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Effect of fermented *Rosa roxburghii* Tratt fruit juice on gut microbiota in a dextran sulfate sodium-induced colitis mouse model

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ABSTRACT

This study mainly investigated the regulatory effect of *Rosa roxburghii* Tratt fruit juice fermented by *Lactobacillus paracasei* SR10-1 (LAB-RRTJ) on modulating gut microbiota in dextran sulfate sodium (DSS)-induced ulcerative colitis in mice. Compared to control group, DSS induction decreased body weight of mice, indexes of Shannon, Simpson, Chao1 and Faith_pd, and increased disease activity index (DAI) and levels of interleukin 1 β (IL-1 β), IL-6, tumor necrosis factor α (TNF- α) and interferon- γ (IFN- γ); And this induction also led to an increase in Proteobacteria, Verrucomicrobia and Actinobacteria at phylum level, harmful bacterial species richness at genus level, and relative richness of *S. sciuri*, *Desulfovibrio* C21_c20, *R. gnavus* and *Akkermansia muciniphila* at species level, and a decrease in Firmicutes at phylum level and relative richness of *B. acidifaciens* in mice. LAB-RRTJ increased body weight of mice with DSS induced ulcerative colitis (UC) and indexes of Shannon, Simpson, Chao1 and Faith_pd, reduced DAI and the content of four inflammatory factors and improved gut microbiota imbalance in DSS induced UC mice. Besides, the number of operational taxonomic units (OTUs) increased, α -diversity and β -diversity were restored and similar to those in mice in the control group after LAB-RRTJ treatment. Compared with the positive drug treatment group, LAB-RRTJ has a better effect on regulating gut microbiota diversity in colitis mice. Correlation analysis showed that inflammatory factors were positively correlated with harmful bacteria and negatively correlated with beneficial bacteria which commonly found in some colitis mice. Taken together, our study demonstrated that LAB-RRTJ could alleviate DSS-induced colitis in mice through the modulation of inflammatory cytokines and gut microbiota composition.

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1. Introduction

Ulcerative colitis (UC), a subtype of inflammatory bowel disease (IBD), usually occurs in the colon and rectum^[1]. The main clinical manifestations of patients with this disease are recurrent abdominal distension, abdominal pain, diarrhea, abnormal stool, and fever^[2], which not only brings great physical and psychological burden to

UC patients, but also brings huge economic pressure and medical burden to families and society^[3]. As a global intestinal disease^[4], UC does not have a good treatment method, and some drug therapies (such as aminosalicic acid, hormones and immunosuppressants, etc.) also bring many adverse effects to the human body, such as excessive immune suppression system, high risk of infection and osteoporosis^[5-6]. With people's attention and research, more adjuvant treatment methods have been proposed, including dietary intervention, immunization and probiotic preparations. Although there is no clear and effective pathogenesis and treatment, many scholars have proposed that the interference of the dysbiosis of gut microbiota plays an important role in the pathophysiology of IBD^[7]. Both the reduction of commensal bacteria and the increase of pathogenic bacteria will

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affect UC. Changes of microbial structure and diversity in the gut of the host will correspondingly affect the integrity of intestinal mucosa. Gut microbiota imbalance may be a predisposing factor for UC, and UC may exacerbate the gut microbiota imbalance. The mechanism of UC caused by intestinal microbiota imbalance mainly manifested as the increase or decrease of species and quantity^[8]. Gut microbiota imbalance also increases the expression and release of inflammatory factors^[9]. The intervention of healthy food can regulate the balance of gut microbiota and prevent various diseases caused by gut microbiota without side effects^[10]. Therefore, the use of food intervention methods may be able to assist in the treatment of UC.

Many vegetables and fruits are rich in a variety of nutrients, and it has proved that they can prevent and treat many diseases. *Rosa roxburghii* Tratt fruit, known as cranberry fruit, is a perennial deciduous shrub of the family Rosaceae, and grows widely at altitudes of 500–2 500 m in mountainous regions in southwest and south-central China, particularly in Guizhou Province^[11–12]. It contains a variety of nutrients, including vitamins, terpenes, organic acids, and antioxidant enzymes, especially rich in vitamin C (1 300–3 500 mg/100 g), superoxide dismutase (SOD) (7 000–10 000 U/g), flavonoids (6.8–8.0 mg/g) and so on^[12–14]. A large number of studies have shown that *R. roxburghii* Tratt fruit has outstanding performance in antioxidant, blood lipid lowering, anti-aging, anti-tumor, and diabetes improvement^[15–17]. *R. roxburghii* Tratt fruit also has good effects in reducing the risk of inflammation^[18]. In order to achieve the effect of coexistence of taste and function, *R. roxburghii* Tratt fruit processed products came out at the historic moment, and especially *R. roxburghii* Tratt fruit fermented products, including *R. roxburghii* Tratt fruit jiaosu and *R. roxburghii* Tratt fruit wine, were favored by the public^[11].

Lactic acid bacteria (LAB) can regulate the gut microbiota in the body and treat intestinal diseases. LAB have been widely used in food fermentation due to their function and safety properties^[19]. Most of the LAB are essential and have important physiological functions in the human body. They are widely present in the human intestine and become an important part of the gut microbiota^[20]. In the treatment of colitis, LAB show outstanding abilities^[21–22], and have been used in clinical treatment. Many studies have confirmed the mechanism of action of LAB in the treatment of IBD without side effects^[23]. Ren et al.^[24] screened a probiotic (*Lactocaseibacillus casei*) with anti-inflammatory activity that can improve colitis by reducing macrophages and the inflammatory factors. Saraiva et al.^[25] found that 15-LOX-1-producing *Lactococcus lactis* was effective in preventing intestinal damage associated with IBD.

