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Cite this: DOI: 10.1039/d1fo01314a

Probiotic yeast BR14 ameliorates DSS-induced colitis by restoring the gut barrier and adjusting the intestinal microbiota†

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The probiotic *Saccharomyces boulardii* has been widely used in colitis treatment; however, the beneficial effects of other yeast species are rarely studied. *Saccharomyces cerevisiae* with great stress tolerance and potential in colitis treatment was investigated in this study. Among 16 yeast strains, BR14, BR54, and BR174 strains showed good stress-resistant capacity, anti-inflammatory activity, and little toxicity to macrophages. As for the colitis mice, BR14 inhibited weight loss the most, as well as the disease activity index and colon shortening. After treatment with BR14, the expression levels of genes related to histological damage were all upregulated. BR14 significantly attenuated the expression levels of TNF-α and IL-6, while the expression of IL-10 was upregulated. Additionally, BR14 rebalanced the intestinal microbial composition of colitis mice by increasing the abundance of *Muribaculaceae*, *Lactobacillus* and *Rikenellaceae* and decreasing the abundance of *Turicibacter*, *Escherichia-Shigella*, *Desulfovibrio*, and *Lachnospiraceae*. In summary, BR14 exhibited great potential in alleviating colitis through restoring the gut barrier and adjusting the intestinal microbiota.

Received 27th April 2021,
Accepted 18th July 2021

DOI: 10.1039/d1fo01314a

rsc.li/food-function

Published on 22 July 2021. Downloaded on 8/12/2021 8:19:57 AM.

1. Introduction

Yeasts are microorganisms that are widespread in natural environments. Yeasts have become highly important microorganisms and are used for processing many food products, such as wine,[1] bread,[2] and other foods.[3] They have a major impact on food safety and organoleptic characteristics. Yeasts can oxidize and ferment simple sugars into CO_2, H_2O, and ethanol, and are also capable of transforming peptides, amino acids, and sugars into flavorful compounds, such as higher fusel alcohols and organic acids.[4] In addition, bakers and *S. cerevisiae* are available as dietary supplements because of their high nutrition and mineral content.[5]

In past decades, probiotic yeasts have been widely employed in the processing of functional products;[6] the functional foods, which could be fermented with probiotic yeasts are yogurt,[7] cheese,[6] fermented juice,[8] and beers.[9] Parrella *et al.*[10] demonstrated that *S. boulardii* could assure the stability of *Lactobacillus* throughout storage and improve the antioxidant properties of the final fermented products. Banik *et al.*[11] reported that *S. cerevisiae* could be used as a starter culture to ferment traditional Indian dishes, whereby the product would exhibit a significant improvement in the increment of the protein, fiber, starch, total phenolic, and total flavonoid contents. The interest in probiotic effects of yeasts has risen in recent years not only for its fermentation characteristics but also for human health. Many researchers have demonstrated that yeast might have a potential probiotic function with human beings; however, studies on its probiotic effects have rarely been reported, except for *S. boulardii*.[6,12] Thus, studying the probiotic functions of other yeasts is of great significance in the development of yeast strains for the application of functional foods.

Inflammatory bowel disease (IBD) is a nonspecific chronic intestinal inflammatory disease with recurrent, refractory, and uncontrolled inflammatory responses.[13] Pharmaceuticals, such as immunosuppressive drugs, biological agents, and antibiotics, are commonly used to treat IBD. Among these, 5-aminosalicylic acid (5-ASA) and its derivatives are the mainstay of therapy for ulcerative colitis (UC). However, some adverse events may appear during the treatment period, including diar-

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†Electronic supplementary information (ESI) available. See DOI: 10.1039/d1fo01314a

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rhea, abdominal pain, headache, and nasopharyngitis.[14] *S. boulardii* is one of the most successful commercial yeast strains due to its effectiveness in the prevention and treatment of IBD.[15] A lot of studies have indicated that *S. boulardii* plays a positive role in human health.[16,17] For example, *S. boulardii* exerts trophic effects, which appear to be mediated by the release of polyamines,[16] and it can also be used to treat IBD by maintaining the intestinal epithelial barrier function.[15] Numerous studies have indicated that yeasts or products containing yeast cells exhibit significantly anti-inflammatory effects.[18–20] Terciolo *et al.*[15] reported that *S. boulardii* inhibited the growth of a number of microbial pathogens, such as *Escherichia coli* and *Candida albicans*. Kondo *et al.*[18] reported that an aqueous extract suspension of rice bran fermented by *S. cerevisiae* and *Lactobacillus plantarum* showed protective effects on IBD model mice, and inhibited the nitric oxide (NO) secretion of RAW264.7 cells. In addition, the β-glucan of yeast could decrease colonic mucosal damage and exhibited a strong inhibition of pro-inflammatory mediators.[21] According to molecular biology techniques, the probiotic yeast *S. boulardii* has actually been identified as a strain of *S. cerevisiae*.[22–24] However, compared with *S. cerevisiae*, *S. boulardii* is sporulation deficient[25] and has exhibited a higher acid resistance,[26] thicker cell wall,[6] and better ability of adhesion,[25] as well as antibacterial properties.[27] Thus, these are named differentially. These results raise the question of whether the distinguished molecular and phenotypic characteristics of *S. boulardii* that make it perform more effectively as a probiotic strain are only present in it in comparison with other strains of *S. cerevisiae*. However, few studies have reported that other *S. cerevisiae* strains are beneficial to the host.[12]

Currently, the most applied probiotic microorganisms are bacteria, such as *Lactobacillus acidophilus*, *Bifidobacterium*, and *Bacillus*, which are commonly present in the intestinal tract of humans. Compared with bacteria, some *Saccharomyces* offert the following advantages: they do not colonize the host intestinal tract,[28–30] they have an antibiotic resistant ability, and they do not exchange the DNA of resistance genes with that of the bacteria.[17] Although *S. boulardii* exhibits good performance in the amelioration of colitis, whether other yeasts have potential for the treatment of colitis remains to be explored. Therefore, the aim of this study was to obtain some potential probiotic yeasts and to explore the potential their mechanisms of action by analyzing gut microbiota and intestinal relevant gene expression.

2. Materials and methods

2.1. Chemicals and reagents

The following reagents were used: D (+)-glucose, peptone from soya, yeast extract, and agar powder as obtained from Beijing Land Bridge Technology Co., Ltd (Beijing, China). LPS, simulated gastric fluid, and simulated intestinal fluid were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) culture medium, penicillin–streptomycin, and fetal bovine serum (FBS) were from obtained Wisent Corporation (Nanjing, China). The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell proliferation and cytotoxicity assay kit, and nitric oxide (NO) detection kit were obtained from Beyotime Biotechnology (Shanghai, China). Trizol and phosphate buffered saline (PBS) were purchased from Sangon Biotech Co., Ltd (Shanghai, China). DSS was obtained from MP Biomedicals (Aurora, USA). The PrimeScript RT reagent kit was purchased from Takara Biomedical Technology Co., Ltd (Beijing, China). The Myeloperoxidase (MPO) assay kit was from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China).

