paliurus* polysaccharide and *Camellia nitidissima* polysaccharide.[35,36]

3.4.2. Cytokines levels in LPS-induced inflammatory mice. To investigate the anti-inflammatory effect of WPSPP-1 on LPS-treated mice, the levels of serum cytokines were evaluated. The inflammatory cytokines, such as TNF-α, IL-1β and IL-6, arise at early stages of the inflammatory response that can contribute to the defense against harmful stimuli. LPS can stimulate overproduction of inflammatory cytokines, leading to excessive inflammatory responses.[37] The imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines is a hallmark of the pathogenesis of inflammation.[38] The anti-inflammatory effect of WPSPP-1 on LPS-induced inflammatory mice is shown in Fig. 3B. As compared to the normal control group, the levels of IL-1β, IL-6, TNF-α and IL-10 were significantly increased in the LPS model group ($p < 0.05$). However, as compared with the LPS model group, the levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) decreased while the level of IL-10 increased in the LPS + WPSPP-1 treatment group. In a previous study, a water-soluble polysaccharide isolated from *Sedum dendroideum* exhibited anti-inflammation activity through reducing IL-1β and TNF-α production.[39] Another study also found that the alkali-soluble polysaccharide from purple sweet potato significantly restrained pro-inflammatory agents while enhancing anti-inflammatory mediators.[40] These results indicated that WPSPP-1 had potential *in vivo* anti-inflammatory activity.

