Saccharomyces cerevisiae Fermentation Product Did Not Attenuate Clinical Signs, but Psyllium Husk Has Protective Effects in a Murine Dextran Sulfate Sodium–Induced Colitis Model

Ching-Yen Lin,[1] Anne H Lee,[2] Karen K Chiu,[1,3] Miranda D Vieson,[3] Andrew J Steelman,[1,2] and Kelly S Swanson[1,2]

[1]Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [2]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA; and [3]College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

ABSTRACT

Background: Yeast products and psyllium husk may provide relief from clinical signs of colitis due to their ability to promote gut integrity, modulate gut microbiota, or positively affect immune responses, which have been demonstrated in several species.

Objective: The objective of this study was to investigate the effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) and psyllium husk (PH) on cecal and fecal microbiota, colonic gene expression and histopathology, and mesenteric lymph node (MLN) immune cells in a dextran sulfate sodium (DSS)–induced colitis model.

Methods: Male C57BL/6J mice ($n = 54$) were assigned to a control, 5% SCFP, or 5% PH diet. After 2 wk of diet adaptation, mice were provided distilled water or 3% (wt:vol) DSS for 5 d ad libitum. Body weight, food and water intakes, and disease activity index (DAI) were recorded daily during the treatment period. Fresh fecal samples were collected before and during treatment for microbial analyses. After treatment, mice were killed, followed by tissue collection. Tissues were stored in proper solutions until further analyses. Data were analyzed using the Mixed Models procedure of SAS 9.4 (SAS Institute).

Results: Consumption of SCFP increased ($P < 0.05$) species richness of the gut microbiota and relative abundance of *Butyricicoccus* in fecal and cecal samples compared with control or PH mice. PH mice had greater ($P < 0.05$) gene expression of claudin (*Cldn*) 2, *Cldn3*, *Cldn8*, and *occludin* (*Ocln*) compared with control mice. DAI, MLN immune cell populations, colonic histopathology, and colonic gene expression were not affected ($P > 0.05$) by SCFP in DSS mice. DSS mice consuming PH had lower ($P < 0.05$) DAI compared with control or SCFP mice.

Conclusions: Results suggest that, despite the modest changes it had on cecal and fecal microbiota, SCFP did not attenuate clinical signs associated with DSS-induced colitis in mice, while PH showed protective effects.   *Curr Dev Nutr* 2020;4:nzaa159.

Introduction

Inflammatory bowel diseases (IBD) are a group of disorders characterized by chronic and relapsing inflammation of the gastrointestinal (GI) tract. The exact etiology of IBD remains unclear. However, it is known that genetic, immunologic, and environmental factors are involved in IBD pathogenesis (1). Patients usually have dysregulated immune responses that are mediated by T cells, resulting in elevated proinflammatory cytokines such as interferon gamma (*Ifng*) and tumor necrosis factor (*Tnf*) (2, 3). Hence, IBD patients have shifts in the GI microbial composition compared with healthy individuals (4–6).

Yeast products are ingredients containing yeast cells or yeast derivatives. Several studies have demonstrated that yeast products may promote GI integrity and positively affect host immune response. For example, yeast products have been reported to help maintain the barrier integrity of infected epithelial cells in vitro (7–9), reduce inflammation of the gut in rats and mice (10, 11), and modify intestinal microbiota composition in dogs and pigs (12–14). As such, yeast products may be an effective strategy to improve in-

testinal health and relieve clinical signs of intestinal diseases such as IBD.

Previous research from our laboratory evaluated the effects of a *Saccharomyces cerevisiae* fermentation product (SCFP), a dry product produced via *S. cerevisiae* fermentation which contains residual yeast cells and fermentation metabolites, in dogs. Results revealed lower immune cell responsiveness and increased relative abundance of beneficial fecal bacteria (i.e., *Bifidobacterium*) in dogs consuming SCFP (15). These outcomes suggest that SCFP may be a potential approach to relieve clinical episodes in IBD patients with dysregulated immune responses and imbalanced GI microbiota. In addition, *Bifidobacterium* spp. are used as a probiotic to prevent and treat IBD (16). Studies have shown that yeast product supplementation with live *Saccharomyces boulardii* improved clinical signs of IBD patients (17, 18) in terms of disease activity index (DAI) and gut permeability. In a study with a murine colitis model, live *S. boulardii* administration improved clinical signs, modulated fecal microbiota, and decreased expression of colonic inflammatory genes (19).

Psyllium husk (PH) is a dietary fiber derived from *Plantago ovata* ripe seed husk and consists of insoluble and soluble fibers that are viscous and nonfermentable (20, 21). With these physicochemical characteristics, PH has a long history of use as a treatment for intestinal disorders such as constipation or diarrhea (22, 23). More recently, PH has been shown to be an approach to ameliorate various symptoms or disorders, including hypercholesterolemia, diabetes, obesity, and colon cancer (24). Additionally, several clinical studies have reported the effectiveness of using PH in the management of IBD, where consumption of PH improved the clinical signs of ulcerative colitis (25, 26). Two animal studies with a DSS-colitis murine model demonstrated that administration of PH positively influenced intestinal microbiota, provided protection from inflammation, and maintained GI integrity (27, 28).

Therefore, the objective of this study was to evaluate the effects of the SCFP investigated in our previous research (15), as well as PH, on alleviating signs of inflammation and disease in a murine dextran sulfate sodium (DSS)–induced colitis model. This aim included the measurement of multiple indices, including intestinal histology, intestinal gene expression, mesenteric lymph node (MLN) immune cells, and fecal and cecal microbiota.

We hypothesized that both SCFP and PH would improve the signs associated with DSS-induced colitis more effectively than a control (diet only) treatment, with mice displaying lower DAI, more balanced immune cell profile, decreased expression of genes related to inflammation, increased expression of genes related to gut integrity, and less disrupted gut microbial composition.

Methods

Animals, diets, and study design

All animal care and use procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation (protocol no. 18218). Male C57BL/6J mice (6 wk of age; Jackson Laboratory, Bar Harbor, ME) were purchased and housed individually in standard shoebox cages in an environmentally controlled room, with a 12-h light:12-h dark cycle. Upon arrival, mice were fed a standard AIN93G diet ad libitum with free access to fresh water for 2 wk

to acclimate to the facility. After acclimation, mice were randomly assigned to 3 diet groups ($n = 18$/diet): *1*) standard AIN93G (control), *2*) modified AIN93G containing 5% SCFP (Diamond V Mills, Inc., Cedar Rapids, IA), or *3*) modified AIN93G containing 5% PH (NOW foods, Bloomingdale, IL) and fed for 2 wk (d 1–d 14). Diets were produced by Research Diets, Inc. (New Brunswick, NJ) and the ingredient and nutrient composition are presented in **Supplemental Table 1**. After the 2-wk feeding period, mice within each diet group were randomly assigned to receive distilled water ($n = 8$/group) or water containing 3% DSS ($n = 10$/group) for 5 d (d 15–19). Therefore, mice were allotted into 1 of 6 groups: *1*) control + water, *2*) control + DSS, *3*) SCFP + water, *4*) SCFP + DSS, *5*) PH + water, and *6*) PH + DSS. Animal numbers were based on the outcomes and variability derived from a previous DSS study conducted in our laboratory (T.-W. Liu, 2017, unpublished data). Body weight (BW), food and water intakes, and DAI were recorded during the DSS/water treatment period. Mice were feed deprived for 4 h and then killed via carbon dioxide inhalation, followed by cervical dislocation after 5 d of DSS/water treatment (d 20). Tissues including colon, cecum, spleen, and MLN as well as cecal contents were collected for analyses.

