Journal of Functional Foods 113 (2024) 106048

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Paraprobiotics 5-PA and 6-PA restore intestinal homeostasis by inhibiting NF-κB/NLRP3 signaling pathway and alleviating dysbiosis in mice with chronic ulcerative colitis

Chunhong Liu [a], Xiaofen Qi [a], Kaifang Guan [a], Haoran Chen [a], Qiming Li [b], Kaidong Mao [c], Guiqi Shen [c], Ying Ma [a,*]

[a] Harbin Institute of Technology, Harbin, China
[b] Sichuan New Hope Dairy Co., Ltd, Sichuan, China
[c] Jiangsu HOWYOU Biotechnology Co., Ltd, Jiangsu, China

ARTICLE INFO

Keywords:
Paraprobiotics
Intestinal homeostasis
Gut microbiota
Metabolism
NF-κB/NLRP3
Tight junction

ABSTRACT

Research on the role of paraprobiotics in ameliorating the pathology of ulcerative colitis and its intestinal homeostatic imbalance is still limited. In this study, we investigated the effects of the anti-inflammatory probiotic HF05 and HF06 derived paraprobiotics 5-PA and 6-PA on mice with ulcerative colitis and their intestinal homeostasis. 5-PA and 6-PA alleviated inflammation, intestinal damage, and weight loss in mice, suppressed the activation of the NF-κB/NLRP3 signaling pathway, and upregulated the expression of intestinal tight junction proteins (ZO-1, occludin, and claudin-1). Notably, the two paraprobiotic regulated the gut microbiota and their metabolic interactions with the host. Specific taxa of gut microbes, including *unclassified_f_Muribaculaceae*, *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus*, as well as bile acids and glycerophospholipid metabolites, were closely associated with gut homeostasis. In conclusion, this study confirms the beneficial effects of 5-PA and 6-PA in alleviating ulcerative colitis and promoting intestinal homeostasis.

1. Introduction

The intestine, as a complex ecosystem, maintains dynamic homeostasis by interacting with a large number of symbiotic microbiotas (Shen, Huang, Yao, & Jin, 2022). According to research, a complex interplay between microbiota, intestinal epithelial barrier, and the host immune system is crucial for maintaining gut balance (Maloy & Powrie, 2011). The gut microbiota impacts both the integrity and function of the intestinal barrier, while the immune system strives to maintain an equilibrium between both defensive and adaptive immune responses. The gut microbiota, also referred to as the gut microbiome, is composed of over 100 trillion microorganisms residing in the intestinal tract, performing critical functions in host immune regulation, nutrient metabolism, intestinal barrier integrity maintenance, and the prevention of pathogenic invasion (Gentile & Weir, 2018; Rinninella et al., 2019). The gut microbiota is a vital system that plays a crucial role in extracting, synthesizing, and absorbing various nutrients and metabolites, such as short-chain fatty acids (SCFAs), vitamins, amino acids, bile acids, lipids (Jandhyala et al., 2015). The dynamic crosstalk between intestinal epithelial cells (IECs), gut microbiota, and local immune cells at the cellular level is one of the essential characteristics of gut homeostasis (Artis, 2008; Hooper & Macpherson, 2010). Thus, maintaining gut homeostasis requires the coordinated action of multiple mechanisms. Dysregulation of gut homeostasis can result in chronic inflammatory pathology, highlighting the severity of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and crohn's disease (CD) (Barbara et al., 2021).

Ulcerative colitis, an idiopathic chronic inflammatory disease primarily affecting the colon and rectum, has the potential to extend to the cecum in patients with proctitis or left-sided colitis (Ordás, Eckmann, Talamini, Baumgart, & Sandborn, 2012). It is characterized by the presence of bloody diarrhea and follows a cyclical pattern of remission and relapse in its clinical course (Ungaro, Mehandru, Allen, Peyrin-Biroulet, & Colombel, 2017). Treatment of UC mainly consists of

Abbreviations: HF05, *Lactiplantibacillus plantarum*; HF06, *Limosilactobacillus fermentum*; 5-PA, HF05-derived paraprobiotic; 6-PA, HF06-derived paraprobiotic; NF-κB, Nuclear factor kappa-B; NLRP3, NOD-like receptor protein 3 inflammasome; ZO-1, Zonula occludens protein 1.

* Corresponding author at: No. 92 Xidazhi Street, Nangang District, Harbin 150001, China.
E-mail address: maying@hit.edu.cn (Y. Ma).

https://doi.org/10.1016/j.jff.2024.106048
Received 23 July 2023; Received in revised form 16 January 2024; Accepted 24 January 2024
Available online 3 February 2024

drugs such as mesalazine, corticosteroids, immunosuppressive drugs and TNF-α monoclonal antibodies (Ordás et al., 2012). These drugs can potentially cause severe side effects, such as opportunistic infections, abdominal pain, acne,diabetes, weight gain, and high blood pressure (Li, Kim, Sandhu, Gao, & Gu, 2017; Toruner et al., 2008). Accordingly, the development of novel colitis treatment drugs with enhanced efficacy and a reduced incidence of adverse effects is imperative. Probiotic therapy has proven to be an effective intervention for alleviating UC and restoring intestinal homeostasis by modulating the intestinal flora, intestinal barrier, and immune system (Fusco et al., 2023; Kaur & Ali, 2022; Shang et al., 2022; Xu et al., 2023). However, there are risks associated with their actual usage, such as the potential for live bacteria displacement, sepsis, and the potential transfer of toxic and resistant genes, especially in patients with weaker immune functions (Boyle, Robins-Browne, & Tang, 2006; Porfiri et al., 2022).

As a preferred alternative to probiotics, paraprobiotics, also known as "inactivated" or "ghost" probiotics, comprise whole or disrupted non-viable microbial cells or primitive cell extracts with complex chemical compositions (Barros et al., 2020). Compared to live probiotics, inactivated paraprobiotics largely avoid the negative effects of live bacteria on the host. These possible adverse effects encompass: posing an increased risk of bacterial infection in individuals with compromised immune systems, such as those who are immunocompromised; administering probiotics to susceptible populations, for instance critically ill patients and newborns, could lead to adverse events such as bacteremia; specific probiotic strains have the potential to transfer genes associated with antibiotic resistance to the host microbiota (Batista et al., 2022; Lee et al., 2022; Lim, Jung, Joo Suh, Choi, & Kim, 2022; Maehata, Arai, Iwabuchi, & Abe, 2021; Sawada et al., 2016). Research has shown that paraprobiotics have health benefits including immune regulation, pathogen inhibition, microbiota modulation, promoting intestinal injury repair, reducing bacterial translocation risk, and protecting intestinal barrier (de Almada, Almada, Martinez, & Sant'Ana, 2016). Paraprobiotics have potential for ulcerative colitis but research is still in early stages with many questions unexplored. The strains *Lactiplantibacillus plantarum* HF05 and *Limosilactobacillus fermentum* HF06 are probiotic strains with anti-inflammatory capabilities that we selected from the traditional Chinese fermented milk product "Qula" (Liu et al., 2023). By co-culturing the candidate strains with LPS-induced RAW264.7 inflammatory cells, we screened HF05 and HF06 based on their ability to significantly suppress inflammation in RAW264.7 cells, as indicated by the inhibition of inflammatory factors (NO, IL-6, IL-1β, and TNF-α). Moreover, during the preliminary evaluation of the strains' probiotic characteristics, both HF05 and HF06 exhibited good antimicrobial and antioxidant activities. The HF05 strain demonstrated strong growth capability, tolerance to high concentrations of bile salts, and a high adhesion rate to 31.31 % to Caco-2 cells. On the other hand, the HF06 strain showed the ability to tolerate low pH environments. Both HF05 and HF06 strains exhibited prominent anti-inflammatory capabilities and probiotic characteristics, which were further confirmed in animal models of intestinal inflammation (unpublished). However, it remains unknown whether the paraprobiotics prepared from these two strains, have the capacity to alleviate intestinal inflammation in *vivo* and whether the different sources of paraprobiotics exhibit the same level of efficacy.

Therefore, in order to understand the positive effects of paraprobiotics on the host and to promote their application, this article investigated and compared the therapeutic effect of two paraprobiotics, 5-PA and 6-PA, derived from *Lactiplantibacillus plantarum* HF05 and *Limosilactobacillus fermentum* HF06, respectively, which were screened by us for their anti-inflammatory capacity, in chronic ulcerative colitis and its imbalance of intestinal homeostasis. We further examined the pathways of action of the paraprobiotics on the intestinal microbiota and host intestinal homeostasis, with a specific focus on the gut microbiota analysis and the metabolic interactions between the intestinal microbiota and the host. By investigating the health effects and mechanisms of action of probiotics 5-PA and 6-PA, we expect to provide a theoretical basis for the utilization of paraprobiotics as functional products.

2. Materials and methods

2.1. Preparation of paraprobiotics

Anti-inflammatory strains *Lactiplantibacillus plantarum* HF05 and *Limosilactobacillus fermentum* HF06 were isolated from the Chinese traditional dairy product Qula, and identified by 16 s rDNA sequencing (Liu et al., 2023). They were then stored in the China General Microbiological Culture Collection Center (CGMCC). Preparation of paraprobiotics was carried out based on the methodology outlined by Trindade et al. (2021). The HF05 and HF06 strains were inoculated at a 2 % concentration into liquid MRS (De Man Rogosa Sharpe, Basebio, Hangzhou, China) medium for activation and then passaged twice. The bacterial cells were obtained by centrifugation (10000*g*, 5 min). After washing the bacterial cells twice with sterile physiological saline solution and adjusting their concentration to 5×10^9 CFU/mL, they were subsequently harvested by centrifugation at 10000*g* for 5 min. The bacterial cells were sterilized by autoclaving them at 121 °C for 15 min after rinsing them twice with sterile saline, resulting in the formation of paraprobiotics.

2.2. Mice and experimental design

Healthy male C57BL/6 mice (6–8 weeks, 20–22 g) were purchased from Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China). All mice were allowed to drink, eat, and move freely in a 12 h light–dark cycle. Following one week of adaptive feeding and weighing, mice were randomized into four groups, including Control, DSS, 5-PA, and 6-PA groups.

Dextran sulfate sodium (DSS) (Rhawn Chemical Technology Co., Ltd, Shanghai, China) was dissolved in sterile water to a final concentration of 2 %. Mice were then provided ad libitum access to the 2 % DSS-containing water for 7 consecutive days, followed by 7 days of standard drinking water. This cycle of DSS exposure and standard water was repeated three times to establish a chronic ulcerative colitis model, as shown in Fig. 1a. On day 18 of the study, mice in the 5-PA and 6-PA groups received 200 μL of 5-PA and 6-PA samples containing 1×10^9 CFU bacteria respectively, administered via oral gavage. This administration continued until the end of the experiment. Meanwhile, mice in the Control and DSS groups were administered sterile PBS via oral gavage. Throughout the experiment, the weight of the mice, and presence of fecal occult blood and diarrhea score were recorded (Table S1). At the end of the experiment, the mice were euthanized using cervical dislocation and their colon and fecal samples were collected.

