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RESEARCH ARTICLE

Effects of *Saccharomyces cerevisiae* Pretreatment on Intestinal Immune Function in Mice with *Salmonella*-Induced Colitis

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Abstract

Salmonella is a major foodborne pathogen, and Saccharomycetes may offer preventive potential. This study evaluated the protective effect of *Saccharomyces cerevisiae* pre-gavage in mice infected with *Salmonella enteritidis*. Forty-eight ICR mice were randomly assigned to four groups: control group (Control), Saccharomycetes cerevisiae group (JM), *Salmonella* infection group (SM), and Saccharomycetes cerevisiae pre-gavage followed by *Salmonella* infection group (JM+SM). During a four-week prevention phase, JM and JM+SM mice received daily Saccharomycetes suspension (1×10^{10} CFU/mL), while Control and SM mice received saline. In the fifth week, SM and JM+SM mice were challenged with *Salmonella* suspension (5.19×10^{9} CFU/mL). Body weight was monitored throughout. At the end, spleen index, serum cytokines, colonic pathology, cytokine mRNA expression, and colonic immune factors were assessed. Compared with the Control group, SM mice showed significant weight loss, colonic damage, elevated spleen index, increased pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and their mRNA, and higher $CD3^+$ and $sIgA^+$ cells ($P<0.05$). JM mice showed no significant changes in any measured parameters compared with the Control group ($P>0.05$). Compared with SM mice, JM+SM mice had attenuated weight loss and significantly improved all measured parameters ($P<0.05$). In conclusion, *S. cerevisiae* pre-gavage effectively alleviates *Salmonella*-induced colitis in mice, likely by mitigating intestinal pathology, balancing pro-/anti-inflammatory factors, and modulating local immune responses.

Keywords: *Saccharomyces cerevisiae*, *Salmonella*, Colitis, Immunomodulation, Intestinal barrier

INTRODUCTION

Salmonella is one of the important foodborne pathogens worldwide, posing a particularly severe threat in the livestock and poultry breeding industry. Its infection can lead to a series of clinical symptoms in the host, such as acute gastroenteritis, bacteremia, subsequently causing impaired intestinal health, hindered animal growth, and decreased immune function [1]. Given the escalating challenge of antibiotic resistance, identifying safe and effective alternative control strategies has become a research priority. Probiotics, with their inherent advantage in regulating intestinal health, have emerged as a core focus in this field. As live microbial supplements, probiotics improve animal health and enhance production performance by modulating gut microbiota balance and strengthening the intestinal barrier, leading to their widespread application in livestock and poultry production [2,3,4]. Among numerous probiotics, Saccharomycetes (such as *Saccharomyces cerevisiae)* have become highly valuable strains due to their unique nutritional composition and immune-modulating potential. This strain's cells are rich in amino acids, vitamins, and various growth-promoting factors. These bioactive substances help improve the intestinal micro-environment, maintain microbial homeostasis, and enhance intestinal barrier function by regulating host immune responses [5,6]. Based on its dual regulatory effects on the intestinal microecology and immune function, Saccharomycetes are considered to have unique application value in preventing intestinal pathogenic microbial infections and alleviating intestinal damage, providing a new potential approach for the prevention and control of *Salmonella*-induced colitis.

The intestine, as the largest digestive organ and a core immune organ in the animal body, is the first line of