DSS treatment

Previous studies have shown that providing 3% DSS water for 5 d is sufficient to induce colitis in C57BL/6J mice (29, 30). During the DSS/water treatment period, 3% (wt:vol) DSS (36,000–50,000 molecular weight; MP Biochemicals, Santa Ana, CA) in sterile water was provided instead of regular drinking water for 5 d. Freshly made DSS water was refilled on d 3 after initial administration. Water intake was recorded every day.

Disease Activity Index

During the DSS/water treatment period (d 15–19), disease severity was assessed using a DAI (31), which is the composite value of scores for weight loss (%), stool consistency, and bleeding (**Supplemental Table 2**). For the bleeding score, a hemoccult kit (Beckman Coulter, Brea, CA) was used to determine the presence of occult blood.

Fecal and cecal sample collection, DNA extraction, amplification, and sequencing

Fresh fecal samples (within 15 min of defecation) were collected on d 14 and 19 of the study. Cecal contents were collected during killing. Fecal and cecal samples were frozen in liquid nitrogen immediately after collection and stored at −80°C until analyses. DNA extraction, amplicon library preparation, and sequencing were conducted as described previously by Lin et al. (15).

Bioinformatics

Sequence data were processed using QIIME 2 (version 2019.4) (32). Trimmomatic was used to remove sequencing adaptors (33) and then imported into the QIIME2 environment. DADA2 was used to remove low-quality reads, denoise, and filter chimeras. Amplicon sequence variants were generated used DADA2 and then taxonomically assigned using scikit-learn classifier (34) against the Greengenes 13_8 reference database (35). An even sampling depth was used for assessing α- and β-diversity measures [28,306 sequences for fecal samples before colitis induction (d 14); 1467 sequences for fecal samples after DSS/water treatment (d 19); 4040 sequences for cecal samples]. α-Diversity
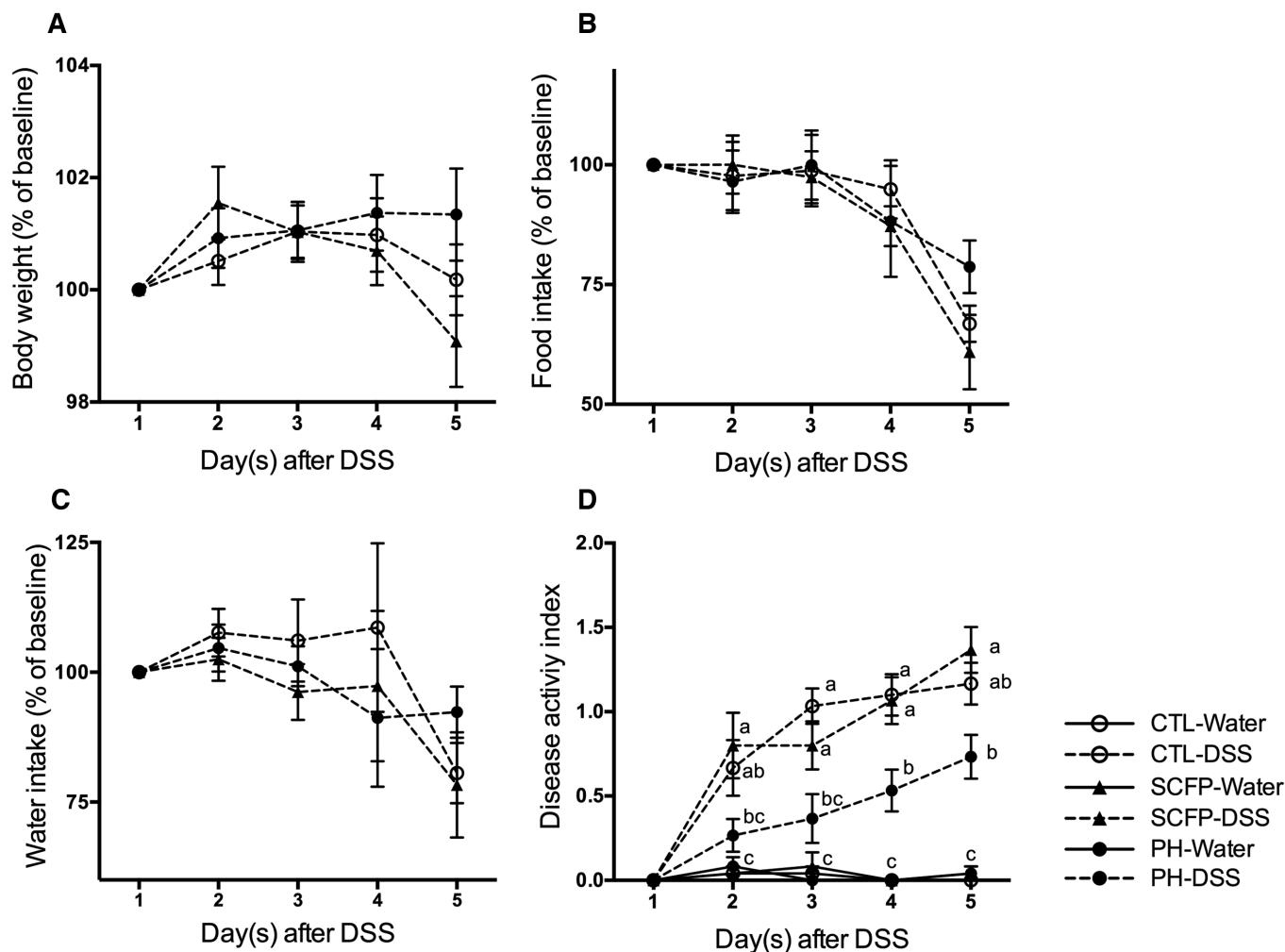
FIGURE 1 Body weight (A), food intake (B), and water intake (C) of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS, and disease activity index (D) of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. Values represent means ± SEMs, n = 8–10/group. Interactions of diet and treatment are denoted by superscript letters. Means at a time without a common letter differ, $P < 0.05$. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

was calculated using phylogenetic diversity whole tree. β-Diversity was calculated using weighted and unweighted UniFrac (36) distance measures and presented by principal coordinates analysis (PCoA) plots.