3.4.3. Levels of SOD, T-AOC and MDA in LPS-induced inflammatory mice. The inflammatory reaction induced by LPS may give rise to oxidative stress. The overproduction of ROS can destroy the antioxidant defense system and eventually exacerbate the damage of organs.[41,42] SOD is the forefront of the antioxidant enzyme system against ROS, which converts superoxide into oxygen and hydrogen peroxide.[43] As for the T-AOC, it is a non-enzymatic antioxidant indicator of the defense system in organs.[44] As presented in Fig. 4, the SOD and T-AOC levels in liver tissue were strikingly decreased but the MDA level was distinctly elevated in the LPS model group as compared with the normal control group ($p < 0.05$). However, as compared with the LPS model group, the T-AOC and SOD levels were enhanced after LPS + WPSPP-1 treatment. Meanwhile, the MDA level was significantly decreased by LPS + WPSPP-1 treatment ($p < 0.05$). A previous study also showed that LPS damaged the endogenous antioxidant defense system (*e.g.* SOD and T-AOC) and increased the production of MDA.[45] Increasing evidence demonstrated that polysaccharides ameliorated LPS-induced oxidative damages.[37,46–48] Our results further demonstrated that WPSPP-1 could alleviate LPS-induced inflammatory and oxidative damages.
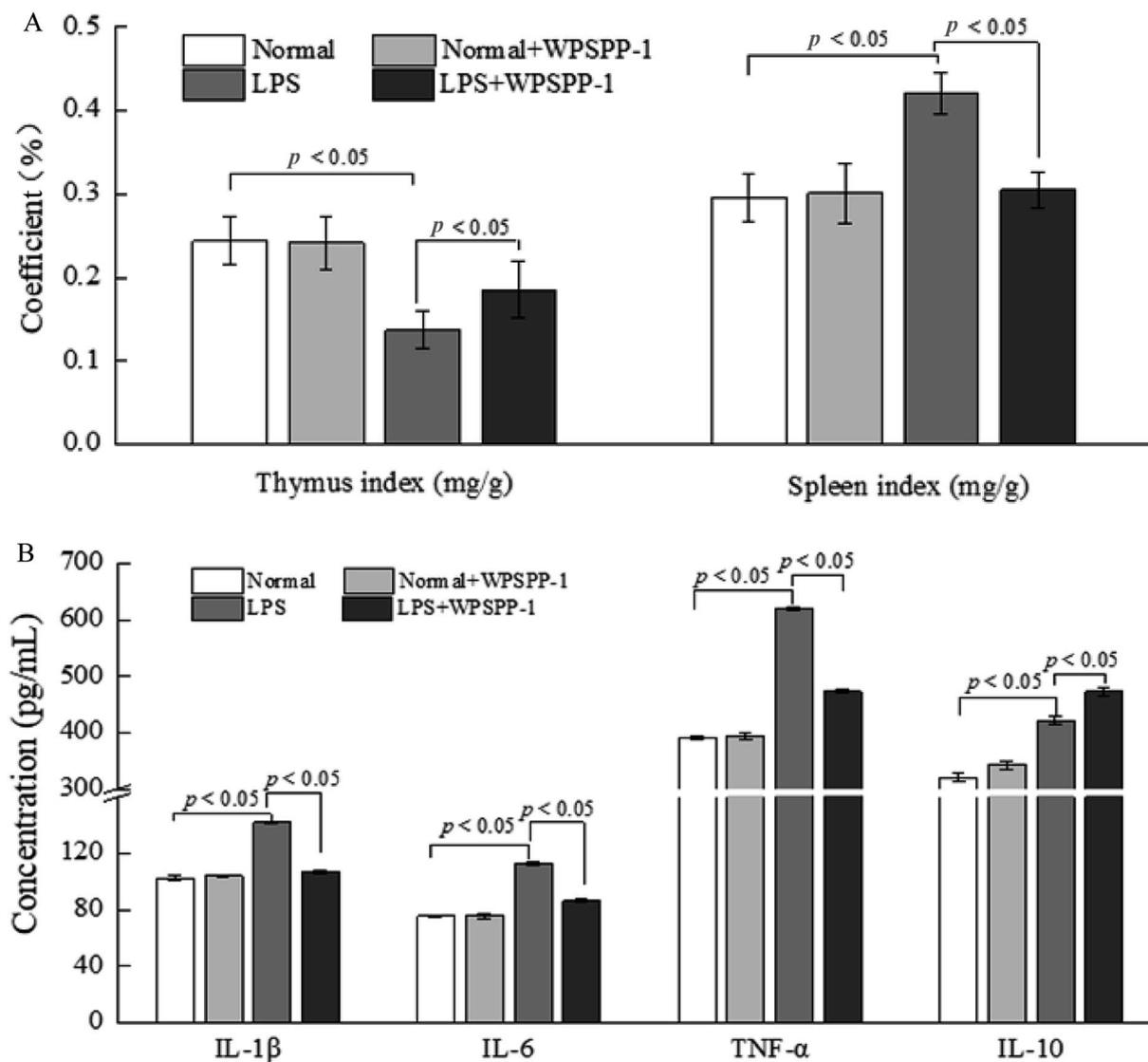
Fig. 3 Effect of WPSPP-1 on immune organ (thymus and spleen) indices (A) and the secretion of cytokines (B) in LPS-induced inflammatory mice. Data are expressed as mean $\pm$ SD ($n = 10$). Significant difference is presented at the level of $p < 0.05$ among different groups.