In our preliminary study, the strain of *Lactocaseibacillus paracasei* SR10-1 was isolated from traditional fermented sour meat products of Dong nationality in China. It has been found that the strain of *L. paracasei* SR10-1 has good antioxidant properties^[26]. In addition, fermentation with *L. paracasei* SR10-1 could significantly increase hypoglycaemic and hypolipidaemic activities of *R. roxburghii* Tratt fruit juice *in vitro* based on the α -glucosidase inhibition rate, glucose dialysis delay index and bile acid salt binding capacity^[27]. We believe that the combination of *L. paracasei* SR10-1 and *R. roxburghii* Tratt fruit juice may have synergistic effect. However, the anti-inflammatory activity of *L. paracasei* SR10-1 fermented *R. roxburghii* Tratt fruit juice and the role of modulating the gut microbiota in mice with dextran sulfate sodium (DSS)-induced colitis remain unclear. The purpose of this study was to investigate the regulation of

R. roxburghii Tratt fruit juice fermented by *L. paracasei* SR10-1 on gut microbiota in UC mice, and to provide support for the application of fermented *R. roxburghii* Tratt fruit juice in alleviating UC.

2. Materials and methods

2.1 Preparation of fermented *R. roxburghii* Tratt fruit juice

The *R. roxburghii* Tratt fruit was planted in Guizhou, China, and the fresh *R. roxburghii* Tratt fruit juice was aseptically filled after beating, filtration, and ultra-high temperature instantaneous sterilization (UHT). *L. paracasei* SR10-1 was isolated from the fermented sour meat products of the Dong nationality in China. *L. paracasei* SR10-1 was inoculated in MRS broth medium under sterile conditions, and cultured at 37 °C for 48 h to a concentration of 9.00 (lg (CFU/mL)). *R. roxburghii* Tratt fruit juice was mixed with 4 volumes of distilled water, filtered, and then added 9.7% sucrose. After pasteurization, it was inoculated with 4.3% *L. paracasei* SR10-1, and fermented at 32 °C for 64.5 h^[28].

2.2 Experimental design and sample collection

Age (5 weeks old mice) and gender (male) matched Kunming (KM) mice from Speifu (Beijing) Biotechnology Co., Ltd. (China) were placed in a sterile isolation cage (with a temperature of 22–24 °C, a 12 h/12 h light/dark cycle and ad libitum access to a basic diet and water). The study was approved (11/03/2022) by the Animal Experiment Ethics Office of Guizhou University (approval number: EAE-GZU-2022-E003). *In vivo* experiment, animals were divided into 5 groups of 10 mice: (i) the normal control group (Control), (ii) the DSS model group (DSS), (iii) the positive control group (Mesalazine), (iv) lactic acid bacteria fermented *R. roxburghii* Tratt fruit juice group (LAB-RRTJ), and (v) *R. roxburghii* Tratt fruit juice group (RRTJ). Except for the Control group, the other groups were administered 3% (m/V) DSS (36–50 kDa, MP Biomedicals, USA) dissolved in drinking water for 5 days for modeling and then started administration. On the 8th and 15th day after the start of administration, the model was maintained by replacing drinking water with 3% (m/V) DSS^[29]. Meanwhile, the mice in group (i) were given normal drinking water. Free access to basic feed from Speifu (Beijing) Biotechnology Co., Ltd. (China) for all animals during rearing. During the period of gastric, the mice in groups (i) and (ii) were fed by 0.85% normal saline, and the mice in group (iii) were fed by 0.30 g/kg mesalazine enteric-coated tablets, whereas the mice in groups (iv) and (v) received a basic diet with drinking water containing LAB-RRTJ or RRTJ for the same period of time. The volume given daily by gavage was 0.10 mL per 10 g body weight. After 21 days of administration, the mice were anesthetized with diethyl ether. Colon tissues and cecal contents from each mouse were collected, immediately frozen in liquid nitrogen and retained at –80 °C for next analysis. All mice were analyzed for body weight and disease activity index (DAI). Among each group, mice were randomly divided into 3 samples, each sample contains caecal contents of 2–3 animals and the cecal contents of each samples were mixed for 16S rRNA detection, 6 mice were randomly selected for enzyme linked immunosorbent assay (ELISA).

2.3 Assessment of DSS-induced colitis

The feeding status of mice was recorded on time, and the DAI was calculated based on weight loss, fecal consistency and blood in the stool score^[30]. A scoring system which includes three parts: body weight loss (0–4), degree of intestinal bleeding (0–4) and stool consistency (0–4). The DAI corresponds to the sum of different scores.

2.4 Measurement of inflammatory cytokines level in the colon

Ten mg of colon tissue was added to 100 μ L PBS (pH = 7.4), and the tissues mixtures were ground with an automatic sample fast grinder (Shanghai Jingxin JxFSTPRP-24, China) (Grinding ball diameter: 0.2 mm (2 pieces), 1 mm (1 piece); homogenization frequency: 90 Hz; time: 90 s) and centrifuged ($3\,000 \times g$, 15 min) under micro high-speed centrifuge (TG16W, Changsha Xiangzhi Centrifuge Instrument Co., Ltd., China). Then, take the supernatant for detection at 450 nm (Rayto, RT-6100, Shenzhen Leidu Life Science Co., Ltd., China). The supernatant was collected for ELISA using a specific kit (Ruixin Biotechnology Co., Guangzhou, China) to measure interleukin 1 β (IL-1 β), IL-6, tumor necrosis factor α (TNF- α) and interferon γ (IFN- γ) levels. The experimental steps were strictly in accordance with the commercial kit requirements.

2.5 Gut microbiome analysis

Analysis of mouse cecal contents was assessed by 16S rRNA amplification and sequencing on the Illumina NovaSeq platform. DNA was extracted from the samples using TGuide S96 Magnetic Bead Soil Kit (Tiangen Biochemical Technology (Beijing) Co., Ltd., China, and model: DP812) according to its instructions. Concentration detection of nucleic acids by the microplate reader (Gene Co., Ltd., China, and model: synergy HTX). After amplification, polymerase chain reaction (PCR) products were detected by electrophoresis using 2.0% agarose (120 V, 40–45 min) (Beijing Bomei Fuxin Technology Co., Ltd., China). The primers selected for bacterial 16S rRNA gene sequencing were 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3')^[31]. The PCR parameters of 16S full-length primer samples were as follows: pre-denaturation at 95 °C for 5 min; 30 cycles, total denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s, and extension at 72 °C for 7 min. In addition, the T_m of the primers is 55 °C and the size of the products is 1.4 kb. PCR products were normalized to equal concentrations. The library was then established, and the sequencing service was provided by Shenzhen Weikemeng Technology Group Co., Ltd., China. In order to ensure the accuracy of operational taxonomic units (OTUs) clustering and subsequent analysis, the original sequencing data will be filtered first. Filtering of processed data to obtain valid data. All raw sequences (input) of all samples were quality controlled (filtered), denoised (sequences corrected for sequencing errors), merged, and non-chimeric), form OTUs using the DADA2 plugin in QIIME2 software. Subsequently, bioinformatic analysis was performed on the cloud platform (<https://www.bioincloud.tech>) of Shenzhen Weikemeng Technology Group Co., Ltd., China.