Tissue collection

Mice were feed deprived for 4 h and then killed by carbon dioxide asphyxiation followed by cervical dislocation. The spleen was dissected and weighed. Intestinal regions, including the ileum, colon, and cecum, were dissected. The length of the colon was measured. Colon and cecum tissues were flushed with sterile PBS and weighed. Midcolon tissues were fixed in 10% neutral-buffered formalin for histopathology analysis and stored in RNAlater stabilization solution (Invitrogen, Carlsbad, CA) at −20°C until further analyses for gene expression. Finally, MLN were collected and placed in Roswell Park Memorial Institute media for determination of immune cell populations by flow cytometry.

Immune cell populations in MLN

Mechanical digestion of the MLN was achieved by a syringe plunger. After mechanical digestion, single cells were obtained by passing the cells through a 70-μm cell strainer (VWR International, Radnor, PA). Red blood cells (RBC) were lysed using RBC lysis buffer (BioLegend, San Diego, CA). Cells from 2 mice in the same experimental groups were combined to increase cell numbers for analyses. For T-cell populations, cells (1 × 10^6 cells) were stained with antibodies anti–CD3e-V450 (500A2; BD Biosciences, Franklin Lakes, NJ) and anti–CD4-PE-Cy5 (RM4-5; BD Biosciences). For B-cell populations, cells (1 × 10^6 cells) were labeled with anti–CD19-PE-Cy7 (1D3; BD Biosciences) and anti–MHC-II-FITC (2G9; BD Biosciences). Populations of T and B cells then were determined by a BD LSR flow cytometer (BD Biosciences). Flow cytometry data were analyzed using FCS Express 6 Flow Cytometry Software (De Novo Software, Glendale, CA). The gating strategy used to determine immune cell populations is presented in **Supplemental Figure 1**.
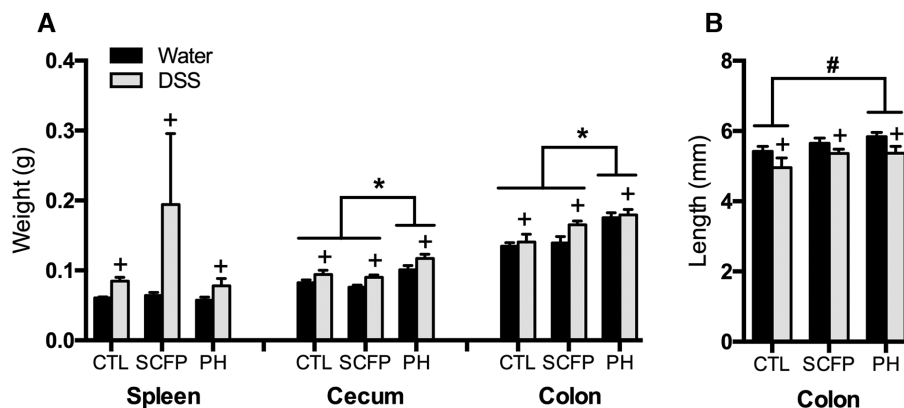
FIGURE 2 Organ weights (A) and colon length (B) of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. Values represent means ± SEMs, $n = 7–10$/group. *Main diet effect, $P < 0.05$. #Trends in diet effect, $P < 0.10$. +Main DSS treatment effect from water control, $P < 0.05$. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

Colon histopathology

Midcolon tissues were fixed in 10% neutral-buffered formalin for 24 h and then transferred to 70% ethanol until tissue processing. The tissues were embedded in paraffin (Tissue Tek VIP). Formalin-fixed paraffin-embedded tissue specimens were sectioned at 7-μm thick using a microtome (Microm HM 310; MICROM Laborgeräte GmbH, Berlin, Germany) and mounted on glass slides (Surgipath® X-Tra® Microscope Slides; Leica Biosystems, Buffalo Grove, IL). Colon samples were stained with Harris' hematoxylin and eosin. Histopathology was scored by a blinded pathologist at the University of Illinois (MDV). The scoring systems used for histological analysis are modified from a published scoring system (37), which considers stromal collapse, mucinous hyperplasia, ulceration, and degree of neutrophil and monocyte infiltration (**Supplemental Table 3**).

Colon gene expression

Colon gene expression was investigated according to methods described in Liu (38). Briefly, total RNA was extracted from colon tissue using RNeasy Mini Kits (Qiagen, Hilden, Germany), followed by assessment of RNA quality and quantity using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). cDNA (180 ng) was synthesized using a reverse-transcription kit (Invitrogen, Carlsbad, CA). Expression of genes related to gut inflammation, immune cells, integrity, and SCFA receptors was measured by real-time quantitative PCR using a Fluidigm Biomark HD Real-Time PCR device (Fluidigm Corporation, South San Francisco, CA). The full list of genes investigated is presented in **Supplemental Table 4**. Evagreen Supermix (BioRad Laboratories, Hercules, CA) was used for real-time amplification and detection. The thermal protocol was 70°C/40 min, 60°C/30 s, and 95°C/1 min, followed by 30 two-segment amplification cycles (96°C/5 s and 60°C/20 s). Melting temperature was measured from a dissociation curve for each primer that was produced from 60–95°C in 2 min. Data were analyzed using Fluidigm Real-Time PCR Analysis Software 4.5.2. All data presented were obtained by using the comparative threshold cycle ($\Delta\Delta$CT) method and were normalized to housekeeping genes (B-actin, peptidylprolpyl isomerase A, and ribosomal protein S13) as internal controls. Data are expressed as fold-change relative to the control + water group.

Statistical analysis

For BW, food and water intakes, and DAI, data were analyzed using the Mixed Models procedure of SAS version 9.4 (SAS Institute, Cary, NC) with time as the repeated measure. The main effects of diet, water, and time as well as the interaction of diet and water treatments were tested, with animal as a random effect. Other data were analyzed using the Mixed Models procedure of SAS version 9.4 (SAS Institute) with diet and water treatments as fixed effects and animal as a random effect. The interaction of diet and water treatment was also tested. Post hoc differences between groups were identified using Tukey's procedure. Statistical significance was set as $P \leq 0.05$ and a trend was set as $0.05 < P < 0.10$.

Results

BW, food intake, water intake, and DAI

Before the 5-d DSS/water treatment phase, BW (control: 23.5 ± 0.6 g; SCFP: 24.0 ± 0.3 g; PH: 24.0 ± 0.3 g) and average daily food intakes (control: 2.8 ± 0.3 g; SCFP: 2.9 ± 0.2 g; PH: 2.8 ± 0.2 g) were not affected ($P > 0.05$) by diet. BW, food intake, and water intake throughout the DSS/water treatment phase were expressed as change from baseline (d 1 post–DSS induction) and were not affected ($P > 0.05$) by diet (**Figure 1**A–C, **Supplemental Figure 2**). For the DAI, an interaction of diet and water treatment ($P < 0.05$) was noted, where control-DSS and SCFP-DSS had a greater DAI than PH-DSS. All water groups had a low DAI near zero (Figure 1D).