defense against *Salmonella* invasion. Its mucosal immune system concentrates over 70% of the body's immune cells, forming a triple defense system through physical barriers, chemical barriers, and immune barriers [7]. Among these, the intestinal immune barrier serves as the first line of defense against pathogenic microorganisms, with its integrity directly determining the host's resistance to *Salmonella* and overall health status [8]. Modulating the intestinal immune system not only maintains intestinal homeostasis and prevents pathogenic microbes from invading the internal environment but also synergizes with beneficial microorganisms in the gut. By participating in metabolic processes and immune regulation, this system collectively safeguards animal health [9]. However, *Salmonella* infection directly damages intestinal morphology, disrupts intestinal flora homeostasis, and consequently impairs intestinal immune barrier function. Research indicates that *Salmonella* can destroy tight junction proteins between intestinal epithelial cells by secreting toxins, degrade antimicrobial peptides in the mucus layer, and simultaneously induce excessive activation of Th17 cells leading to a cytokine storm, ultimately causing a systemic breakdown of barrier function [10]. Furthermore, *Salmonella* Typhimurium infection can lead to gastrointestinal dysfunction, intestinal mucosal damage, and systemic inflammatory responses [11]. Although the functional properties of Saccharomycetes in regulating immune responses and repairing the intestinal mucosal barrier have been preliminarily recognized, and their active components can specifically alleviate intestinal damage, the molecular mechanisms by which they regulate intestinal immune barrier repair through specific components in the context of *Salmonella* infection remain unclear. Moreover, key issues such as the effectiveness of pre-gavage intervention, long-term safety, and dosage optimization have not been resolved. Enhancing intestinal immune function and inhibiting *Salmonella* colonization and proliferation are precisely the core keys to preventing and controlling *Salmonella*-induced colitis, ensuring livestock and poultry health, and improving breeding efficiency.

In recent years, the positive role of Saccharomycetes in preventing and treating intestinal inflammation has been preliminarily confirmed, providing a theoretical basis for its application in preventing and controlling *Salmonella*-induced infectious colitis. Existing research indicates that Saccharomycetes, as probiotic biological agents, can effectively alleviate autoimmune inflammation (such as ulcerative colitis). The core mechanism lies in regulating immune responses and repairing the intestinal mucosal barrier, compensating for the limitations of traditional drugs, and offering advantages such as safety, high efficiency, and minimal side effects [12]. Concurrently, active Saccharomycetes can also improve the intestinal micro-ecological environment, promote intestinal development, and further enhance intestinal resistance to *Salmonella* by consuming intestinal oxygen, regulating the proportion of intestinal flora, and lowering intestinal pH [13]. In probiotic application research, *S. cerevisiae* has shown broad application prospects, and its value in alleviating intestinal inflammation has been experimentally confirmed. For instance, Marco Gentili et al.[14] found that a *S. cerevisiae* extract with prebiotic properties significantly improved tissue damage and diarrhea symptoms in colitis mice, a mechanism closely related to enhancing intestinal barrier function. This further supports the feasibility of Saccharomycetes alleviating intestinal inflammation by regulating the intestinal barrier. Currently, research on Saccharomycetes intervention mostly focuses on post-onset treatment of colitis, and the research system is relatively mature. However, there is still a lack of in-depth exploration regarding *Salmonella*-induced colitis, particularly the regulatory effect and mechanism of Saccharomycetes pre-gavage on intestinal immune function in this mice model [15]. As a commonly used experimental animal model, mice exhibit physiological and immune systems highly similar to other mammals [16].

Addressing this research gap, this study employs mice as an experimental model. By establishing a *Salmonella*-induced colitis model through *S. cerevisiae* pre-treatment, we systematically investigate the regulatory effects of Saccharomycetes on intestinal immune function in these mice and explore the underlying molecular mechanisms. The results of this study can not only enrich the immunomodulatory theory of Saccharomycetes in *Salmonella* infection prevention and control, clarify the application value of its pre-gavage method, but also provide practical experimental basis and new ideas for developing novel probiotic preparations based on Saccharomycetes and optimizing *Salmonella* prevention and control strategies in livestock and poultry, ultimately contributing to the healthy development of the livestock and poultry breeding industry.

Material and Methods

Ethical Statement

All experimental procedures were approved by the Animal Protection and Ethics Committee of the Southwest University of Science and Technology (Approval No. [L2024032]).