2.6 Statistical analysis

Data are shown as mean $\pm$ standard deviation of three replicates for each assay. The statistical significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests using IBM SPSS Statistics 25 ($P < 0.05$). Origin 2018 was used for graphical processing.

3. Results

3.1 Body weight and DAI in DSS-colitis mice

DSS-induced colitis led to a significant decrease in body weight in all mice groups ($P < 0.05$) when compared to the Control group at day 6, and the body weight of mice in DSS and RRTJ groups continued to decline at day 9 (Fig. 1a). In addition, the mice showed poor mental status and hair. From the 9th day, the body weight of mice fed with LAB-RRTJ and Mesalazine gradually increased until it was similar to that of the Control group. At the end of the gavage, the weight of mice in DSS group was significantly different from that in Control group ($P < 0.05$) (Fig. 1a). More precisely, the consumption of LAB-RRTJ significantly limited weight loss ($P < 0.05$). As expected, DSS-induced colitis increased the DAI score, mice developed severe blood in the stool and loose stools, and some mice suffered severe weight loss. The DSS group reached the maximum DAI score on day 9^[32] (Fig. 1b). On the last day, the DAI score of the DSS group was still the highest, and the higher the score, the more severe the enteritis. After drug intervention, the DAI score of LAB-RRTJ and Mesalazine groups were lower than that of the DSS group ($P < 0.05$) (Fig. 1b). Altogether, these results showed that the LAB-RRTJ reduced the severity of DSS-induced colitis.

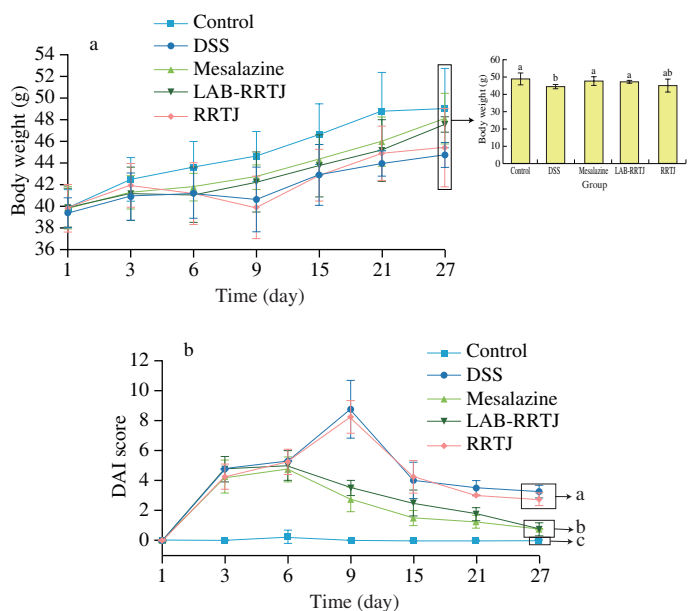


Fig. 1 Impact of LAB-RRTJ and RRTJ on body weight and the DAI of DSS-induced colitis mice. (a) Time-course of mice body weight monitoring. (b) Time-course of mice DAI score monitoring. The data represent the mean $\pm$ SD of 10 mice per group. Multiple comparisons were performed; Values with different superscripts (a, b, c) are significantly different among groups according to ANOVA with Duncan's multiple range test at $P < 0.05$.

3.2 LAB SR10-1 fermented RRTJ modulates cytokines in mouse colon

ELISA kits were used to test colonic cytokines in mice, and it was found that the levels of colonic IL-1 β , IL-6, TNF- α and IFN- γ were increased in the DSS group compared with the Control group ($P < 0.05$) (Figs. 2a–d), increased by 32.27%, 49.55%, 44.12% and 57.85% respectively, indicating that DSS can induce colitis. Compared with the DSS group and the RRTJ group, the levels of IL-6, IL-1 β , TNF- α and IFN- γ in the colon of mice in LAB-RRTJ group were decreased ($P < 0.05$). Overall, the effect of LAB-RRTJ group in reducing inflammatory factors was better than that of Mesalazine group, but did not significantly affect these inflammatory cytokines in the RRTJ-fed mice.

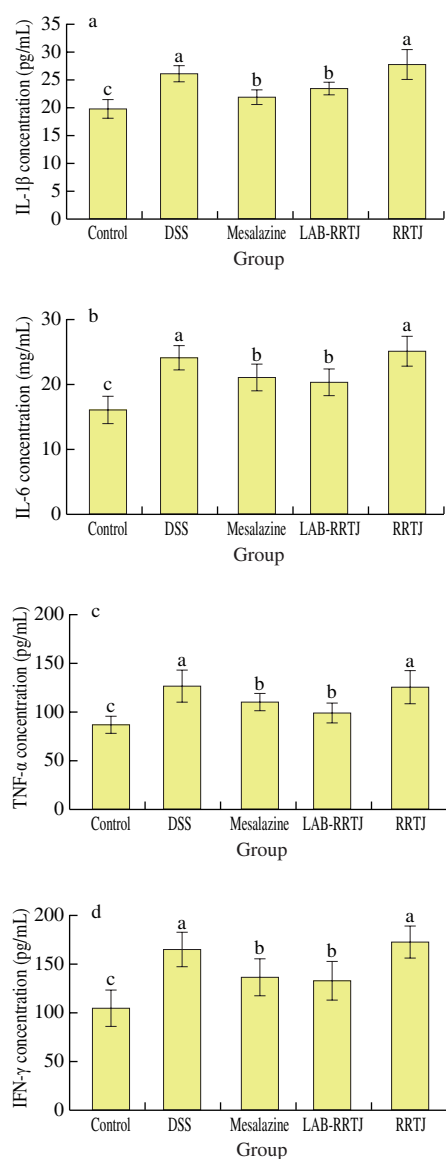


Fig. 2 Supplementation with lactic acid bacteria-fermented *R. roxburghii* Tratt fruit juice can relieve colon inflammation. Cytokines concentration of (a) IL-1 β , (b) IL-6, (c) TNF- α and (d) IFN- γ were quantified by ELISA. The data represent the mean $\pm$ SD of 6 mice per group. Multiple comparisons were performed; Values with different superscripts (a, b, c) are significantly different among groups according to ANOVA with Duncan's multiple range test at $P < 0.05$.