Organ weights and colon length

Spleen, cecum, and colon weights were greater ($P < 0.05$) in DSS groups compared with water groups, whereas the colon length was shorter ($P < 0.05$) in DSS groups compared with water groups. Mice consuming PH had greater ($P < 0.05$) cecum and colon weights compared with control
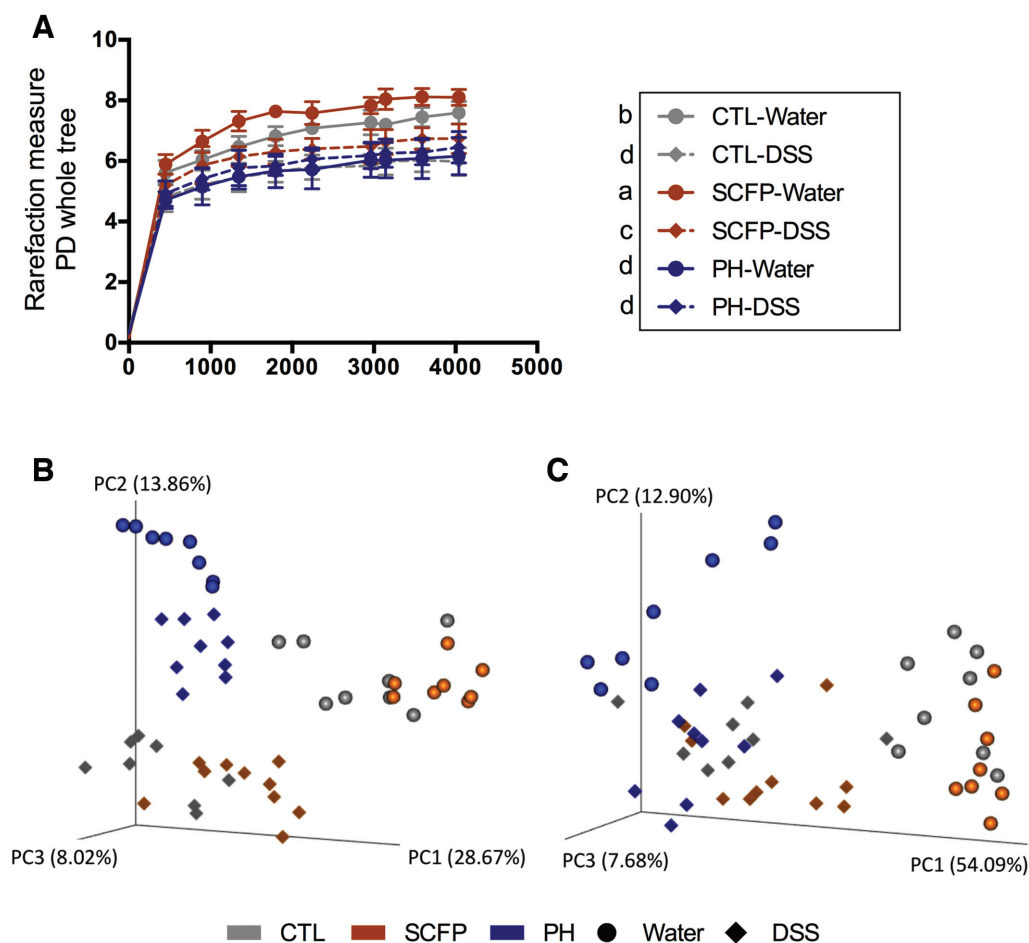
FIGURE 3 Cecal microbiota communities of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. α-Diversity measures (A), including PD whole tree (shown), suggested that there were interactions of diet and treatment on species richness ($P < 0.05$). Different superscript letters denote interactions of diet and water treatment. Means of an experimental group without a common letter differ, $P < 0.05$. PCoA plots of unweighted (B) and weighted (C) UniFrac distances of fecal microbial communities show a distinct separation between water and DSS groups ($P < 0.05$). The PCoA plot of unweighted UniFrac distances revealed distinct separations between the 3 diet groups ($P < 0.05$). The PCoA plot of weighted UniFrac distance showed that PH mice were clustered together and away from other diet groups ($P < 0.05$). Circles are for water-treated and diamonds are for DSS-treated. CTL, control; DSS, dextran sulfate sodium; PC, principal component; PCoA, principal coordinates analysis; PD, phylogenetic diversity; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

or SCFP mice (**Figure 2**A). PH mice tended to have a longer ($P = 0.08$) colon than control mice (Figure 2B).

Fecal and cecal microbiota

Before colitis induction (d 14), measurements of bacterial α-diversity indicated that SCFP mice had greater ($P < 0.05$) species richness than control mice or PH mice (**Supplemental Figure 3**A). PCoA plots of weighted and unweighted UniFrac distances showed that PH mice were clustered together ($P < 0.05$) and away from control and SCFP mice (Supplemental Figure 3B, C). For taxonomy at the phylum level (**Supplemental Figure 4**A), PH mice had greater ($P < 0.05$) relative abundance of Proteobacteria and a lower ($P < 0.05$) relative abundance of Bacteroidetes than control or SCFP mice. Mice treated with SCFP had a greater ($P < 0.05$) relative abundance of Tenericutes than control or PH mice. At the genus level, PH mice had a distinct change in the relative

abundances of a few genera (Supplemental Figure 4B). Mice fed the PH diet had greater ($P < 0.05$) relative abundances of *Bacteroides*, *Clostridium*, *Coprobacillus*, *Enterococcus*, *Sutterella*, and *Turicibacter* and lower ($P < 0.05$) relative abundances of *Dehalobacterium*, *Lactobacillus*, and *Oscillospira*. Finally, SCFP mice had a greater ($P < 0.05$) relative abundance of *Butyricicoccus* than control or PH mice. Mice fed the SCFP diet also had greater *Coprococcus* than control mice, with PH mice being intermediate.