### 3.5. SCFAs content in LPS-induced inflammatory mice

SCFAs are the by-products of dietary fibre fermented by the intestinal microorganisms.[49] A large proportion of ingested foods including dietary fibers, resistant starch, proteins and fats cannot be digested and absorbed in the small intestine and finally reach the colon.[50] Most of these unabsorbed compounds are fermented by gut microbiota in the colon. Intestinal flora degrade undigested polysaccharides to produce various SCFAs, such as acetate, propionate, and butyrate. These SCFAs are easily absorbed and can be utilized by colonic epithelial cells as sources of energy and carbon.[51] As shown in Fig. 5, acetic acid (AA), propionic acid (PA) and butyric acid (BA) were all dramatically reduced in the LPS model group as compared with the normal control group ($p < 0.05$). However, the WPSPP-1 + LPS treatment group effectively promoted SCFA production as compared to the LPS model group ($p < 0.05$). Numerous studies have revealed that SCFAs have the potential to suppress inflammatory responses. On one hand, SCFAs can reduce the translocation of inflammatory stimulus into systemic circulation by strengthening the intestinal epithelial barrier. On the other hand, SCFAs can act on FOXP3$^+$ regulatory T cells to produce anti-inflammatory factors TGF-β and IL-10 and inactivate the NF-κB signaling pathway.[52] Therefore, WPSPP-1 might ameliorate LPS-induced inflammation partly through promoting SCFA production. Previous studies showed that linear polysaccharides, such as inulin, xylan and glucan, could elevate SCFAs levels in animals or humans under inflammatory conditions.[53,54] Our results suggested WPSPP-1 had a similar effect on SCFAs production in LPS-induced inflammatory mice.
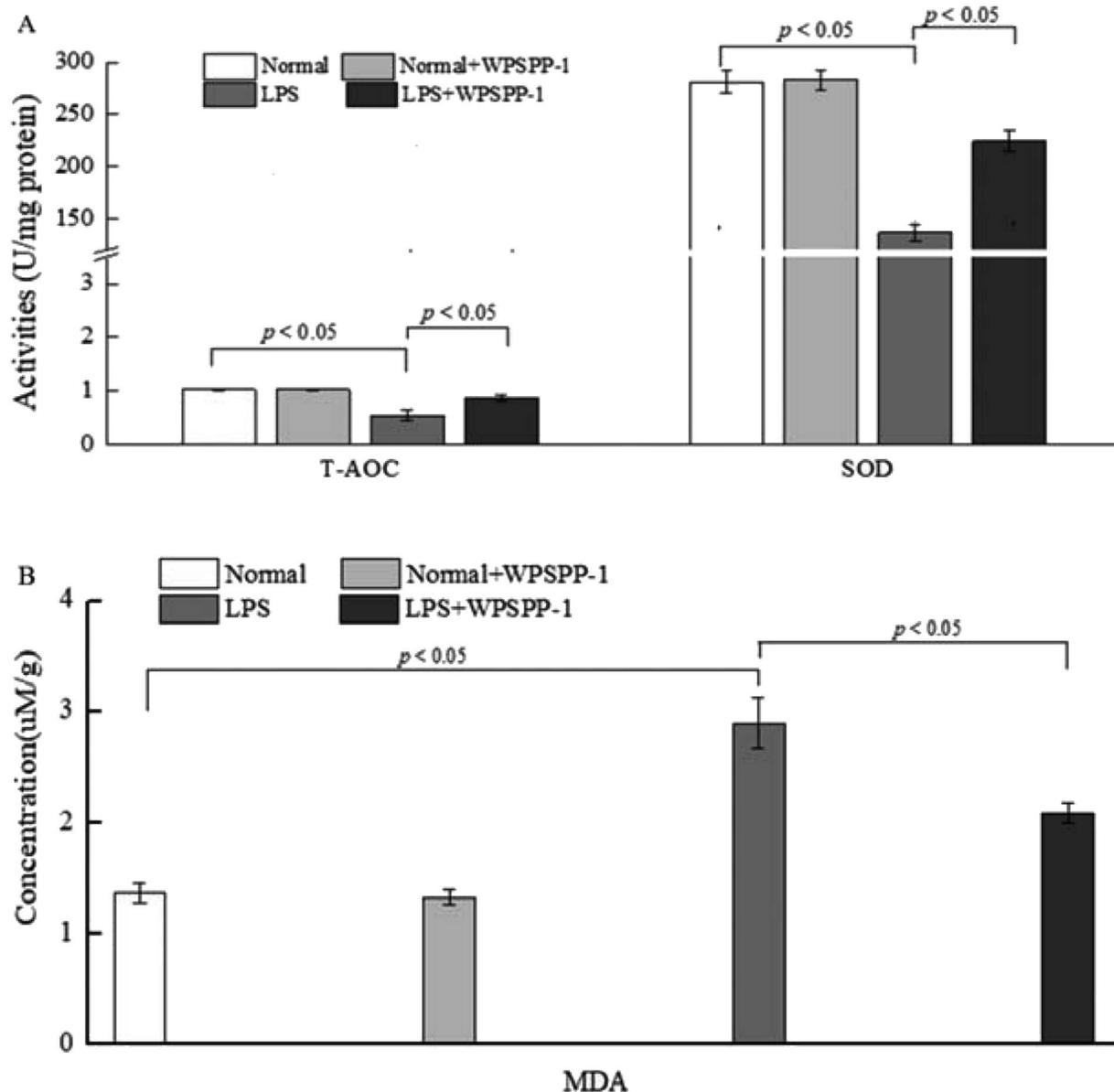
Fig. 4   Effect of WPSPP-1 on T–AOC and SOD activity (A) and MDA level (B) in LPS-induced inflammatory mice. Data are expressed as mean ± SD ($n = 10$). Significant difference is presented at the level of $p < 0.05$ among different groups.

3.6.   Diversity of gut microbiota in LPS-induced inflammatory mice

OTUs can reflect the microbial diversity and abundance of different microorganisms in the gut.[55] The total number of OTUs in four treatment groups are displayed in Fig. 6A. The total number of OTUs in the LPS model group was significantly reduced as compared with the normal control group. However, LPS-treated mice administered with WPSPP-1 had a distinctly elevated total number of OTUs as compared to the LPS model group. These indicated that administration of LPS had a negative effect while WPSPP-1 had a positive effect on gut microbiota. The same total number of OTUs in these four groups was 585, indicating that most of the bacteria were the core microbiota in these groups.