Experimental Materials

The primary materials used in this experiment included *Salmonella* Typhimurium (ATCC 14028, Haibo Bio), *S. cerevisiae* (a laboratory-owned strain, isolated and cultured from a local farm, and identified via sequencing as

S. cerevisiae), and 3-week-old SPF-grade healthy male ICR (Institute of Cancer Research) mice (Beijing Speifu Bio Co.). The yeast strain was identified by molecular methods as follows: genomic DNA was extracted from pure cultures, and the internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR products were sequenced via Sanger sequencing, and the obtained sequences were compared against the NCBI GenBank database using BLAST analysis. The sequence showed 99-100% identity with known *S. cerevisiae* sequences. The ITS sequence has been submitted to GenBank; the accession number will be provided upon manuscript acceptance. The full ITS sequence is also available as supplementary material.

Animal Husbandry and Management

A total of 48 three-week-old healthy male ICR mice were randomly divided into 4 groups: control group (Control), *S. cerevisiae*-only group (JM), *Salmonella* infection group (SM), and Saccharomyces cerevisiae pre-gavage followed by *Salmonella* infection group (JM+SM), with 12 mice per group. The mice were acclimated for one week with standard feeding. The formal experiment consisted of two phases: Weeks 1~4 served as the prevention phase, during which the Control and SM groups were daily gavaged with physiological saline (0.2 mL/mice/day), while the JM and JM+SM groups received an equal volume (0.2 mL/mice/day) of yeast suspension (1×10^{10} CFU/mL). The yeast concentration of 1×10^{10} CFU/mL and the gavage volume of 0.2 mL/mouse/day were selected based on previous studies demonstrating that daily oral administration of *S. cerevisiae* at concentrations of 10^9-10^{10} CFU/mL effectively modulates intestinal immunity and protects against pathogen infection in mouse models [17]. Specifically, Milani et al.[18] reported that daily gavage of *S. cerevisiae* at 10^9CFU/mL significantly reduced inflammation in a dose-dependent manner, while other studies have used similar volumes for yeast administration in mice. Based on these precedents, we selected 1×10^{10} CFU/mL to ensure sufficient yeast colonization and immunomodulatory effects during the four-week prevention phase. Week 5 was the colitis induction phase, during which the Control and JM groups were daily gavaged with physiological saline (0.2 mL/mice/day), while the SM and JM+SM groups received an equal volume (0.2 mLmice/day) of *Salmonella* Typhimurium suspension (5.19×10^9 CFU/mL). Bedding was changed, and cages and water bottles were cleaned every 3 days. The ambient temperature was maintained at 25±2°C.

Determination of Mice Growth Performance

During the experimental period, the body weight of mice in each group was measured and recorded weekly. Body weights were measured in grams (g) to assess growth dynamics throughout the study period. Data are presented as the mean±standard deviation, and trend charts of body weight changes over time for each group were plotted. Furthermore, the differences in body weight among groups at the end of the experiment were compared to analyze the impact of *Salmonella* infection on mice growth and the potential protective effect of yeast pre-treatment.

Determination of Organ Index and Histopathological Observation

After the experiment, mice were weighed, anesthetized with ether, and subjected to retro-orbital blood collection for serum separation. Mice were then euthanized by cervical dislocation. The spleens were collected, surface connective tissue was removed, and after washing, surface moisture was absorbed using filter paper. The spleen weight was measured, and the spleen index was calculated using the formula: Organ Index = (Organ Weight (mg)/Body Weight (g))×100%.

Subsequently, the mid-colon segments were collected and fixed in 4% paraformaldehyde. After thorough fixation, tissues were dehydrated through a graded alcohol series, embedded in paraffin, and sectioned at 5 μm thickness. Sections were rehydrated, sequentially stained with hematoxylin and eosin (H&E), dehydrated, cleared, and mounted with neutral resin. Colonic histological changes were observed under a microscope.

