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Original Article

Fermented *Spirulina platensis* alleviated DSS-induced ulcerative colitis by regulating gut microbiota and MyD88/TLR4 signaling pathway

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Abstract

Spirulina platensis (*S. platensis*) produces a variety of biologically active compounds that exhibit antioxidative, anti-inflammatory, antibacterial, and immunoregulatory properties. Here, dextran sulfate sodium (DSS) was used to develop an animal model of ulcerative colitis (UC) to evaluate the potential protective benefits of fermented *S. platensis* against DSS-induced colitis in mice. Gut microbiota alterations were investigated using 16S ribosomal RNA (rRNA) gene sequencing. Real-Time Quantitative PCR (RT-qPCR) was used to detect the expression of mRNA of inflammatory factors and pathway-related molecules in the inflammatory process. The results showed that fermented *S. platensis* could reverse the DSS-induced weight loss and colon length shortening in mice. The study of the 16S rRNA sequence showed that treatment with fermented *S. platensis* changed the gut microbiota of mice, with an increase in the relative abundance of beneficial bacteria such as Lachnospira. According to RT-qPCR and histopathological analyses, fermented *S. platensis* also improved the loss of goblet cells and neutrophil infiltration induced by DSS, while improving anti-inflammatory capacity. In addition, compared with the model group, the fermentation group significantly downregulated the relative expression of MyD88/TLR4 signaling pathway genes compared with the non-fermentation group. Overall, this investigation demonstrated that fermentative *S. platensis* can reduce DSS-induced UC by regulating gut microbiota composition, and the MyD88/TLR4 signaling pathway.

Keywords: *Spirulina platensis*, *Rhizopus oligosporus*, Ulcerative colitis, Gut microbiota

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Introduction

Ulcerative colitis (UC) is a chronic intestinal disorder that is characterized by mucosal submucosal superficial ulceration, hematochezia, diarrhea, and weight loss (Yu et al., 2019). Most of the UC lesions are found in the colon and rectum, with recurring intestinal infections linked to factors like the environment, diet, genetics, and changes in mucosal permeability (Tan et al., 2022). The inflammation of the intestinal mucosa is caused by a defective immune response (Yang et al., 2021). UC treatments include surgery and microbial therapy (Li et al., 2020). The drugs for UC treatment include corticosteroids, immunosuppressants, and therapeutic medicines. However, these drugs are only effective for temporary symptomatic relief and are associated with toxic side effects, drug dependence, adverse reactions, immune function decline, and cancer risks. Microbiota treatment options include antibiotics, probiotics, postbiotics, and fecal microbiota transplantation, but there is still controversy surrounding their safety and effectiveness. Therefore, natural remedies that can both prevent and treat UC are so desperately needed.

Intestinal flora disturbance is one of UC's essential characteristics, and disruption to the intestinal mucosal barrier is the disease's primary pathological alteration (Pan et al., 2020). Intestinal microbiota plays a crucial role in the pathophysiology of UC by regulating intestinal mucosal immunological and inflammatory responses to restore the damaged intestinal mucosal barrier. Under normal physiology, helpful bacteria, and pathogenic bacteria in the intestine balance each other to maintain the dynamic equilibrium of the immune inflammatory system. Intestinal flora imbalance will encourage an abnormal immune response of the intestinal mucosa, which will damage the mucosal barrier and increase the permeability of the intestinal epithelium, cause an accumulation of bacteria and endotoxins in the mucosal lamina propria, ultimately leading to UC (Jergens et al., 2021). Therefore, the integrity of the intestinal mucosal barrier can be repaired by managing the gut microbiota and reestablishing its homeostasis, which can prevent UC from progressing further.

Currently, *S. platensis* is used increasingly as a functional food and a supplement to human nutrition (Yousefi et al., 2019). Due to *S. platensis'* considerable nutritional and therapeutic value as well as the functional variety of its pharmacologically active components, which are often utilized in the medical field and the food sector (Kose et al., 2017). It is rich in nutrients, composed of 60%-70% protein, 6%-12% polysaccharide, vitamins, fatty acids, and minerals (Abdul Rahim et al., 2021). Proteins in *S. platensis* are hydrolyzed into bioactive peptides that have weight-loss, anti-inflammatory and antibacterial properties (Luo and Song, 2021; Wang and Zhang, 2017). Furthermore, *S. platensis* contains a variety of useful bioactive substances that have anti-inflammatory and antioxidant properties, such as phenols, phycobiliproteins, and C-phycocyanin (Finamore et al., 2017).