The whole structural changes of gut microbiota in four treatment groups were described by the unsupervised multivariate statistical method. As shown in Fig. 6B, the normal
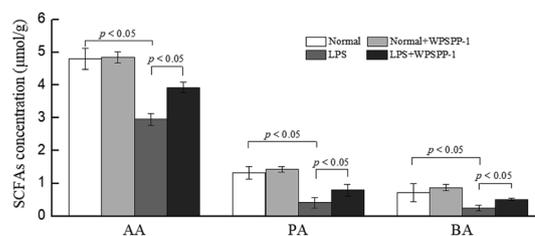
Fig. 5   Effect of WPSPP-1 on the content of SCFAs (AA, PA and BA) in mice. AA, acetic acid; PA, propionic acid; BA, butyric acid. Data are expressed as mean ± SD ($n = 7$). Significant difference is presented at the level of $p < 0.05$ among different groups.
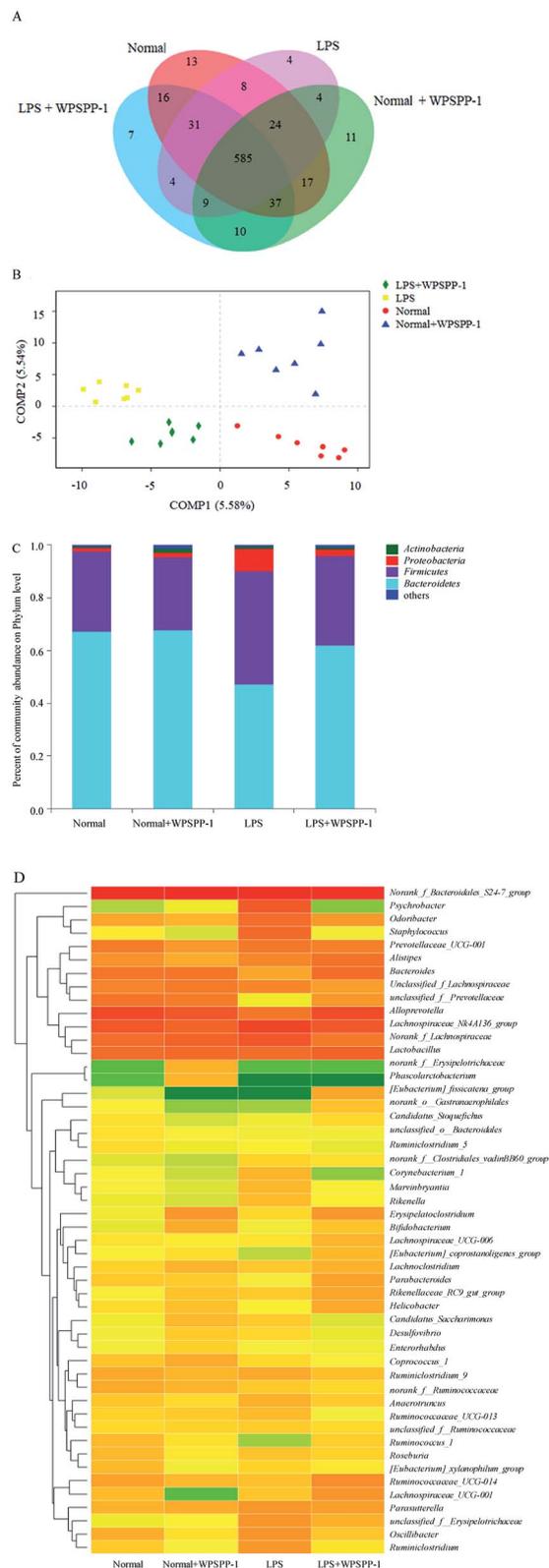
Fig. 6 Effects of WPSPP-1 on the gut microbiota compositions and relative abundances in mice. (A) Venn diagram, (B) PCA, (C) relative abundances of phylum level, (D) heat map of relative abundances on genus level.

control group and normal + WPSPP-1 treatment group were separated from LPS and LPS + WPSPP-1 treatment groups in the first component (COMP1). For LPS-treated mice, the whole structure of gut microbiota was obviously altered. By comparison, the composition of intestinal flora in the LPS + WPSPP-1 treatment group was close to that in the normal control group, suggesting that the administration of WPSPP-1 could adjust gut microbiota in LPS-induced inflammatory mice.