2.3. Histological analysis of colitis

The slices of the distal colon were prepared using the Zhou et al. protocol (Zhou et al., 2022). The distal colon tissue was fixed in 4 % paraformaldehyde (Biotopped, Beijing, China) for 24 h, and then the colon tissue wax block was prepared by paraffin embedding. The paraffin blocks were sectioned into 4 μm thick slices to prepare colon tissue slides. The colon tissue slices were submerged in the hematoxylin solution to stain the nuclei, rinsed, and then immersed in the eosin staining solution to stain the cytoplasm. Finally, the samples were examined under an optical microscope.

2.4. Determination of the content of inflammatory factors in the serum

Whole blood was harvested from the canthal orbital vein and placed in a 1.5 mL EP tube. After standing for 3 h, the whole blood samples were centrifuged at 3000 rpm at 4 °C for 10 min, and the supernatant was collected to obtain the serum. The serum (10 μL serum was used) levels

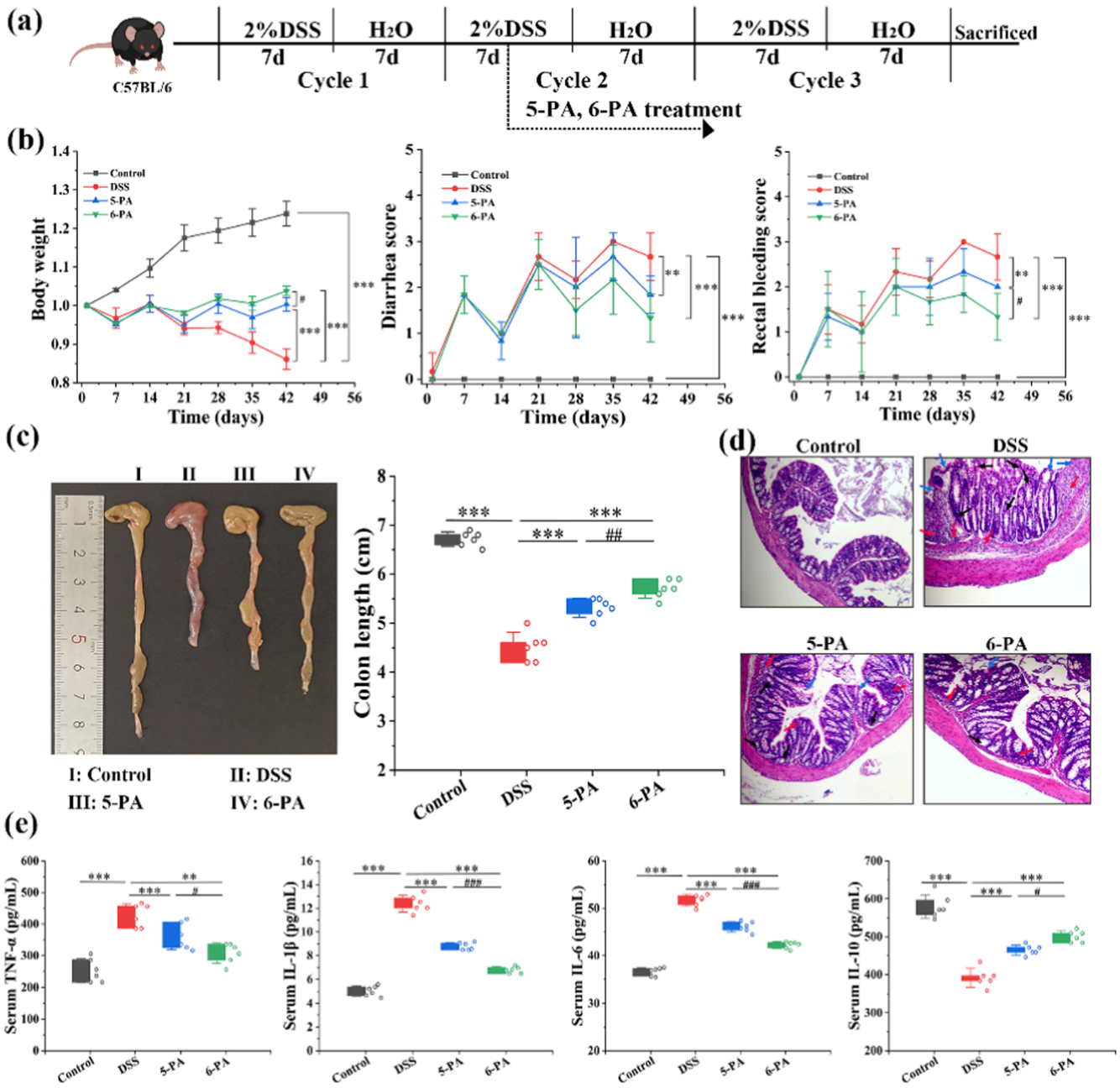

Fig. 1. 5-PA and 6-PA improved symptoms of chronic ulcerative colitis. (a) Experimental schedule of animal treatment. (b) Result of body weight, diarrhea score, and rectal bleeding score. (c) The representative images and length of the colon from each group. (d) Representative images of colon staining from each group of mice. Blue arrows represent mucosal epithelial damage; black arrows represent atrophy of the crypts and reduction of cup cells; red arrows represent inflammatory cell infiltration. (e) Concentrations of TNF-α, IL-1β, IL-6 and IL-10 in serum. N = 6 for each group. Data presented as mean ± SEM. Statistically significant differences between intact DSS group and other groups are indicated *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Statistically significant differences between 5-PA group and 6-PA group are indicated #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of pro-inflammatory cytokines including Tumor necrosis factor-α (TNF-α), Interleukin- 6 (IL-6), and Interleukin- 1β (IL-1β) (minimum detection limits of 25 pg/mL, 3.0 pg/mL, 2.5 pg/mL, respectively) as well as the anti-inflammatory cytokine, Interleukin- 10 (IL-10, minimum detection limits of 30 pg/mL), were determined using ELISA kits (Mouse) and following the manufacturer's instructions (MEIMIAN Biotechnology, Jiangsu, China).

2.5. RNA isolation and quantitative real-time PCR

Colon tissue was extracted by an RNA extraction kit (Tiangen Biochemical Technology Co., Ltd, Beijing, China), i.e., it was homogenized with Trizol, extracted by chloroform, precipitated with isopropanol, and washed with 75 % ethanol, and dissolved in DEPC water. After the concentration of RNA extracted from colon tissue was determined, cDNA was synthesized using a reverse transcriptase kit (Vazyme, Nanjing, China). mRNA expression was quantified using qPCR with comparative quantification ($2^{-\Delta\Delta Ct}$) method, and β-actin was used as a reference gene to determine the fold changes. Refer to Table S2 for a list of the mRNA primers that were used.

2.6. Western blot analysis

The total protein of colon tissue was extracted using radioimmunoprecipitation assay (RIPA, Beyotime, Beijing, China) buffer with phosphatase inhibitor (Beyotime, Beijing, China), and the protein concentration was adjusted to 4 μg/μL after determining the total protein content using a BCA assay kit (Beyotime, Beijing, China). The protein was mixed with 5 × loading buffer, boiled at 100 °C for 10 min, and then separated by SDS-PAGE gel electrophoresis (Solarbio Technology Co., Ltd, Beijing, China) with consistent loading amounts in each lane. After electrophoresis, the separated proteins were transferred onto a 0.22 μm PVDF membrane. The membrane was blocked in QuickBlock™ western Closing solution (Beyotime, Beijing, China) for 40 min, and then incubated overnight at 4 °C with diluted primary antibodies p65 (1:1000, Affinity Biosciences, Jiangsu, China), p-p65 (1:300, Senta Cruz Biotechnology, USA), IkBα (1:1000, Proteintech, Rosemont, USA), p-IkBα (1:300, Senta Cruz Biotechnology, USA), NLRP3 (1:1000, Affinity Biosciences, Jiangsu, China), ASC (1:500, Affinity Biosciences, Jiangsu, China), caspase-1 (1:2000, Proteintech, Rosemont, USA), IL-1β (1:1000), IL-18 (1:2000, Proteintech, Rosemont, USA), ZO-1 (1:5000, Proteintech, Rosemont, USA), occludin (1:5000, Proteintech, Rosemont, USA), claudin-1 (1:2000, Proteintech, Rosemont, USA), and β-actin (1:5000, Proteintech, Rosemont, USA). The membrane was then washed three times with TBST buffer. The bands were incubated with HRP-conjugated secondary antibodies against mouse (1:2000, Proteintech, Rosemont, USA) or rabbit (1:2000, Proteintech, Rosemont, USA) for 2 h at room temperature, followed by washing with TBST buffer and visualization of the proteins using 3,3′-diaminobenzidine (DAB). The bands were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and β-actin was used to calculate the relative expression levels of each protein.

2.7. Analysis of fecal microbiome and non-targeted metabolomics

According to the instructions of the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.), fecal microbial community genomic DNA extraction was performed. The quality of the extracted genomic DNA was checked using 1 % agarose gel electrophoresis, and the DNA concentration and purity were determined using NanoDrop 2000 (Thermo Scientific, USA). Using the extracted DNA as a template, the V3-V4 variable region of the 16S rRNA gene was amplified by PCR using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with a Barcode sequence. The purified PCR products were then used to construct libraries using the NEXTFLEX Rapid DNA-Seq Kit, followed by sequencing on the Illumina Miseq PE300/NovaSeq PE250 platform.

For the extraction method, 50 mg of fecal sample was added to 400 μL of extraction solution (methanol: water = 4:1 (v:v)) containing 0.02 mg/mL of internal standard (L-2-chlorophenylalanine) and frozen grinding (−10 °C, 50 KHz) was performed using a 6 mm diameter grinder head, followed by low-temperature ultrasonic extraction for 30 min (5 °C, 40 KHz). Subsequently, the sample was allowed to stand at −20 °C for 30 min, centrifuged (4 °C, 13,000*g*) for 15 min, and the supernatant was collected for LC-MS/MS analysis. 3 μL of the sample was separated on an HSS T3 chromatographic column (100 mm × 2.1 mm i.d., 1.8 μm) and entered the mass spectrometry detection. The mobile phase A consisted of 95 % water + 5 % acetonitrile (containing 0.1 % formic acid), and the mobile phase B consisted of 47.5 % acetonitrile + 47.5 % isopropanol + 5 % water (containing 0.1 % formic acid). The mass spectrometry signals of the samples were acquired using positive and negative ion scanning modes, with a mass scanning range of *m/z*: 70–1050. The ion spray voltage was set at 3500 V for positive ions and 2800 V for negative ions, the sheath gas pressure was 40 psi, the auxiliary heating gas pressure was 10 psi, the ion source heating temperature was 400 °C, collision energies of 20–40-60 V were applied in a cycle, and the MS1 resolution was set to 70,000 and the MS2 resolution was set to 17500.

The fecal microbiome analysis and non-targeted metabolomics were performed using the Illumina MiSeq platform from Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) and Thermo UHPLC-Q Exactive system respectively. The species annotation and evaluation, differential analysis, environmental factor correlation analysis, gene functional prediction, and annotation and differential analysis for fecal metabolites were all performed on the Majorbio cloud platform (cloud.majorbio.com).