2.2. Yeast strains properties and culture conditions

The studied regions of the evaluated strains in this study are listed in Table S1.† All the strains were grown on yeast peptone dextrose (YPD) agar plates at 28 °C for 12 h. The yeast strains were cultured in YPD fluid medium with 10^8 CFU mL^{-1} for 12 h at 28 °C, and then the medium were centrifuged at 4500*g* for 10 min (4 °C) to obtain the sediment (yeast cells) or supernatant (metabolites of yeast). To test the ethanol production, the supernatant was analyzed by a biosensors analyzer (Shenzhen Sieman Technology Co., Ltd, Shenzhen, China). All the strains were stored in a 40% glycerol tube at −80 °C and kept at the China General Microbiological Culture Collection Center (Shanghai, China), as *S. cerevisiae* BR14 (preservation number, CGMCC No. 20352).

2.3. Survival of yeast under *in vitro* gastrointestinal and high-temperature conditions

For testing the survival rates of the yeasts under gastric and intestinal conditions, each strain was exposed to simulated gastric fluid and simulated intestinal juice for 4 h, respectively. After 4 h, the samples were diluted in sterile water and surface-plated on YPD agar plates (n = 3). To test the heat-resistant ability of the yeasts, each strain was incubated in YPD fluid medium at 37 °C for 12 h. After 12 h, the tubes containing the yeasts were vortexed for 30 s, and then subjected to a series of 10-fold dilutions with 9.0 ml sterile water, and then surface-plated on YPD agar plates (n = 3).

2.4. Cell cultures

The RAW264.7 cells were purchased from Zhong Qiao Xin Zhou Biotechnology (Shanghai, China). The cells were cultured in DMEM supplemented with 5% FBS and 1% penicillin–streptomycin at 37 °C inside a 5% controlled CO_2 incubator.

2.5. Cell viability

The RAW264.7 cells were seeded at 1×10^4 cells per well in 96-well plates and incubated for 4 h at 37 °C. The old medium was removed and the cells were washed with PBS twice before the experiments.

To study the effects of the yeasts and their metabolites on cell viability, Raw264.7 cells were incubated with different strains or their metabolites in fresh DMEM for 24 h (n = 6). After 24 h, the cell viability was analyzed by the MTT assay kit. The absorbance at 570 nm was measured.

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To study the anti-inflammation activity of the yeasts and their metabolites, 10 μL (10 μg mL^{-1}) LPS was added to the cells in each well and incubated for 4 h. Then, the Raw264.7 cells were incubated with yeasts and their metabolites for 24 h (n = 6). After 24 h, the cell medium was harvested to analyze NO production. The absorbance at 540 nm was measured.

2.6. Colitis model and animal experiments

Male C57BL/6J mice 6 weeks old (18–20 g) were obtained from Shanghai Jiesijie Experimental Animal Co., Ltd (Shanghai, China) and housed under an autoregulated temperature (22 ± 2 °C) and humidity (50% ± 5%) in a 12 h light/dark cycle room. The animal studies were approved and performed following the guidelines of the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University in accordance with the animal protocol 201801130.

After acclimatization for 7 days, the mice were randomly divided into six groups (control, model, cure, DSS + BR14, DSS + BR54 and DSS + BR174, n = 8). As shown in Fig. 4A, the mice in all groups (except the control group) were given 3.5% DSS in fresh water for 7 days. From 5 days to 13 days, the DSS group and control group orally received 200 μL day^{-1} sterile water, while the cure group orally received 200 μL day^{-1} 5-ASA (2.5%), and the yeast-treatment group orally received 200 μL day^{-1} yeast cells (10^8 CFU mL^{-1}).

During the experiments, the body weight, stool consistency and occult blood in the stool were measured daily. At the termination of the study, after 12 h of food deprivation, the excrement of the mice was collected. Then, each mouse was sacrificed, and the colon length and weight were measured. Colon tissues and fecal contents from each mouse were also collected, and then immediately frozen in liquid nitrogen, and retained at −80 °C for further analysis.

2.7. Assessment of disease activity

The disease activity index (DAI) was assessed by a standard scoring system.[31] The body weight loss, stool consistency, and occult blood in the stool were recorded daily (Table S2†). The body weight loss was defined as the percentage difference between the initial body weight (day 0) and at each day. The activity of MPO in the colon tissue was measured using an MPO assay kit according to the manufacturer's instructions.

2.8. Hematoxylin and eosin (H&E) staining

After sacrificing the mice, 5 mm colon tissue was cut off and fixed in neutral buffered formalin for 24 h, then dehydrated with graded alcohol (75%–100%) solutions, and finally embedded in paraffin wax for hematoxylin and eosin staining. The stained slices of colon tissue were scanned with a Pannoramic MIDI digital section scanner and photographed.

2.9. RNA isolation and quantitative real-time polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from the intestinal samples using Trizol according to the manufacturer's instructions. The concentration and purity of the extracted RNA were measured using a NanoDrop ND2000C spectrophotometer (Thermo Scientific, DE) at an A_{260}/A_{280} ratio. RNA integrity was determined by agarose gel electrophoresis using a Gel Doc XR + system (Bio-Rad, Hercules, Canada). One microgram of total RNA was reverse transcribed using a PrimeScript RT reagent kit following the manufacturer's protocol and amplified by real-time quantitative polymerase chain reaction (RT-qPCR) using the SYBR Premix Ex TaqII system (TaKaRa Bio) and the appropriate primers. The reactions were performed as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. Fluorescence was measured at the end of each annealing step, and the melting curves were monitored to confirm the specificity of the PCR products. Data were analyzed according to the 2−ΔΔCt method and normalized to an endogenous reference (β-actin gene). The primers used in this experiment are listed in Table S3.†

2.10. Analysis of the intestinal microbial diversity

DNA was extracted from the mice feces utilizing the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The hypervariable regions V3–V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Then, the purified PCR products were sequenced on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd, (Shanghai, China). Further sequence reads processing in the present study was performed by barcodes using a QIIME pipeline and included additional quality trimming, demultiplexing, and taxonomic assignments. Operational taxonomic unit (OTU) classifications were reported as the percentage of reads that were classified within a given species at a bootstrap value of 97% or greater using Mothur. The representative sequences were classified using the RDP classifier method and SILVA database.

2.11. Statistical analysis

All the experimental data are expressed herein as the mean ± SD. The Statistical Package for Social Sciences (SPSS) software version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. One-way analysis of variance (ANOVA) with Duncan's multiple range test and Student's test was used to compare the data among the different groups. A value of $p < 0.05$ was considered statistically significant, $^{*}P < 0.05$; $^{**}P < 0.01$.