After treatment (d 19), a diet by treatment interaction was noted for α-diversity (**Supplemental Figure 5**A). Both control and SCFP mice had lower ($P < 0.05$) species richness in DSS groups compared with water control groups. However, for PH mice, no difference in species richness was noted between water and DSS groups. The PCoA plot of unweighted UniFrac distances (Supplemental Figure 5B) revealed distinct separation ($P < 0.05$) among the 3 diet groups. The PCoA plot
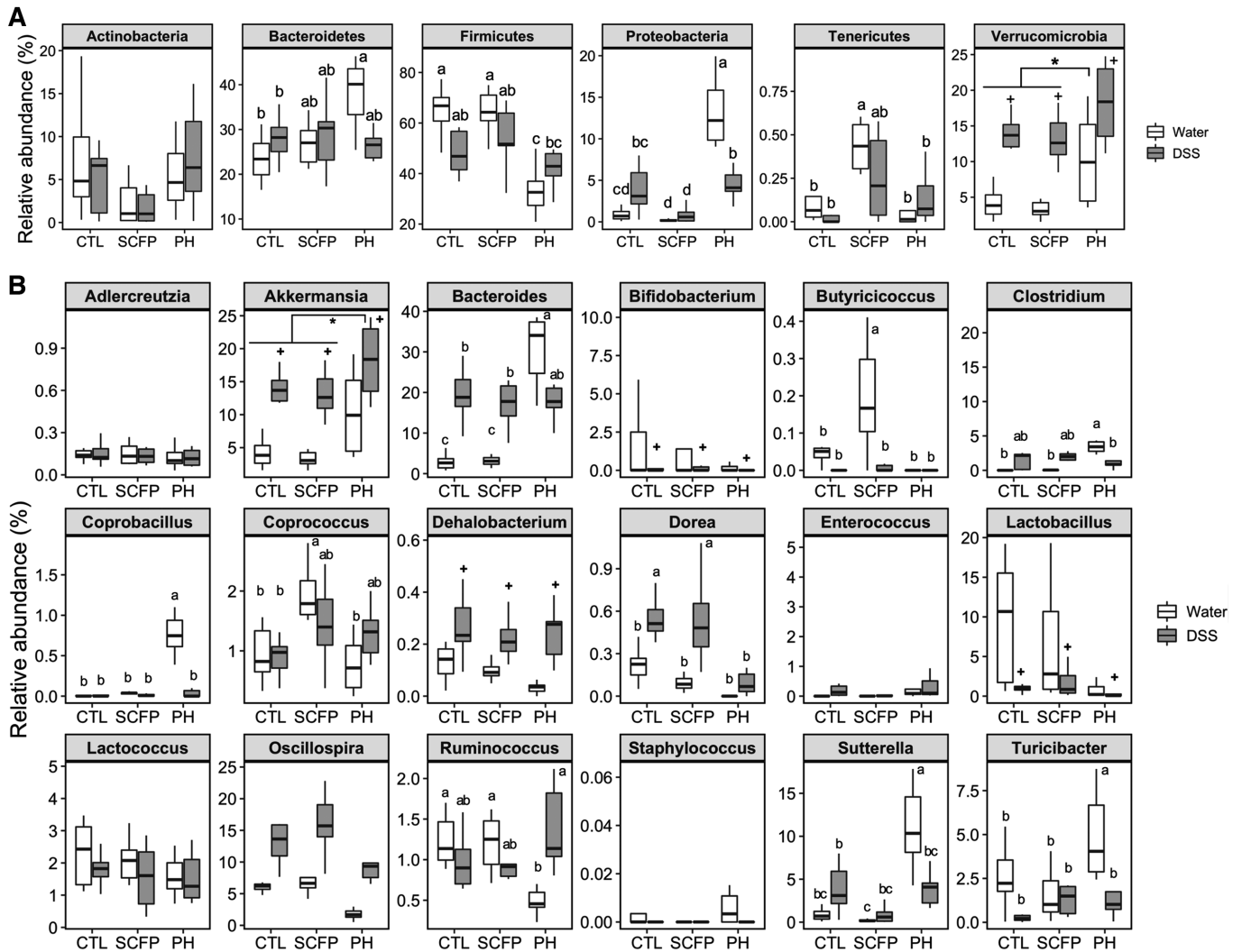
FIGURE 4 Cecal bacterial phyla (A) and genera (B) of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. *Main diet effect, $P < 0.05$. +Main DSS/water treatment effect from water control, $P < 0.05$. Different superscript letters denote interactions of diet and water treatment. Means of an experimental group without a common letter differ, $P < 0.05$. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

of weighted UniFrac distances (Supplemental Figure 5C) showed that PH mice were clustered together and away from ($P < 0.05$) the other diet groups. Moreover, PCoA plots of weighted and unweighted UniFrac distances suggested a distinct separation ($P < 0.05$) between water and DSS groups. For taxonomy, at the phylum level (**Supplemental Figure 6**A), an interaction of diet and water treatment ($P < 0.05$) was noted for the relative abundance of Proteobacteria. Mice fed the SCFP diet had a greater ($P < 0.05$) relative abundance of Tenericutes than control or PH mice as well as a greater ($P < 0.05$) relative abundance of Bacteroidetes than PH mice, with control mice being intermediate. At the genus level (Supplemental Figure 6B), interactions of diet and water treatment ($P < 0.05$) were observed for the relative abundances of *Bacteroides*, *Butyricicoccus*, *Clostridium*, *Coprobacillus*, and *Sutterella*. Mice fed the PH diet had a greater ($P < 0.05$) relative abundance of *Enterococcus* than control or SCFP mice.

