Different Drugs in Modulating Gut Microbiome of Colitis Mice

Xinjun Chen¹, Pingshun Song²,³, Pingrong Yang²,³, Yaping Jing⁴,⁵,⁶, Chenzhi Li⁴,⁵,⁶, Junshu Wei⁴,⁵,⁶, Anping Li²,³, Xiao Ma²,³, Tuanjie Che⁵,* and Chunjiang Zhang⁴,⁵,⁶,*

¹Laboratory of Pathogenic Biology and Immunology, Hainan Medical University, China
²Gansu Institute of Drug Control, China
³Gansu Tibetan Herbal Medicine Engineering Laboratory of Testing and Detection, China
⁴School of Life Sciences, Lanzhou University, China
⁵Gansu Key Laboratory of Functional Genomics and Molecular Diagnosis, China
⁶Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution, Lanzhou University, China

Submission: February 22, 2019; Published: March 11, 2019

*Corresponding author: Chunjiang Zhang, School of Life Sciences, Lanzhou University, Tianshui Road 222, Lanzhou, Gansu province, China

Tuanjie Che, Gansu Key Laboratory of Functional Genomics and Molecular Diagnosis, Lanzhou 730000, China

Abstract

Background: The gut microbiome plays a key role in Inflammatory Bowel Disease (IBD), which has spurred the development of novel therapeutics aimed at restoring microbial community structure. Saccharomyces boulardii is a new probiotic that has not been commonly used to treat IBD. Although the traditional Chinese herbal medicine Sijunzi Tang (SJZT) decoction has been widely used to alleviate the symptoms of ulcerative colitis (UC), its underlying mechanism remains unknown.

Methods: The efficacy of four drugs named S. boulardii, SJZT, Dangshen (Codonopsis pilosula) polysaccharide or S. boulardii in combination with Dangshen polysaccharide were tested during treatment with Dextran Sulphate Sodium (DSS)-induced mice colitis. Weight loss, colon shortening, and ameliorated histological damage as well as DSS-induced dysbiosis in mice. Beneficial bacteria—especially Short Chain Fatty Acid (SCFA)-producing genera were remarkably enriched in the treated mice. Roseburia, Anaerovorax and Lactobacillus were selectively enriched by S. boulardii, while Butyribrio, Quinella and Anaerotruncus were selectively enriched by SJZT. Most notably, Dangshen polysaccharide selectively enriched Bifidobacterium, Arthrobacter, Akkermansia, Anaerovorax, Roseburia, Prevotella, Dialister, Megamonas, Faecalibacterium and Subdoligranulum.

Results: All four drugs profoundly inhibited weight loss, colon shortening, and ameliorated histological damage as well as DSS-induced dysbiosis in mice. Beneficial bacteria—especially Short Chain Fatty Acid (SCFA)-producing genera were remarkably enriched in the treated mice. Roseburia, Anaerovorax and Lactobacillus were selectively enriched by S. boulardii, while Butyribrio, Quinella and Anaerotruncus were selectively enriched by SJZT. Most notably, Dangshen polysaccharide selectively enriched Bifidobacterium, Arthrobacter, Akkermansia, Anaerovorax, Roseburia, Prevotella, Dialister, Megamonas, Faecalibacterium and Subdoligranulum.

Conclusion: S. boulardii, Sijunzi decoction, and its main component, Dangshen polysaccharide, are effective in alleviating DSS-induced colitis. Modulating the gut microbiome may be the common mechanism whereby these compounds improve colonic health. Furthermore, Dangshen polysaccharide is a powerful prebiotic that selectively promotes probiotic growth, especially SCFA-producing bacteria.

Keywords: Saccharomyces boulardii; Polysaccharide; Gut microbiome; Ulcerative colitis

Abbreviations: IBD: Inflammatory Bowel Disease; UC: Ulcerative Colitis; CD: Crohn’s Disease; CAMS: Complementary and Alternative Medicines; SJZT: Sijunzi Tang; DSS: Dextran Sulfate Sodium; OTUS: Operational Taxonomic Units

Introduction

Inflammatory Bowel Disease (IBD) is the term used to describe chronic intestinal inflammatory conditions, including Ulcerative Colitis (UC) and Crohn’s Disease (CD). While IBD is especially common among western populations, the incidence of IBD in China has increased rapidly in recent years because of the widely adopted westernized lifestyle [1]. The etiology and pathogenesis of IBD are not fully understood, and effective therapeutics are lacking. A healthy, balanced intestinal microbiome plays a key role in maintaining whole-body homeostasis. Significant alterations to the gut microbiome, referred to as dysbiosis, are present in most chronic inflammatory disease. Intestinal dysbiosis is a defining feature of IBD, with some even considering it to be causative. Indeed, manipulating the gut microbiome has been shown to greatly alleviate the symptoms of IBD [2]. Pro- and prebiotics are two
widely adopted therapeutic strategies that are also great examples of Complementary and Alternative Medicines (CAMs) being used in clinic. Traditional Chinese medicines have also shown remarkable curative effects as CAMs in IBD patients. SijuNZi Tang (SJZT) decoction is a classical herbal formula composed of Danshen [Codonopsis pilosula (Franch.) Nannf], Baizu (Atractylodes macrocephalae Koidz), Fuling [Poria cocos(Schw.) Wolf] and Gancao (Glycyrrhiza uralensis Fisch.). Related researches claimed that SJZT were efficiently in treating UC patients in China. Its mechanism of action however remains unknown. Recent study suggests that Chinese herbal medicines could potentially be used to modulate the intestinal microbiome. Treatment with Gegen QInlian decoction for example promotes the growth of beneficial bacteria, especially Faecalibacterium prausnitzii, to alleviate type II diabetes [3]. We therefore hypothesized that SJZT decoction could also relieve IBD symptoms by altering the gut microbiome.