In addition to supplementing the special components of probiotics, fermentation can transform and metabolize plant substances into forms that are easily absorbed (Dimidi et al., 2019), improving their antioxidant and anti-inflammatory activities (Gabriele et al., 2023). In comparison to non-fermented *S. platensis*, the amino acid content, and the ratio of essential amino acids to total amino acids were significantly enhanced through lactic acid bacteria fermentation (Yu et al., 2020). Mixed fermentation of microorganisms can effectively improve the biological activity and immunomodulatory activity of *S. platensis* (An et al., 2020). *Rhizopus oligosporus* has a strong enzyme production system and a strong decomposing ability on proteins, polysaccharides, while fats and produces many active ingredients (Zhang et al., 2022). The fermentation of *S. platensis* with *Rhizopus oligosporus* may increase its potential value. To explore the effect of *S. platensis* fermented by *Rhizopus oligosporus* on UC, the animal model of UC induced by DSS was established in this experiment. 16S rRNA analysis and RT-qPCR were used to explore the relationship between *S. platensis* and UC. This study aimed to investigate whether fermenting *S. platensis* could provide a protective effect against DSS-induced UC and to understand the mechanism behind it. This offers theoretical backing and a foundation for the advancement and usage of *S. platensis.*

Material and Methods

Preparation and fermentation of *Spirulina platensis* solution

S. platensis powder (Fuqing King Dnarmsa *Spirulina* Co., Ltd) was prepared into a solution with distilled

water (1:1.5, w/v), and then sterilized (121 °C, 30 min). Firstly, culture *Rhizopus oligosporus* (Strain was preserved in Food Quality and Health Laboratory of Sichuan University of Science & Engineering) in PDB (Hangzhou Baisi Biotechnology Co., Ltd) medium at 37 °C and 180 rpm for 24 h. Then 1 mL of it was inoculated into the sterilized *S. platensis* solution and fermented at 37°C for 5 days. The same amount of sterile water was used to inoculate the non-fermented group. The *S. platensis* sample was stored in −20 °C refrigerator for further use.

Ethical statement

Mice were obtained from SPF Biotechnology Co. Ltd in Beijing, China. All experimental procedures were conducted in accordance with the guidelines provided by the Care and Use of Animals Committee at Southwest Medical University (Luzhou), after obtaining appropriate approval (swmu20220137).

Experimental setup for model animal study

Following a week of adapted feeding, 32 C57BL/6J mice (20-24 g, male) were randomized into four groups at random: control group (C), UC model group (D), UC + non-fermented *S. platensis* group (P), and UC + fermented *S. platensis* group (R). Mice in groups C and D oral gavage an equal volume of sterile water for the first two weeks, whereas mice in groups P and R oral gavage an equal volume of non-fermented and fermented *S. platensis*, respectively. Except for group C, the drinking water of the other groups was changed to sterile water containing 2.5% DSS (w/v) on day 14, after which the mice were given unlimited access to food and water for 5 days. As in the preceding two weeks, gavage was administered to all groups during the modeling period. The Disease Activity Index (DAI) was computed throughout the experiment by recording body weight, stool blood, and stool consistency three times each day. On day 19, mice were sacrificed, and colonic tissue was removed to measure length. It was then rinsed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, and used for histological investigation. For further examination, blood, colonic tissue, and intestinal contents are collected and kept in storage at –80 °C.

Histopathological observation of colon

The transverse rings of the colons were fixing in 4% paraformaldehyde, embedded in paraffin, then stained with hematoxylin and eosin (H&E) to observe histomorphological changes under a microscope (Leica Biosystems, Wetzlar, Germany). Alcian blue/periodic acid-Schiff (AB/PAS) straining was used to see goblet cells (BX53M; Olympus, Tokyo, Japan). Based on the degree of epithelial hyperplasia, mucosal ulceration, lamina propria neutrophil infiltrate, and lamina propria mononuclear infiltrate, these sections were given histologic inflammatory ratings ranging from 0 to 10.

DNA sequencing

DNA sequencing was performed according to the method described in the literature (Wang et al., 2022). Amplification of V4 hypervariable region (F: 5'-GTGCCAGCMGCCGCGGTAA-3'; R: 5'-GGACTACHVGGTWTCTAAT-3') of 16S Ribosome RNA gene using DNA as template and sequenced by Novogene Bioinformatics Technology Co. Ltd. (Beijing, China).

Sequence analyses

The 16S rRNA sequencing was performed on the intestinal contents, based on Illumina MiSeq platform. To enhance the accuracy and reliability of information analysis findings, the raw reads were subjected to quality control, splicing, then filtering procedures to obtain clean data. Subsequent analysis was performed using the effective tags. Uparse algorithm (Edgar, 2013) was used to cluster all Effective Tags from the samples. These sequences were then grouped into Operational Taxonomic Units (OTUs) with a default 97% agreement. For species annotation analysis, the Mothur method was used in conjunction with the SILVA138.1 SSUrRNA database, setting the threshold at 0.8-1 (threshold set at 0.8-1) (Quast et al., 2013; Wang et al., 2007). QIIME software was employed to calculate the α and β diversity indices of the OTUs. Chao1 and Shannon indices were calculated using Qiime software (version 1.9.1) with a LDA Score screening value set to 4. To visualize the results, PCoA plots were generated using R software (version 2.15.3). This analysis aimed to identify genera with significant differences between groups. After the table of OTUs with annotation was produced, α-diversity and β-diversity analysis was performed. The raw sequence data mentioned in this paper have been stored in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics, 2017) in National Genomics Data

Center (Nucleic Acids Res 2021), China National Center for Bioinformation/ Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number CRA008241 that are publicly accessible at https://bigd.big.ac.cn/gsa.