The results for cecal microbiota were similar to those obtained from fecal microbiota on d 19. An interaction of diet and treatment ($P < 0.05$) was observed for α-diversity (**Figure 3**A). Mice treated with DSS had lower ($P < 0.05$) species richness than water groups for control and SCFP mice. Species richness was not different between water and DSS groups for PH mice. The PCoA plot of unweighted UniFrac distances (Figure 3B) revealed distinct separations ($P < 0.05$) between the 3 diet groups. The PCoA plot of weighted UniFrac distances (Figure 3C) showed that PH mice were clustered together and away from ($P < 0.05$) the other diet groups. Additionally, PCoA plots of weighted and unweighted UniFrac distances showed distinct separations ($P < 0.05$) between water and DSS groups. For taxonomy at the phylum level (**Figure 4**A), interactions of diet and water treatment ($P < 0.05$) were observed for relative abundances of Bacteroidetes, Firmicutes, Proteobacteria, and Tenericutes. At the genus level (Figure 4B), diet by
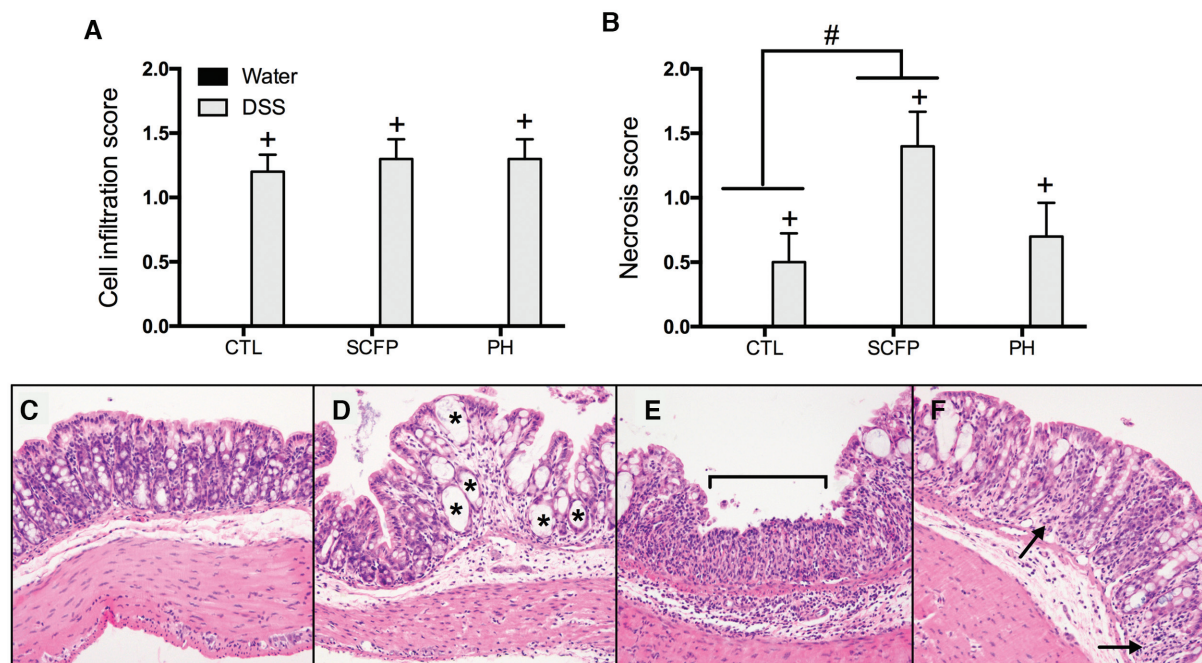
FIGURE 5 Histopathology scores for immune cell infiltration (A) and degree of necrosis (B) in the colon of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. Values represent means ± SEMs, $n = 6$–10/group. #Trends in diet effect, $P < 0.10$. +Main DSS/water treatment effect from water control, $P < 0.05$. Representative hematoxylin and eosin stains of colon tissues of male C57BL/6J mice treated with water (C), control diet + DSS (D), SCFP diet + DSS (E), or PH diet + DSS (F). Asterisks represent multifocal mucinous change within the mucosa. The bracket represents mucosal erosion with loss of colonic glands and stromal collapse. Arrows represent mild loss of colonic glands and replacement by stromal collapse. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

treatment interactions ($P < 0.05$) were noted for relative abundances of *Bacteroides*, *Butyricicoccus*, *Clostridium*, *Coprobacillus*, *Coprococcus*, *Dorea*, *Ruminococcus*, *Sutterella*, and *Turicibacter*. Hence, PH mice had a greater ($P < 0.05$) relative abundance of *Akkermansia* than control or SCFP mice.

Immune cell populations in MLN

We examined the ability for treatment to modulate lymphocyte populations in the MLN. We found that CD3+ (total T cells), CD3+CD4+ [T-helper (Th) cells], CD19+ (B cells), and CD19+MHC II+ cells were not altered ($P > 0.05$) by diet (**Supplemental Figure 7**). Mice treated with DSS had a lower ($P < 0.05$) percentage of Th cells in MLN than the water group.

Colon histopathology

Administration of DSS resulted in greater ($P < 0.05$) immune cell infiltration and necrosis, indicating greater colon damage and cell infiltration in the lamina propria (**Figure 5**). No dietary effects were observed, except that SCFP mice tended to have higher ($P = 0.081$) necrosis scores than control mice. No diet by water treatment interactions were noted.

Colon gene expression

Relative expressions of genes associated with inflammation, immune cells, intestinal integrity, and SCFA receptors were assessed in colon tissue. For genes related to inflammation (**Figure 6**), mice consuming the