3. Results

3.1. Physicochemical properties of different strains

To confer healthy benefits on the host, probiotics must be able to survive passage through the stomach and the intestine, and

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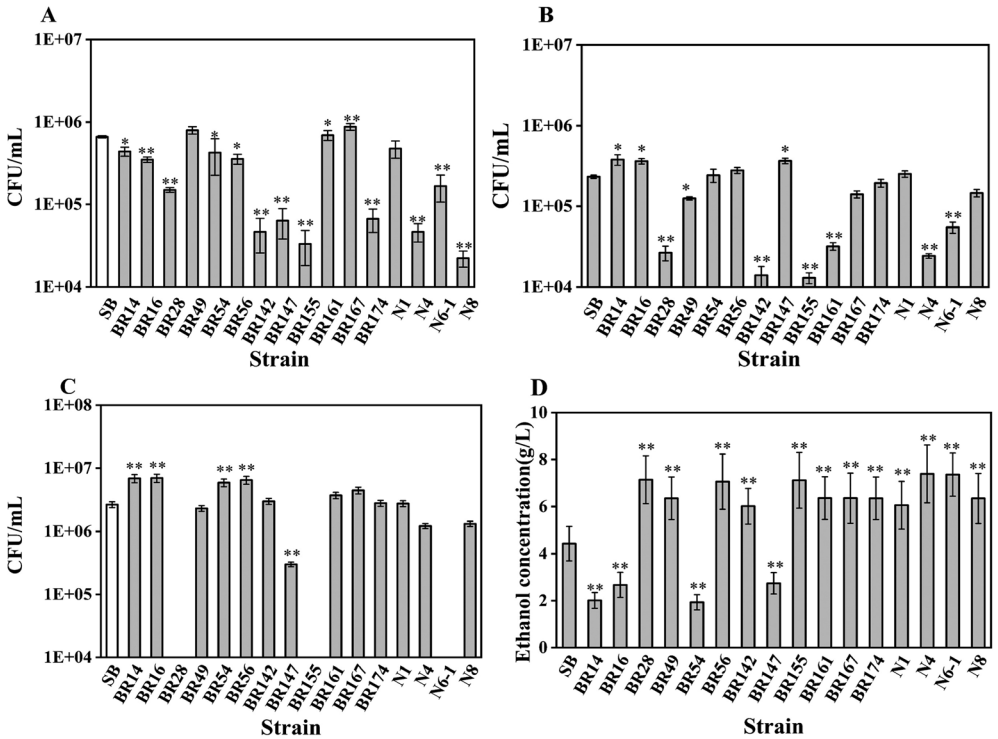

Fig. 1 Effects of different treatments on the cell growth of the yeast strains. (A) Effect of simulated gastric fluid on the viability of the yeasts. (B) Effect of simulated small intestinal juice on the viability of the yeasts. (C) Effect of temperature on the viability of the yeasts. (D) Ethanol-production capacity of 18 yeasts strains. Results are expressed as the mean ± SD for each experimental group. $*p < 0.05$, $**p < 0.01$.

to be present in sufficient numbers to impact the colonic environment.[32] The gastrointestinal tolerance, temperature tolerance, and ethanol production of different yeast strains are shown in Fig. 1, in which SB (*Saccharomyces boulardii*) was set as the control.

In order to test the survival of the yeasts under human gastrointestinal conditions, the viability of the yeasts under the simulated stomach condition and the small intestinal-simulated condition were evaluated (Fig. 1A and B). The tolerance of probiotics to acid and bile can be species and strain specific.[33] Under the acid-stress of gastric conditions, the yeast strains exerted different tolerance abilities; whereby BR161 and BR167 showed the greatest acid-resistance ability, and the biomass of these strains were higher than 5.85 log CFU mL^{-1}, while the biomass of BR142, BR147, BR155, BR174, N4, and N8 ranged from 4.35 to 4.8 log CFU mL^{-1} (Fig. 1A). BR14, BR16, and BR147 displayed the greatest bile resistance, and the biomass of these three strains were about twice that of SB (Fig. 1B). The heat-resistance results revealed that most of the yeast strains were unable to grow at 37 °C, except for the BR14 strain (0.744 ± 0.15 log CFU mL^{-1}) and BR16 strain (0.748 ± 0.15 log CFU mL^{-1}) (Fig. 1C). The ethanol production results revealed that BR14, BR16, BR54, and BR147 were low ethanol-producing strains, while the ethanol production of the other strains was higher than that of the SB strain (Fig. 1D). These results showed that BR14, BR16, BR54, BR56, BR167, and BR174 exhibited better physiological and tolerance activities than the other yeast strains. Therefore, we selected these yeast strains for the subsequent experiments.

3.2. Effects of the yeast strains and their metabolites on the viability of RAW264.7 macrophages

To determine whether the yeasts and metabolites were detrimental to RAW264.7 macrophages, the cell viability was analyzed by MTT assay. After incubating the RAW264.7 macrophages with the yeasts or their metabolites, it was found that only the metabolites promoted the growth of Raw264.7. As shown in Fig. 2A, the SB strain (fourfold change), BR14 strain (352% ± 3.87%), and N1 strain (340% ± 6.54%) showed a stronger stimulation effect on cell growth in comparison with the control group. In Fig. 2B, the stimulation effect of diluted yeast metabolites was weakened, but the effect was almost unchanged in the BR16 group (210% ± 10.91% to 193% ± 6.01%) and BR167 group (183% ± 8.53% to 207% ± 8.89%). In our study, suspension of the yeasts decreased the viability of RAW264.7 (Fig. 2C). After the suspension dilution, the BR14 and BR54 strains showed no cytotoxic effects on RAW264.7 macrophages, while the other strains still had little cytotoxic effect (Fig. 2D).

3.3. NO production in LPS-stimulated RAW264.7 cells regulated by different yeast

The immune cells will produce excessive NO during inflammation, and the overproduction of NO induces pro-