Detection of Serum Cytokine Concentrations

The serum collected and separated above was used to detect the concentrations of interleukins (IL-1β, IL-4, IL-6, IL-10) and tumor necrosis factor-α (TNF-α) using commercial ELISA kits, Specifically, the following kits were used: Mouse IL-1β ELISA Kit (Elabscience, E-EL-M0037), Mouse IL-4 ELISA Kit (Elabscience, E-EL-M0043), Mouse IL-6 ELISA Kit (Elabscience, E-EL-M0044), Mouse IL-10 ELISA Kit (Elabscience, E-EL-M0046), and Mouse TNF-α ELISA Kit (Elabscience, E-EL-M3063). All procedures were performed strictly according to the manufacturers' instructions.

Determination of Cytokine mRNA Expression Levels in Colonic Tissues

The middle 2 cm of the colon (approximately 1 cm from the cecum) was collected from mice under ether anesthesia, rinsed with physiological saline to remove intestinal contents, and then snap-frozen in liquid nitrogen. For analysis, the frozen tissue was removed from liquid nitrogen, ground into powder under liquid nitrogen, and an appropriate amount of tissue powder was taken. Total RNA was extracted using a Trizol reagent kit. RNA integrity was assessed by 1% agarose gel electrophoresis, and RNA concentration was measured using a UV spectrophotometer. RNA was reverse-transcribed into

cDNA using a reverse transcription kit, and then the mRNA expression levels of cytokines (IL-1β, IL-4, IL-6, IL-10, TNF-α) were analyzed using a real-time quantitative PCR instrument. The specific primer sequences for the corresponding genes are shown in *Table 1*. The relative mRNA expression levels of each target gene in the colonic tissue were calculated using the $2^{-}\Delta\Delta Ct$ method.

Fluorescence Staining of Paraffin Sections

Immunofluorescence staining for sIgA, CD3, and $FOXP_3$ was performed on colonic paraffin sections, with DAPI used for nuclear localization. The main steps were as follows: colonic samples were fixed, embedded, sectioned, deparaffinized, rehydrated, subjected to antigen retrieval, decolorized (if necessary), blocked, incubated with primary and secondary antibodies, developed with DAB, counterstained, dehydrated, and mounted. The slides were then scanned using a fluorescence microscope. Based on the scanned images, positive cell rates were quantified and calculated using the HALO platform (Indica Labs, USA).

Statistical Analyses

Differences between groups in the result data were analyzed using one-way analysis of variance (ANOVA). Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test. All data met the assumptions for parametric analysis. When a significant overall difference was detected by ANOVA, Tukey's honest significant difference (HSD) test was performed as the post-hoc multiple comparison procedure to determine significant differences between specific groups (Control, JM, SM, and JM+SM). Visualization graphs were generated using GraphPad Prism 10 software. Data are presented as the mean ± standard deviation, with P=0.05 set as the threshold for statistical significance. The sample size of n=12 per group was determined based on a priori power analysis using G*Power software (version 3.1). Assuming a significance level of α=0.05, a statistical power of 80% (1-β=0.80), and an expected medium-to-large effect size (Cohen's f = 0.40) based on previous *Salmonella* colitis studies, the calculated minimum sample size was 8-10 mice per group. We therefore selected 12 mice per group to account for potential mortality, excluded outliers, and inter-individual variability, while ensuring adequate statistical power for all planned comparisons.

Results

Effect of *S. cerevisiae* on Body Weight Changes in *Salmonella*-Infected Mice

As shown in *Fig. 1*, during the 1-4 week prevention phase (0-28 days), the body weight of mice in all four groups showed a steady upward trend, with no significant differences observed among the groups (P>0.05). Upon entering the Week 5 enteritis induction phase (29-35 days), significant differences in body weight among the groups became apparent. Compared to the Control group, the body weight of the JM group was significantly higher, while that of the SM group was significantly lower. Simultaneously, the body weight of the JM+SM group showed no significant difference compared to the Control and JM groups, but was significantly higher than that of the SM group (P<0.05).

Effects of *S. cerevisiae* on Spleen and Colon Tissue in *Salmonella*-Infected Mice

As shown in *Fig. 2-A, B*, compared to the Control group, the spleens of the JM and JM + SM groups appeared larger in size; however, the spleens of the SM group were larger than those of the JM and JM + SM groups. The spleen index of SM mice was significantly higher than that of the Control, JM, and JM + SM groups (P<0.05). Meanwhile, the spleen index of JM + SM mice was significantly higher than that of the Control group (P<0.05).