3.3 LAB SR10-1 fermented RRTJ supplementation attenuates DSS-induced disruption of gut microbiota

The proportions of shared and unique OTUs are illustrated by the Venn diagram in Fig. 3a, the number of OTUs in Control, DSS, Mesalazine, LAB-RRTJ and RRTJ groups is 6 367, 654, 98, 9 613, and 3 326, respectively. The number of OTUs in the DSS and Mesalazine groups were much lower than that in the Control group. However, the number of OTUs in LAB-RRTJ and RRTJ groups were increased compared with the DSS group (Fig. 3b). The Shannon-Wiener curve was constructed based on the number of extracted sequences and their corresponding diversity indices. With the increase of the amount of sequencing data, the curve tended to be flat, indicating that the amount of sequencing data was large enough to reflect the vast majority of microbial information in the sample^[33] (Figs. 3c and d). α -Diversity analysis is the main method to reflect the richness, which was examined using the Shannon, Simpson, Chao1 and Faith_pd indices (Table 1). After DSS intervention, the Shannon, Simpson, Chao1 and Faith_pd indices were all significantly decreased ($P < 0.05$), but the LAB-RRTJ and RRTJ groups have reversed this situation. In addition, the richness and diversity of LAB-RRTJ bacteria were significantly higher than the RRTJ ($P < 0.05$).

Table 1
Alpha diversity of gut microbiota in mice with colitis.

Group	Shannon index	Simpson index	Chao1 index	Faith_pd index
Control	10.92 $\pm$ 0.17 ^a	0.99 $\pm$ 0.00 ^a	3 262.33 $\pm$ 228.13 ^a	353.44 $\pm$ 19.44 ^a
DSS	5.41 $\pm$ 0.40 ^c	0.96 $\pm$ 0.01 ^b	82.33 $\pm$ 16.50 ^c	17.92 $\pm$ 3.12 ^c
Mesalazine	5.69 $\pm$ 0.16 ^c	0.97 $\pm$ 0.01 ^b	87.67 $\pm$ 20.13 ^c	17.26 $\pm$ 2.79 ^c
LAB-RRTJ	10.40 $\pm$ 1.32 ^a	0.98 $\pm$ 0.01 ^{ab}	3 097.00 $\pm$ 1 137.36 ^a	332.66 $\pm$ 102.18 ^a
RRTJ	8.06 $\pm$ 0.71 ^b	0.96 $\pm$ 0.03 ^b	1 121.67 $\pm$ 188.00 ^b	154.50 $\pm$ 17.65 ^b

Note: Data represent the mean $\pm$ SD of 3 samples per group. Values with different superscripts (a, b, c) are significantly different among groups according to ANOVA with Duncan's multiple range test at $P < 0.05$.

3.4 β -Diversity analysis

β -Diversity analysis can intuitively reflect the differences in the composition of gut microbiota between different groups. Non-metric multidimensional scaling (NMDS) and principal co-ordinates analysis (PCoA) three-dimensional coordinate map analysis were used to explore the influence of the composition of gut microbiota between different groups. It could see from Fig. 4a that the DSS group and other groups are clearly discrete, showing that there is a large difference in the community structure between the DSS group and each group. As shown in Fig. 4b, the stress value of the NMDS results is 0.094 3, which can accurately reflect the degree of difference between samples. Furthermore, NMDS revealed that the microbial composition in the Control, LAB-RRTJ and RRTJ groups was different from the DSS group. In particular, the samples LAB-RRTJ and RRTJ were similar to the Control group. To sum up, no significant differences were found between the LAB-RRTJ group and the RRTJ group from the β -diversity analysis.