2.8. Statistical analysis

Mean ± standard deviation values were analyzed using IBM SPSS Statistics 26 for Windows through a one-way ANOVA test with Duncan post-hoc multiple comparisons. The data was plotted using Origin 2023.

3. Results

3.1. 5-PA and 6-PA improved symptoms of chronic ulcerative colitis

To assess the impact of probiotics 5-PA and 6-PA on chronic UC, mice were administered sterile water containing 2 % DSS for a total of three cycles to induce chronic UC. As illustrated in Fig. 1b, DSS consumption led to weight loss, bloody stools, and diarrhea, indicating successful establishment of the colitis model. In addition, the colon of the DSS group mice was significantly shortened, which was related to the severity of colitis (Fig. 1c). After the intervention with 5-PA and 6-PA, colon length significantly recovered, and the intervention effect of 6-PA was significantly greater than that of 5-PA (Fig. 1c, $p < 0.01$). The H&E staining demonstrated that the Control group revealed an intact and clear colonic mucosal epithelium, with orderly and regular crypts, without any inflammatory cells, whereas the DSS group exhibited a damaged mucosal epithelium (indicated by the blue arrow), with a disorderly and reduced crypt structure (indicated by the black arrow), infiltrated with a significant number of inflammatory cells in the mucosa and submucosa (indicated by the black arrow, Fig. 1 d). In the DSS group, the concentration of inflammatory cytokines TNF-α, IL-1β, and IL-6 significantly increased, while that of the anti-inflammatory cytokine IL-10 decreased significantly. The intervention using 5-PA and 6-PA resulted in improvement of colon pathological changes and a remarkable reduction in the inflammatory cytokine levels (Fig. 1e). Based on the data analysis, the intervention of 5-PA and 6-PA significantly improved the symptoms of ulcerative colitis in mice and restored the gut stability. Moreover, the therapeutic effect of 6-PA was superior to that of 5-PA.

3.2. Expression of inflammatory signaling pathways in colonic tissue

To investigate the effect of paraprobiotics on intestinal inflammation, we evaluated the expression of genes and proteins associated with inflammation. The experiment demonstrated that the expression levels of mRNA for IL-1β, IL-18, IL-17A, IL-22, TLR4, NLRP3, caspase-1, NF-κB, and IκBα were considerably elevated within the colonic tissue of mice with colitis (Fig. 2a, $p < 0.001$). Conversely, treatment with 5-PA and 6-PA significantly decreased the aforementioned mRNA expression levels. The level of protein expression of NLRP3, caspase-1, ASC, IL-1β, and IL-18 was remarkably augmented in the DSS group, and levels of p-p65/p65 and p-IκBα/IκBα were dramatically elevated, as depicted in Fig. 2b and c. Administration of 5-PA and 6-PA mitigated these changes, and 6-PA had a more potent inhibitory impact on the NF-κB/NLRP3 signaling pathway than 5-PA.

3.3. Effects of 5-PA and 6-PA on the intestinal barrier

We first measured mRNA expression of ZO-1, MUC-2, occludin, claudin-1, E-cadherin related to the intestinal barrier in the mouse

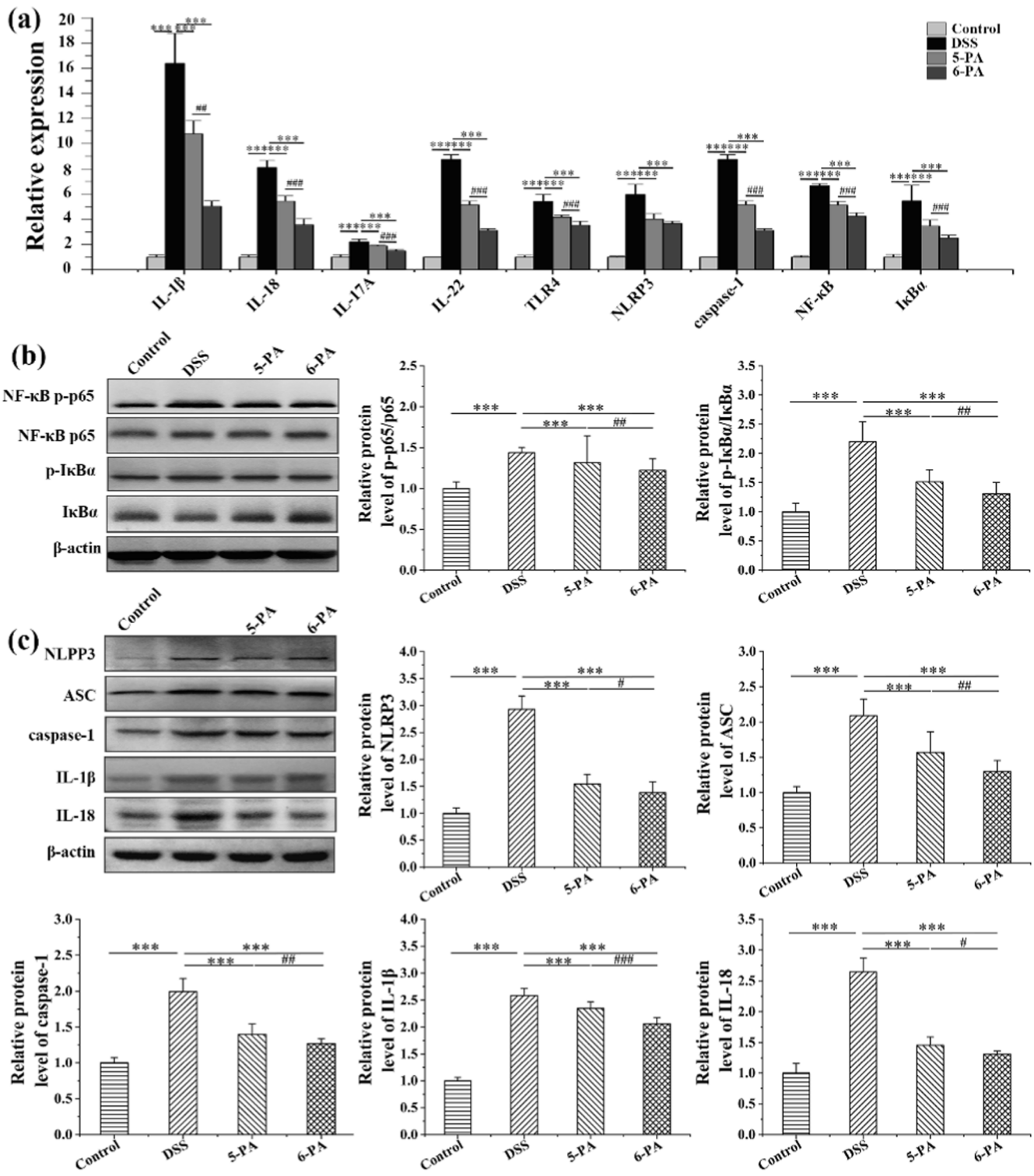

Fig. 2. Effects of 5-PA and 6-PA on the expression of inflammation related genes and proteins in colon tissue of mice in each group. (a) Changes in mRNA expression levels of inflammation-related genes. (b) NF-κB p65 and IκBα protein levels in the colonic tissue analyzed by Western blotting. (c) Protein levels of NLRP3, ASC, caspase-1, IL-1β and IL-18 were determined in colonic tissues of mice. N = 6 for each group. Data presented as mean ± SEM. Statistically significant differences between intact DSS group and other groups are indicated $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Statistically significant differences between 5-PA group and 6-PA group are indicated $\#p < 0.05$, $\#\#p < 0.01$ and $\#\#\#p < 0.001$. NF-κB: Nuclear factor kappa-B; IκBα: Inhibitor kappa B alpha; NLRP3: Nucleotide- binding oligomerization domain; ASC: Apoptosis-associated speck-like protein; IL-1β: Interleukin-1β; IL-18: Interleukin-18.

intestine following exposure to DSS. These results demonstrated that the mRNA expression of these genes significantly decreased after exposure to DSS, whereas intervention with 5-PA and 6-PA mitigated these changes and brought the values closer to that of the Control group (Fig. 3a, $p < 0.01$). As shown in Fig. 3b, the protein levels of ZO-1, occludin, and claudin-1 in the colon tissue of the DSS group were significantly reduced compared to those in the Control group ($p < 0.01$). However, there was a significant recovery in the protein expression

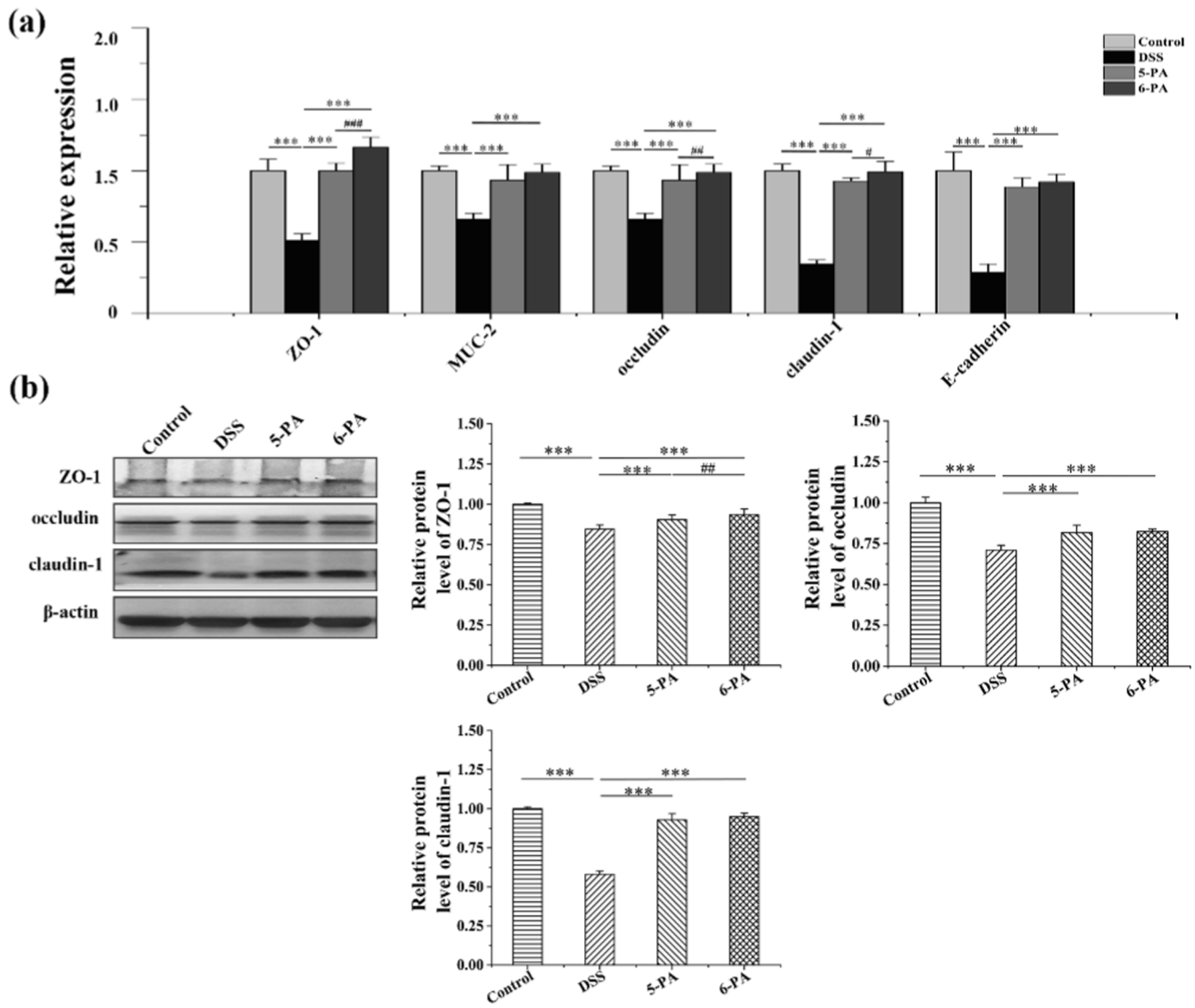

Fig. 3. Effects of 5-PA and 6-PA on the intestinal barrier. (a) mRNA of intestinal barrier in colon for each group. (b) Western blot analysis of ZO-1, occludin, and claudin-1 in colon tissue. N = 6 for each group. Data presented as mean ± SEM. Statistically significant differences between intact DSS group and other groups are indicated *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Statistically significant differences between 5-PA group and 6-PA group are indicated #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$. ZO-1: Zonula occludens protein 1.

levels after interventions with 5-PA and 6-PA. It is noteworthy that there was no significant difference in the expression levels of occludin and claudin-1 proteins between the 5-PA and 6-PA groups. However, 6-PA led to a significantly higher up-regulation level of the ZO-1 protein compared to 5-PA ($p < 0.01$).