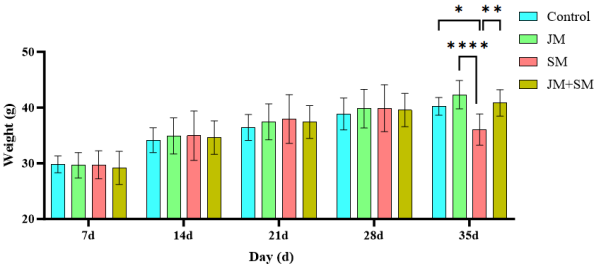

Fig 1. Mice body weight. At each time point, pairwise comparisons among the four groups show no significant differences (P>0.05) and are not labeled in the figure. Only those with significant differences (P<0.05) are annotated. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001

Table 1. *Primer sequences of cytokines*

Target Gene	Forward (5'-3')	Reverse (3'-5')
IL-1β	TCGGCAAAGAAATCAAGATGGC	GTGCAAGTCTCATGAAGTGAGC
IL-6	ACAGAAGGAGTGGCTAAGGA	AGGCATAACGCACTAGGTTT
TNF-α	CGTCGTAGCAAACCACCAAG	TTGAAGAGAAACCTGGGAGTAGACA
IL-4	CTTCCAAGGTGCTTCGCATA	GATGAATCCAGGCATCGAAA
IL-10	AATTCCCTGGGTGAGAAAGCTGAAG	CTGCTCCACTGCCTTTGCTCTTAT

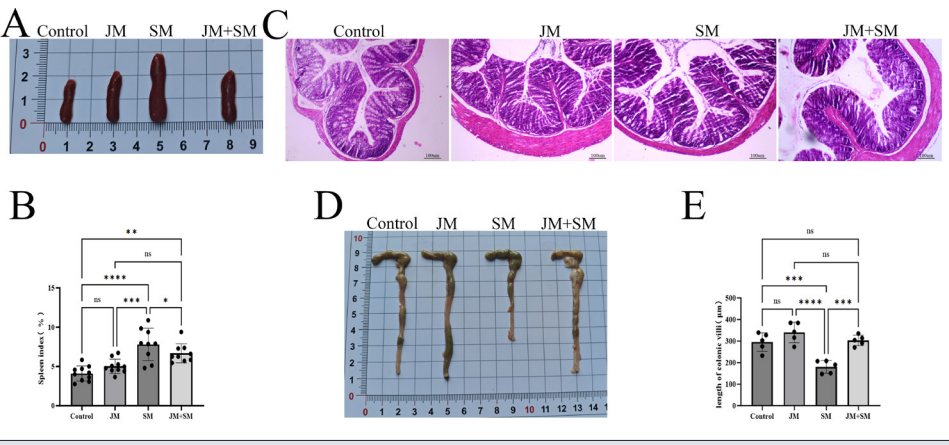

Fig 2. Histological morphology observation and quantitative analysis. **A.** Spleen appearance, **B.** Spleen organ index, **C.** Colon H&E staining (100×), **D.** Colon appearance, **E.** Colon villus length. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, ns P>0.05, The same notation applies subsequently

Morphological and histological alterations in mice colon are depicted in *Fig. 2-C, D, E*. The colons of SM mice showed a reduced number of goblet cells, obvious villus damage, and villus length was significantly shorter compared to the Control, JM, and JM + SM groups *(Fig. 2-C, E)*. The gross morphology also exhibited shortening in length. The degree of villus damage in the JM+SM group was lower than that in the SM group.