PH diet had greater ($P < 0.05$) C-C chemokine receptor *(Ccr) 2* expression compared with control or SCFP mice. Mice fed the PH diet also tended to have greater ($P = 0.08$) C-C motif chemokine ligand *(Ccl)5* expression than control mice. Mice treated with DSS had greater ($P < 0.05$) expression of *Ccl2*, *Ccl3*, *Ccl5*, *Ccr2*, *Ccr5*, *Tnf*, interleukin *(Il) 1b*, *Ifng*, *Il10*, *Il10ra*, *Il6*, C-X-C motif ligand *(Cxcl)* 2, mannose receptor C *(Mrc)* 1, and nitric oxide synthase *(Nos)* 2 than those treated with water. For expression of immune cell marker genes (**Figure 6**), a diet by water treatment interaction ($P < 0.05$) was noted for the expression of cluster of differentiation *(Cd) 19*. Overall, PH mice had greater ($P < 0.05$) expression of *Cd68* than control mice, with SCFP mice being intermediate. The expression of *Cd3e*, *Cd4*, *Cd8a*, and *Cd68* was greater ($P < 0.05$) in mice treated with DSS compared with water groups. For the expression of intestinal integrity markers (**Figure 7**), diet by water treatment interactions ($P < 0.05$) were observed for the expression of claudin *(Cldn)1*, *Cldn2*, and *Cldn4*. In addition, PH mice had a greater ($P < 0.05$) expression of *Cldn8* than control or SCFP mice. PH mice also had a greater ($P < 0.05$) *Cldn3* expression than control mice, with SCFP mice being intermediate. Administration of DSS decreased ($P < 0.05$) the expression of *Cldn3*, *Cldn8*, and occludin *(Ocln)*. Last, for SCFA receptors (**Figure 7**), PH mice had greater ($P < 0.05$) free fatty acid receptor *(Ffar)* 2 expression than control mice, with SCFP mice being intermediate. The DSS-treated mice overall had a lower ($P < 0.05$) *Ffar2* expression than the water group.
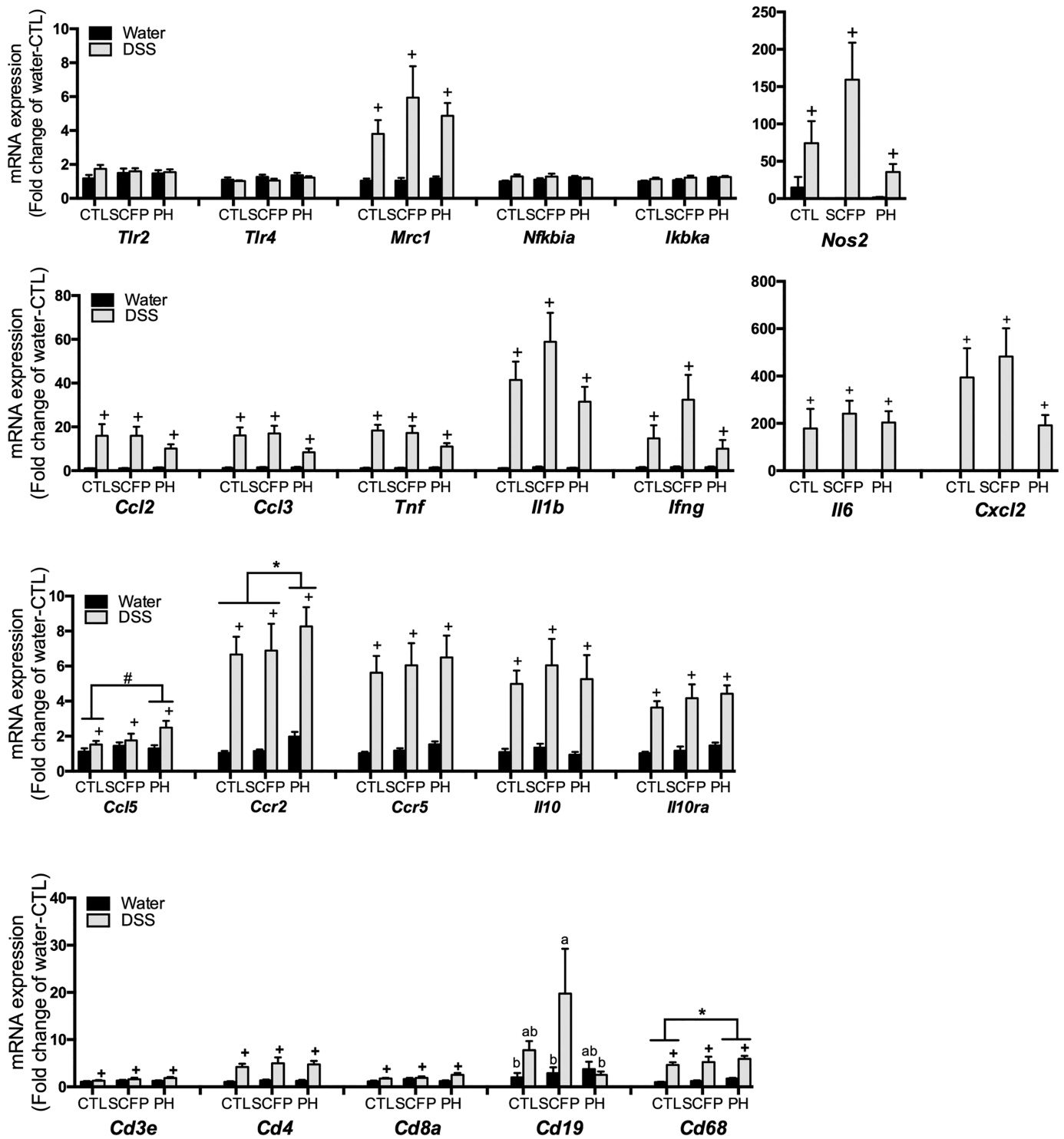
FIGURE 6 Relative expression of genes related to inflammation and immune cells in the colon of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. Values represent means ± SEMs, $n = 7$–10/group. *Main diet effect, $P < 0.05$. #Trends in diet effect, $P < 0.10$. +Main DSS/water treatment effect from water control, $P < 0.05$. Different superscript letters denote interactions of diet and water treatment. Means of an experimental group without a common letter differ, $P < 0.05$. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.
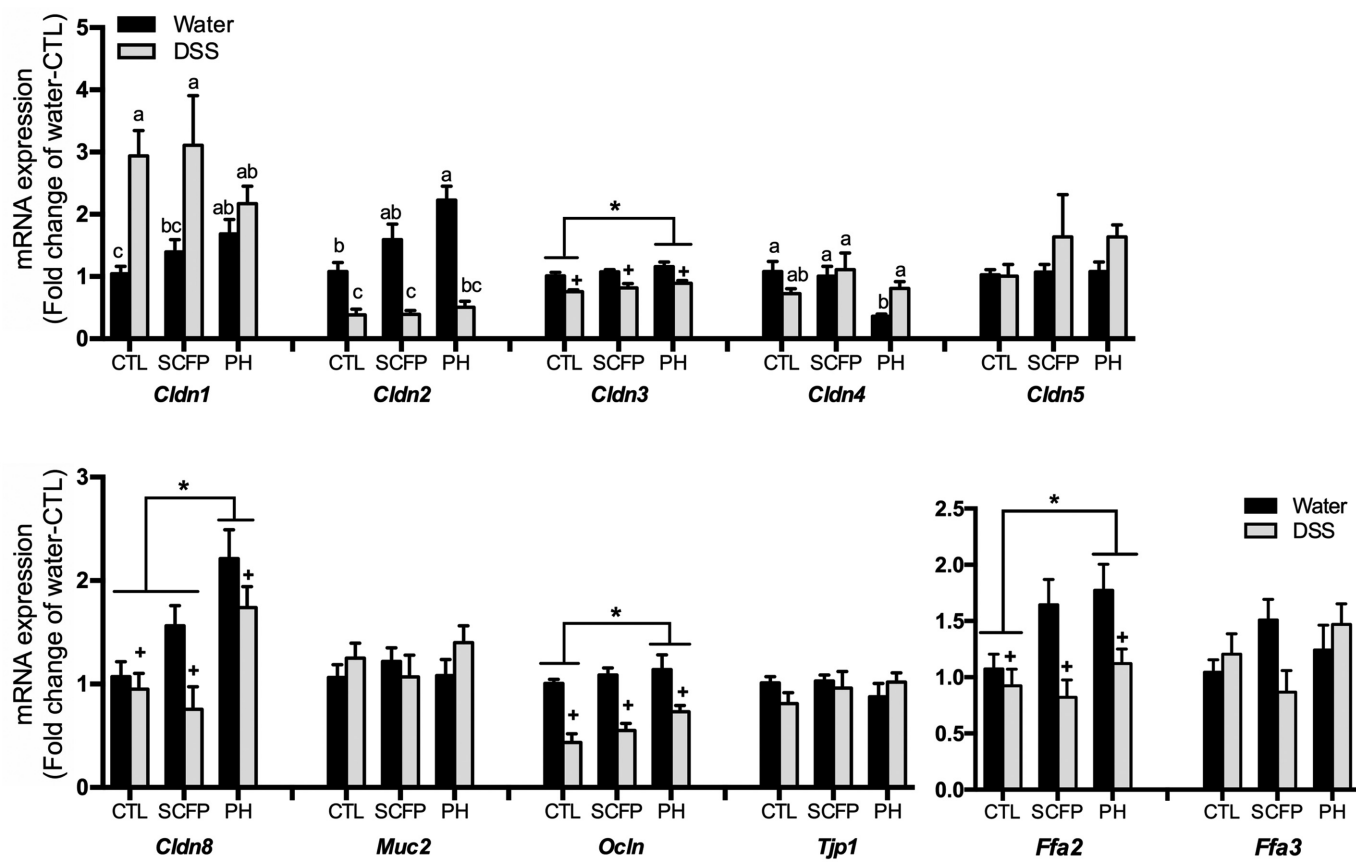
FIGURE 7 Relative expression of genes related to gut integrity and SCFA receptors in the colon of male C57BL/6J mice fed a CTL, SCFP, or PH diet and treated with 3% DSS or distilled water. Values represent means ± SEMs, $n = 7–10$/group. *Main diet effect, $P < 0.05$. +Main DSS/water treatment effect from water control, $P < 0.05$. Different superscript letters denote interactions of diet and water treatment. Means of an experimental group without a common letter differ, $P < 0.05$. CTL, control; DSS, dextran sulfate sodium; PH, psyllium husk; SCFP, *Saccharomyces cerevisiae* fermentation product.