Histograms were employed to display the differences in species and relative abundance of gut microbiota among different treatment groups. As shown in Fig. 6C, the phylum-level distribution features of the four treatment groups were distinct. *Firmicutes* and *Bacteroidetes* are two predominant populations at the phylum level, accounting for approximately 90% of total microbes, which play important roles in regulating carbohydrate, lipid and bile acid metabolisms.[56] When compared to the normal control group, a significant increase in the relative abundance of *Firmicutes* and a significant decrease in the relative abundance of *Bacteroidetes* were observed in the LPS model group ($p < 0.05$) (Table 1). However, mice administered with LPS + WPSPP-1 exhibited an evident decrease in the relative abundance of *Firmicutes* and a significant increase in the relative abundance of *Bacteroidetes* as compared to the LPS model group ($p < 0.05$). In addition, the *Proteobacteria* level in the LPS model group was strikingly increased as compared with that of the normal control group. The treatment of WPSPP-1 could strikingly reduce the relative abundance of *Proteobacteria* in LPS-treated mice. No distinct differences were found in the levels of *Bacteroidetes*, *Firmicutes* and *Proteobacteria* between the WPSPP-1 group and normal control group. The ratio of *Bacteroidetes* to *Firmicutes* is often used to evaluate the distribution of gut flora. The increased ratio of *Bacteroidetes* to *Firmicutes* is responsible for protecting the host against systemic inflammatory diseases.[57] The *Bacteroidetes* to *Firmicutes* ratio in the LPS + WPSPP-1 group was increased as compared with that of the LPS model group. The abnormal dilatation of *Proteobacteria* is generally considered to be a key feature of the intestinal microbiota imbalance.[17] WPSPP-1 treatment reduced the level of *Proteobacteria* in LPS-treated mice. All these results revealed that WPSPP-1 exhibited a potential role in regulating intestinal flora in LPS-treated inflammatory mice.

To further verify the differences in the intestinal flora among different treatment groups, the genus level of fecal microflora in different treatment groups was contrasted. As shown in Fig. 6D, the microbial structure of different treatment groups was different. At the genus level, the dominant intestinal flora in four groups mainly included *nonrank_f_Bacteroidales S24-7 group*, *Alloprevotella*, *Lachnospiraceae NK4A136 group*, *Norank_-f_Lachnospiraceae* and *Lactobacillus*. The relative abundances of *Psychrobacter* and *Staphylococcus* were remarkably elevated, while the levels of *Bacteroides*, *Lactobacillius* and *Parabacteroides* were significantly inhibited in LPS-stimulated inflammatory mice as compared with the normal control group (Table 2). However, the relative abundances of *Bacteroides*, *Lactobacillius*, *Bifidobacterium* and *Parabacteroides* in the LPS + WPSPP-1

Table 1 Differences in gut microbiota at phylum levels of each group[a]

Group	Bacteroidetes	Firmicutes	Proteobacteria	Actinobacteria
Normal	67.35 ± 5.87[a]	30.36 ± 3.77[bc]	1.22 ± 0.36[c]	0.63 ± 0.21[b]
Normal + WPSPP-1	67.73 ± 6.04[a]	27.76 ± 5.11[c]	1.80 ± 0.51[c]	1.36 ± 0.43[a]
LPS	47.26 ± 7.31[b]	42.96 ± 2.13[a]	8.42 ± 1.01[a]	0.84 ± 0.26[b]
LPS + WPSPP-1	61.97 ± 2.45[a]	34.07 ± 1.86[b]	2.49 ± 0.27[b]	0.71 ± 0.19[b]

[a] One-way ANOVA and Duncan test were used to evaluate significant differences in gut microbiota at phylum levels. Values are presented as mean ± SD ($n = 7$). Different lowercase alphabet letters are significantly different at the level of $p < 0.05$.

treatment group were increased as compared with the LPS model group. *Bacteroides* are important members of human intestinal microorganisms that bring countless benefits to the host, devoting more than 20% of their genomes to transport and degrade polysaccharides.[58,59] *Lactobacillus* is a member of lactic acid-producing microorganisms that promote health.[60] *Bifidobacterium* can generate butyrate, which provides an anti-inflammatory effect.[61] *Parabacteroides* has been demonstrated to possess metabolic benefits on decreasing weight gain, hyperglycemia and hepatic steatosis in mice fed with high-fat diet.[62] Our results indicated that WPSPP-1 might ameliorate inflammation by increasing the relative abundance of *Bacteroides*, *Lactobacillus*, *Bifidobacterium* and *Parabacteroides*, and thus enhance the content of beneficial metabolites in the feces, such as SCFAs.