3.4. Influence of 5-PA and 6-PA on the composition of gut microbiota in colitis mice

As shown in Fig. 4a, 64 unique genera were detected in the Control group, while 52, 108, and 34 unique genera were detected in the DSS group, 5-PA group, and 6-PA treatment group respectively. The colonic microbiota is composed of five major phylum: Bacteroidota, Firmicutes, Proteobacteria, Actinobacteria, and Uroviricota. In mice with colitis, treatment with DSS significantly increased the relative abundance of Proteobacteria and Uroviricota. Conversely, a significant inhibition of the proliferation phenomenon was shown with 6-PA treatment (Fig. 4b, $p < 0.05$). There are 10 major families with clear information, including *Muribaculaceae, Lachnospiraceae, Bacteroidaceae, Prevotellaceae, Oscillospiraceae, Lactobacillaceae, Rikenellaceae, Clostridiaceae, Erysipelotrichaceae and Tannerellaceae* (Fig. 4c). The dominant families, *Muribaculaceae* and *Lactobacillaceae*, were significantly decreased in the DSS group, while the abundance of *Oscillospiraceae* was significantly increased. The relative content of *Muribaculaceae* increased from 27.55 % (DSS group) to 32.07 % (6-PA group) after treatment with 6-PA and the relative content of *Lactobacillaceae* increased from 0.19 % to 0.43 % after treatment with 5-PA or 6-PA. Fig. 4d shows the results of Linear Discriminant Analysis (LDA) at Genus level, which demonstrated that 5-PA and 6-PA can modify the abundance and diversity of the gut microbiome in mice with DSS-induced colitis. Compared to the Control group, the DSS group exhibited significantly reduced levels of *Lactobacillus* alongside significantly increased levels of *Clostridium, Escherichia, Shigella, Butyrivibrio, Candidatus_Coprovivens, Alkaliphilus*, and *Etanoligenens*. Following treatment with 5-PA and 6-PA, the trends were reversed (Fig. 4e).

3.5. Metabolomic analysis of fecal samples

A total of 2497 *m/z* peaks were detected in the metabolomics data, representing 1571 valid peaks in the positive mode and 926 valid peaks

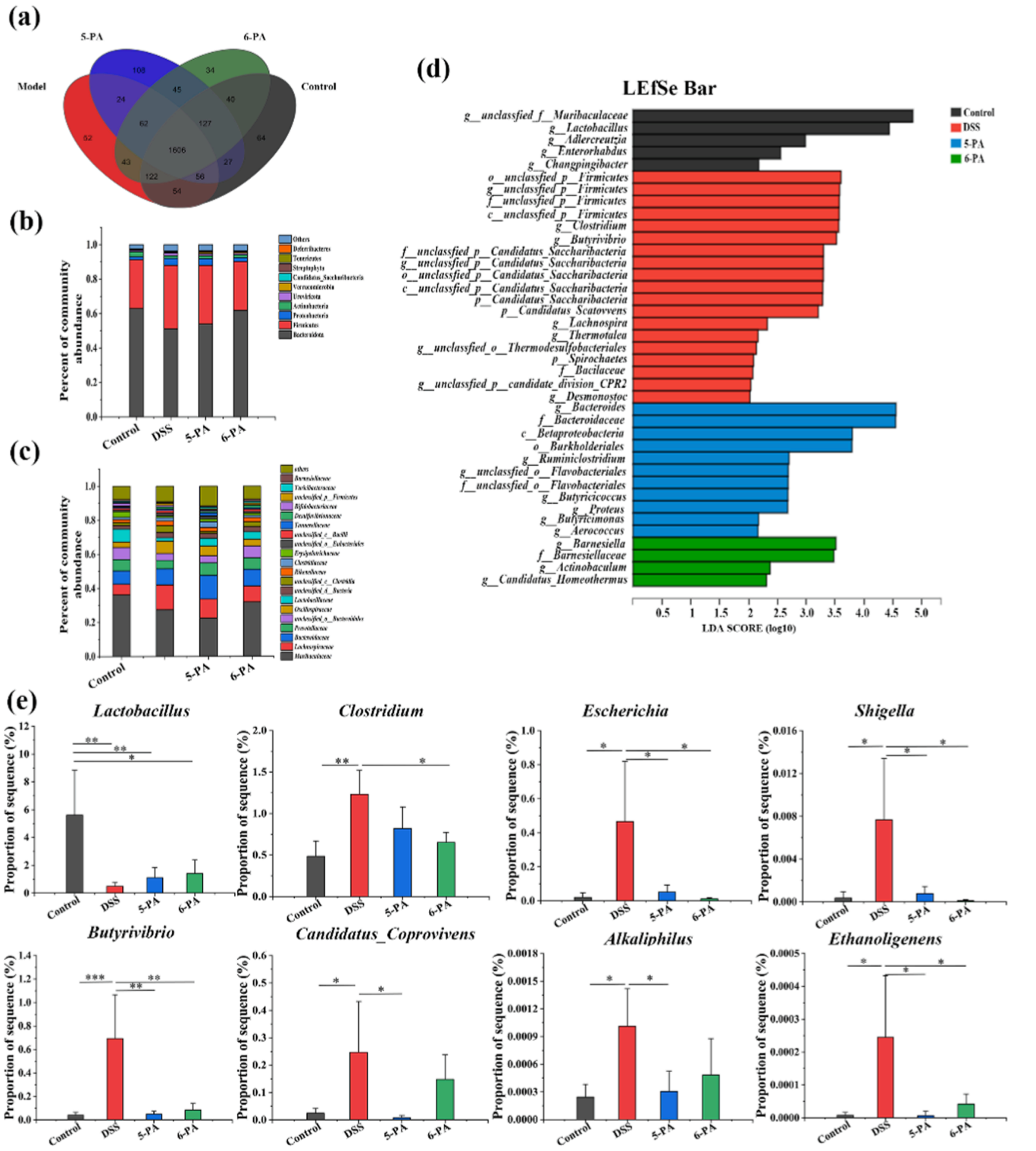

Fig. 4. Influence of 5-PA and 6-PA on the Composition of gut Microbiota in Colitis Mice. (a) Venn graph of genus of colonic microbiota. Relative abundance of (b) Phylum and (c) Family Genus level in colitis mice. (d) Distribution histogram based on LDA. LDA score > 2.0. (e) The relative abundance of specific Genus. N = 6 for each group. Data presented as mean ± SEM. Statistically significant differences between intact DSS group and other groups are indicated $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

in the negative mode. PLS-DA analysis revealed that the identified metabolites in samples from the Control, DSS, 5-PA, and 6-PA groups varied significantly in both the positive and negative modes (Fig. 5a). The Kruskal-Wallis H test was performed to identify potential biomarkers among the Control, DSS, 5-PA, and 6-PA groups. Furthermore, VIP values were utilized to screen for biomarkers that showed a significant difference between two groups. According to the results, lipids and lipid-like molecules were identified as the most abundant metabolites, accounting for nine of the top 20, with the remainder being benzenes, organic heterocyclic compounds, organic acids and their derivatives, organic oxygen compounds, phenylpropanoids, polyketides and hydrocarbons (Fig. 5b). In the DSS group, the abundance of seven metabolites