Effects of *S. cerevisiae* on Peripheral Blood Cytokine Levels in *Salmonella*-Infected Mice

The cytokine levels in the peripheral blood of mice are shown in *Fig. 3-A,B,C,D,E*. Compared to the Control group, the levels of IL-6, IL-10, IL-1β, and TNF-α were significantly increased in SM mice (P<0.05), while no significant difference was observed for IL-4 (P>0.05). No significant differences were found in the levels of IL-4, IL-6, IL-10, IL-1β, and TNF-α between the JM group and the Control group (P>0.05). The levels of IL-6, IL-10, IL-1β, and TNF-α in the JM+SM group were significantly higher than those in the Control group (P<0.05), but the level of IL-4 showed no significant difference compared to the Control (P>0.05). Compared to the SM group, the levels of IL-6, IL-1β, and TNF-α were significantly lower in the JM+SM group (P<0.05), while no significant differences were observed for IL-4 and IL-10 (P>0.05).

Effects of *S. cerevisiae* on Relative mRNA Expression Levels in Colon Tissue

Fig. 4-A, B, C, D, E present the relative mRNA expression levels of cytokines in mice colon. Compared to the Control group, the relative expression levels of IL-10, IL-6, IL-1β, and TNF-α were significantly increased in

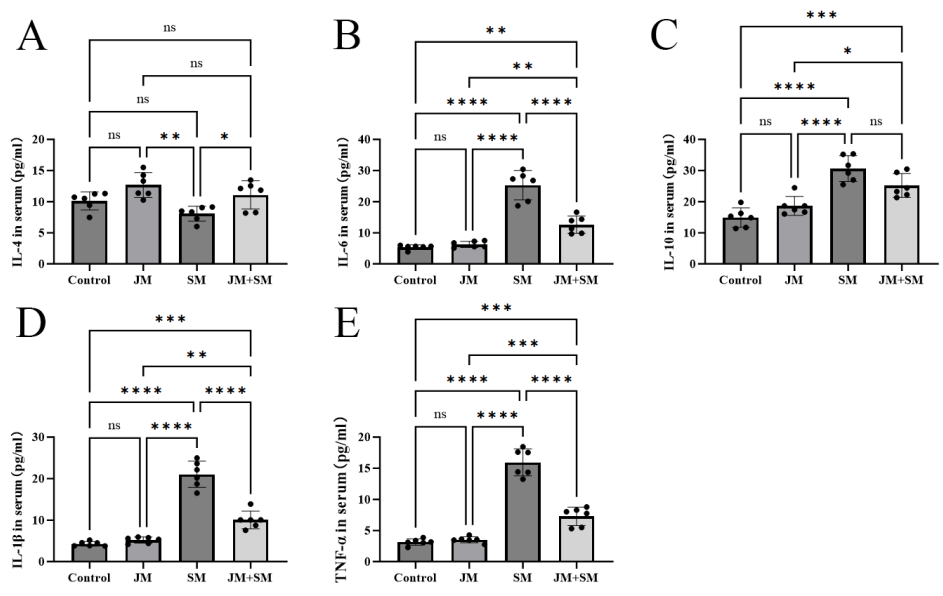

Fig 3. Cytokine levels in murine peripheral blood

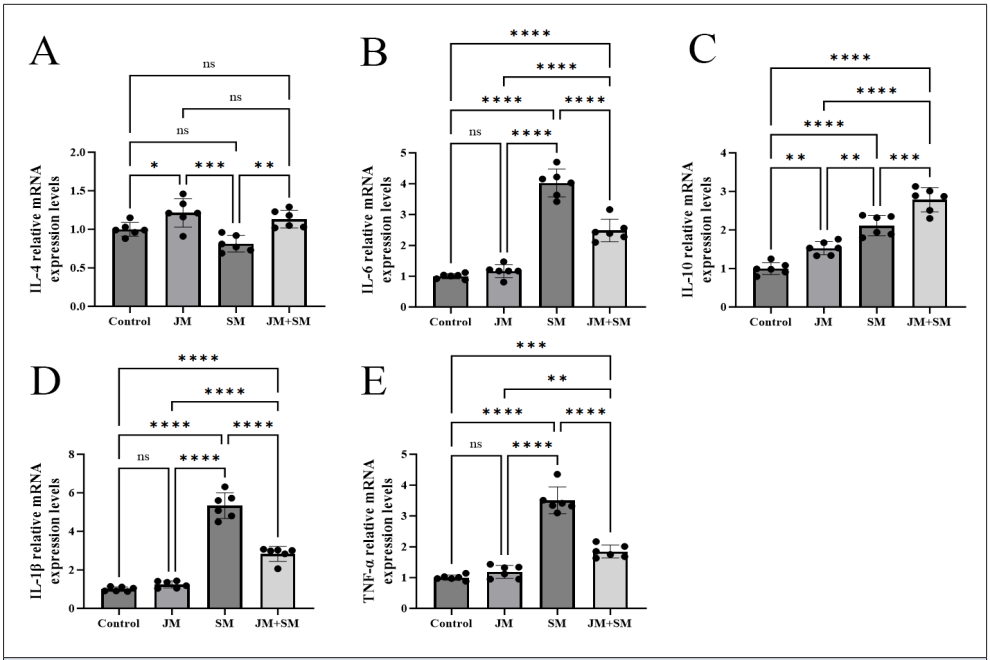

Fig 4. Relative mRNA expression levels of cytokines in murine colonic tissue