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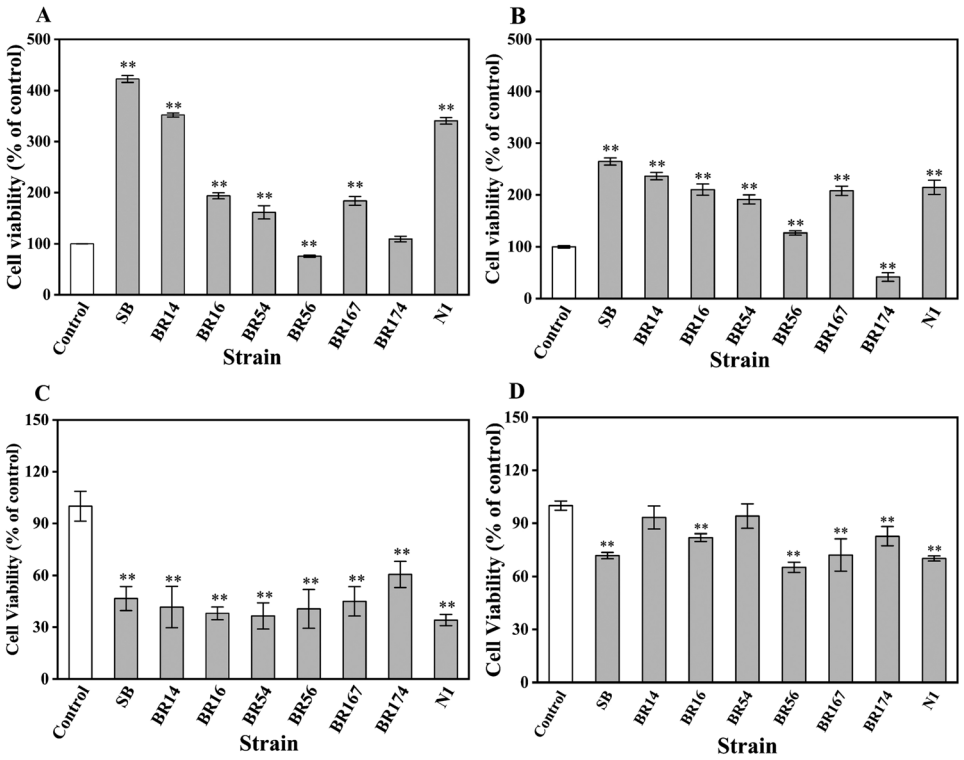

Fig. 2 Effect of the yeast strains and their metabolites on the activity of RAW264.7 macrophages. (A) Effect of the metabolites on RAW264.7. (B) Effect of the dilution metabolites on RAW264.7. (C) Effect of the strain suspensions on RAW264.7. (D) Effect of the dilution strain suspensions on RAW264.7. Results are expressed as the mean ± SD for each experimental group. *$p < 0.05$, **$p < 0.01$.

inflammatory responses, hence it is important to reduce NO production. The effect of the yeast and its metabolite on the regulation of NO production in LPS-stimulated macrophages were examined (Fig. 3). All the yeast strains could reduce NO production compared to the LPS group, whereby the NO concentration of all the groups ranged from 4.29 ± 1.69 μm mL^{-1} to 11.41 ± 1.3 μm mL^{-1} (Fig. 3A). The metabolites from different yeast strains also reduced the NO production; however, the metabolites from the BR56 strain could hardly decrease the concentration of NO (21.33 ± 2.46 μm mL^{-1}) in comparison with the LPS group (18.07 ± 1.99 μm mL^{-1}) (Fig. 3B). The macrophage assays showed that BR14, BR54, and BR174 had little cytotoxic effects on the cells, and thus these three strains were selected to evaluate the probiotic properties by an animal assay.

3.4. BR14 alleviates the symptoms of DSS-induced colitis

To assess the effects of the different yeast strains and 5-ASA on the colitis mice, the body weight loss, decrease of the colon length, MPO, and DAI index were determined (Fig. 4). As pre-

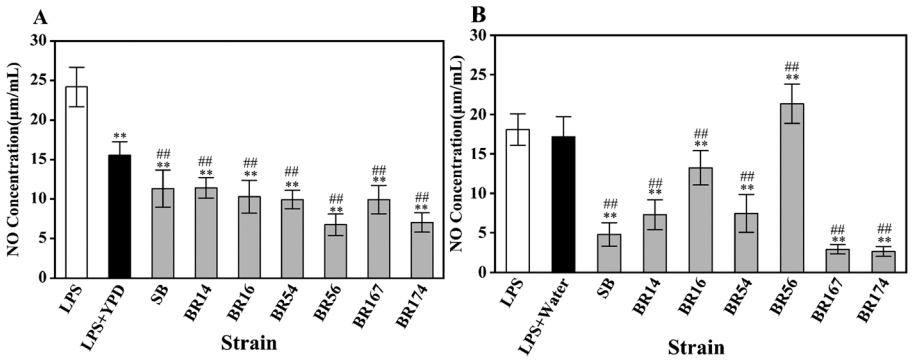

Fig. 3 Effects of the yeasts and metabolites on the NO production in LPS-stimulated RAW264.7 cells. (A) RAW264.7 cells were incubated with metabolites from the yeast. (B) RAW264.7 cells were incubated with the yeasts. Results are expressed as the mean ± SD for each experimental group. *$p < 0.05$, **$p < 0.01$ *vs*. LPS group; #$p < 0.05$, ##$p < 0.01$ *vs*. SB group.

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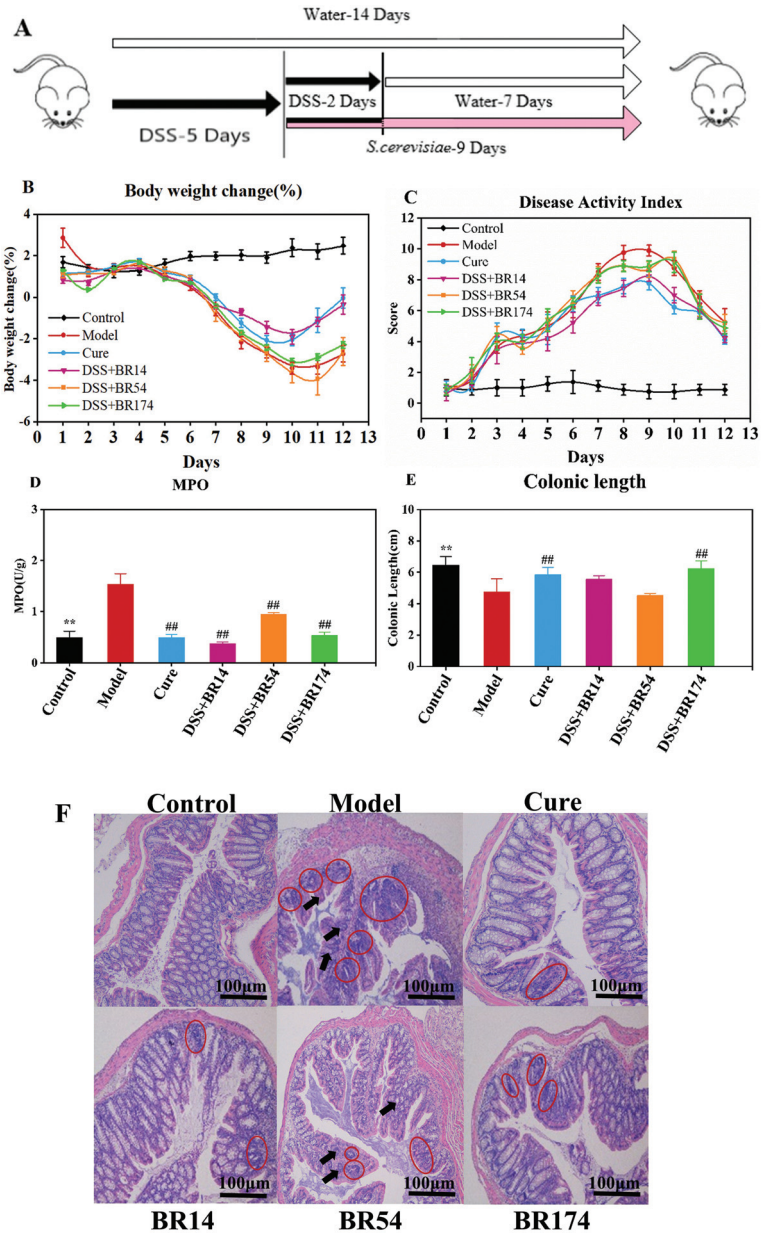