Linear discriminant analysis (LDA) was performed to identify specialized microbial communities. Four treatment groups were shown in cladograms and LDA scores of more than 2 were confirmed by linear discriminant analysis effect size (LEfSe) (Fig. 7). In the LPS model group, eight bacteria were predominant, namely *Clostridiales_vadinBB60_group* (from family to species), *Aerococcaceae* (the family and genus of *Facklamia* to species), *Atopostipes* (from genus to species), *Pseudogracilibacillus* (from genus to species), *Planococcaceae* (the family and genus of *Sporosarcina* to species), *Bacilli* (from order to species), *Paenalcaligenes* (from genus to species), and *Psychrobacter* (from genus to species). For the LPS + WPSPP-1 treatment group, five bacteria were significantly enriched, namely *Lachnospiraceae_UCG_001* (genus), *Streptococcaceae* (from family to species), *Parabacteroides* (from order to species), *Alphaproteobacteria* (the class and order of *Rhodospirillales* to genus), and *Cyanobacteria* (the phylum and its class, order of *Gastranaerophilales* to species). For the normal control group, the bacteria of *Anaerovorax* (from genus to species) and *Alloprevotella* (from genus to species) were enriched. But no microbiota were

detected at a significant level in the WPSPP-1 treatment group. Therefore, there was an obvious difference in gut microbiota composition among different treatment groups. Notably, WPSPP-1 increased the relative abundance of *Lachnospiraceae_UCG_001* and *Streptococcaceae*. It has been reported that *Lachnospiraceae* is able to breakdown polysaccharides and produce SCFAs, which might disintegrate WPSPP-1.[63] *Streptococcaceae* belongs to the order of *Lactobacillales*, which is a dominant bacterial species in the gut and is widely used as a probiotic.[64]

A phylogenetic investigation of communities by the reconstruction of unobserved states (PICRUSt) method was adopted to predict metagenomic functions; a total of 262 KEGG pathways were detected in all samples. A significant difference in KEGG pathways is shown in Fig. 8. Compared to the normal control group, 34 functional modules (7 increased and 27 decreased) were obviously detected in the LPS model group ($p < 0.05$). However, the intervention of WPSPP-1 improved the abundance of these pathways. Metabolic activities of intestinal microbiota were the most important functions in mammals and avians.[65] Our results suggested that metabolism accounted for 48.56% on average of KEGG pathways in four different groups. Energy metabolism was remarkably enhanced in the LPS model group, and carbohydrate metabolism, amino acid metabolism, glycan biosynthesis and metabolism, and cofactor and vitamin metabolisms were strikingly elevated in the LPS + WPSPP-1 treatment group ($p < 0.05$). Besides, WPSPP-1 could lower the disease risk, such as infectious diseases. Nevertheless, it is plausible that microbiota in the LPS model group were involved in maintaining fundamental activities, while microbiota in the LPS + WPSPP-1 treatment group may ameliorate metabolic disorders and inhibit inflammatory reactions.[66] Our results suggest that WPSPP-1 could suppress LPS-induced inflammation by improving the gut microbiota composition, enhancing the anti-oxidative

Table 2 Differences in gut microbiota at genus levels of each group[a]

Group	Psychrobacter	Staphylococcus	Bacteroides	Lactobacillius	Bifidobacterium	Parabacteroides
Normal	0.03 ± 0.01[b]	0.21 ± 0.08[b]	3.34 ± 0.91[b]	4.52 ± 0.57[b]	0.09 ± 0.03[c]	0.43 ± 0.13[b]
Normal + WPSPP-1	0.11 ± 0.03[b]	0.06 ± 0.02[b]	3.22 ± 0.67[b]	4.80 ± 0.72[b]	0.74 ± 0.21[a]	0.38 ± 0.08[b]
LPS	6.84 ± 1.09[a]	4.33 ± 1.35[a]	0.81 ± 0.23[c]	3.90 ± 0.43[c]	0.12 ± 0.04[c]	0.14 ± 0.03[c]
LPS + WPSPP-1	0.01 ± 0.01[b]	0.15 ± 0.04[b]	4.67 ± 1.16[a]	6.55 ± 1.11[a]	0.49 ± 0.12[b]	1.06 ± 0.35[a]