the colonic tissues of both the SM and JM+SM groups (P<0.05). In contrast, the relative expression of IL-4 in the SM and JM+SM groups showed no significant difference compared to the Control (P>0.05). No significant differences were observed in the expression of IL-4, IL-10, IL-6, IL-1β, and TNF-α between the JM group and the Control group (P>0.05). The expression levels of IL-4 and IL-10 in the JM+SM group were significantly higher than those in the SM group (P<0.05), while the levels of IL-6, IL-1β, and TNF-α were significantly lower than those in the SM group (P<0.05).

Effects of *S. cerevisiae* on Cytokines in the Colonic Tissue of *Salmonella*-Infected Mice

Fig. 5 shows the results of immunofluorescence staining and positive cell rate analysis for $FOXP_3$, sIgA, and CD3 in mice colonic tissue. The $FOXP_3$-positive cell rates showed no significant differences among the groups. The positive rates for sIgA and CD3 in the SM group were significantly higher than those in the Control, JM, and JM + SM groups (P<0.05); however, no significant differences in sIgA positive rates were observed among the remaining groups (P>0.05). Furthermore, the CD3-positive rate in the JM+SM group was significantly lower than that in the Control and JM groups (P<0.05).

Discussion

As a commonly used probiotic, *S. cerevisiae* possesses a robust survival capability within the intestinal tract, which forms the foundation for their immunomodulatory functions. Recent reviews have systematically summarized the immunomodulatory mechanisms of *S. cerevisiae*, highlighting its capacity to modulate both innate and adaptive immune responses through interactions with pattern recognition receptors on intestinal epithelial cells and immune cells. Studies have shown that Saccharomycetes are not easily inactivated after entering the intestine and can maintain high activity within 2-4 h [19]. Furthermore, their survival rate in simulated