Furthermore, based on the theory of Chinese medicine, water-soluble polysaccharides are the key compounds in decoctions. Danshen polysaccharide, a macromolecular substance that is hard to digest, consists of monosaccharides like D-galactose, D-rhamnose and D-arabinose and their derivatives, a backbone of (1→3)-linked-β-D-galactopyranosyl, (1→2,3)-linked-β-D-galacto- pyranosyl and (1→3)-linked-α-D-rhamnopiranoyl residues [4]. Based on the fact that herbal polysaccharides such as Lentinula edodes [5] and Ganoderma lucidum [6] are very effective at modulating the gut microbiome, we hypothesized that non-digestible Danshen polysaccharide might favor the growth of specific, beneficial microbes, i.e. act as a prebiotic. The fungi Saccharomyces boulardii is a new probiotic that is resistant to antibiotics and low pH and grows very well at 37°C [7]. Although widely used in preventing and treating diarrhea, it is rarely used to treat IBD. Most studies on S. boulardii in animal colitis models focus mainly on immune-related mechanisms [8-9]. Recently administration of S. boulardii has been reported to exert its therapeutic effects by altering the gut microbiome in obese and type 2 diabetic mice [10]. Whether S. boulardii can change microbial community structure to relieve IBD symptoms remains to be seen. The aim of this study was to determine the effect of SijuNZi decoction, Danshen polysaccharides and Saccharomyces boulardii on microbial diversity and composition during the treatment of DSS-induced acute colitis in mice, using high-throughput 16S-based sequencing.

Material and Methods

S. Boulardii, sijuNZi decoction and danshen polysaccharide preparation

S. boulardii sachets were purchased from Laboratoires BIO-CODEX (Bio flor®, France). In order to remove any contaminants a pure S. boulardii culture was obtained by inoculating the lyophilized yeast in YPD broth with shaking at 37°C for 18 hours; S. boulardii was then harvested and concentrated tenfold to 10⁵ CFU/ml. The yeast was centrifuged and re-suspended in PBS solution for oral administration. The four dried raw materials used to prepare the SJZT decoction (Danshen, Baizu, Fuling, and Gancao) were all purchased from Xukang pharmaceutical co., ltd (Lanzhou, China). The quality were all met the requirement of Chinese Pharmacopoeia (2015 Version). The voucher specimens number were DS-20150721, BZ-20150701, FL-20150718, GC-20150726 respectively. All specimens were identified by senior engineer Pingrong Yang and deposited in the Herbarium of Chinese patent medicine test laboratory of Gansu Institute of Drug Control, Lanzhou, China. Four materials mixed in a ratio of 9:9:9:6 and herbs were decocted twice with boiling water (10 times of the material’s weight) for 60 min then filtered, pooled and concentrated to 1g/ml. Mice were orally administered 10ml/kg (convert from clinical dose). Quality control of the SJZT decoction was performed by high performance liquid chromatography (HPLC) analysis of glycyrrhizic acid and liquiritin from Gancao, according to an established method [11]. Briefly, chromatographic separation was performed on a Zobax SB-C18 column (4.6mm×150mm, 5µm); the mobile phase consisted of methyl alcohol -0.5% acetic acid; the flow rate was 1ml/min; the column temperature was 30°C; liquiritin was detected at 276nm, and glycyrrhizic acid at 250nm. The crude polysaccharide extract was obtained from Danshen by water decoction and alcohol precipitation. Total carbohydrate content was then determined by phenol-sulfuric acid assay based on a standard curve determined from D-glucose at six different concentrations (µg/ml).