[a] One-way ANOVA and Duncan test were used to evaluate significant differences in gut microbiota at genus levels. Values are presented as mean ± SD ($n = 7$). Different lowercase alphabet letters are significantly different at the level of $p < 0.05$.
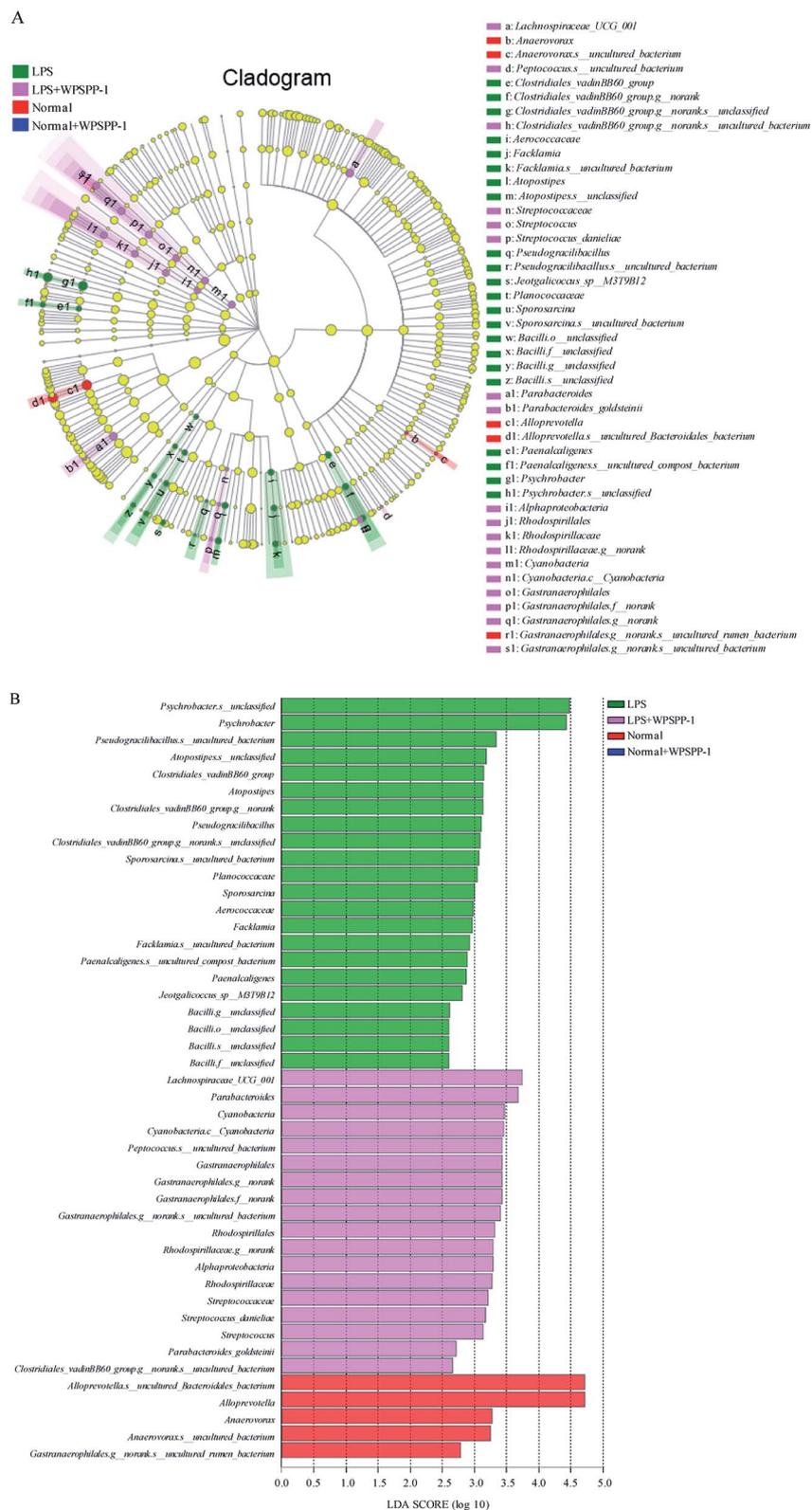
Fig. 7 LEfSe analysis of WPSPP-1 intervention on gut microbiota. (A) Phylogenetic distribution cladogram of the bacterial lineages, (B) LDA scores with 2 or greater of indicator bacteria. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group.
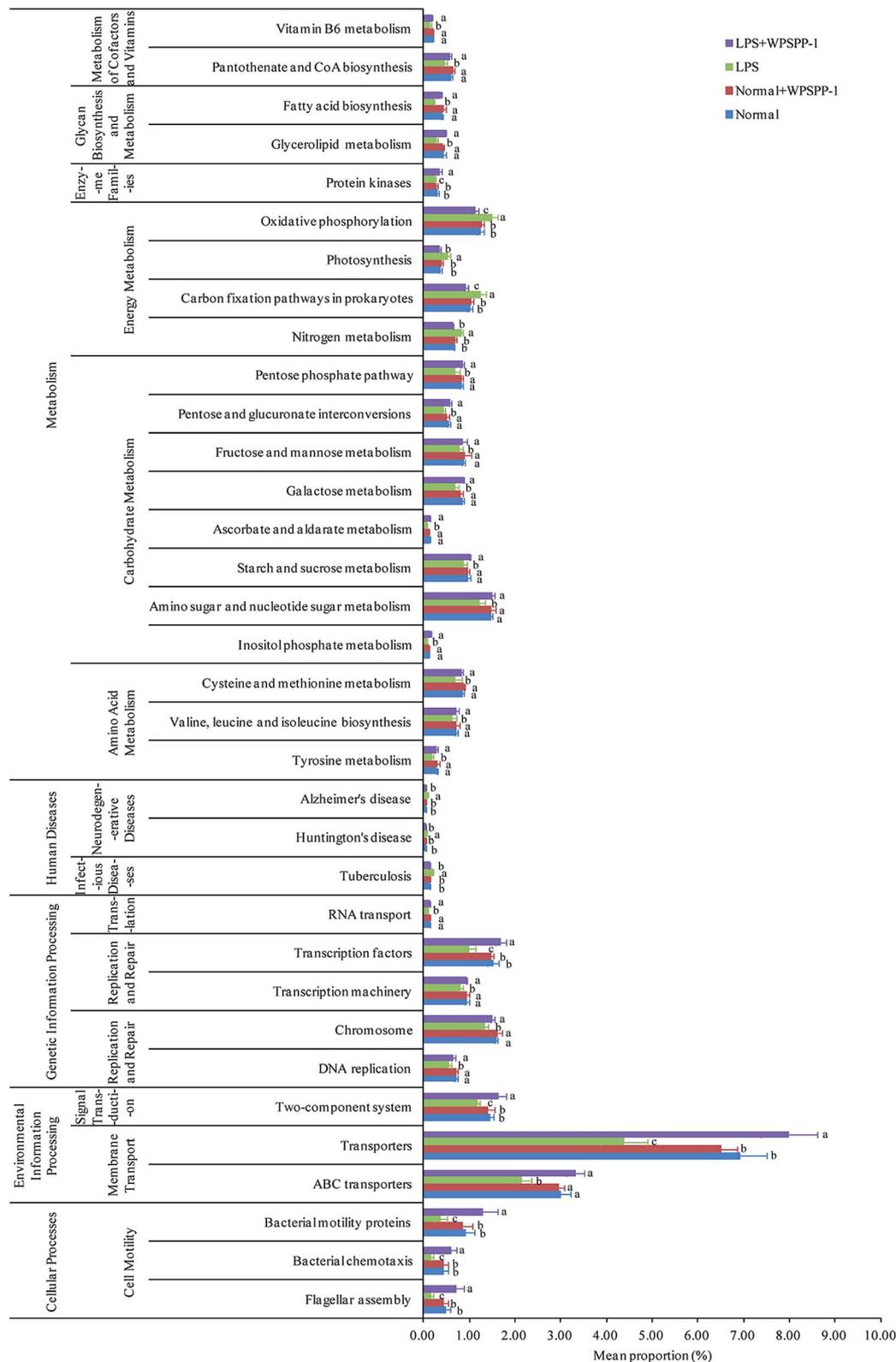
Fig. 8 Effect of WPSPP-1 on gut microbiota function predicted by PICRUSt. Data are expressed as mean ± SD (n = 7). Different lowercase alphabet letters are significantly different at the level of $p < 0.05$.

defense system and stimulating the production of anti-inflammatory metabolite SCFAs.

4. Conclusion

In summary, WPSPP-1 effectively regulated the levels of pro-inflammatory cytokines and anti-inflammatory cytokines, suppressed the growth of *Psychrobacter* and *Staphylococcus*, and promoted the growth of health-promoting microorganisms including *Lachnospiraceae*, *Lactobacillales* and *Parabacteroides*. In addition, WPSPP-1 significantly elevated the production of SCFAs in the gut of inflammatory mice and enhanced the anti-oxidative defense system against oxidative stress. Therefore, WPSPP-1 could effectively alleviate LPS-induced inflammation both *in vitro* and *in vivo*. In the future, more in-depth studies can be carried out to reveal the anti-inflammatory mechanisms of WPSPP-1 at molecular levels.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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