Fig. 4 Effect of different yeasts on the symptoms of DSS-induced colitis in mice. (A) Experimental design for the administration of fungi and DSS administration in conventional mice. (B) Weights of the different groups. (C) DAI of the different groups. (D) MPO activity in colon homogenates. (E) Colons lengths of the different groups. (F) Representative histological examination micrographs of the colonic slides. Red circle indicates inflammatory cell infiltration, black arrow indicates a severe reduction in goblet cells. Results are expressed as the mean ± SD for each experimental group (n = 8). *p < 0.05, **p < 0.01 *vs*. control group; #p < 0.05, ##p < 0.01 *vs*. DSS group.

viously described,[31] DSS treatment led to a loss of body weight in the mice, but the ratio decrease in body weight was lower than in the 5-ASA group and BR14 group (Fig. 4B). The administration of BR54 induced an increased severity of colitis, characterized by a significant weight loss (Fig. 4B).

The inflammatory activity of neutrophils showed that the MPO activity of the DSS-treated mice group (1.53 ± 0.2 U g^{-1}) was significantly higher than that of the control group (0.49 ± 0.13 U g^{-1}), while in contrast, 5-ASA (0.5 ± 0.02 U g^{-1}), BR14 (0.38 ± 0.03 U g^{-1}), BR54 (0.94 ± 0.03 U g^{-1}), and BR174 (0.54 ± 0.07 U g^{-1}) treatment inhibited the MPO activity induced by DSS (Fig. 4D). Colon shortening can be used as a marker of inflammation.[21] After the mice were euthanized, the results of the colonic length confirmed the beneficial effects observed in the 5-ASA group and BR174 group. The 5-ASA, BR14, and BR174 groups prevented DSS-induced shortening of the colon, whereby the colonic length of these three groups were 5.83 ± 0.48 cm, 5.54 ± 0.24 cm, and 6.05 ± 0.62 cm, respectively, but the difference with the BR14 group was not significant (Fig. 4E).

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To further assess the symptoms of DSS-induced colitis, the histological injury was measured. As shown in Fig. 4F, the colon of mice from the model group showed extensive mucosal necrosis, marked inflammatory infiltrate, abundant neutrophils and destroyed goblet cells compared to the control colon group. In contrast, even though some distortions could still be found in the cure and BR14-administrated groups, most the crypt structures were normal. Vast numbers of goblet cells could be observed in the cavities of the crypts, with minimal inflammatory cell infiltration. The colon of mice treated with BR54 and BR174 showed the most goblet cells destroyed, and abundant neutrophils could be observed in the epithelium. The H&E results showed that BR14 and BR174 alleviated effectively colitis symptoms and had q protective effect against tissue damage in terms of the epithelium structure. *S. cerevisiae* BR14 could recover their body weight and decrease the DAI level more rapidly than with BR54 and BR174.

3.5. Changes of inflammatory gene expression and protein secretion

To determine the effect of different yeasts on DSS-induced colitis, secretion of the pro-inflammatory cytokines TNF-α and IL-6, and the anti-inflammatory markers IL-10 were evaluated (Fig. 5). DSS treatment increased the expression of pro-inflammatory cytokines, where TNF-α was increased 8.43-fold and IL-6 increased 3.41-fold as compared to with the control group. Among the yeast-treatment groups, the BR14 strain exhibited the best treatment performance, whereby the levels of TNF-α and IL-6 were significantly lower than in the DSS group ($p < 0.01$) (Fig. 5A and B). Also, 5-ASA administration had little effect on TNF-α expression, while 5-ASA significantly downregulated the mRNA expression of IL-6. There was no significant difference in anti-inflammatory cytokine IL-10 between the model group and cure group (Fig. 5C). However, the administration of BR14 increased the expression level of IL-10, while BR54 and BR174 strains decreased IL-10 expression (Fig. 5C).

Intestinal proteins, such as ZO-1, MUC-2, and MUC-3, are considered to be markers in the evaluation of intestinal injury. As Fig. 5D and F show, compared with the control group, the expression of the genes-encoded intestinal proteins were downregulated in the DSS group ($p < 0.01$). Notably, in all the groups of yeast administration, only the BR14 group upregulated the expression of ZO-1, MUC-2, and MUC-3 (1.68 ± 0.12-, 1.36 ± 0.1-, and 1.38 ± 0.39-fold changes, respectively). Also, 5-ASA administration stimulated the expression of ZO-1 and MUC-3, but there was no statistical significance in the gene expression of MUC-2 between the cure and DSS groups. Several studies have shown that the decrease of mucin and TJP are key factors in inducing IBD.[19,34] These results showed that BR14 treatment could improve the expression of mucin and TJP to ameliorate colitis symptoms.

3.6. Effects of *S. cerevisiae* on the gut microbiome structure

Previous evidence has demonstrated that the reduction of gut microbiota diversity is related to the development of colitis.[19,35] The richness and diversity of intestinal microbiota were assessed by α-diversity analysis using Chao and Shannon indices.[36] As shown in Fig. 6A and B, the community richness and diversity of the DSS group was changed significantly compared to that of the control group (Shannon, $p < 0.01$; Chao, $p < 0.05$). Although the diversity of mice treated with BR14 recovered to the same level as that of the control group (Shannon index, $p < 0.05$), the richness of the mice gut microbiota showed no significant change ($p > 0.05$). In Fig. 6B, the Chao index of the cure group was much lower than that of the other groups, demonstrating that 5-ASA could destroy the richness