Animal experiments and treatment design

The present animal study was conducted according to protocols approved by the Ethics Committee of Animal Experiments of Lanzhou University. Female C57BL/6 mice were purchased from the Gansu university of Chinese medicine. After acclimatization for one week, mice were housed individually in plastic cages at constant temperature (21±2°C), with an alternating 12h light/dark cycle, and animal chow and water were provided ad libitum. mice were divided into six groups and treatments lasted for 30 days

A. Danshen polysaccharide (dissolved in PBS solution, 300mg/kg)

B. Saccharomyces boulardii (re-suspended in PBS to a concentration 10⁵ CFU/ml)

C. Danshen + S. boulardii, (Danshen polysaccharide dissolved in an S. boulardii suspension)

D. SijuNZi (SJZT decoction (1g/ml) at 10ml/kg; dose selection was based on that used in clinic patients)

E. Colitis (DSS, PBS solution)

F. Normal (no DSS, PBS solution)

After 21 days of treatment, acute colitis was induced by administering 2.5% DSS solution to groups A-E for 7 days, followed by distilled water for two days. All drugs (groups A-D) were adminis-
tered throughout the study (see experimental design in Figures). Fresh fecal samples were collected immediately upon defecation on days 1(blank) and 21(before colitis induction), placed in sterile centrifuge tubes and stored at-80°C. On day 30 (post-colitis) mice were sacrificed and intestinal fecal samples were collected from the colon. To evaluate the therapeutic effects of the four different treatments, mice body weight was monitored daily, and following a final assessment of colon length, inflamed colon tissues were treated with formalin and stained with hematoxylin and eosin. Inflammation was graded according to an established system [12].

DNA isolation and illumina pyrosequencing of the V3 16S rRNA gene region

Microbial DNA was extracted from fecal samples using the Qian Gen#Stool DNA kit (Beijing, China) according to the manufacturer’s instructions. DNA concentration and purity were quantified on a Nano Drop 1000 spectrophotometer (Thermo Scientific) and DNA integrity was confirmed by agarose gel electrophoresis. Universal primers were selected to span the V3-V4 hypervariable region of the 16S rRNA gene: 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). In brief, PCRs were performed in 20μl reactions containing 5x Fast Pfu Buffer (4μl), 10 ng template DNA, 2μl dNTPs, 0.8μl forward and reverse primer (5μl), 0.4μl Fast Pfu Polymerase and ddH₂O to 20μl. The PCR parameters were: 95°C for 3 min, 27 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 45 seconds, followed by a final extension of 72°C for 10 min. PCR products were detected on a 2% agarose gel and quantified by Quanti Fluor™-ST and all samples were pooled at mean concentrations. Library preparation and pyrosequencing were performed on an Illumina Mi Seq PE 300 platform by Shanghai Majorbio Technology Company.

Bioinformatic and statistical analyses

High quality sequences were assigned to each sample (demultiplexed) and clustered as Operational Taxonomic Units (OTUs) at a threshold of ≥ 97% similarity. OTU abundance data was used to calculate alpha diversity (Shannon index), richness and rarefaction estimates using Mothur [13]. Community structure was assessed using Uni Frac Principal Coordinate Analysis (PCoA), Nonmetric Multidimensional Scaling (NMDS) based on Bray-Curtis distance, and hierarchical clustering, using R packages vegan. Heatmaps were created using Mothur and R package heatmap [14] to visualize treatment-specific changes in the microbiome. All results are presented as the mean (±SE). Group differences were assessed by ANOVA and the Mann-Whitney test in SPSS19.0. P-values < 0.05 were considered significant.

Results

Preparation of probiotic, Sijunzi decoction, Dansheng polysaccharide

An S. boulardii culture was prepared from a commercial product and typical morphologic characteristics of yeast was observed (Figure 1A). Dansheng polysaccharide constituted 38.7% of the crude, brown Dansheng polysaccharide powder (Figure 1B). HPLC-based quality control of the Sijunzi decoction was performed using glycyrrhizic acid and liquiritin as indicator components (Figure 1C & 1D). The content of glycyrrhizic acid in SJZT (1g/ml) was 13.5μg/ml while that of liquiritin was 5.8μg/ml.

Different treatments alleviate DSS-induced colitis in mice

An outline of the animal experiments and treatment design is shown in Figure 2. Before induction of colitis, four different treatments were administered orally for three weeks. Body weight was measured daily (from day 1) and all groups showed a steady increase in body weight. However, Dansheng and especially SJZT attenuated weight gain compared vs normal group (SJZT P<0.05). By day 20, body weight was increased in the S. boulardii and D+S groups (Figure 3A). After induction of colitis (2.5% DSS solution for 7 days) the four treatments were continued administered until the end of study. All treatments significantly inhibited weight loss by day 30 relative to the DSS-only group (P<0.05), especially S. boulardii and D+S significantly (P<0.001) (Figure 3B). Total colon length decreased in all DSS-treated mice relative to controls, while treatment groups A-D showed a slight increase in colon length relative to the DSS-only group (P<0.05, Figure 3C & 3D).
Figure 2: Outline of experimental design. DSS solution was administered for 7 days to induce acute colitis.

Figure 3: Different treatments alleviate DSS-induced colitis in mice.
A): Daily body weight increase (relative to controls) before DSS administration, *P< 0.05. B): Daily body weight loss post-colitis treatment, *P< 0.05. C): ***P< 0.001 vs DSS, ###P< 0.001 vs controls. D): Colon length after each treatment relative to DSS administration, *P< 0.05, # #P< 0.01. Differences were assessed by ANOVA (n=6/group).