RNA extraction and Real Time Quantitative PCR

In accordance with the manufacturer's instructions, the extracted colon RNA using the E.Z.N.A® Total RNA Kit I (Omega Bio-Tek, Norcross, GA, USA). Spectrophotometry was used to assess RNA purity (A260/A280 = 1.8-2.0). RNA reverse transcription kit instructions were followed to synthesize cDNA. To determine the key genes and pathways that could be responsible for the anti-inflammatory effects of fermented *S. platensis*, the relative mRNA expression levels of inflammation-related genes (TNF-α, IL-6, IL-1β, MyD88, NF-κB, TLR4) and tight junction proteins (Claudin-1, Occludin, ZO-2) were measured by RT-qPCR using β-actin as an internal reference gene. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Table-1).

Table-1. Primers sequences for Real Time Quantitative PCR

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
IL-1β	CTGAACTCAACTGTGAAATGC	TGATGTCTGCTGCGAGA
IL-6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
MyD88	GCATGGTGGTGGTTGTTTCTG	GAATCAGTCGCTTCTGTTGG
NF-κB	ACACTGGAAGCACGGATGAC	TGTCTGTGAGTTGCCGGTCT
TLR4	ATCGCCTATGGTTGTTGACC	GGTTTCACGACTGGAGGTTC
TNF-α	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
Occludin	TGGCTATGGAGGCGGCTATGG	AAGGAAGCGATGAAGCAGAAGGC
ZO-2	CCGTAGAGCCCATCCAGAGACC	GTGTTCATGCCGTGACCTAGACTC
Claudin-1	GCTGGGTTTCATCCTGGCTTCTC	CCTGAGCGGTCACGATGTTGTC
β-actin	AATCGTGCGTGACATCAA	GCTCGTTGCCAATAGTGA

Statistical analyses

The statistical data are given as mean ± standard deviation after three replications of each experiment. The mean and standard deviation were determined using a one-way analysis of variance (ANOVA). Duncan's approach was applied in the Statistical Program for Social Sciences version 20.0 after determining the statistical significance between the groups (Chicago, IL, USA). Column diagrams and line charts were created with OriginPro version 2021 (OriginLab, Northampton, MA, USA). Differences between test groups are significant when the p-values<0.05.

Results

Effects of fermented *Spirulina platensis* on ulcerative colitis

By measuring the weight of mice (Figure-1. B), it was found that compared with group C, the weight of groups D, P, and R decreased significantly, with a weight loss of 5.96% in group D, 4.28% in group P, and 3.59% in group R. The DSS administration resulted in a shorter colonic length of mice in the D group (6.3 ± 0.59 cm) as compared to mice in the C group (8.7 ± 0.95 cm). However, both fermented and non-fermented *S. platensis* exhibited an inhibitory effect on the colon length shortening of Group D mice (Figure-1. C). According to the scoring results, the DAI score of Group D was significantly higher than that of Group C, with some mice exhibiting colon adhesions and sparse fecal content. The scores of Group P and Group R were lower than those of Group D, with Group R having the lowest DAI score among these three groups. These data indicate that *S. platensis* can alleviate the symptoms of fecal occult blood and diarrhea in mice and has the effect of alleviating UC (Figure-1. D).

Histological effects of fermented *Spirulina platensis* on ulcerative colitis

According to the colons' histological analysis, the epithelium, crypts, and submucosa of group C colonic mucosa were all in good condition. Epithelial and goblet cell loss, significant neutrophil infiltration, crypt structural deformation, the development of crypt abscesses, and the development of ulcers were all seen in the D, P, and R groups (Figure-2). Interestingly, the severity of gastrointestinal bleeding caused by DSS was reduced in mice treated with *S. platensis*. After 5 days of DSS administration, the HE scores in P and R groups were 5.41 ± 0.73, and 4. 84 ± 0.81, respectively. Compared with the D group induced by DSS, mice oral gavage fermented, and non-fermented *S. platensis* reduced inflammation, with the R group showing the most significant decrease in histological scores (Figure-1. E).