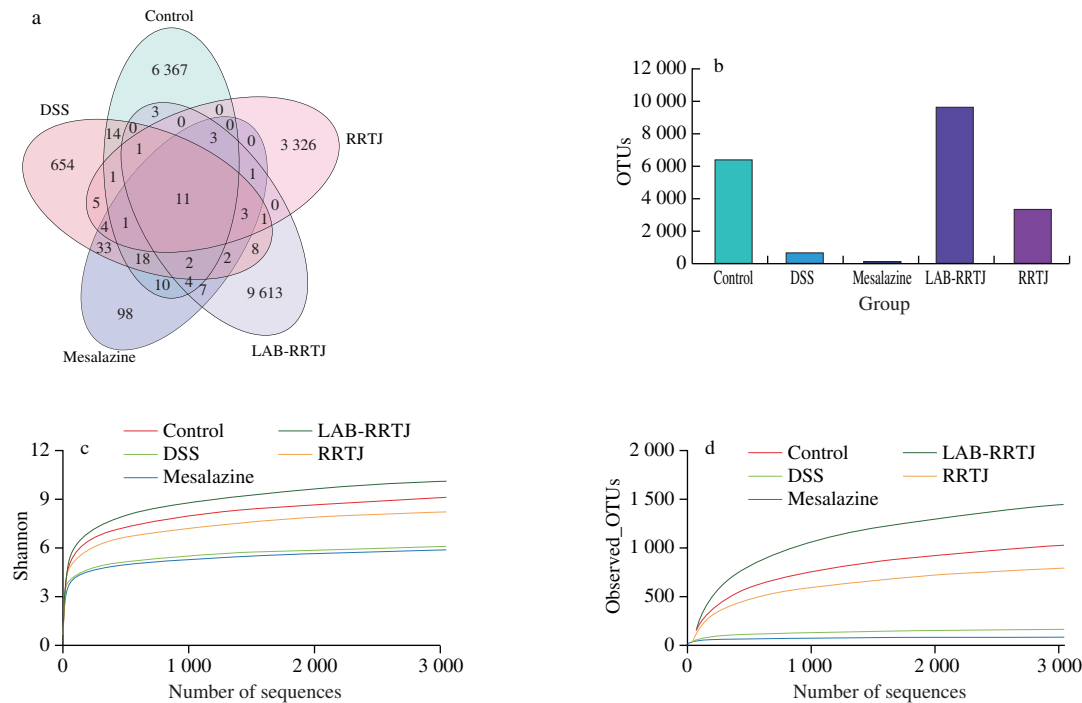


Fig. 3 Supplementation of LAB-RRTJ can improve the richness and diversity of gut microbiota in mice with colitis. After the intervention, (a) Venn diagram, (b) OTUs, (c) Shannon exponential dilution curve and (d) 16S rRNA gene sequence dilution curve were determined. Data represent the mean $\pm$ SD of 3 samples per group.

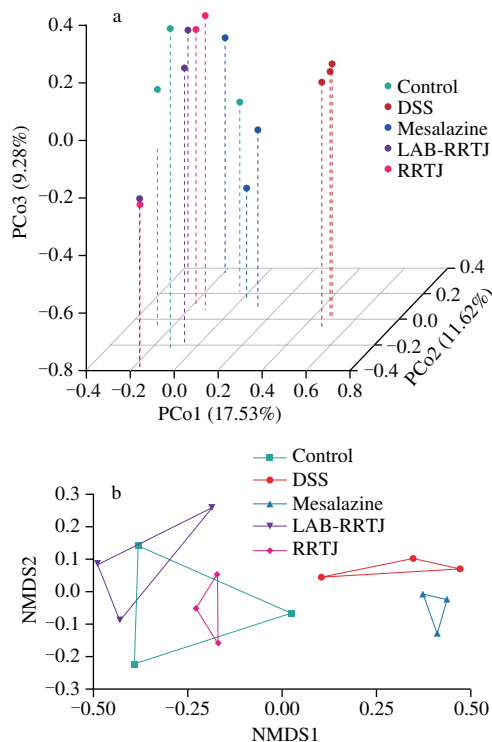


Fig. 4 Impact of LAB-RRTJ and RRTJ on β -diversity of DSS-induced colitis mice. (a) Principal coordinate analysis (PCoA) plot analysis from each sample. (b) NMDS plot analysis from each sample. Data represent the mean $\pm$ SD of 3 samples per group.

3.5 Species composition analysis at different levels

Analysis of community composition with relative abundance

at the phylum level in the top 20 was in Fig. 5a. The 5 groups are mainly composed of Firmicutes, Bacteroidetes, Proteobacteria and Verrucomicrobia. The abundance of Firmicutes was decreased but Proteobacteria, Verrucomicrobia, and Actinobacteria in the DSS group were increased compared with the Control group, which was consistent with previous studies. These changes were reversed by LAB-RRTJ administration in which Firmicutes increased from 48.98% to 74.51%, while Proteobacteria decreased from 17.96% to 1.59%, Verrucomicrobia decreased from 6.05% to 0.42%, and Actinobacteria decreased from 0.88% to 0.30%. However, neither the Mesalazine nor RRTJ group could increase the level of Firmicutes, and the RRTJ group had little effect on the changes of Proteobacteria (Fig. 5b).

Analysis of community composition with relative abundance at the genus level in the top 20 was in Fig. 5c. It was found that the abundance of harmful bacteria genera such as *Staphylococcaceae_Staphylococcus*, *Desulfovibrio*, *Akkermansia*, *Ruminococcus*, *Odoribacter*, *Candidatus_Arthromitus* and *Sedimentobacteria* were the highest in the DSS group, while beneficial bacteria such as *Bacteroides* and *Lactobacillus* were decreased, compared to the Control group. The abundance of a series of harmful bacteria in the DSS group were restored by LAB-RRTJ. However, the levels of *Ruminococcus* and *Prevotellaceae_Prevotella* of Mesalazine group were increased, and there was no trend toward a decrease in the colitis-specific harmful bacteria *Acinetobacter* in the RRTJ group. Analysis of community composition with relative abundance at the species level in the top 10 was in Fig. 5d. Compared with the Control group, the relative abundances of *S. sciuri*, *Desulfovibrio* C21_c20, *R. gnavus* and *Akkermansia muciniphila* (in the phylum

Verrucomicrobia) were higher in the DSS group but *B. acidifaciens* was reduced. The LAB-RRTJ could restore DSS induced intestinal microbial species except *sciuri* in mice. The relative abundances of *Ruminococcus gnavus* were high in the Mesalazine group. The dominant species in the RRTJ group was *Acinetobacter lwoffii*.

According to the heat map of classification and clustering, at the phylum and genus levels, the LAB-RRTJ clustered into one group with the Control group, and far away from the DSS group (Figs. 6a and b). LEfSe can be used to distinguish key bacteria between groups. Bacteria, *Ruminococcus* and Planococaceae were the key bacteria in the Control, Mesalazine and LAB-RRTJ groups, respectively (Figs. 6c and d). However, the key bacteria in the DSS group included Actinobacteria, *Sediminibacterium*, Chitinophaga, *Methylovirgula* (from family level to genus level), *Bradyrhizobium* (from order level to genus level), *Hydrogenophaga* (from class level

to genus level), *Pseudomonas* (from family level to genus level), and most of them were bacteria that had outstanding contributions to the pathogenesis of colitis, including Bradyrhizobiaceae, Betaproteobacteria, Actinobacteria, Pseudomonadaceae.