Figure 4: Different treatments attenuated the pathology associated with DSS-induced colitis.
A): Histological data summary. B): Representative histological images (magnification 200×) are shown Differences were assessed by ANOVA, *P<0.05, **P< 0.01, # #P< 0.01.
The degree of colonic inflammation was further confirmed by histological analysis. While control mice had intact surface epithelia, stroma, cryptal glands, and submucosae, DSS-treated mice showed surface epithelium damage, cryptal gland disruption and infiltration of lymphocytes. All treatment groups had significantly lower histology scores than DSS-treated mice (P<0.05). In the S. boulardii and Dangshen + S. boulardii groups, surface epithelium and cryptal glands were more intact than in the Dangshen and SJZT groups (Figure 4A). There was no obvious infiltration of inflammatory cells in any of the treated groups, especially not in the D+S group (Figure 4B).

**Gut microbiome structure in response to different treatments in DSS-induced colitis**

---

**Figure 5:** Diversity and richness of the gut microbiome in different treatments groups

A): Rarefaction curves on days 1, 21 and 30, respectively. B-C): OTU estimates of each group on day 21 and day 30, respectively (*P< 0.05 vs normal, where normal values represent the data from all mice at day 1). D): Shannon indices on days 1, 21 and 30, respectively. E-F): Shannon index of each group before colitis (*P< 0.05 vs controls) and after colitis (*P< 0.05 vs DSS).
Sequencing the V3-V4 16S hypervariable region produced 2387053 high quality sequences from fecal samples collected on days 1, 21 and 30, with a mean of 43400±1901 sequences per sample. The mean number of OTUs per sample was 373. Rarefaction and diversity analysis show that the majority of the gut microbial diversity was captured at the current sequencing depth, with few new OTUs anticipated at increased sequencing depths (Figure 5A). All treatments increased gut microbiome richness in mice by day 21 relative to baseline levels at day 1 (Figure 5B), especially D+S (P<0.01), Dangshen and S. boulardii (P<0.05), and to a lesser extent SJZT (P>0.05, Figure 5C). Post-treatment (day 30), all groups had slightly more unique OTUs than DSS-only mice, but this was not significant (Figure 5D). In terms of diversity, the two polysaccharide-rich groups (Dangshen, D+S) had significantly higher Shannon indices than control mice before colitis (Figure 5E P<0.05), while the SJZT group appeared unchanged. Acute colitis rapidly decreased diversity however, the four treatments all significantly increased diversity (Figure 5F, P<0.05). The overall gut microbiome structure in response to the various treatments were further analyzed by unifrac distance-based PCoA, which revealed that control mice had distinct microbiomes compared to all the other groups. All four treatments, except for a few outlier samples, modulated the colitis-associated microbiome, and the different treatments tended to form separate clusters (Figure 6A). NMDS analysis (Bray-Curtis distance) supported the PCoA-based result and further showed that bacterial communities in the Dangshen group are more homogenous than other groups, followed by the SJZT group (Figure 6B). These findings were confirmed by hierarchical clustering analysis and suggest that the four treatments alter the microbiome in the inflamed colon (Figure 6C).

**Gut microbiome composition in response to different treatments in DSS-induced colitis**

At baseline (day 1), Firmicutes, Bacteroidetes and Proteobacteria were the three most abundant phyla, with Firmicutes and Bacteroidetes accounting for about 91% of reads. Following treatment, both Dangshen (74.97% vs 79.35%) and Sijunzi (75.43% vs 79.82%) groups had increased proportions of Bacteroidetes. Meanwhile, Bacteroidetes were decreased in the S. boulardii (81.98% vs 65.48%) and D+S (75.48% vs 70.97%) groups. Firmicutes were less abundant in the Dangshen (13.10% vs 12.88%) and SJZT groups (16.86% vs 16.37%) and D+S (12.62% vs 16.52%) groups had an increased proportion of Firmicutes. Proteobacteria was only decreased in the Dangshen group (9.03% vs 6.92%), and increased in all three other groups, especially in the S. boulardii group, where it was increased more than three-fold (4.62% vs 17.14%, P<0.05). Verru-
comicrobia was significantly decreased (P<0.01) in all treatment groups (Figure 7A). DSS administration led to a remarkable decrease in Firmicutes (17.77% vs 2.36%, P<0.05) and Proteobacteria (5.18% vs 2.52%, P<0.05), with a significant increase in Bacteroidetes (59.65% vs 78.98%, P<0.05) relative to controls. These changes could however be mitigated by the treatments, especially by Danshen polysaccharide which increased the proportion of Firmicutes (group A: 31.54%, group C: 32.90% vs DSS-only: 17.77%, P<0.05) and decreased the proportion of Bacteroidetes (group A: 57.11%, group C: 61.66% vs DSS-only: 78.98%, P<0.05), Figure 7B. Relative to the DSS-only group, the Danshen group had a significantly higher proportion of Proteobacteria (9.65% vs 2.52%, P<0.05), while Verrucomicrobia was slightly increased by Danshen and SJZT (0.54%, 0.047% vs 0, P<0.05).