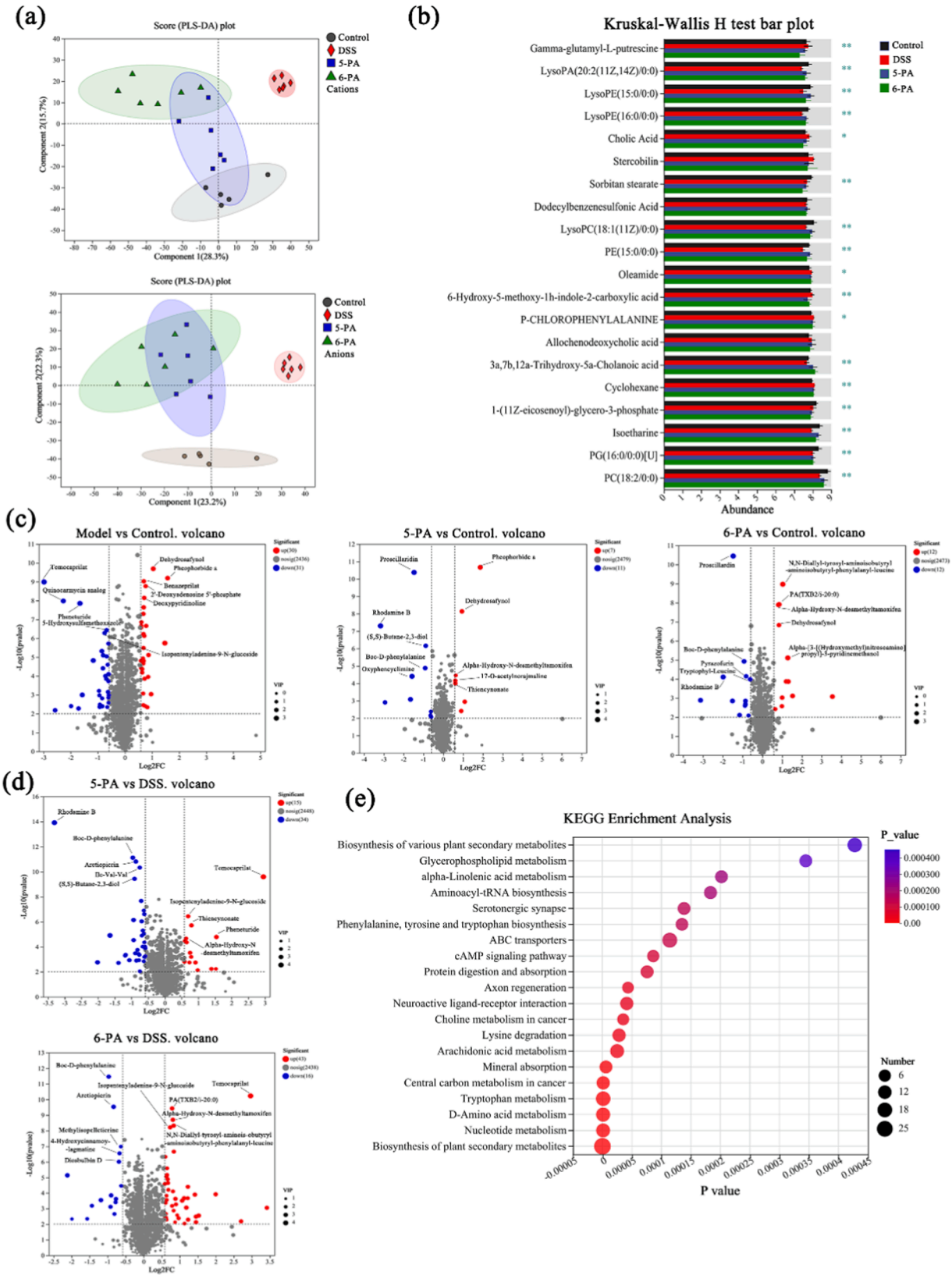

Fig. 5. Metabolomic analysis of fecal samples. (a) PLS-DA score plot of cationic metabolites and anionic metabolites in different groups. (b) Kruskal-Wallis H test bar plot of metabolites in Control group, DSS group, 5-PA group, and 6-PA group. (c) Volcano plot of the metabolite comparison with Control group ($p < 0.01$, VIP ≥ 1, Variance multiplier 1.5). (d) Volcano plot of the metabolite comparison with DSS group ($p < 0.01$, VIP ≥ 1, Variance multiplier 1.5). up indicates up regulation, down indicates down regulation, and nosing indicates no statistical. (d) KEGG pathway enrichment plot of differentially expressed metabolites among the four groups. N = 6 for each group. Data presented as mean ± SEM. Statistically significant differences between four groups are indicated *$p < 0.05$ and **$p < 0.01$.

including Gamma-glutamyl-L-putrescine, Cholic acid, Stercobilin, Oleamide, 6-Hydroxy-5-methoxy-1 h-indole-2-carboxylic acid, P-CHLOROPHENYLALANINE, and Cyclohexane showed a significant increase ($p < 0.05$). However, the treatments with 5-PA and 6-PA caused a reversal of the changes of three metabolites: Cholic acid, 6-Hydroxy-5-methoxy-1 h-indole-2-carboxylic acid, and Cyclohexane ($p < 0.05$). Additionally, the levels of LysoPA(20:2(11Z,14Z)/0:0), LysoPE(16:0/0:0), LysoPE (15:0/0:0), PE(15:0/0:0), and 3a,7b,12a-Trihydroxy-5a-Cholanoic acid were significantly higher in the 5-PA and 6-PA groups compared to the DSS group, which suggests that the 5-PA and 6-PA treatments lead to elevated levels of these metabolites.

The levels of Dehydrosafynol were significantly increased in the DSS, 5-PA, and 6-PA treatment groups compared to the Control group, whereas the levels of Proscillaridin, Rhodamine B, and Boc-D-phenylalanine were significantly decreased in the 5-PA and 6-PA treatment groups (Fig. 5c). Furthermore, in comparison to the DSS group, the levels of Temocaprilat and Alpha-Hydroxy-N-desmethyltamoxifen were both significantly upregulated in the 5-PA and 6-PA treatment groups, while the levels of Boc-D-phenylalanine and Arctiopicrin were significantly downregulated (Fig. 5d). Within the Control group, DSS group, 5-PA group, and 6-PA groups, there were a total of 1562 different metabolites. As shown in Fig. 5E, based upon the significance level of $p \leq 0.00045$, the different metabolites were enriched in KEGG, with a total of 20 metabolic pathways including nucleotide metabolism, tryptophan metabolism, mineral absorption, cAMP signaling pathway, arachidonic acid metabolism, and others (Fig. 5e).

3.6. Correlation analyses of colitis index, microbiota communities and fecal metabolites

In order to provide additional insights into the therapeutic mechanism of paraprobiotics on colitis, Spearman's correlation analysis was performed on various indicators, including colitis symptoms, inflammatory cytokines, intestinal barrier function, intestinal microbiota, and fecal metabolites. The results indicated that *unclassified_f_Muribaculaceae*, *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus* were positively associated with colon length, the anti-inflammatory factor IL-10, and intestinal tight junction proteins ZO-1, occludin, and claudin-1 (Fig. 6, $p < 0.05$). In contrast, *Clostridium*, *Butyrivibrio*, *Ruminiclostridium*, *Butyricicoccus*, *Lachnospira*, and *Alkaliphilus* were significantly positively correlated with pro-inflammatory factors TNF-α, IL-6, and IL-1β ($p < 0.05$). Additionally, *Clostridium*, *Butyrivibrio*, *Escherichia*, *Lachnospira*, and *Shigella* were significantly positively correlated with p-p65/p65, p-IκBα/IκBα, NLRP3, ASC, and IL-18 ($p < 0.05$). Based on the correlation analysis of gut bacteria and potential fecal biomarkers, PC(18:2/0:0), LysoPC(18:1(11Z)/0:0), Isoetharine, LysoPE(16:0/0:0), and LysoPA(20:2(11Z,14Z)/0:0) had a significant positive correlation with *Lactobacillus* ($p < 0.01$). On the other hand, the compound also exhibited a significant negative correlation with *Clostridium*. Moreover, Cholic acid was negatively correlated with *unclassified_f_Muribaculaceae*, *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus*. Dehydrosafynol had significant negative correlations with *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus*, but showed significant positive correlations with *Clostridium*, *Butyrivibrio*, *Lachnospira*, and *Shigella*. Proscillaridin exhibited a significant negative correlation with *Ruminiclostridium* ($p < 0.05$), while Boc-D-phenylalanine and rhodamine B exhibited significant negative correlations with *Barnesiella* ($p < 0.05$). Alpha-Hydroxy-N-desmethyltamoxifen had a significant positive correlation with *Bacteroides* ($p < 0.05$). In addition, Temocaprilat exhibited significant positive correlation with *Candidatus_Homeothermus* ($p < 0.01$), but negative correlations with *Clostridium*, *Alkaliphilus*, and *Ethanoligenens* ($p < 0.05$). Lastly, arctiopicrin exhibited significant negative correlation with *Adlercreutzia* ($p < 0.05$).

4. Discussion

The stability of the intestinal homeostasis relies on the complex interplay between the microbiota, the intestinal epithelial barrier, and the host immune system (Maloy & Powrie, 2011; Saez et al., 2021; Zuo, Kuo, & Turner, 2020). To achieve intestinal homeostasis, it is crucial to ensure the stability of the microbiota, barrier, and immune system in the intestine (Stolfi, Maresca, Monteleone, & Laudisi, 2022). Probiotics *Limosilactobacillus fermentum* HF06 and *Lactiplatibacillus plantarum* HF05 have been demonstrated to provide protection for the intestinal barrier, regulate the intestinal microbiota, and suppress the excessive inflammatory responses (unpublished). Owing to the potential hazards of administering live probiotics, there has been a growing inclination

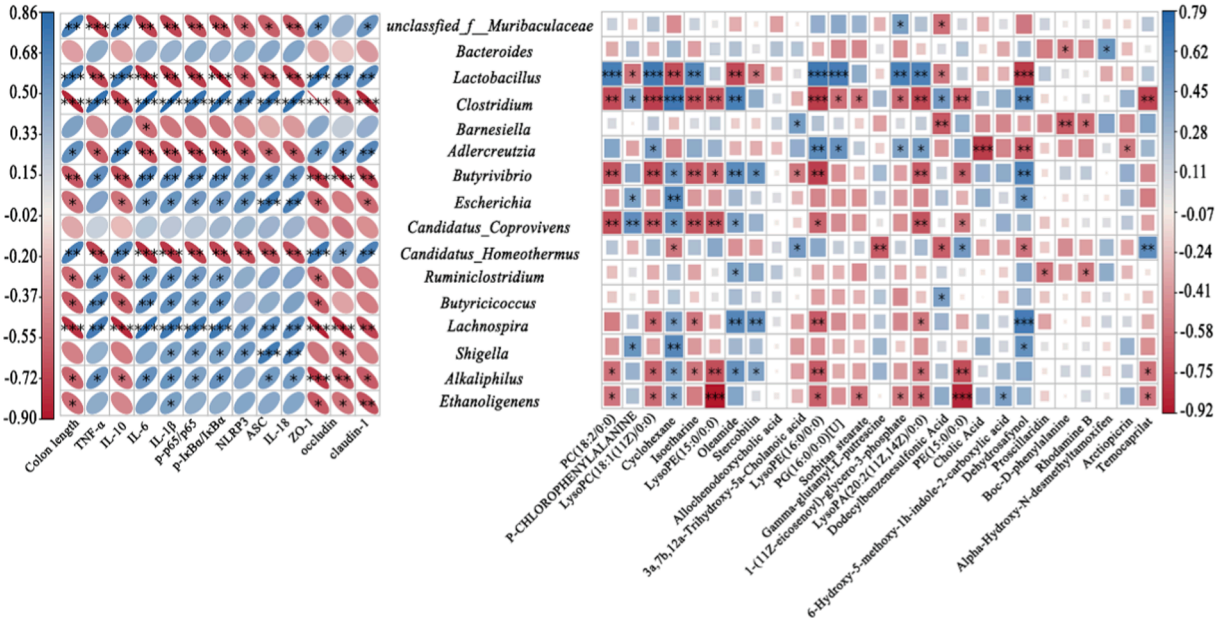

Fig. 6. Correlation analyses of colitis index, microbiota communities and fecal metabolites. *, **, and *** respectively represent p-values less than 0.5, 0.1, and 0.01. IκBα: Inhibitor kappa B alpha; NLRP3: Nucleotide- binding oligomerization domain; ASC: Apoptosis-associated speck-like protein; TNF-α: Tumour necrosis factor alpha; IL-1β: Interleukin-1β; IL-18: Interleukin-18; IL-6: Interleukin-10; IL-10: Interleukin-10; ZO-1: Zonula occludens protein 1.

towards studying nonviable probiotics commonly referred to as paraprobiotics (Barros et al., 2021). Nonetheless, the positive impacts of paraprobiotics in maintaining intestinal homeostasis have not been widely reported, and their mechanisms are still not fully understood. This study aimed to investigate the effects and mechanisms of two paraprobiotics, 5-PA and 6-PA, derived from HF05 and HF06, respectively, on maintaining intestinal homeostasis. Following the administration of 5-PA and 6-PA, the mice with chronic ulcerative colitis displayed significant reduction in weight loss, diarrhea, and occult fecal levels. Additionally, an increase in colon length was observed, as well as a decrease in the degree of pathological changes in colon tissue and levels of pro-inflammatory factors.