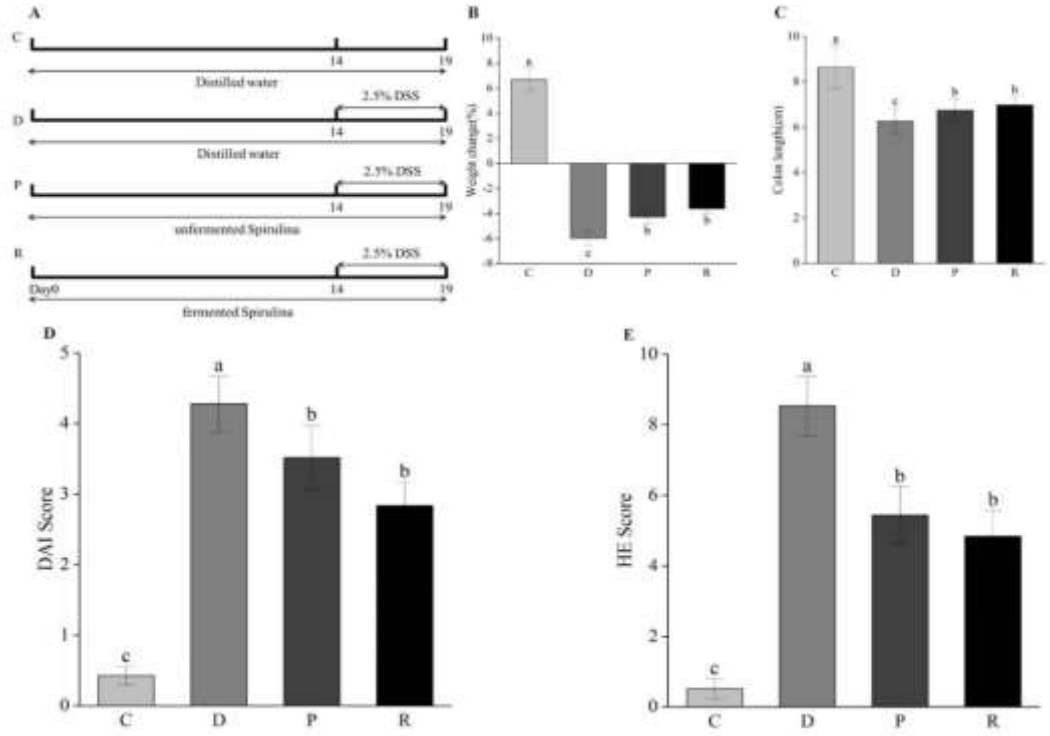

Figure-1. Effect of *S. platensis* on the colon tissues of dextran sodium sulfate (DSS)-induced UC in mice. (a) Evaluation of fermented *S. platensis's* effect on colon tissue in DSS-induced UC mice (n=8); Experimental procedures and animal grouping; (b) body weight change; (c) DAI scores; (d) colon length; (e) Hematoxylin-eosin score. The different lowercase letters indicate significant differences (p<0.05).

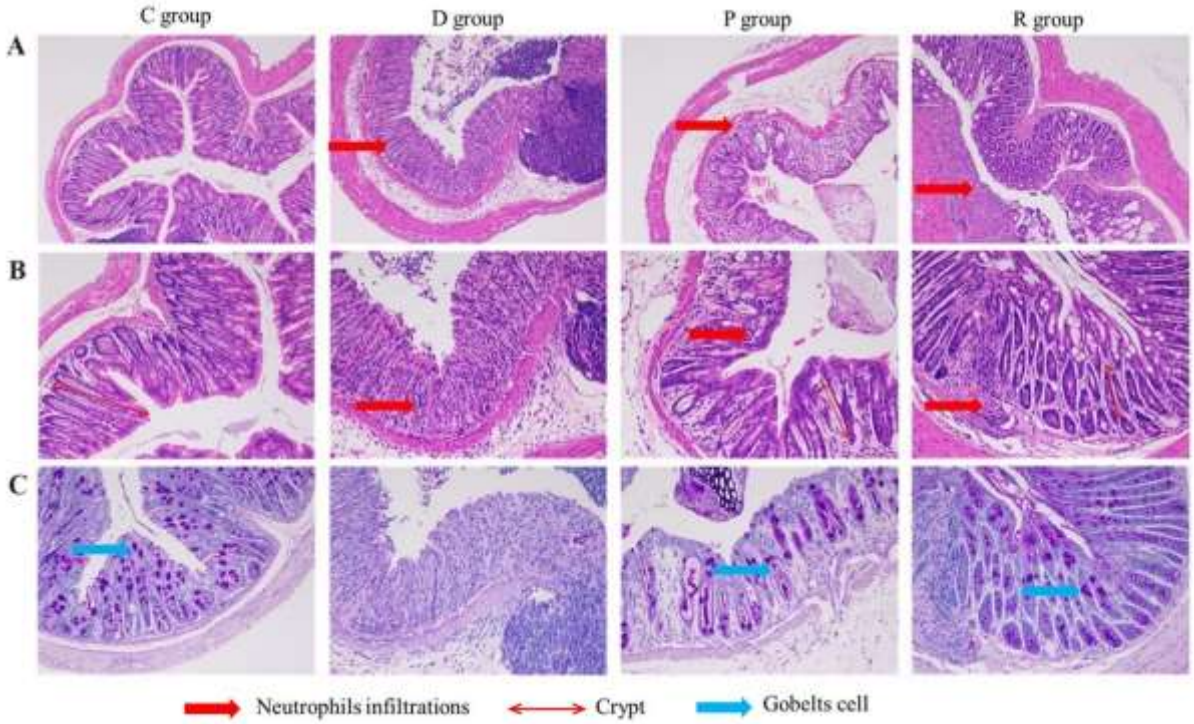

Figure-2. Histopathological analysis of colon segments in mice. after staining the tissue with Hematoxylin-eosin (a) 100×magnifcation, (b) ×200×magnifcation; (c) AB-PAS staining was used to observe goblet cells.