The Firmicutes to Bacteroidetes ratio (F/B) is used as an indicator of the gut microbial composition and was used to evaluate colitis remission and relapse. Post colitis treatment, F/B ratios decreased in the Danshen and SJZT groups and increased in the S. boulardii and D+S groups (Figure 7C). Induction of colitis (DSS-only) significantly decreased the F/B ratio relative to normal mice (0.55 vs 0.24, P<0.05), while all four preventative treatments increased the F/B ratio (Figure 7D), which was significant in the Danshen and D+S groups (P<0.05), where F/B ratios were close to that of controls (0.57, 0.56 vs 0.55, respectively). We next compared community differences at family level (Figure 8A & 8B), which again highlighted the modulating effect of preventative treatment on gut microbial composition. Relative to day 1, all four treatments decreased the proportion of S24-7, especially Danshen (53.27% vs 30.52%, P<0.05) and S. boulardii (44.37% vs 23.50%, P<0.05). Prevotellaceae was increased in all four treatment groups, and significantly so in the Danshen (16.27% vs 45.67%, P<0.05) and D+S (18.05% vs 42.84%, P<0.05) groups. S. boulardii promoted Helicobacteriaceae growth (3.54% vs 14.87%, P<0.05); SJZT decreased Rikenellaceae (5.37% vs 1.39%, P<0.05), Porphyromonadaceae (1.52% vs 0.72, P<0.05) and Bacteroidaceae (1.92% vs 0.84%, P<0.05); and D+S decreased Verrucomicrobiaceae (1.16% vs 0.001%, P<0.05). Compared with control mice, DSS-induced colitis led to a drastic decrease in S24-7 (35.07% vs 15.18%, P<0.05), Lactobacillaceae (4.98% vs 0.21%, P<0.05) and Helicobacteriaceae (3.17% vs 0.27%, P<0.05), while Prevotellaceae (19.27% vs 50.37%, P<0.05) and Bacteroidaceae (1.34% vs 8.67%, P<0.05) were obviously increased. In the treatment groups, S24-7 recovered in the SJZT group compared vs DSS-only (15.18% vs 30.25%, P<0.05); Prevotellaceae was inhibited by all four treatments, especially by Danshen and SJZT (P<0.05), with Prevotellaceae levels returning to baseline in the SJZT group. Verrucomicrobiaceae was restored in the Danshen and SJZT groups, while Danshen and S. boulardii increased Helicobacteriaceae abundance compared vs DSS-only (5.23%, 2.22% vs 0, P<0.05).
Specific treatment-associated taxa in colitic mice

To further compare treatment-specific changes to gut microbiota, the top 60 most abundant genera (omitting a few rarely reported genera) were selected for comparison between groups (Figure 9). Mice with DSS-induced acute colitis (Group E) showed signs of dysbiosis, marked by a significant decrease in beneficial bacteria and an increase in pathogenic bacteria. Lactobacillus (5.07% vs 0.17%, *P*<0.05), Bacillus (0.38% vs 0, *P*<0.05) and Lactococcus (0.32% vs 0, *P*<0.05) were all decreased in DSS-only vs control mice, while potentially harmful IBD-associated bacteria including Bacteroides (1.37% vs 8.97%, *P*<0.05), Paraprevotella (0.16% vs 1.78%, *P*<0.05), Escherichia_Shigella (0.03% vs 0.13%, *P*<0.05), and Alistipes (0.67% vs 2.40%, *P*<0.05) were all notably increased in DSS-only treated mice. Preventative treatment could however alter colitis-associated dysbiosis. Lactobacillus, a commonly used probiotic, was significantly increased in the two *S. boulardii*-containing groups (*P*<0.05) yet was only slightly increased in the Dangshen and SJZT groups relative to the DSS-only group, but still far from the levels in the normal group. *Bifidobacteriaceae_ unclassified*, *Bifidobacterium* and a new probiotic, Arthrobacter, were all significantly increased in the Dangshen group, and increased (without significance) in the D+S group. *Bacillus* and *Lactococcus* could not however be restored in any of the preventative treatment groups. *Akkermansia* - a new beneficial microbe that plays a key role in combating metabolic disorders [15] - was significantly increased in SJZT and Dangshen groups (Table 1). Group differences in potentially harmful bacteria are summarized in Table 1.
(Table 2). All preventative treatments led to a decrease in the relative abundance of Paraprevotella, Parasutterella, and Prevotello-
acael_uncultured; Bacteroides was significantly decreased only in the Dangshen group (P<0.05); Escherichia,Shigella was significantly
decreased in all treatment groups except SJZT (P<0.05); Desulfo-
vibrio was decreased in the S. boulardii and SJZT groups; Interest-
ingly, there were significant increases in certain SCFA-producing
bacteria, with 9 of the 13 identified genera significantly enriched
in at least one treatment group relative to the DSS-only group (D)
(Table3); Butyrivibrio, Quinella, and Anaerotruncus were signifi-
cantly enriched in the SJZT group (P<0.05). Allobaculum Blau-
tia and Odoribacter were marginally increased in all treatment
groups (P=0.05); Faecalibacterium, Megamonas, Prevotella, Sub-
doligranulum and Dialister were increased in the Dangshen and
D+S groups, but not in the S. boulardii-only group, which suggests
that Dangshen polysaccharide (and not S. boulardii) may be mitig-
egating these effects; Roseburia was markedly increased in all treat-
ment groups, especially in the Dangshen and S. boulardii groups,
which also promoted Anaerovorax growth.