3.6 Correlation analysis of gut microbes and inflammatory factors in mice

Redundancy analysis (RDA) showed that the influence degree of environmental factors affecting the distribution of gut microbes in each group were: $IL-1\beta > IFN-\gamma > TNF-\alpha > IL-6$. Changes in the number and types of microbiotas in the gut of UC patients are involved in the initiation of endogenous intestinal infection and the suppression of the immune system^[34]. It could be seen that only the DSS group had a positive correlation with $IL-1\beta$, $IFN-\gamma$, $TNF-\alpha$, and

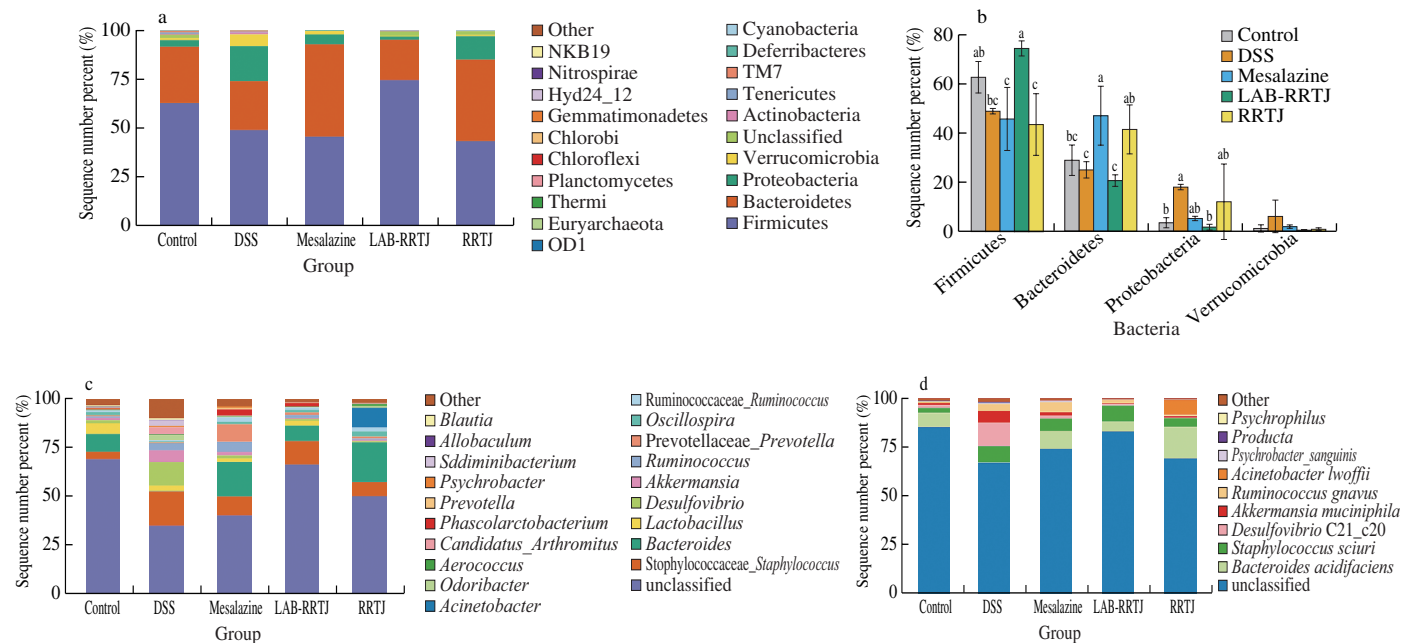


Fig. 5 Supplementation of LAB-RRTJ modulates gut microbiota in mice with colitis. (a) Percent of community abundance on phylum level. (b) Percent of community abundance on Firmicutes, Bacteroidetes, Proteobacteria and Verrucomicrobia. (c) Percent of community abundance on genus level. (d) Percent of community abundance on species level. Data represent the mean $\pm$ SD of 3 samples per group.

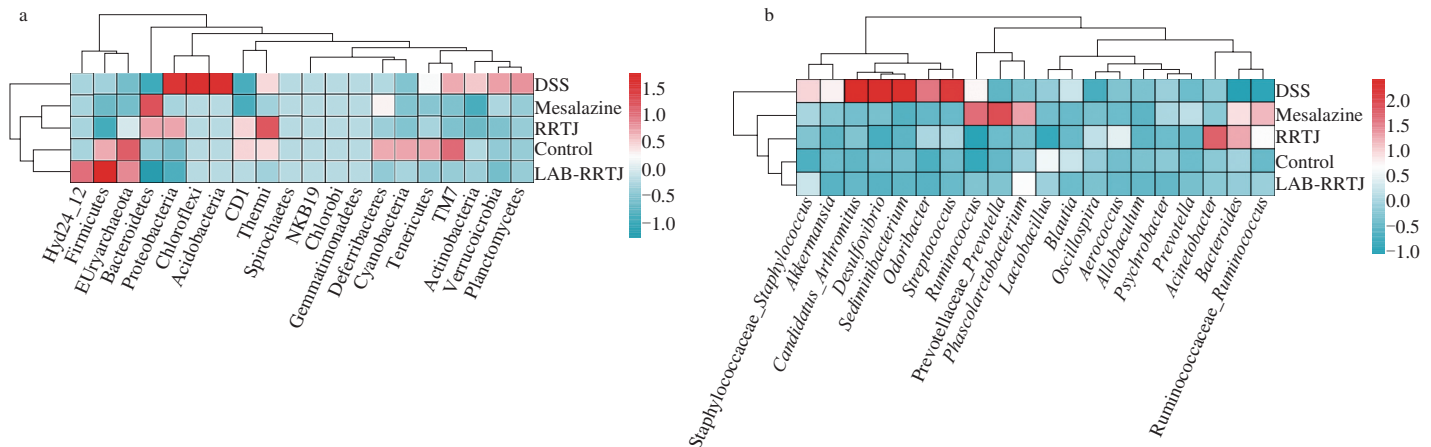


Fig. 6 Supplementation of LAB-RRTJ modulates gut microbiota in mice with colitis. (a) The categorical clustering heatmap of phylum level. (b) The categorical clustering heatmap of genus level. (c, d) LEfSe cladogram represents differentially abundant taxa. Only taxa with LDA scores of more than 2 are presented. Data represent the mean $\pm$ SD of 3 samples per group.