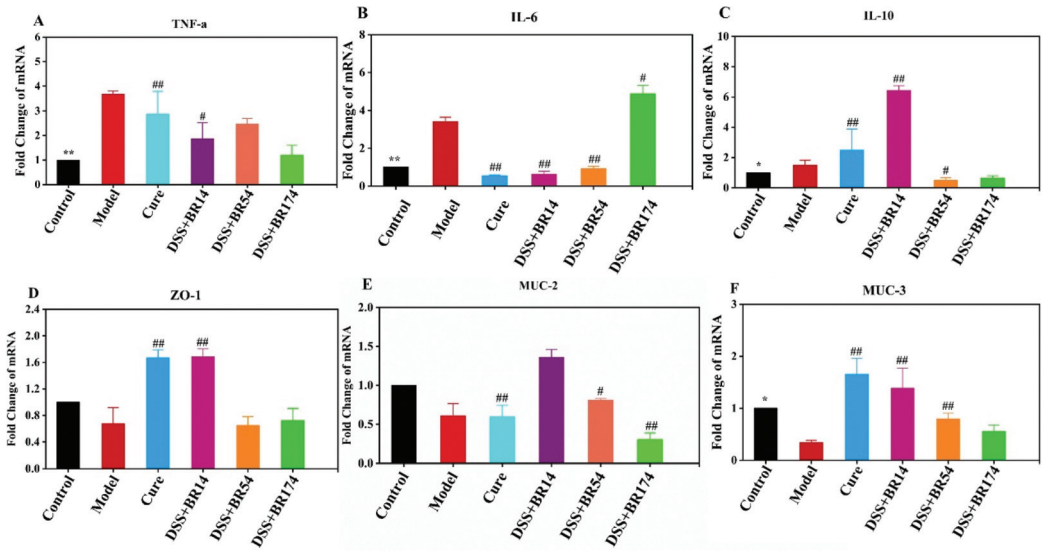

Fig. 5 Effects of different yeasts on the inflammatory responses in the colon of DSS-induced colitis mice. (A–C) mRNA expression of inflammatory cytokine in the colon for each group (TNF-α, IL-6, IL-10). (D–F) mRNA expression of proteins in the colon for each group (ZO-1, MUC-2, MUC-3). Data are presented as the mean ± SD, $n \geq 5$. *$p < 0.05$, **$p < 0.01$ *vs.* control group; #$p < 0.05$, ##$p < 0.01$ *vs.* DSS group.

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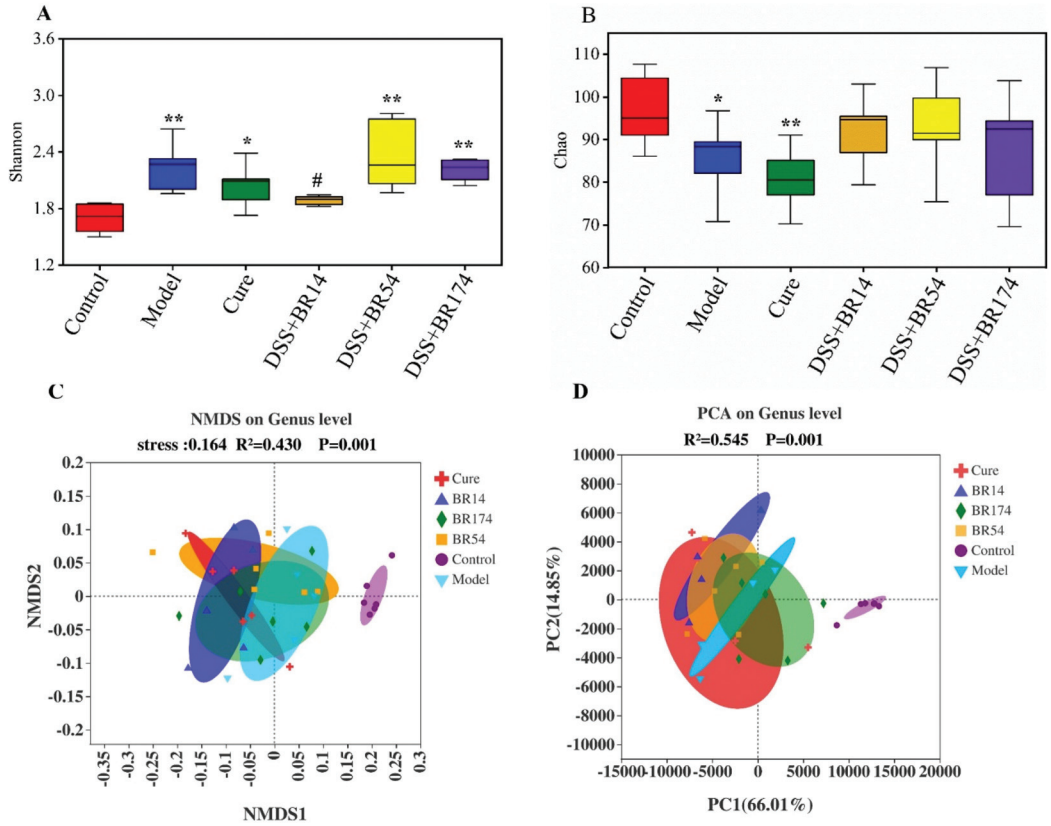

Fig. 6 Effects of the yeasts on the diversities of the gut microbiota. (A) Shannon index; (B) Chao index; (C) NMDS analysis; (D) PCA analysis. Data are presented as the mean ± SD, $n \geq 5$. *$p < 0.05$, **$p < 0.01$ *vs.* control group.

of the gut microbial community to some extent.[37] Although BR14 exhibited no significant effect in recovering the amount of intestinal microbiota, the recovery trend of the gut microbiota amount was increased overall.

Besides, β-analysis, including PCA and NMDS, was used to investigate the gut microbial community structure. As shown in NMDS (Fig. 6C) and PCA (Fig. 6D), the samples of each treatment group were distinctly clustered. In all the DSS-induced groups, only the BR14 treatment group and model group were apparently separated into two clusters, indicating that BR14 treatment modulated dysbiosis of the gut microbiota in the DSS-induced mice to some extent. These analyses confirmed the effect of BR14 on the microbiome structure remodeling in the colitis mice.