The gut microbiota and its metabolites have been reported to be closely linked to the stability of intestinal homeostasis (Zhou et al., 2021). *Muribaculaceae* promotes the production of anti-inflammatory cytokines and short-chain fatty acids, while preserving the integrity and balance of the intestinal barrier and suppressing the colonization of *Clostridium perfringens* in the gut (Hiraishi et al., 2022). The relative abundance of *Muribaculaceae* in the intestinal flora of mice in the DSS group was significantly lower compared to the Control group, which aligns with findings from previous studies (Zhang et al., 2022). *Lactobacillaceae* and its genus-level bacteria *Lactobacillus* offer several health benefits to humans. They act as an immunoregulatory agent that relieves experimental colitis, suppress pathogenic organisms, and promote nutrient absorption, which contributes to restoring gut homeostasis (Guo et al., 2021; Shi et al., 2022). At the family level, the abundance of *Lactobacillaceae* was observed to be lower in the DSS group, which is in line with previous research (Huang et al., 2021). The research has shown that *Oscillospiraceae*, which is considered a primary pathogenic organism associated with inflammation in colitis development, exhibited a substantial increase in the DSS group of mice subjected to experimental colitis (Wang, Guo, Chang, & Gui, 2022; Yang, Chen, Wu, Liao, & Yen, 2023). In this study, treatment with 5-PA and 6-PA resulted in increased abundances of *Muribaculaceae* and *Lactobacillaceae* and decreased abundance of *Oscillospiraceae* in the colon compared to the DSS group (Fig. 4c). Both *unclassified_f_Muribaculaceae* and *Candidatus_Homeothermus* belong to the *Muribaculaceae* family and are high-dimensional microorganisms for the Control and 6-PA groups, respectively. At the same time, *Lactobacillus* is also one of the high-dimensional microorganisms for the Control group, significantly decreased in the DSS group, and its abundance increased after the intervention of 5-PA and 6-PA (Fig. 4d).

Changes in the composition of the intestinal microbiota result in modifications to their metabolic outputs and pathways (Yi et al., 2019). Cholic acid is a primary bile acid that is synthesized in the liver and secreted into the gut (Li et al., 2022). High levels of bile acids are associated with intestinal inflammation in comparison to healthy individuals (Wohlgemuth et al., 2011). Bile acids can directly affect intestinal inflammation through the NF-κB signaling pathway and NLRP3 inflammasome activity (Hang et al., 2019). Furthermore, bile acids influence the development of intestinal inflammation through their interaction with the gut microbiota (Guzior & Quinn, 2021). Consistent with previous studies, a significant increase in cholic acid was observed in the DSS group (Chen et al., 2022; Kajiura, Ohkusa, & Okayasu, 1998). The cholic acid levels decreased in both the 5-PA and 6-PA groups when compared to the DSS group (Fig. 5b). We also found a significant negative correlation between cholic acid and *Adlercreutzia* ($p < 0.01$), and *Adlercreutzia* was positively associated with colonic length, IL-10, and tight junction proteins (ZO-1, occludin, claudin-1) (Fig. 6). *Adlercreutzia* is a specific beneficial bacterium that acts as a potential biomarker for inflammatory bowel disease and is decreased in fecal samples of patients with this disease (Čipčić Paljetak et al., 2022; Nan et al., 2023). In addition to cholic acid, *Adlercreutzia* is also negatively correlated with two other compounds; dehydrosafynol and arctiopicrin ($p < 0.05$). The gut microbiota and bile acids interact with each other and may together promote intestinal inflammation in the case of dysbiosis (Ke et al., 2020). The gut microbiota can utilize bile salt hydrolase (BSH) in the distal colon to deconjugate taurine or glycine from bile acids and it can further convert the unconjugated bile acids to secondary bile acids (Ridlon, Kang, & Hylemon, 2006). 3a,7b,12a-Trihydroxy-5a-Cholanoic acid, also known as allocholic acid (ACA), is a secondary bile acid whose levels were significantly reduced in the DSS group but were significantly restored by interventions with 5-PA and 6-PA. 3a,7b,12a-Trihydroxy-5a-Cholanoic acid is significantly positively correlated with *Candidatus_Homeothermus*. Six glycerophospholipid metabolites positively correlated with *Lactobacillus*: PC(18:2/0:0), PG(16:0/0:0), LysoPC(18:1(11Z)/0:0), LysoPE(16:0/0:0, 1-(11Z-eicosenoyl)-*glycero*-3-phosphate (PA(20:1(11z)/0:0)), LysoPA(20:2(11Z,14Z)/0:0) (Fig. 6, $p < 0.01$). Except for PA(20:1(11z)/0:0), the abundance of the other five metabolites decreased significantly in the DSS group (Fig. 5b, $p < 0.01$). Nonetheless, the administration of 5-PA and 6-PA interventions partially mitigated this effect. The study showed that glycerophospholipid (GP) levels in patients with inflammatory bowel disease (IBD) are altered (Horta et al., 2021). Glycerophospholipids are essential structural components of cell membranes and are involved in various cellular processes (Xie, Zhong, Wu, & Chen, 2023). They are also biologically active molecules involved in protein recognition and signal transduction, which are associated with inflammation and metabolic syndrome (Liu et al., 2020). Glycerophospholipids, as components of the lipid bilayer, may help protect the intestinal epithelial barrier against damage and maintain the stability of the intestinal microbiota (Nicolson & Ash, 2014). Enrichment analysis was conducted on the KEGG metabolic pathway in this study that identified significant enrichment of the glycerophospholipid metabolism pathway, suggesting a crucial role of this pathway in the development of ulcerative colitis.

NF-κB is a key mediator in the inflammatory response, regulating innate and adaptive immune function (Liu, Zhang, Joo, & Sun, 2017). NF-κB is a vital transcription factor in M1 macrophages, inducing the expression of numerous inflammatory genes like TNF-α, IL-1β, IL-6, IL-12p40, and COX-2 (Wang, Liang, & Zen, 2014). Research has indicated that NF-κB has a vital role in the UC's pathogenesis, is a hallmark of chronic inflammatory diseases, and focused inhibition can relieve colitis (Wei & Feng, 2010). Prior research has demonstrated that NF-κB is involved in inflammasome activation, further promoting the development and onset of inflammatory diseases (Guo, Callaway, & Ting, 2015). Upregulating the transcription of pro-IL-1β and NLRP3 is dependent on the significant role that NF-κB plays (Sutterwala, Haasken, & Cassel, 2014). The NLRP3 inflammasome is an intact molecular platform activated by various pathogen- and damage-related molecular patterns (Franchi, Eigenbrod, Muñoz-Planillo, & Nuñez, 2009). The inflammasome binds with apoptosis-associated speck-like protein containing an apoptosis-associated speck-like protein adapter (ASC), leading to the translocation and activation of pro-caspase-1 that, in turn, causes the maturation and secretion of IL-1β (Lu et al., 2014). Research has shown that the maturation of IL-1β mediated by NLRP3/ASC/caspase-1 is crucial for DSS-induced experimental colitis (Christian et al., 2010). The critical protein of the NLRP3 inflammasome increased significantly in UC mice; however, the severity of colitis was significantly reduced in mice that underwent NLRP3 gene knockout and treated with DSS (Zhang et al., 2017). Our study revealed a significant decrease in the expression of NF-κB/NLRP3 signal pathway genes and proteins in the 5-PA and 6-PA groups, in comparison to the DSS group. Among them, p-p65/p65, p-IκBα/IκBα, NLRP3, ASC, and IL-18 were significantly negatively correlated with *unclassified_f_Muribaculaceae*, *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus*, which have the beneficial effect of relieving colitis.

Our study confirms a strong association between changes in colon length, levels of pro-inflammatory factors (TNF-α, IL-6, IL-1β), levels of anti-inflammatory factor (IL-10), and levels of intestinal tight junction proteins (ZO-1, occludin, and claudin-1) with alterations in the composition of intestinal flora and its metabolites in individuals with

chronic UC. The differential effects of 5-PA and 6-PA on chronic UC can be attributed to their distinct impacts on the composition and structure of the gut microbiota, consequently influencing the metabolic communication between the gut microbiota and the host. The varying regulatory effects of 5-PA and 6-PA on the gut microbiota may arise from their specific effects on high-dimensional organisms in mouse gut microbiota. Following the administration of 6-PA, there is a notable shift in the high-dimensional microbial composition of the murine fecal microbiota, with *Barnesiella* dominating the community (Fig. 4d). Previous studies have demonstrated the anti-inflammatory effects of *Barnesiella* in DSS-induced intestinal inflammation in mice (Weiss, Chassard, & Hennet, 2014). The levels of *Barnesiella* in the gut microbiota correlate with decreased susceptibility to DSS, suggesting that *Barnesiella* has the ability to create an intestinal environment that is less prone to inflammation (Presley Laura, Wei, Braun, & Borneman, 2010). Moreover, 5-PA and 6-PA mediate metabolic crosstalk with the host through the action of bile acids (Cholic acid, 3a, 7b, 12a Trihydroxy-5a Cholanoic acid) and glycerol phospholipids, affecting intestinal homeostasis. Following the intervention of 5-PA and 6-PA, the composition of the intestinal flora develops divergently, with both compounds exerting inhibitory effects on the NF-κB signaling pathway. As a result, NLRP3 inflammasome activation is suppressed, leading to a reduction in intestinal inflammation in mice. The contribution of the gut microbiota to immune system development and immune responses has been extensively documented (W. Yang & Cong, 2021). Restoring a balanced gut microbiota normalizes the development of the immune system and immune responses, while the metabolites produced by the microbiota continue to regulate local and systemic immune cells (Macpherson & Harris, 2004).

Probiotics have demonstrated their ability to restore the intestinal microenvironment in colitis through the modulation of redox homeostasis, immune responses, and the composition of the gut microbiome (Xu et al., 2023). In practical implementation, the use of probiotic therapy with live microorganisms is not without risks, as it has the potential to induce translocation and sepsis, particularly in individuals with weakened immune systems (Boyle et al., 2006). Our findings revealed that mice with DSS-induced colitis experienced an inflammatory response, accompanied by disruptions in the intestinal barrier and disturbances in the intestinal flora. This led to the imbalance of intestinal homeostasis. However, the administration of paraprobiotics 5-PA and 6-PA effectively regulated the inflammatory response in the intestines and strengthened the intestinal barrier. This was achieved through modulating the composition of the intestinal flora and their associated metabolites, ultimately restoring the intestinal homeostasis to some extent in mice with ulcerative colitis. Our findings revealed that mice with DSS-induced colitis experienced an inflammatory response in the intestines, accompanied by disruptions in the intestinal barrier and disturbances in the intestinal flora. This led to the imbalance of intestinal homeostasis. However, the administration of paraprobiotics 5-PA and 6-PA effectively regulated the inflammatory response in the intestines and strengthened the intestinal barrier. This was achieved through modulating the composition of the intestinal flora and their associated metabolites, ultimately restoring the intestinal homeostasis to some extent in mice with UC. One notable advantage of paraprobiotics 5-PA and 6-PA, compared to traditional live microorganism-based probiotics, is their increased safety. Paraprobiotics do not contain live microorganisms that may pose a risk of infection in vulnerable individuals, nor do they carry the potential for overgrowth (Sawada et al., 2016). Additionally, probiotics in the form of inactivated bacteria possess favorable characteristics such as ease of processing, heat resistance, and shelf stability (Siciliano et al., 2021).