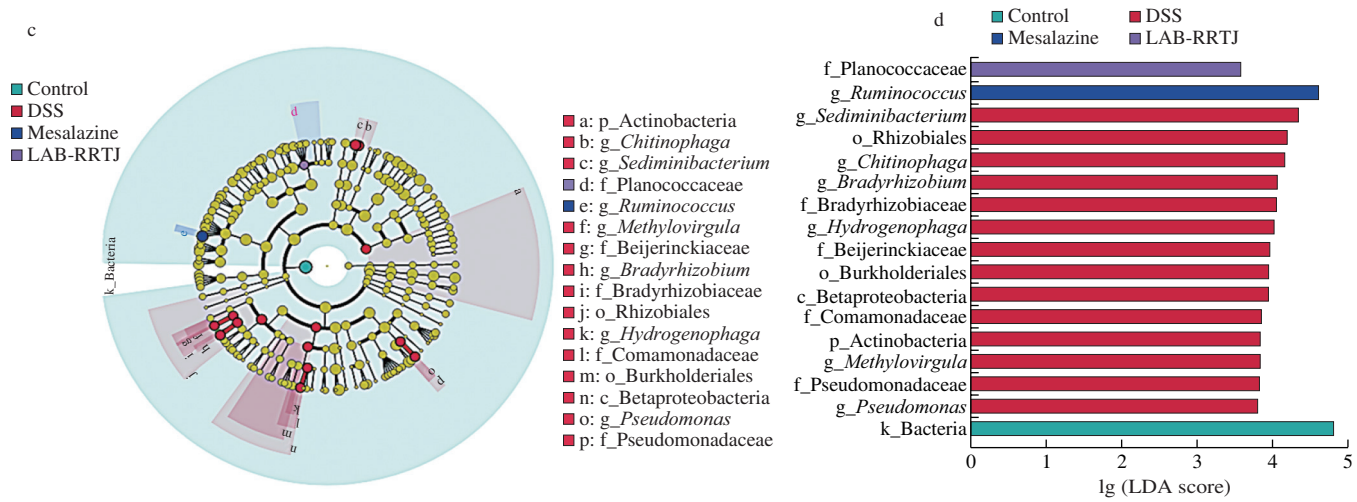


Fig. 6 (Continued)

IL-6. Compared with the LAB-RRTJ group, the RRTJ group was positively correlated with IL-1 β and IL-6 (Fig. 7a). The IL-1 β , IL-6, TNF- α and IFN- γ were positively correlated with *Oscillospira*, *Staphylococcaceae_staphylococcus* and *Acinetobacter*, but negatively correlated with *Lactobacillus* and *Prevotellaceae_Prevotella* (Fig. 7b). Both inflammatory factors and gut microbiota are influencing factors of colitis in mice, and there is also a correlation between the two. Fig. 7c showed the heat map analysis of the correlation between the top 20 species in relative abundance at the genus level and inflammatory factors, with positive correlation in red and negative correlation in blue. Further analysis showed that IL-6, TNF- α and IFN- γ were positively correlated with *Burkholderia*, *Brevundimonas* ($P < 0.05$), IL-1 β was positively correlated with *Staphylococcaceae_Staphylococcus*, *Odoribacter*, etc. ($P < 0.01$), All four inflammatory factors were positively correlated with *Stenotrophomonas*. All four inflammatory factors were negatively correlated with *Anaerotruncus*, *Weissella*, *Erwinia*, etc. ($P < 0.05$).

4. Discussion

Both *R. roxburghii* Tratt fruit and lactic acid bacteria have anti-inflammatory effects. At present, many studies have proved that some strains of LAB have a certain preventive and therapeutic effect on colitis, but there are few studies on the effect of *R. roxburghii* Tratt fruit on colitis. Some studies have found that *R. roxburghii* Tratt fruit polysaccharide has a therapeutic effect on colitis^[35]. Therefore, we envisaged that the use of *L. paracasei* SR10-1 to ferment *R. roxburghii* Tratt fruit juice can not only synthesize the properties of *R. roxburghii* Tratt fruit, but also the combination of them can supplement and improve the biological activity of the two to achieve the effect of preventing and treating colitis. Therefore, this study compared the intervention effect of *R. roxburghii* Tratt fruit juice and *R. Roxburghii* Tratt fruit juice after *L. paracasei* SR10-1 fermentation on colitis. The results showed that LAB-RRTJ ameliorated DSS-induced colitis by regulating gut microbes.

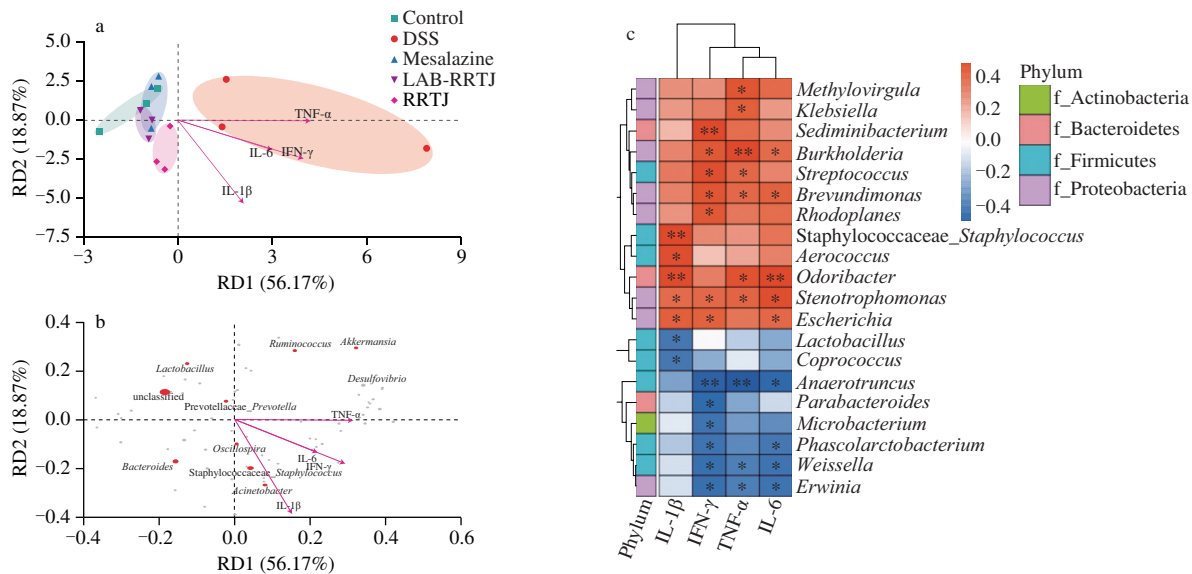


Fig. 7 Correlation analysis of inflammatory factors and gut microbiota. (a, b) Redundancy analysis (RDA) from each group. (c) Correlation heat map analysis. Data represent the mean $\pm$ SD of 3 samples per group, * $P < 0.05$, ** $P < 0.01$.