3.7. *S. cerevisiae* alters the composition of the gut microbiota

To evaluate the effects of the yeast treatment on the bacterial microbiota during DSS-induced colitis, the 16sRNA sequence method was used to study the change in the gut microbiota. The histograms in Fig. 6A show the relative abundance of major gut microbes in different groups. Compared with the control group, DSS treatment lowered ($p < 0.01$) the abundance of *norank_f_Muribaculaceae*, Clostridium, while it increased ($p < 0.01$) the abundance of *Lactobacillus*, *Rikenellaceae*, and *Esherichia-Shihella*, which had a positive correlation with the pathological characteristics of colitis.[19] As shown in Fig. 6B, further comparison of the relative abundance of gut microbes among the four groups at the genus level revealed that the abundance of *norank_f_Muribaculaceae*, *Clostridium_sensu_stricto_1*, and *norank_f_norank_o_Clostridia_U GG-014* were significantly decreased ($p < 0.01$), while that of *Lactobacillus*, *Turicibacter*, *Rikenellaceae_RC9_gut_group* and *Escherichia-Shigella* were significantly increased in the DSS-treated mice group ($p < 0.05$). By contrast, BR14 treatment could ameliorate these DSS-induced changes in bacterial abundance to a certain extent. For example, BR14 intervention increased ($p < 0.01$) the abundance of *norank_f_Muribaculaceae* by at least 11.79% and of *Lactobacillus* by at least 80.47% and decreased ($p < 0.01$) the abundance of *Turicibacter* and *Clostridium_sensu_stricto_1* by at least 50.79% and 87.21%, respectively, compared with the corresponding abundances in the model group. *Lactobacillus* is a probiotic bacterium that contributes to preventing IBD.[31] These results supported the previous study of Shao *et al.*[19]

As shown in Fig. 7C and D, correlation analysis was performed to evaluate the association between colitis severity and taxa shift in the fecal microbiota at the genus level. The aim was to identify specific fecal bacterium populations that are potentially associated with colitis. In Fig. 7C, 11 genera were found to be correlated with at least one colitis

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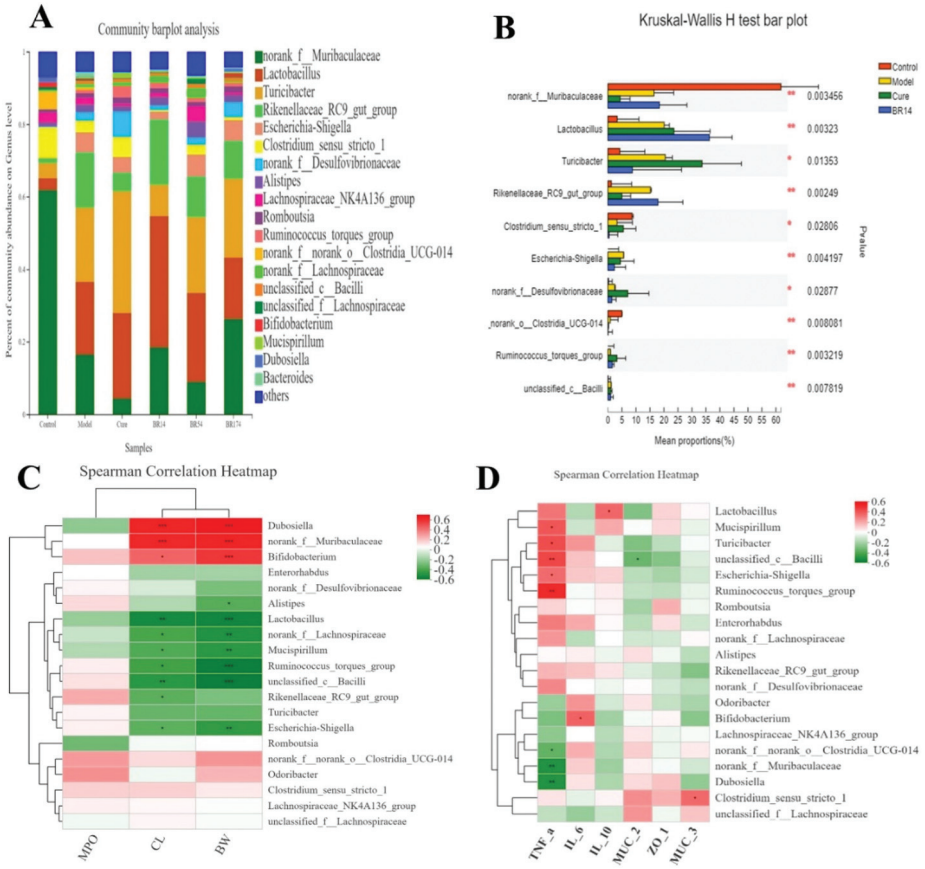

Fig. 7 16s rDNA sequencing revealed an altered microbiota composition after yeast treatment. (A) Relative abundance (%) at the genus level of each group. (B) Phylotypes significantly different between the control, model, cure, and BR14 groups. (C) Correlation analyses between the relative abundance (%) of fecal microbiota and the clinical parameters at the genus level. (D) Correlation analyses between the relative abundance (%) of fecal microbiota and mRNA expression of inflammatory cytokine and epithelial proteins at the genus level. Results are expressed as the mean ± SD. The correlation analyses were performed by Spearman's correlation analyses, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

parameter. The genera *Dubosiella*, *norank_f_Muribaculaceae*, and *Bifidobacterium* showed significant positive correlations with some colitis parameters, including colon length and body weight. In contrast, the genera *Lactobacillus*, *norank_f_Lachnospiraceae*, *Mucispirillum*, *Escherichia-Shigella*, *Ruminococcus_torques_group*, and *unclassified_c_Bacilli* showed significant negative correlations with the colonic length and body weight. As shown in Fig. 7D, the genera *Lactobacillus* and *Bifidobacterium* showed significant positive correlations with the expression of IL-10. Also, the genera *Escherichia-Shigella*, *Turicibacter*, and *Ruminococcus_torques_group* showed significant positive correlations with the expression of TNF-α.

4. Discussion

Potentially a probiotic, especially one that exerts beneficial effects on human health, should possess the ability to survive in the gastrointestinal tract (to tolerate acid pH and bile salts). Resistance to acid and bile is a probiotic property that is essential for yeasts to have protection systems to survive in stress environments. Most of probiotic microorganisms belong to the bacteria (like *Lactobacillus*), while fungi (like *S. cerevisiae*) that exist in dairy food and fermented products also exhibit probiotic activity.[38] A variety of yeast species are used in different productions, such as bread,[2] alcoholic beverages,[39] and fermented fruit juices.[40] However, except for *S. boulardii*, few reports have focused on *Saccharomyces cerevisiae* and its probiotic activity. In this study, commercially probiotic *S. boulardii* as well as potential probiotic yeasts isolated from starter cultures and food were investigated for their probiotic properties. First, 17 yeast strains were evaluated for their resistant ability to gastrointestinal juice, temperature, and ethanol production ability. Probiotics from different sources are strain specific. Six strains, namely BR14, BR16, BR54, BR56, BR167, and BR174, showed a relatively strong resistant ability to simulated gastrointestinal juice, in agreement with the results reported in other studies.[11,41,42] Campana *et al.*[43] reported that the main decrease in viability was found in the simulated conditions of the small intestinal. As suggested by other researchers, the possible use of yeasts as probiotics is encouraged by the observation of the ability of *S. cerevisiae* members to

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survive passage through the intestinal tract.[44] In addition, the resistance to temperature and ethanol production are valuable characteristics for probiotics. The heat-resistance and ethanol-production abilities of the yeasts were determined. BR14, BR16, and BR54 grew well at 37 °C and showed a low production ability.