**Table 2:** The relative abundance of representative harmful bacterial genera in different groups. Difference were assessed by Kruskal-Wallis test followed by the Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Genus name</th>
<th>Relative_abundance (%)</th>
<th>Kruskal wallis test</th>
<th>P value_Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F% E% A% B% C% D%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alitipes</td>
<td>0.67 2.4 1.7 5.2 1.2 3.2</td>
<td>0.059</td>
<td>0.043 0.386 0.564 0.77 0.386</td>
</tr>
<tr>
<td>Bilophila</td>
<td>0.02 0.03 0.03 0 0 0</td>
<td>0.534</td>
<td>0.776 0.554 0.384 0.77 1</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>1.37 8.97 6.1 13 12 16</td>
<td>0.022</td>
<td>0.02 0.56 0.77 0.56 0.08</td>
</tr>
<tr>
<td>Coprococcus</td>
<td>0.04 0.11 0.04 0.1 0.1</td>
<td>0.154</td>
<td>0.245 0.245 0.773 0.25 0.11</td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>1.21 1.19 2.27 1 0.6 0.4</td>
<td>0.069</td>
<td>0.386 0.248 0.773 0.89 0.386</td>
</tr>
<tr>
<td>Escherichia,Shigella</td>
<td>0.03 0.13 0.03 0 0 0.3</td>
<td>0.014</td>
<td>0.021 0.019 0.021 0.02 1</td>
</tr>
<tr>
<td>Prevotello,Paraprevotella</td>
<td>19.44 37.7 34.3 31 24 18</td>
<td>0.377</td>
<td>0.248 0.564 0.564 0.56 0.248</td>
</tr>
<tr>
<td>Paraprevotella</td>
<td>0.16 1.78 1.21 1.7 1.2 0.8</td>
<td>0.017</td>
<td>0.021 0.248 1 0.25 0.083</td>
</tr>
<tr>
<td>Panbacteroides</td>
<td>1.06 1.18 1.26 0.5 1.3 0.8</td>
<td>0.557</td>
<td>0.773 0.773 0.083 0.66 0.248</td>
</tr>
<tr>
<td>Parasutterella</td>
<td>0.41 1.49 0.76 0.6 0.6 0.7</td>
<td>0.227</td>
<td>0.083 0.083 0.083 0.08 0.149</td>
</tr>
<tr>
<td>Oscillibacter</td>
<td>0.35 1.69 1.89 2 1.2 0.7</td>
<td>0.011</td>
<td>0.021 0.386 0.564 0.77 0.043</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24.09 54.27 47.91 49.54 40.34 38.6</strong></td>
<td><strong>0.014</strong></td>
<td><strong>0.021 0.019 0.021 0.02 1</strong></td>
</tr>
</tbody>
</table>

**Table 3:** Group-specific relative abundances of SCFA-producing bacteria. Difference were assessed by Kruskal-Wallis test followed by the Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Genus name</th>
<th>Relative_abundance (%)</th>
<th>Kruskal wallis test</th>
<th>P value_Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F% E% A% B% C% D%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerotruncus</td>
<td>0.78 0.13 0.37 0.3 0.3 0.4</td>
<td>0.014</td>
<td>0.021 0.083 0.149 0.08 0.021</td>
</tr>
<tr>
<td>Allobaculum</td>
<td>0.04 0.01 0.02 0 0.1 0</td>
<td>0.269</td>
<td>0.08 0.659 0.882 0.11 0.081</td>
</tr>
<tr>
<td>Anaerovorax</td>
<td>0 0 0.05 0 0 0</td>
<td>0.001</td>
<td>1 0.019 0.036 1 0.096</td>
</tr>
<tr>
<td>Butyrivibrio</td>
<td>0 0 0 0 0 0.1</td>
<td>0.006</td>
<td>1 1 1 1 0.047</td>
</tr>
<tr>
<td>Blautia</td>
<td>0 0 0.02 0 0 0</td>
<td>0.416</td>
<td>1 0.307 1 1 1</td>
</tr>
<tr>
<td>Dialister</td>
<td>0 0 0.02 0 0 0</td>
<td>0.001</td>
<td>1 0.014 1 0.01 1</td>
</tr>
<tr>
<td>Faecalibacterium</td>
<td>0.03 0 1.36 0 1 0</td>
<td>0.002</td>
<td>1 0.014 1 0.01 1</td>
</tr>
<tr>
<td>Megamonas</td>
<td>0 0 1.05 0 0.8 0</td>
<td>0.002</td>
<td>1 0.014 1 0.01 1</td>
</tr>
<tr>
<td>Odoribacter</td>
<td>1.18 0.56 0.83 0.8 0.6 0.6</td>
<td>0.572</td>
<td>0.248 0.386 0.386 0.77 0.663</td>
</tr>
<tr>
<td>Prevotella</td>
<td>0 0 2.67 0 1.3 0</td>
<td>0.001</td>
<td>1 0.014 1 0.01 1</td>
</tr>
<tr>
<td>Quinella</td>
<td>2.31 1.43 2.53 3.6 2.1 3.8</td>
<td>0.547</td>
<td>0.773 0.564 0.248 0.25 0.043</td>
</tr>
<tr>
<td>Roseburia</td>
<td>0.48 0 0.16 0 0.1 0</td>
<td>0.007</td>
<td>0.014 0.014 0.047 0.01 0.047</td>
</tr>
<tr>
<td>Subdoligranulum</td>
<td>0 0 0.18 0 0.2 0</td>
<td>0.001</td>
<td>1 0.014 1 0.01 1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4.82 2.13 9.26 4.78 6.4 4.85</strong></td>
<td><strong>0.014</strong></td>
<td><strong>1 0.014 1 0.01 1</strong></td>
</tr>
</tbody>
</table>