The balance of gut microbes is an important factor in maintaining body health, and previous studies have demonstrated that the pathogenesis of UC is related to gut microbiota^[36]. The fecal microbiota composition of patients with ulcerative colitis differs from healthy individuals, and different bacterial species contribute to the pathogenesis of UC^[37]. Modulating the gut microbiota can improve the IBD series of diseases, which has become a research hotspot. In IBD patients, microbial transfer due to an increase in Gram-negative bacteria is accompanied by a decrease in bacterial diversity, which leads to abnormal inflammatory processes^[38]. In the present study, LAB-RRTJ improved the richness and diversity of DSS-induced mice gut microbiota better than RRTJ, and much better restored the level of gut microbiota. LAB-RRTJ has a positive impact on the richness and diversity of gut microbiota community. Alpha diversity was decreased in Mesalazine group, that the induction of mesalamine might have certain side effects on the gut microbiota. Finding microbial markers of colitis can be used to assess the severity of the disease. Previous studies have found that the decrease of Firmicutes^[39–40] and the increase of Proteobacteria and Verrucomicrobia are associated with the pathogenesis of UC^[41–43]. In addition, *Staphylococcus*, *Desulfovibrio*, etc. have also confirmed could disrupt the intestinal tract after DSS induction^[44]. In recent years, many studies have also found that *Akkermansia*, which are beneficial to human health, have opposite effects in colitis patient^[45]. The decreased abundance of Firmicutes in the IBD patient group may be related to the presence or persistence of inflammation^[37]. The medical importance of various members of the Proteobacteria phylum in human gastrointestinal diseases has well documented, and some of these groups can cause diarrhea and can cause acute inflammatory bowel disease^[46]. *Desulfovibrios*, Gram-negative bacteria, are able to produce LPS, that is a key mediator of gut dysbiosis and inflammation. When gut bacteria generate huge amounts of LPS, the production of IL-6, IL-1 β and TNF- α will be triggered^[47]. *Staphylococci* can invade inhalation and bloodstream infections causing widespread infection^[48]. However, LAB-RRTJ could restore the abundance of some harmful bacteria related to colitis, which is similar to the Control group.

In this experiment, the species level of caecum content in mice was analyzed. The abundance of *S. sciuri*, *Desulfovibrio* C21_c20, *R. gnavus* and *Akkermansia muciniphila* were significantly increased in the DSS group. *S. sciuri* was found in the colon tissues of patients with colitis and was reported as an oral bacterium that promoted intestinal inflammation in colitis^[49]. *Desulfovibrio* subspecies are sulfate-reducing anaerobic Gram-negative bacteria associated with increased gut permeability^[50]. The increase in the abundance of *Desulfovibrio* C21_c20 and *A. muciniphila* may reduce intestinal mucosal permeability, increased intestinal mucosal inflammation and systemic inflammation^[51]. A growing number of metagenomics studies are reporting an association between *R. gnavus* and a range of diseases including inflammatory bowel disease (IBD), and it was IBD related bacteria^[52–54]. Research showed *B. acidifaciens* was significantly correlated with IBD disease severity^[55]. To sum up, our research found that these situations could be reversed by LAB-RRTJ intervention.

Imbalances in the species composition and metabolic state of the gut microbiota can disrupt the balance between inflammatory factors and trigger an inflammatory response^[56]. Studies have shown that the content of pro-inflammatory factors in the intestinal tract of UC mice

increased, while the content of anti-inflammatory factors decreased^[57], and there was a positive correlation between beneficial bacteria and anti-inflammatory factors, and a positive correlation between harmful bacteria and pro-inflammatory factors^[58]. Our study found that the pro-inflammatory factors IL-1 β , IL-6, TNF- α and IFN- γ were elevated after DSS induction but decreased to normal levels after LAB-RRTJ intervention. According to the correlation analysis between inflammatory factors and gut microbiota, *Lactobacillus* and Prevotellaceae_*Prevotella* were negatively correlated with inflammatory factors, and *Oscillospira*, Staphylococcaceae_*Staphylococcus* and *Acinetobacter* were positively correlated with the four inflammatory factors. Potential pathogenic microorganisms such as *Acinetobacter* multiply and colonize in the host, which will threaten intestinal health, and can mediate cytokine accumulation, reduce intestinal colonization resistance and increase the risk of inflammation in the body^[59]. Due to the increased metabolic activity of *Lactobacillus*, it leads to the large production of lactic acid and the excessive secretion of bacteriocin, which reduces the pH value of the intestinal environment, creates unfavorable conditions for the growth of pathogenic bacteria, and plays an important role in controlling the colonization of pathogenic bacteria such as *Stenotrophomonas*. And *Lactobacillus* can reduce the production of proinflammatory cytokines and increase the production of anti-inflammatory cytokines^[60–61]. It can be concluded that LAB-RRTJ can alleviate UC by maintaining gut microbiota and restoring the imbalance of inflammatory factors.

5. Conclusion

This study found that feeding a diet supplemented with LAB-RRTJ regulated the gut dysbiosis of UC mice induced by DSS and maintained the balance of the gut microbial ecology. The number of OTUs increased, alpha diversity and beta diversity were restored and similar to those in mice in the Control group when received LAB-RRTJ treatment. It could also be observed that the abundance of Firmicutes increased, while Proteobacteria, Verrucomicrobia and Actinomyces decreased dramatically. In addition to this, there is a significant correlation between inflammatory factors and changes in gut microbiota, and they interaction in UC mice. In conclusion, the research revealed that the LAB-RRTJ have great potential as an immunological and anti-inflammatory drug modulator for the treatment of UC, and theoretical support was provided for the future application of the LAB-RRTJ in medical or functional food. Nevertheless, the influencing mechanism of the inflammatory factors and gut microbiota needs to be further explored.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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