5. Conclusion

In conclusion, the present study demonstrated that 5-PA and 6-PA alleviated the symptoms of mice with ulcerative colitis and effectively inhibited the activation of the NF-κB/NLRP3 signalling pathway. Additionally, these compounds upregulate the expression of intestinal tight junction proteins, which enhances the intestinal barrier. The most important thing is that compared with the DSS group, 5-PA and 6-PA can restore intestinal homeostasis to some extent in mice with ulcerative colitis. We found that homeostasis is intricately linked with specific bacterial groups in the gut microbiome, such as the *unclassified f_Muribaculaceae*, *Lactobacillus*, *Adlercreutzia*, and *Candidatus_Homeothermus*. In addition, the gut microbiome, as well as the metabolic byproducts resulting from host-microbe interactions, namely bile acids and glycerophospholipids, play critical roles in maintaining gut homeostasis.

6. Ethics statement

The authors declare that all animal experiments were approved by the Ethics Committee for Experimental Animal Welfare of Harbin Institute of Technology (IACUC-202047), and following the ARRIVE Guidelines for the report of in *vivo* studies.

Funding

This study was supported by the Natural Science Foundation of Sichuan Province (2023NSFSC0018).

CRediT authorship contribution statement

Chunhong Liu: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Xiaofen Qi:** Resources, Investigation, Formal analysis. **Kaifang Guan:** Validation, Resources, Data curation, Conceptualization. **Haoran Chen:** Validation, Resources, Data curation, Conceptualization. **Qiming Li:** Supervision, Project administration. **Kaidong Mao:** Supervision, Project administration. **Guiqi Shen:** Supervision, Project administration. **Ying Ma:** Project administration, Methodology, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgement

The authors thank Dr. Ying Ma for helping to solve the difficulties encountered in the research.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106048.

References

Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Reviews Immunology, 8*(6), 411–420. https://doi.org/10.1038/nri2316

Barbara, G., Barbaro, M. R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., … Stanghellini, V. (2021). Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. *Frontiers in Nutrition, 8*, Article 718356. https://doi.org/10.3389/fnut.2021.718356

Barros, C. P., Guimarães, J. T., Esmerino, E. A., Duarte, M. C. K. H., Silva, M. C., Silva, R., … Cruz, A. G. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. *Current Opinion in Food Science, 32*, 1–8. https://doi.org/10.1016/j.cofs.2019.12.003

Barros, C. P., Pires, R. P. S., Guimaraes, J. T., Abud, Y. K. D., Almada, C. N., Pimentel, T. C., … Cruz, A. G. (2021). Ohmic heating as a method of obtaining

paraprobiotics: Impacts on cell structure and viability by flow cytometry. *Food Research International, 140*, Article 110061. https://doi.org/10.1016/j.foodres.2020.110061

Batista, V. L., De Jesus, L. C., Tavares, L. M., Barroso, F. L., Fernandes, L. J., Freitas, A. D., … Azevedo, V. (2022). Paraprobiotics and Postbiotics of *Lactobacillus delbrueckii* CIDCA 133 Mitigate 5-FU-Induced Intestinal Inflammation. *Microorganisms, 10*(7), 1418. https://doi.org/10.3390/microorganisms10071418

Boyle, R. J., Robins-Browne, R. M., & Tang, M. L. K. (2006). Probiotic use in clinical practice: What are the risks? *The American Journal of Clinical Nutrition, 83*(6), 1256–1264. https://doi.org/10.1093/ajcn/83.6.1256

Chen, L., Jiao, T., Liu, W., Luo, Y., Wang, J., Guo, X., … Xie, C. (2022). Hepatic cytochrome P450 8B1 and cholic acid potentiate intestinal epithelial injury in colitis by suppressing intestinal stem cell renewal. *Cell Stem Cell, 29*(9), 1366–1381. https://doi.org/10.1016/j.stem.2022.08.008

Christian, B., Peter, D., Christine, M., Hans Anton, L., Katherine, A. F., Marc, D., … Max, S. (2010). Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut, 59*(9), 1192. https://doi.org/10.1136/gut.2009.197822

Čipčić Paljetak, H., Barešić, A., Panek, M., Perić, M., Matijašić, M., Lojkić, I., … Verbanac, D. (2022). Gut microbiota in mucosa and feces of newly diagnosed, treatment-naïve adult inflammatory bowel disease and irritable bowel syndrome patients. *Gut Microbes, 14*(1), 2083419. https://doi.org/10.1080/19490976.2022.2083419

de Almada, C. N., Almada, C. N., Martinez, R. C. R., & Sant'Ana, A. S. (2016). Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. *Trends in Food Science & Technology, 58*, 96–114. https://doi.org/10.1016/j.tifs.2016.09.011

Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., & Nuñez, G. (2009). The inflammasome: A caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nature Immunology, 10*(3), 241–247. https://doi.org/10.1038/ni.1703

Fusco, A., Savio, V., Cimini, D., D'Ambrosio, S., Chiaromonte, A., Schiraldi, C., & Donnarumma, G. (2023). In *Vitro* Evaluation of the Most Active Probiotic Strains Able to Improve the Intestinal Barrier Functions and to Prevent Inflammatory Diseases of the Gastrointestinal System. *Biomedicines*, *11*(3), Article 865. https://doi.org/10.3390/biomedicines11030865.

Gentile, C. L., & Weir, T. L. (2018). The gut microbiota at the intersection of diet and human health. *Science, 362*(6416), 776–780. https://doi.org/10.1126/science.aau5812

Guo, F., Tsao, R., Li, C., Wang, X., Zhang, H., Jiang, L., … Xiong, H. (2021). Green Pea (Pisum sativum L.) Hull Polyphenol Extracts Ameliorate DSS-Induced Colitis through Keap1/Nrf2 Pathway and Gut Microbiota Modulation. *Foods*, *10*(11), Article 2765. https://doi.org/10.3390/foods10112765.

Guo, H., Callaway, J. B., & Ting, J. P. Y. (2015). Inflammasomes: Mechanism of action, role in disease, and therapeutics. *Nature Medicine, 21*(7), 677–687. https://doi.org/10.1038/nm.3893

Guzior, D. V., & Quinn, R. A. (2021). Review: Microbial transformations of human bile acids. *Microbiome, 9*(1), 140. https://doi.org/10.1186/s40168-021-01101-1

Hang, S., Paik, D., Yao, L., Kim, E., Trinath, J., Lu, J., … Huh, J. R. (2019). Bile acid metabolites control TH17 and Treg cell differentiation. *Nature, 576*(7785), 143–148. https://doi.org/10.1038/s41586-019-1785-z

Hiraishi, K., Zhao, F., Kurahara, L.-H., Li, X., Yamashita, T., Hashimoto, T., … Hirano, K. (2022). Lactulose Modulates the Structure of Gut Microbiota and Alleviates Colitis-Associated Tumorigenesis. *Nutrients, 14*(3), 649. https://doi.org/10.3390/nu14030649

Hooper, L. V., & Macpherson, A. J. (2010). Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nature Reviews Immunology, 10*(3), 159–169. https://doi.org/10.1038/nri2710

Horta, D., Moreno-Torres, M., Ramírez-Lázaro, M. J., Lario, S., Kuligowski, J., Sanjuan-Herráez, J. D., … Calvet, X. (2021). Analysis of the Association between Fatigue and the Plasma Lipidomic Profile of Inflammatory Bowel Disease Patients. *Journal of Proteome Research, 20*(1), 381–392. https://doi.org/10.1021/acs.jproteome.0c00462

Huang, Y., Yang, Q., Mi, X., Qiu, L., Tao, X., Zhang, Z., … Wei, H. (2021). Ripened Pu-erh Tea Extract Promotes Gut Microbiota Resilience against Dextran Sulfate Sodium Induced Colitis. *Journal of Agricultural and Food Chemistry, 69*(7), 2190–2203. https://doi.org/10.1021/acs.jafc.0c07537

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Nageshwar Reddy, D. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology, 21*(29), 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Kajiura, K., Ohkusa, T., & Okayasu, I. (1998). Relationship between Fecal Bile Acids and the Occurrence of Colorectal Neoplasia in Experimental Murine Ulcerative Colitis. *Digestion, 59*(1), 69–72. https://doi.org/10.1159/000007469

Kaur, H., & Ali, S. A. (2022). Probiotics and gut microbiota: Mechanistic insights into gut immune homeostasis through TLR pathway regulation. *Food & Function, 13*(14), 7423–7447. https://doi.org/10.1039/D2FO00911K

Ke, J., Li, Y., Han, C., He, R., Lin, R., Qian, W., & Hou, X. (2020). Fucose Ameliorate Intestinal Inflammation Through Modulating the Crosstalk Between Bile Acids and Gut Microbiota in a Chronic Colitis Murine Model. *Inflammatory Bowel Diseases, 26*(6), 863–873. https://doi.org/10.1093/ibd/izaa007

Lee, S.-Y., Lee, B.-H., Park, J.-H., Park, M.-S., Ji, G.-E., & Sung, M.-K. (2022). *Bifidobacterium bifidum* BGN4 Paraprobiotic Supplementation Alleviates Experimental Colitis by Maintaining Gut Barrier and Suppressing Nuclear Factor Kappa B Activation Signaling Molecules. *Journal of Medicinal Food, 25*(2), 146–157. https://doi.org/10.1089/jmf.2021.K.0150

Li, L., Liu, T., Gu, Y., Wang, X., Xie, R., Sun, Y., … Cao, H. (2022). Regulation of gut microbiota-bile acids axis by probiotics in inflammatory bowel disease. *Frontiers in Immunology, 13*, Article 974305. https://doi.org/10.3389/fimmu.2022.974305

Li, R., Kim, M.-H., Sandhu, A. K., Gao, C., & Gu, L. (2017). Muscadine Grape (Vitis rotundifolia) or Wine Phytochemicals Reduce Intestinal Inflammation in Mice with Dextran Sulfate Sodium-Induced Colitis. *Journal of Agricultural and Food Chemistry, 65*(4), 769–776. https://doi.org/10.1021/acs.jafc.6b03806

Lim, J.-J., Jung, A. H., Joo Suh, H., Choi, H.-S., & Kim, H. (2022). *Lactiplantibacillus plantarum* K8-based paraprobiotics prevents obesity and obesity-induced inflammatory responses in high fat diet-fed mice. *Food Research International, 155*, Article 111066. https://doi.org/10.1016/j.foodres.2022.111066

Liu, C., Liu, X., Sun, Y., Qi, X., Ma, Y., & Wang, R. (2023). Anti-inflammatory probiotic *Lactiplantibacillus plantarum* HF05 screening from Qula: Genomic analysis and alleviating effect on intestinal inflammation. *Food Bioscience, 55*, Article 103002. https://doi.org/10.1016/j.fbio.2023.103002

Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, *2*(1), Article 17023. https://doi.org/10.1038/sigtrans.2017.23.