Discussion

The human gut microbiome plays a vital role in maintaining body homeostasis. Changes in bacterial compositional (i.e. dysbiosis) can profoundly impact human health, which makes the microbiome an important therapeutic target. Both bacterial diversity and abundance is altered in human IBD; however, whether dysbiosis is a consequence or cause of IBD remains controversial. The success of manipulating the gut microbiome to cure or alleviate the symptoms of IBD has encouraged the use of probiotic and prebiotic therapies. IBD-directed therapies however have variable success rates and a high risk of severe side effects with long-term use. Therefore, in recent years, the use of CAMs have become widespread due to their effectiveness and absence of side effects [16]. Besides probiotics and prebiotics, Chinese traditional medicines may be a viable microbiome-manipulating therapeutic option.

In this study, we clearly demonstrated the effectiveness of four different CAMs in relieving DSS-induced colitis. Although most Chinese herbal medicines and their active polysaccharides (e.g. Astragalus membranaceus, Rheum rhabarbarum polysaccharide) have anti-inflammatory effects in a mice colitis model [17], we are the first to report the use of Dangshen [Codonopsis pilosula (Franch.) Nannf] polysaccharide in the treatment of colitis in mice. In fact, Dangsheng polysaccharide was even more effective than the herbal medicine SJZT, which confirms that Dangshen polysaccharide is the key active ingredient in SJZT. In addition, we found that S. boulardii significantly alleviates the symptoms of colitis, which is in agreement with previous research [8]. S. boulardii in combination with Dangshen polysaccharide, was more effective than S. boulardii alone, which suggests a possible synergistic or symbiotic effect. Following 21 days of preventive treatments (before induction of colitis), a high proportion of Bacteroidetes were present in the Dangshen and SJZT groups. Besides Dangshen polysaccharide, the herbal decoction also contains other plant polysaccharides. On the other hand, Bacteroidetes are well equipped for carbohydrate metabolism [18] so it is not surprising that Bacteroidetes are increased in these groups. Enrichment with Bacteroidetes in polysaccharide-treated mice could be related to polysaccharide degradation in the gut. Furthermore, mice body weight was increased in the two S. boulardii-containing groups compared with the Dangshen and SJZT groups. This was associated with increased F/B ratios in S. boulardii-containing groups, which has been associated with enhanced energy harvesting [19].

The F/B ratio is also a key index in evaluating IBD remission and relapse [20]. All treatments inhibited the DSS-induced decrease in the F/B ratio, with significantly increased F/B ratios in the Dangshen and D+S groups (P<0.05) to values similar to that of the normal (non-DSS group). Modulating F/B ratios in DSS-induced colitis may represent an important underlying mechanism of these treatments. Another interesting feature is that Proteobacteria was significantly increased, especially in the S. boulardii group after 21 days of treatment yet increased only in the Dangsheng group post-colitis (day 30). Proteobacteria can induce a specific IgA response to regulate maturation of the immune maturation [21]. It has also been reported that S. boulardii stimulates intestinal IgA production [22], and that total polysaccharide extracted from SJZT can restore IgA production in a cyclophosphor-
mide-induced lymphoid tissue injury model [23]. Therefore, the ability of *S. boulardii* and Dangshen polysaccharide to induce IgA may promote the growth of Proteobacteria. The most important characteristic of a good prebiotic is to selectively stimulate the growth of host-beneficial bacteria such as Bidifobacteria and Lactobacilli. *Bifidobacterium, Bifidobacteriaceae*, uncultured and *Lactobacillus* were only seen in the Dangshen and D+S group. *Arthrobacter* produces hydrolytic enzymes (endo-β-1,4-glucanases) that degrade inulin to common prebiotic fructooligosaccharides. *Arthrobacter* arilaitensis species can produce β-fructofuranosidase, which is used to synthesize the prebiotic kestose [25]. Most *Arthrobacter* species are probiotics and both *Arthrobacter agilis* and *Arthrobacter citreus* have been used to treat IBD [26]. In this study, *Arthrobacter* growth was facilitated by Dangshen polysaccharide, which supports its prebiotic value.