Discussion

Supplementation of yeast products or PH has been shown to improve intestinal health by modulating gut microbiota, reducing intestinal inflammation, or enhancing gut integrity. With these benefits, yeast products and PH may be potential approaches to relieving clinical signs of intestinal diseases such as IBD. The purpose of this study was to evaluate the effects of an SCFP and PH on attenuating signs of inflammation and disease in a murine DSS-induced colitis model.

In agreement with previous studies, PH attenuated the clinical signs in mice with DSS-induced colitis (27, 28). Ogata et al. (28) demonstrated that PH ameliorated DSS-induced colon damage and inflammation by modulating the expression of tight junction proteins. Consumption of 5% and 10% PH increased protein expression of zonula occludens *(Zo)* 2, *Ocln*, *Cldn3*, and *Cldn7* in BALB/c mice treated with 2% DSS. Here, we observed increased gene expression of *Cldn2*, *Cldn3*, *Cldn8*, and *Ocln* in PH mice compared with control mice. The protective effects of PH also may be attributed to its effects on the gut microbiota. Llewellyn et al. (27) noted that mice consuming 15% PH had elevated butyrate concentrations in the colon and an increase in colonic regulatory T cells, which effectively reduced the severity of colitis in a T-cell transfer model. In this study, PCoA revealed that the fecal microbiota of PH mice

formed a distinct cluster from the fecal microbiota of mice fed the control or SCFP diets. PH mice also had greater gene expression of *Ffar2*, an SCFA receptor that is expressed on epithelial cells and immune cells, compared with control mice. *Ffar2* is activated by SCFA and plays an important role in regulating inflammation (39). Therefore, the upregulated expression of *Ffar2* could be beneficial in the prevention of colitis. Finally, PH fiber is a soluble viscous fiber that has high water-holding and gel-forming capacity. When ingested, PH may form a physical barrier in the gut and prevent epithelial damage coming from contact with DSS.

However, supplementation of SCFP did not ameliorate inflammation. Signs associated with colitis, such as DAI, colon length, and colonic gene expression, were not altered by SCFP. These results are in contrast to those reported in previous studies where live *S. boulardii* were supplemented to DSS-treated mice (19, 40). The possible mechanism by which *S. boulardii* consumption is anti-inflammatory is from the production of a soluble anti-inflammatory factor that inhibits nuclear factor kappa beta (*Nfkb*) activation and *Nfkb*–mediated proinflammatory signaling in host cells (7, 41). The expression of *Nfkb* in the colon was not affected by SCFP in this study. However, it may not be surprising as *Nfkb* activation depends on post-translation modification of inhibitor of nuclear factor kappa beta (*Ikb*). Another potential mechanism is that *S. boulardii* may restore the compromised gut microbiota dysbiosis that is shown to

contribute to IBD. In IBD patients, reduced species richness of gut microbiota and depleted Firmicutes and Bacteroidetes have been reported ([42–44]). Rodríguez-Nogales et al. ([19]) demonstrated that administration of *S. boulardii* (5×10^9 CFU/d) for 26 d increased gut microbial species richness and restored the Firmicutes to Bacteroidetes ratio in DSS-treated C57BL/6 mice. Moreover, beneficial bacteria, including *Lactobacillus* and *Bifidobacterium*, were increased by *S. boulardii* treatment. These 2 genera often are associated with a healthy gut in terms of modulated immune function ([45–48]) and reduced pathogenic bacteria ([49–52]). Studies also have demonstrated the ability of these bacteria in the prevention and treatment of IBD ([16]).

In the current study, supplementation of SCFP increased the species richness of gut bacteria when compared with control or PH groups. This effect, however, did not prevent a reduction in species richness resulting from DSS induction. Interestingly, the SCFP increased the cecal and fecal relative abundance of *Butyricicoccus*, a butyrate-producing genus whose numbers are low in IBD patients ([53, 54]). Butyrate not only is the preferred energy source for colonocytes but is also a potent anti-inflammatory mediator that promotes gut integrity and regulates colonic T-cell development ([55–57]). A specific *Butyricicoccus pullicaecorum* strain was demonstrated to reduce inflammation in rats with 2,4,6-trinitrobenzenesulfonic acid (TNBS)–induced colitis ([53]). The elevated abundance of *Butyricicoccus* in fecal and cecal samples by SCFP, however, disappeared upon DSS administration in this study. Taken together, these data may indicate that butyrate production resulting from SCFP supplementation was not sufficient to overcome the inflammatory insult induced by DSS.

The results obtained from this study were contradictory to those reported previously, which could possibly be explained by the nature of the yeast products tested. The SCFP tested in this study was derived from *S. cerevisiae*. It has been reported that *S. cerevisiae* and *S. boulardii* are genetically similar but metabolically and physiologically distinct. Greater growth rate and enhanced resistance to low pH and high temperature are characteristics of importance when considering *S. boulardii* for use as a probiotic, but these characteristics are not noted in *S. cerevisiae* ([58, 59]).The metabolic differences between strains might also contribute to distinct metabolite profiles produced by these 2 strains such as the soluble anti-inflammatory factor. To date, there are no data regarding the secretion of soluble anti-inflammatory factors by *S. cerevisiae*. Furthermore, the SCFP evaluated in this study was composed of a large amount of active components from yeast fermentation and a low amount of yeast cells. The amount of undigested carbohydrates that could be utilized by gut microbiota is also low (∼20%) in SCFP. Given these features, SCFP may not be able to modulate the gut microbiota as dramatically as live *S. boulardii*. Our results and previous reports suggest that viable yeasts rather than yeast active components may be necessary for the management of intestinal inflammation. However, more research on *S. cerevisiae* supplementation is needed to confirm this hypothesis. A dose–response study that tests several inclusion amounts of SCFP may also be of interest.

In conclusion, supplementation of 5% SCFP did not protect mice from DSS-induced colitis, despite slight modulation of the gut microbiota. As reported previously, PH relieved the clinical signs of colitis, which was possibly due to increased gut integrity, modulated gut microbiota, and/or a greater physical barrier coming from the gel-forming characteristics of PH fiber.

Acknowledgments

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