Effects of *Spirulina platensis* on the gut microbiota diversity in ulcerative colitis mice

To elucidate the possible contribution of microbiota to the impacts of fermented and non-fermented *S. platensis*, the gut bacteria of the mice were examined by through 16S rRNA analysis. A total of 2,751,485 reads were obtained through splicing and quality control, resulting in 2,714,181 reads. Following additional filtering, each sample's average effective tags had 65915 readings and a 77% effective rate.

The diversity index group difference analysis was performed to evaluate changes in the organization of the microbial community. The variety and homogeneity of the gut microbiome were assessed using the alpha diversity analysis using the Chao 1 and Shannon indices. Intestinal flora in group D was much less than those in group C, according to the Chao1 and Shannon indices. After feeding *S. platensis* to mice, Chao1 and Shannon indices had a certain correction compared with group D. The top 35 genera, which were separated into 6 phyla, were chosen for heat map and cluster analysis based on species annotation and abundance data at the genus level (Figure-3). Firmicutes and Bacteroidetes dominated the gut microbiota at the phylum level in each group (Figure-4. A). At the genus level, group R had the largest relative abundance of *Lachnospiraceae_NK4A136_group*, which accounted for 15.42% of the group's total bacterial population. The other dominant groups were *Odoribacter* (4.89%), *Alistipes* (3.18%), *Prevotellaceae_UCG-001* (3.09%). Comparison of the microbiota in the caecum of the four groups showed that beneficial microorganisms such as *Lachnospirace_NK4A136_group* and *Prevotellaceae_UCG-001* decreased first and then increased in groups C, D, P and R. Odoribacter's relative abundance in group D was much higher than it was in group C, but it was down-regulated in group R. Notably, group R had a significantly higher relative abundance of the *Lachnospiraceae_NK4A136_group* when compared to D and P groups ($p < 0.05$, Figure-4. B).

The research conducted a PCoA analysis using Weighted Unifrac distance and found evident clustering among the treatment groups, suggesting that groups C and R had distinct bacterial communities (Figure-4. C). LEfSe used linear discriminant analysis (LDA) scores to determine which species had a significant effect on the differences between groups. The *S platensis* group consisted of a total of 12 taxa, with five belonging to group R (*g_Lachnospiraceae_NK4A136_group*, *p_Proteobacteria*, *c_Alphaproteobacteria*, *o_Acetobacterales*, *f_Acetobacteraceae*), which was more prevalent compared to groups D and C. Notably, in group R *Lachnospiraceae_NK4A136_group* was the dominant group and significantly higher than in group D (Figure-4. D).

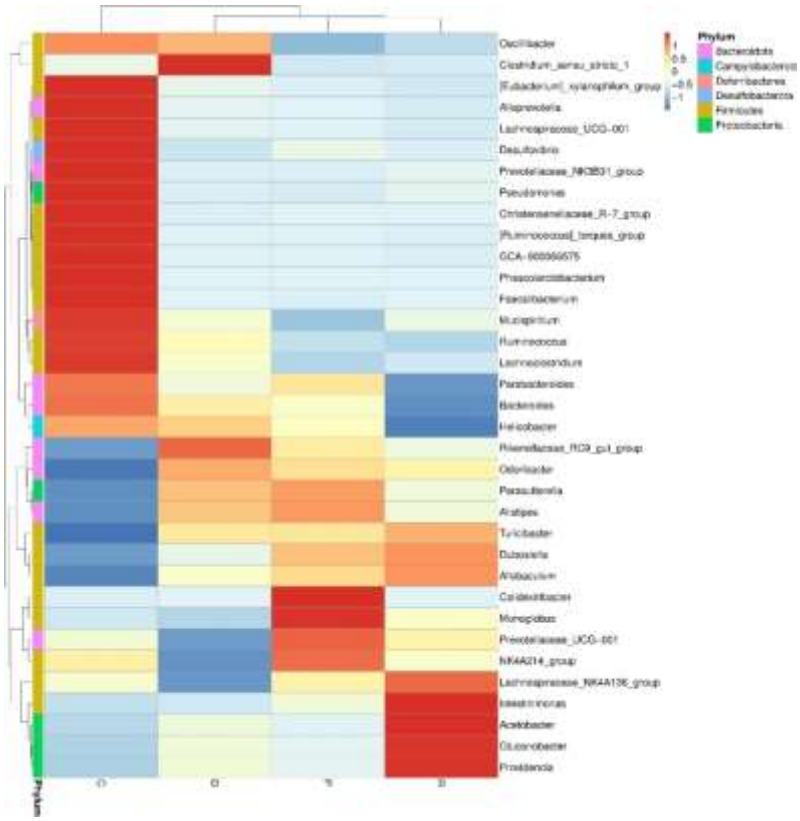

Figure-3. Species abundance cluster map at the genus level.