Macrophages are one of the most important immune cells that are innate in the body and play a leading role in directing host inflammation and other immune processes. The activation of macrophages has been considered to be a primary and necessary step for immune system stimulation.[45] Therefore, the response of macrophages to yeasts and their metabolites is extremely important. In the present study, the yeast strains and their metabolites were co-incubated with macrophages to confirm whether they were detrimental to the macrophages. No decrease in cell viability was observed after treatment with the yeasts' metabolites. The metabolites enhanced the viability of RAW264.7 macrophages by 2–5 times, demonstrating that the yeast metabolites could promote the proliferation of RAW264.7 macrophages. This result was similar to those reported by other researchers.[46] During growth, yeast cells produce the primary metabolites ethanol and CO_2, as well as secondary metabolites, such as organic acids and polysaccharides.[47,48] Glutathione and polysaccharides may occur in the medium through the lysis of dead yeast cells, and they can enhance the viability of RWA264.7 cells.[49] However, the interactions among other metabolites from yeasts and macrophages have been poorly studied to date. Hence, which metabolite stimulates cell growth most obviously in this study required further research. A high concentration of strains' suspension decreased the cell viability, while the cell viability increased after the suspension was diluted. A few studies have reported that yeasts were detrimental to macrophages, but some findings indicated that *S. cerevisiae* could stimulate apoptosis in cancer cell through the nutrient competition.[50,51] Hence, we suspected that a high concentration of yeast suspension decreased the cell viability by competing for available nutrients, like the glucose and amino acids. The therapeutic benefits of fungal β-glucans have been demonstrated as immuno-stimulating agents. Zheng *et al.*[52] pointed out that yeast glucans interacted with complement receptor 3 and toll-like receptor 2 on the surface of RAW264.7 cells, and initiated the activation of RAW264.7 cells, as characterized by the significant production of TNF-α and monocyte chemoattractant protein 1. Probiotics used in foods are expected to be safe and beneficial to human health. Therefore, probiotic characterization must be carried out through standard experiments *in vitro*. Three isolated yeast species (BR14, BR54, and BR174) showed high survival rates under stress conditions, and the proliferation of macrophages was remarkably increased by the stimulation of the metabolites of these three yeast strains. These evidences demonstrated that the isolated yeasts could be classified as potential probiotic yeast species. IBD comprises two types of inflammatory conditions: Crohn's disease and ulcerative colitis.[53] Numerous earlier studies have suggested that impaired intestinal microbiota (dysbiosis), and the upregulation of inflammatory mediators and some cytokines are associated with IBD.[20] Some probiotics can stimulate and regulate innate and adaptive immune responses, such as *L. plantarum* and *B. longum*.[31] The mechanisms of probiotics to cause an alteration in colitis include regulation of the intestinal flora, immunological effects, pathogen-binding, and antitoxic effects.[54–56] Our initial results revealed that the selected *S. cerevisiae* BR14 was able to cause alterations in the colitis symptoms.

In the current study, weight loss, diarrhea, a higher MPO activity, and infiltration with granulocytes appeared after DSS inducement. It has been reported that the appearance of these symptoms during the development of IBD[57] and the decrease in MPO activity may improve IBD treatment. In our study, *S. cerevisiae* BR14 significantly inhibited the increase in MPO activity, suggesting that BR14 had a potent anti-inflammatory effect in colitis. Increased intestinal permeability and decreased expression of TJP and mucin are thought to be closely related to the development of IBD.[58] However, administration with BR14 increased the genes of ZO-1, MUC-2, and MUC-3 expression. Indeed, the expression of these genes in the BR14-administered group was even higher than those in the cure group. Furthermore, BR14 treatment increased the expression of IL-10 and decreased the expression of IL-6 and TNF-α. The changed inflammation in the colon, in turn, further upregulated the expression of TJP and mucosal barrier reconstruction, blocking pathogenic bacteria from passing through the mucus layer. Thus, maintaining the mucosal barrier might be one of the mechanisms by which BR14 alleviates DSS-induced colitis.

Studies suggest that there are two causes of functional disorders of the colonic mucus layer: goblet cells depletion and bacteria over-proliferation.[59] As demonstrated by previous data, one of the obvious differences between the gut microflora in colonic mice and normal mice was the decrease in the diversity of the microbiota components, while the other difference is the change in the overall microbiome structure.[35] As expected, the gut microbiota composition was changed significantly by DSS treatment. At the genus level, *Muribaculaceae*, *Clostridium*, and *Clostridia* were markedly decreased by DSS. *Muribaculaceae* is the primary gut microbiota identified in healthy individuals, which produces succinate, acetate, and propionate.[19] After BR14 intervention, the relative abundance of *Muribaculacea* increased significantly. *Bacteroides*, *Escherichia-Shigella*, and *Lachnospiraceae* exhibited a higher relative abundance in IBD individuals and further led to severe colitis.[60] *Bacteroides* can produce succinic acid and promote inflammation in patients with ulcerative colitis.[61] *Desulfovibrio* is a kind of *Sulfidogenic* bacteria that produce H_2S, and H_2S has been shown to damage DNA, leading to genomic or chromosomal instability. *Lachnospiraceae* has been reported as a potential pathogen in DSS-induced mice.[19] However, *Desulfovibrio*, *Escherichia-Shigella*, and *Lachnospiraceae* were significantly decreased by BR14. It is well know that *Lactobacillus* is a probiotic bacterium and numerous reports indicate that *Lactobacillus* ameliorate inflammation and intestinal barrier in dextran sodium sulfate-induced acute

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colitis.[31,53] *Lactobacillus* colonize the intestines of normal mice, and after DSS treatment maybe increase the abundance of it.[57] However, BR14 treatment significantly increased the abundance of *Lactobacillus* compared with DSS group. Several studies indicated that yeasts can help in the stabilization and proliferation of lactic acid bacterial populations.[62] Thus, *Lactobacillus* might be one of causes of the reduced microbial community in the mice intestine. In summary, the results suggested that BR14 modulated the abundance of these bacteria and thereby suppressed the development of colitis.

In the present study, we obtained a *Saccharomyces cerevisiae* BR14 with great potential in colitis. Compared with *S. boulardii*, BR14 demonstrated an excellent ability against acid, bile, and temperature. Yeast strains and their metabolites also stimulated RAW 264.7 cell growth and inhibited the NO secretion of cells induced by LPS. Moreover, BR14 alleviated DSS-induced colitis model in mice to some extent by improving the gut barrier, inhibiting the inflammatory response, and remodeling the gut microbiome structure.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This study was financially supported by the Shanghai Agriculture Applied Technology Development Program (2019-02-08-00-07-F01152), the National Science Fund for Distinguished Young Scholars (32025029), the Shanghai Engineering Research Center of Food Microbiology Program (19DZ2281100), and the Technical Standard Project of Huangjiu in 2018 (18DZ2200200).

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