Liu, Y., Luo, Y., Wang, X., Luo, L., Sun, K., & Zeng, L. (2020). Gut Microbiome and Metabolome Response of Pu-erh Tea on Metabolism Disorder Induced by Chronic Alcohol Consumption. *Journal of Agricultural and Food Chemistry, 68*(24), 6615–6627. https://doi.org/10.1021/acs.jafc.0c01947

Lu, A., Magupalli, V. G., Ruan, J., Yin, Q., Atianand, M. K., Vos, M. R., … Egelman, E. H. (2014). Unified Polymerization Mechanism for the Assembly of ASC-Dependent Inflammasomes. *Cell, 156*(6), 1193–1206. https://doi.org/10.1016/j.cell.2014.02.008

Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology, 4*(6), 478–485. https://doi.org/10.1038/nri1373

Maehata, H., Arai, S., Iwabuchi, N., & Abe, F. (2021). Immuno-modulation by heat-killed *Lacticaseibacillus paracasei* MCC1849 and its application to food products. *International Journal of Immunopathology and Pharmacology*, *35*, Article 20587384211008291. https://doi.org/10.1177/20587384211008291.

Maloy, K. J., & Powrie, F. (2011). Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature, 474*(7351), 298–306. https://doi.org/10.1038/nature10208

Nan, X., Zhao, W., Liu, W.-H., Li, Y., Li, N., Hong, Y., … Peng, G. (2023). *Bifidobacterium animalis* subsp. *lactis* BL-99 ameliorates colitis-related lung injury in mice by modulating short-chain fatty acid production and inflammatory monocytes/macrophages. *Food & Function, 14*(2), 1099–1112. https://doi.org/10.1039/D2FO03374G

Nicolson, G. L., & Ash, M. E. (2014). Lipid Replacement Therapy: A natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1838*(6), 1657–1679. https://doi.org/10.1016/j.bbamem.2013.11.010

Ordás, I., Eckmann, L., Talamini, M., Baumgart, D. C., & Sandborn, W. J. (2012). Ulcerative colitis. *The Lancet, 380*(9853), 1606–1619. https://doi.org/10.1016/S0140-6736(12)60150-0

Porfiri, L., Burtscher, J., Kangethe, R. T., Verhovsek, D., Cattoli, G., Domig, K. J., & Wijewardana, V. (2022). Irradiated Non-replicative Lactic Acid Bacteria Preserve Metabolic Activity While Exhibiting Diverse Immune Modulation. *Frontiers in Veterinary Science, 9*, Article 859124. https://doi.org/10.3389/fvets.2022.859124

Presley Laura, L., Wei, B., Braun, J., & Borneman, J. (2010). Bacteria Associated with Immunoregulatory Cells in Mice. *Applied and Environmental Microbiology, 76*(3), 936–941. https://doi.org/10.1128/AEM.01561-09

Ridlon, J. M., Kang, D.-J., & Hylemon, P. B. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research, 47*(2), 241–259. https://doi.org/10.1194/jlr.R500013-JLR200

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A., Gasbarrini, A., & Mele, M. C. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms, 7*(1), Article 14. https://doi.org/10.3390/microorganisms7010014

Saez, A., Gomez-Bris, R., Herrero-Fernandez, B., Mingorance, C., Rius, C., & Gonzalez-Granado, J. M. (2021). Innate Lymphoid Cells in Intestinal Homeostasis and Inflammatory Bowel Disease. *International Journal of Molecular Sciences*, 22(14), Article 7618. https://doi.org/10.3390/ijms22147618.

Sawada, D., Sugawara, T., Ishida, Y., Aihara, K., Aoki, Y., Takehara, I., … Fujiwara, S. (2016). Effect of continuous ingestion of a beverage prepared with *Lactobacillus gasseri* CP2305 inactivated by heat treatment on the regulation of intestinal function. *Food Research International, 79*, 33–39. https://doi.org/10.1016/j.foodres.2015.11.032

Shang, J., Yang, S., Tang, Z., Chen, Y., Duan, B., & Meng, X. (2022). Bifidobacterium bifidum H3–R2 and Its Molecular Communication within the Context of Ulcerative Colitis. *Journal of Agricultural and Food Chemistry, 70*(37), 11678–11688. https://doi.org/10.1021/acs.jafc.2c02909

Shen, Q., Huang, Z., Yao, J., & Jin, Y. (2022). Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. *Journal of Advanced Research, 37*, 221–233. https://doi.org/10.1016/j.jare.2021.07.002

Shi, Z., Takeuchi, T., Nakanishi, Y., Kato, T., Beck, K., Nagata, R., … Satoh-Takayama, N. (2022). A Japanese Herbal Formula, Daikenchuto, Alleviates Experimental Colitis by Reshaping Microbial Profiles and Enhancing Group 3 Innate Lymphoid Cells. *Frontiers in Immunology, 13*, Article 903459. https://doi.org/10.3389/fimmu.2022.903459

Siciliano, R. A., Reale, A., Mazzeo, M. F., Morandi, S., Silvetti, T., & Brasca, M. (2021). Paraprobiotics: A New Perspective for Functional Foods and Nutraceuticals. *Nutrients*, *13*(4), Article 1225. https://doi.org/10.3390/nu13041225.

Stolfi, C., Maresca, C., Monteleone, G., & Laudisi, F. (2022). Implication of Intestinal Barrier Dysfunction in Gut Dysbiosis and Diseases. *Biomedicines*, *10*(2), Article 289. https://doi.org/10.3390/biomedicines10020289.

Sutterwala, F. S., Haasken, S., & Cassel, S. L. (2014). Mechanism of NLRP3 inflammasome activation. *Annals of the New York Academy of Sciences, 1319*(1), 82–95. https://doi.org/10.1111/nyas.12458

Toruner, M., Loftus, E. V., Jr., Harmsen, W. S., Zinsmeister, A. R., Orenstein, R., Sandborn, W. J., … Egan, L. J. (2008). Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology, 134*(4), 929–936. https://doi.org/10.1053/j.gastro.2008.01.012

Trindade, L. M., Torres, L., Matos, I. D., Miranda, V. C., de Jesus, L. C. L., Cavalcante, G., … de Vasconcelos Generoso, S. (2021). Paraprobiotic *Lacticaseibacillus rhamnosus* Protects Intestinal Damage in an Experimental Murine Model of Mucositis. *Probiotics and Antimicrobial Proteins., 15*(2), 338–350. https://doi.org/10.1007/s12602-021-09842-z

Ungaro, R., Mehandru, S., Allen, P. B., Peyrin-Biroulet, L., & Colombel, J.-F. (2017). Ulcerative colitis. *The Lancet, 389*(10080), 1756–1770. https://doi.org/10.1016/S0140-6736(16)32126-2

Wang, K., Guo, J., Chang, X., & Gui, S. (2022). Painong-San extract alleviates dextran sulfate sodium-induced colitis in mice by modulating gut microbiota, restoring intestinal barrier function and attenuating TLR4/NF-κB signaling cascades. *Journal of Pharmaceutical and Biomedical Analysis, 209*, Article 114529. https://doi.org/10.1016/j.jpba.2021.114529

Wang, N., Liang, H., & Zen, K. (2014). Molecular Mechanisms That Influence the Macrophage M1–M2 Polarization Balance. *Frontiers in Immunology, 5,* Article 614. https://doi.org/10.3389/fimmu.2014.00614.

Wei, J., & Feng, J. (2010). Signaling pathways associated with inflammatory bowel disease. *Recent Patents on Inflammation & Allergy Drug Discovery, 4*(2), 105–117. https://doi.org/10.2174/187221310791163071

Weiss, G. A., Chassard, C., & Hennet, T. (2014). Selective proliferation of intestinal Barnesiella under fucosyllactose supplementation in mice. *British Journal of Nutrition, 111*(9), 1602–1610. https://doi.org/10.1017/S0007114513004200

Wohlgemuth, S., Keller, S., Kertscher, R., Stadion, M., Haller, D., Kisling, S., … Loh, G. (2011). Intestinal steroid profiles and microbiota composition in colitic mice. *Gut Microbes, 2*(3), 159–166. https://doi.org/10.4161/gmic.2.3.16104

Xie, J., Zhong, Q., Wu, W.-T., & Chen, J.-J. (2023). Multi-omics data reveals the important role of glycerophospholipid metabolism in the crosstalk between gut and brain in depression. *Journal of Translational Medicine, 21*(1), Article 93. https://doi.org/10.1186/s12967-023-03942-w

Xu, J., Xu, J., Shi, T., Zhang, Y., Chen, F., Yang, C., … Nie, G. (2023). Probiotic-Inspired Nanomedicine Restores Intestinal Homeostasis in Colitis by Regulating Redox Balance, Immune Responses, and the Gut Microbiome. *Advanced Materials*, *35*(3), Article 2207890. https://doi.org/10.1002/adma.202207890.

Yang, J.-Y., Chen, S.-Y., Wu, Y.-H., Liao, Y.-L., & Yen, G.-C. (2023). Ameliorative effect of buckwheat polysaccharides on colitis via regulation of the gut microbiota. *International Journal of Biological Macromolecules, 227*, 872–883. https://doi.org/10.1016/j.ijbiomac.2022.12.155

Yang, W., & Cong, Y. (2021). Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cellular & Molecular Immunology, 18*(4), 866–877. https://doi.org/10.1038/s41423-021-00661-4

Yi, W., Fenglei, W., Jihong, Y., Jie, L., Dandan, J., Jingjing, Z., … Duo, L. (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut*, *68*(8), Article 1417. https://doi.org/10.1136/gutjnl-2018-317609.

Zhang, J., Liang, F., Chen, Z., Chen, Y., Yuan, J., Xiong, Q., … Liang, J. (2022). Vitexin protects against dextran sodium sulfate-induced colitis in mice and its potential mechanisms. *Journal of Agricultural and Food Chemistry, 70*(38), 12041–12054. https://doi.org/10.1021/acs.jafc.2c05177

Zhang, Z., Shen, P., Lu, X., Li, Y., Liu, J., Liu, B., … Zhang, N. (2017). In *Vivo* and In *Vitro* study on the efficacy of terpinen-4-ol in dextran sulfate sodium-induced mice experimental colitis. Article 558 *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2017.00558.

Zhou, N., Wu, N., Yao, Y., Chen, S., Xu, M., Yin, Z., … Tu, Y. (2022). Anti-inflammatory effects of tripeptide WLS on TNF-α-induced HT-29 cells and DSS-induced colitis in mice. *Food & Function, 13*(18), 9496–9512. https://doi.org/10.1039/D2FO01235A

Zhou, X., Zhang, D., Qi, W., Hong, T., Xiong, T., Wu, T., … Nie, S. (2021). Exopolysaccharides from *Lactobacillus plantarum* NCU116 facilitate intestinal homeostasis by modulating intestinal epithelial regeneration and microbiota. *Journal of Agricultural and Food Chemistry, 69*(28), 7863–7873. https://doi.org/10.1021/acs.jafc.1c01898

Zuo, L., Kuo, W.-T., & Turner, J. R. (2020). Tight junctions as targets and effectors of mucosal immune homeostasis. *Cellular and Molecular Gastroenterology and Hepatology, 10*(2), 327–340. https://doi.org/10.1016/j.jcmgh.2020.04.001