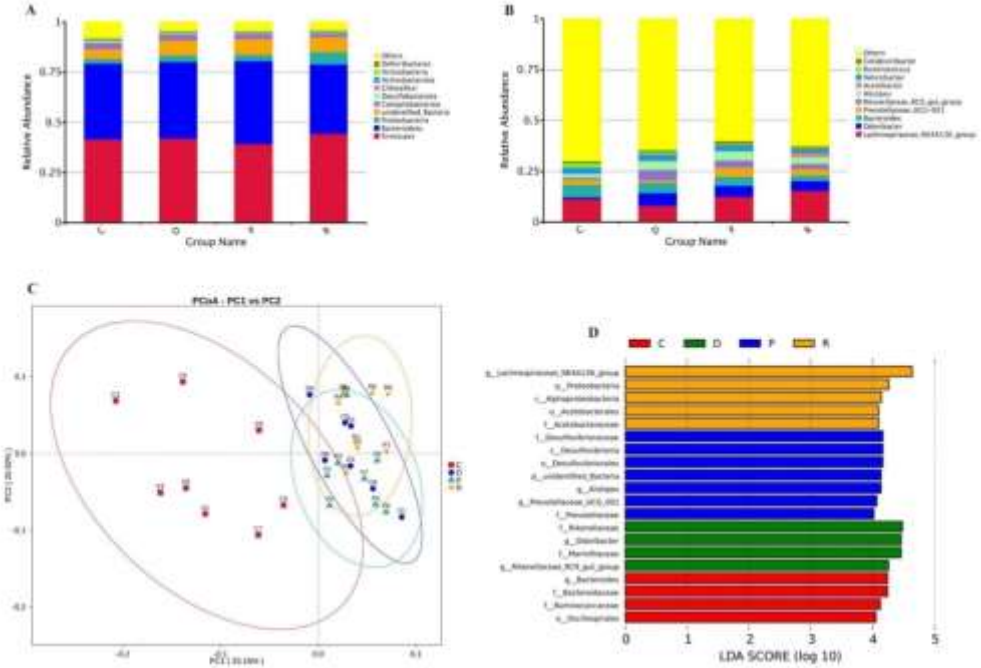

Figure-4. Comparison of the microbial community in different *S. platensis* fed mice. (a) Differences in the gut microbiota of mice Chao1 index; (b) Shannon index; c Phylum level analysis of the microbiota in mice; (d) genus level analysis of the microbiota in mice; (e) Analysis of principal coordinates (PCoA) for DSS-induced UC mice with different treatments; (f) Distribution histogram based on LDA.

Fermented *Spirulina platensis* treatment affected MyD88/TLR4 expression in ulcerative colitis mice

To determine the therapeutic effect of *S. Platensis* on UC through pro-inflammatory cytokines and the MyD88/TLR4 signaling pathway, the measured the relative expression levels of TNF-α, IL-6, IL-1β, TRL4, MyD88, and NF-κB in colon tissue. Compared with group C, the relative mRNA expression of group D was significantly increased, several genes including *TNF-α* (4.5-fold), *IL-6* (22.5-fold), *IL-1β* (7.7-fold), *TLR4* (7.7-fold), *MyD88* (4.3-fold), and NF-κB (1.2-fold) were upregulated. In response to fermented *S. platensis* administration, expression of *TNF-α*, *IL-6*, *IL-1β*, *MyD88*, *TLR4*, and *NF-κB* genes were decreased ($p < 0.05$), where *MyD88* and *TLR4* genes were downregulated by 3.4- and 2.1-fold, respectively. The DSS-induced mice had active MyD88/TLR4 signaling in comparison to the control group. Fermented *S. platensis* suppressed MyD88/TLR4 gene expression more effectively in Group R compared to Group P than non-fermented *S. platensis*. The results showed, indicating that fermenting *S. platensis* could better inhibit MyD88 and TLR4 in colon tissue to prevent UC.

Effects of fermented *Spirulina platensis* on intestinal barrier in colon

It was also examined that how non-fermented and fermented *S. platensis* affect the expression of genes related to the integrity of the intestines was researched. They found that the mRNA levels of *Claudin-1*, *Occludin*, and *ZO-2* in the colon of mice with UC were significantly lower compared to healthy mice. Notably, treatments with *S. platensis* in DSS mice resulted in a significant increase in the expression levels of *Claudin-1* ($p < 0.05$), *Occludin* ($p < 0.05$), and *ZO-2* ($p < 0.05$) genes. Specifically, both the P and R groups of *S platensis* showed a significant increase in the expression levels of these genes compared to the D group (Figure-5).