Bacterially-derived SCFAs are major products of prebiotic metabolism. We found that *Anaerovorax, Roseburia, Prevotella, Megamonas, Dialister, Faecalibacterium* and *Subdoligranulum* were remarkably increased in the Dangshen group. Apart from *Anaerovorax* and *Roseburia*, the other taxa were specifically increased in the Dangshen group. *Prevotella* is associated with consumption of a diet rich in fiber because of its outstanding ability for cellulose and xylan hydrolysis. A long-term fiber-rich diet therefore promotes the growth of *Prevotella*, along with increased production of SCFAs [27]. *Megamonas* and *Faecalibacterium* are known producers of SCFAs, which are decreased in IBD patients [28]. *F. prausnitzii*, the representative species of the *Faecalibacterium* genus, is a commonly administered probiotic in recent years. It produces SCFAs and increases production of IL-10 and TGF-β to control colonic inflammation by regulating the TH17/TREG balance. The common prebiotic inulin greatly facilitates *Faecalibacterium* growth [29]. In this study, when colitic mice were treated with Dangshen polysaccharide, *Prevotella, Megamonas* and *Faecalibacterium* all increased to > 1% (2.67%, 1.05%, 1.36% respectively), which supports the value of Dangshen polysaccharide as a powerful prebiotic.

Other SCFA-producing bacteria (*Butyribifibacterium*, *Quinella* and *Anaerotruncus*) were seen only in the SJZT group, which suggests the presence of small active molecules or medicinal polysaccharides other than Dangshen polysaccharide that promote the growth of these beneficial microbes. The exact composition of SJZT however requires further study. *Roseburia*, which produces SCFAs and is now used to treat UC [30], was increased in all four treatment groups. *Odoribacter* was reduced in DSS-induced colitis, and *Odoribacter splanchus*, a typical representative of the genus *Odoribacter*, is a known producer of acetate and propionate [31]. *S. boulardii* mildly promoted *Odoribacter* growth. *Anaerovorax* metabolizes putrescine to acetate and butyrate [32] and *Anaerovorax* was remarkably increased in the *S. boulardii* group. Taken together, these results suggest that *S. boulardii* is an effective probiotic that facilitates the growth of the SCFA-producing bacteria: *Roseburia, Odoribacter* and *Anaerovorax*. *Lactobacillus* was also increased significantly in the *S. boulardii* group. Other yeasts have been reported to stimulate the growth of lactic acid strains in fermented products [33]. Our result therefore suggests that *S. boulardii* also has the ability to promote *Lactobacillus growth in vivo*. *Akkermansia* plays a key role in preventing obesity and metabolic disease. *A. muciniphila* is decreased significantly in diabetes patients. Recent research suggests that metformin may act by facilitating *Akkermansia* proliferation [34]. *Akkermansia* is also decreased in IBD patients and a previous study reported that extracellular vesicles derived from *Akkermansia muciniphila* protect against DSS-induced colitis [35]. In addition, administration of common oligofructose-containing prebiotics completely restored *Akkermansia* levels in both genetically and high fat-fed obese mice [15]. In this study, we found that Dangshen polysaccharide restored *Akkermansia* abundance in acute colitic mice, which suggests that Dangshen polysaccharide may be useful as a prebiotic in the prevention of obesity and diabetes. *Desulfovibrio* is the most important member of the sulphate-reducing bacteria. It breaks down SCFA and amino acids to produce H₂S that harm intestinal epithelial cells. *Desulfovibrio* is increased in the inflamed colon [36], which can be counteracted by *S. boulardii* and SJZT. Bacteroides was increased in acute colitis, which is in agreement with a previous study [37]. However, only Dangshen polysaccharide was able to control this increase in Bacteroides, while *S. boulardii* even promoted its growth. This is in agreement with a previous report where Bacteroides was dramatically increased by *S. boulardii* administration in type 2 diabetic mice [10]. This may be due to the ability of Bacteroides to utilize t cell walls as, which is made up of β-glucans, as an energy source [10]. However, it remains unclear why SJZT increased the proportion of Bacteroides.

*Prevotellaceae*, uncultured and *Parasutterella*, which are enriched in IBD patients [38] and in colorectal cancer tissue [39], were inhibited by all four treatments at varying degrees. *Escherichia-Shigella* is a common pathogenic bacterium that was induced by DSS administration. All treatments, except SJZT, inhibited *Escherichia-Shigella* growth. Taken together, the common mechanism, shared by all four treatments, was to inhibit harmful bacterial growth.

**Conclusion**

Chinese traditional herbal medicine SJZT, Dangshen polysaccharide and *S. boulardii* were all effective in alleviating DSS-induced colitis. Promoting beneficial and inhibiting pathogenic bacteria may be the shared mechanism of these potential CAM drugs in improving colonic health.

**Acknowledgments**

We thank Professor Hongyu Li for providing help in data analysis. This work was supported by Jiangsu Science and Technology Major Project (BA2016036) and Gansu Science and Technology Major Project (17ZD2FA009).
Conflict of Interest
The author has no conflicts of interest to declare.

References