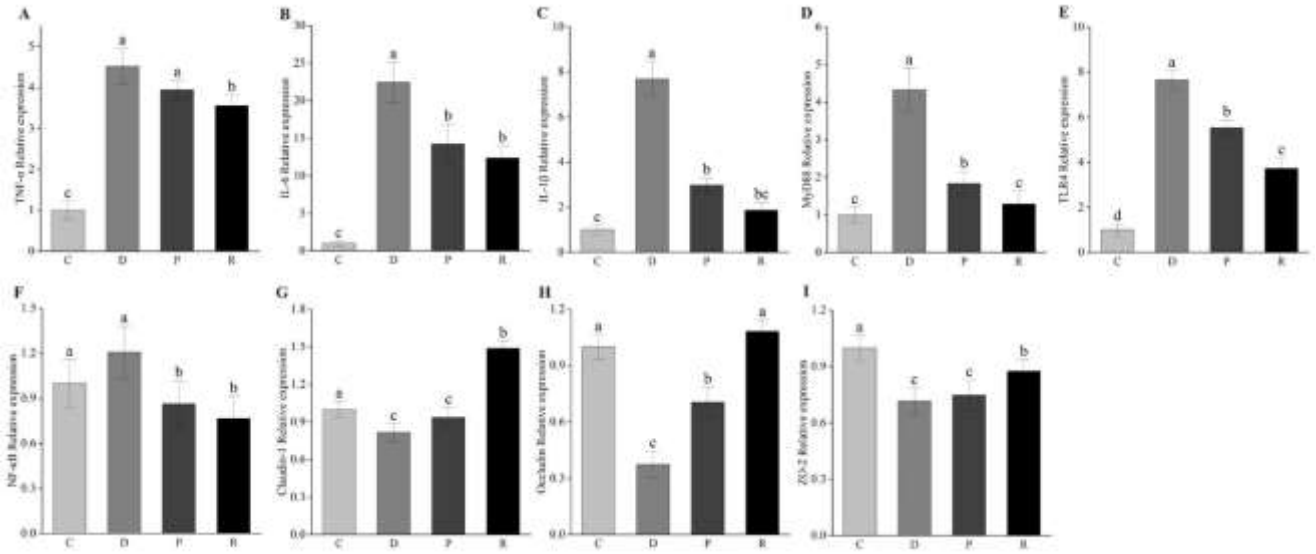

Figure-5. Relative mRNA expression levels. (a) *TNF-α*, (b) *IL-6*, (c) *IL-1β*, (d) *MyD88*, (e) *NF-κB*, (f) *TLR4*, (g) *Claudin-1*, (h) *Occludin*, and (i) *ZO-2* in colon tissue.

Discussion

Animal studies have proven that *S. platensis* has a functional role in preventing various diseases. Protein, amino acids, polysaccharides, and other beneficial compounds are all plentiful in *S. platensis* (AlFadhly et al., 2022). Having been used safely as a food supplement for a very long time, *S. platensis* is a promising addition to a healthy diet. According to studies (Galal et al., 2019; Wang et al., 2022), *S. platensis* formulations exhibit neuroprotective, anti-ulcer and anti-inflammatory properties. *S. platensis* can alleviate symptoms caused by UC, such as weight loss, bloody diarrhea, intestinal damage, and shortened colon (Wang et al., 2022). In the current investigation, mice with DSS-induced colitis had pathological colonic lesions that were alleviated by *S. platensis*. Along with lowering DAI and histology scores, it also decreased body weight and intestinal length. Histological analysis using HE and AB-PAS staining revealed disrupted intestinal epithelial mucosa, crypt loss, decreased goblet cells, and infiltration of inflammatory cells in the DSS-induced model group. However, when mice were given both fermented and non-fermented *S. platensis*, these symptoms were reduced.

There are two potential mechanisms by which *S. platensis* prevents UC. The first mechanism by which *S. platensis* prevents UC is indirect regulation of the gut microbiota. According to several studies, gut flora imbalances are related to UC (Vindigni et al., 2016). Probiotics, the normal composition of the intestinal microbiota, and the restoration of intestinal microbiota tolerance can also have an anti-inflammatory effect in IBD (Lammers et al., 2002). In this experiment, Firmicutes and Bacteroidetes made up most of the gut microflora. When the model mice were given a treatment of fermentative *S. platensis*, the *Lachnospiraceae_NK4A136_group* dramatically increased. The primary generator of short chain fatty acids, which are present in the intestinal cavity from birth, Lachnospira is at the center of the gut microbiome (Vacca et al., 2020). The short chain fatty acid butyric acid helps maintain cell integrity and control intestinal inflammation (Mishiro et al., 2013). Lachnospiraceae_NK4A136 can produce butyrate (Antonissen et al., 2016), while butyrate protects the intestinal mucosa and modulates the immune system, thereby reducing inflammation. Lachnospiraceae_NK4A136 reduction may compromise intestinal mucosal integrity and lead to intestinal inflammation. Research has demonstrated that *S. platensis* may significantly upregulate Lachnospiraceae in UC-induced mice, demonstrating the close relationship between beneficial bacteria and the prevention of colitis. Notably, fermented *S. platensis* enhanced the integrity of the mucosal barrier, increased relative abundance of the *Lachnospiraceae_NK4A136_group*, and effectively mitigated colitis. The utilization of *S. platensis* could potentially have a therapeutic impact on UC by regulating the gut microbiota, a crucial factor in governing immune response and metabolic functions.

The second mechanism is to inhibit the development of colitis by controlling inflammatory factors and regulating intestinal barrier function. TNF-a, IL-6 and IL-1β are proinflammatory cytokines that are crucial in the pathogenesis of UC. As UC colon macrophages are activated, this results in increased secretion of TNF-a, IL-6 and IL-1β (Liu et al., 2021). DSS increases IL-1 production, and the pathophysiology of colitis is significantly influenced by the elevation of IL-1β expression (Wang et al., 2021). These inflammatory factors severely disrupt intestinal barrier function, leading to intestinal epithelial cell damage and ultimately UC. Inflammation and the intestinal mucosal barrier are crucial for the emergence and development of UC (Wargo, 2020). The relative expression of proinflammatory cytokines increases during the development of UC. According to the experimental results, fermented *S. platensis* may have significantly lowered the relative expression levels of *TNF-a*, *IL-6*, and *IL-1* genes in the DSS model group.

The fermented *S. platensis* extract has a preventive effect on DSS-induced UC by detecting colonic tissue inflammation-related signaling pathway genes. The activation of the MyD88-dependent pathway triggers the NF-κB pathway, resulting in the overexpression of downstream inflammatory genes such as *TNF-a*, *IL-6*, and *IL-1β* (Xia et al., 2020). Innate immune system activation by Toll-like receptors (TLRs) results in an inflammatory response. (Alfonso-Loeches et al., 2010). Currently, within the TLR family known in humans, TLR1 to TLR10 are genetically expressed and may be receptor molecules involved in immune function. TLR plays a crucial role in the innate immune system. When TLRs are stimulated, they can influence the production and release of inflammatory factors, relay information about the inflammatory response, and cause inflammatory harm through a signaling

pathway that is reliant on MyD88 (Cheng et al., 2018). The findings demonstrated that fermented *S. platensis* might restrict the production of inflammatory factors to lessen UC by altering the signal pathway mediated by MyD88/TLR4, while reducing the relative expression of MyD88 and TLR4.

The intestinal epithelial cells' tight junction (TJ) core proteins Claudin-1, Occludin and ZO-2 were examined to further support *S. platensis* fermentum's protective activity against DSS. The intestinal barrier can be strengthened by TJ proteins, which are essential for blocking the entry of infections or hazardous elements from the environment (Li et al., 2020). Occludin is crucial for barrier integrity and TJ formation, while Claudin-1, a full membrane protein, contributes to the tight junction protein's structure (Sun et al., 2020). The intestinal mucosal barrier's integrity is preserved by colonic TJ proteins. In this research, it was discovered that *S. platensis* predominantly prevents colitis by lowering inflammatory cytokine release and decreasing intestinal epithelial TJ protein depletion. The above experiments can confirm that the fermented *S. platensis* can reduce the inflammation of UC by regulating intestinal flora and MyD88/TLR4 signaling pathway.

Conclusion

In this study, it was found that Spirulina can alleviate UC, and its beneficial effect may be due to the production after fermentation. Fermented *S. platensis* has anti-inflammatory and intestinal barrier enhancing effects, while also upregulating beneficial microorganisms such as *Lachnospiraceae_NK4A136_group* and *Prevotellaceae_UCG-001*. To increase the possibility for *S. platensis* to be used as a functional food, this study was intended to improve the health advantages of *S. platensis* through fermentation. Based on these findings, it is recommended that further studies should be conducted to explore the specific bioactive compounds and mechanisms involved in the anti-inflammatory and gut health effects of fermented *S. platensis*. Moreover, efforts should be made to develop innovative food products or supplements incorporating fermented *S. platensis* for potential therapeutic applications in managing UC and promoting overall gastrointestinal well-being.

Disclaimer: None.

Conflict of Interest: None.

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Contribution of Authors

Wang N: Designed the experiment and revised the first draft.

Xiang Y: Performed the experiment and wrote the first draft.

Ma Y: Participated in designing study and coordination.

Zhang P, Zhou X & Zhu H: Assisted in the completion of the experiment.

Zhang Z & Li Z: Analyzed the raw data.

Xiao X & He M: Collected the experimental material.

Mehmood MA & Zhu H: Supervised the project and contributed to writing and editing of the manuscript.

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