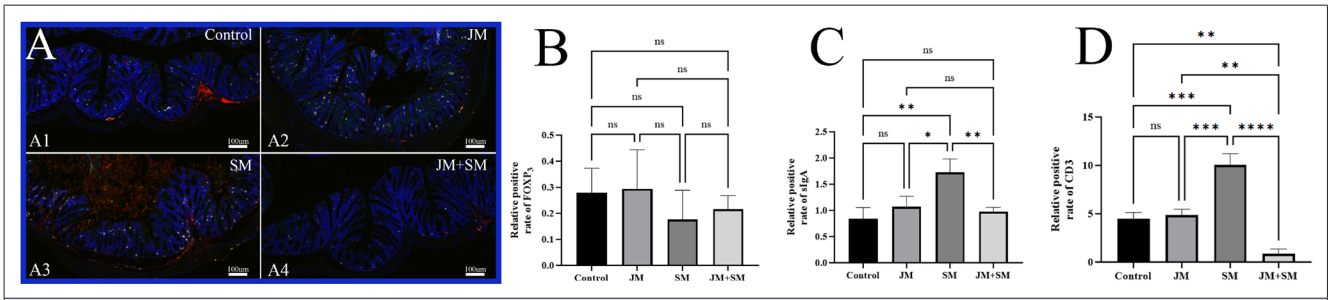

Fig 5. Immunofluorescence staining and quantitative analysis of murine colonic tissue. **A.** Immunofluorescence staining (400× magnification): FOXP3 (green), sIgA (red), CD3 (yellow), nuclear counterstain (blue), **B.** Quantification of FOXP3 cell frequency, **C.** Quantification of sIgA cell frequency, **D.** Quantification of CD3 cell frequency

gastrointestinal environments is significantly higher than that of other microecological agents such as lactobacilli and lactic acid bacteria [20], ensuring their sustained role in immunoregulation and antibacterial activities within the gut. In this study, a probiotic strain of Saccharomycetes was isolated and screened from a local farm. It was identified via 16S rDNA sequencing as *S. cerevisiae*. A suspension of this *S. cerevisiae* was administered via pre-gavage to mice to investigate its regulatory effects on *Salmonella*-induced colitis and intestinal immune function impairment. The results indicated that, compared to the Control group, the JM group showed no significant differences in intestinal immunity and barrier-related indicators. In contrast, the SM group exhibited typical intestinal immune dysfunction, characterized by increased expression of pro-inflammatory factors, decreased expression of anti-inflammatory factors, reduced expression of intestinal tight junction proteins, along with typical colitis symptoms, confirming the successful establishment of the *Salmonella*-induced colitis mouse model. Compared to the SM group, all the aforementioned indicators were significantly improved in the JM+SM group, suggesting that pre-treatment with this *S. cerevisiae* can effectively alleviate *Salmonella*-induced intestinal immune damage and colitis symptoms. This finding is consistent with previous research. Ju et al.[21] found that oral administration of *S. cerevisiae* CNCM I-3856 alleviated inflammatory responses in mice infected with *Salmonella* Typhimurium. Bum Ju Kil et al.[22] also reported that dietary supplementation with S. cerevisiae enhanced host immune responses and competitively inhibited pathogen colonization. Different from the aforementioned studies, the present research employed a pre-treatment intervention strategy, which offers greater preventive advantages compared to post-onset interventions, providing more practical experimental evidence for its application and promotion in livestock and poultry farming.

Intestinal morphology is one of the core indicators for evaluating intestinal health and integrity, playing a pivotal role in maintaining normal physiological functions of the organism. Studies have shown that probiotics can enhance intestinal barrier function by increasing the number of intestinal goblet cells and promoting mucus secretion [23]. In this study, compared to the Control group, SM group mice exhibited significantly shortened colonic villi, reduced goblet cells, and a thinner mucus layer, indicating that Salmonella severely disrupted intestinal structure, accompanied by weight loss. In contrast, JM+SM group mice showed alleviated damage to colonic villi, increased villus length, more orderly arrangement, partial restoration of goblet cells and the mucus layer, and a mitigated trend of weight loss. The results indicate that pre-gavage with the Saccharomycetes suspension significantly alleviated the damage caused by Salmonella to intestinal tissue morphology and markedly reduced the severity of colitis in mice.

Cytokines, as key signaling molecules that regulate immune function, play a crucial role in the body's defense against pathogenic microbial infections and in maintaining immune homeostasis [24]. The cytokines TNF-α, IL-1β, and IL-6 are key pro-inflammatory substances in colitis, while IL-10 and IL-4, as common anti-inflammatory cytokines, play pivotal roles in mediating humoral immune responses and in infectious diseases. Céline Nourrisson et al. found that intervention with *Saccharomyces boulardii* in rats infected with Blastocystis improved the levels of IL-4 and IL-10, thereby normalizing the intestinal microbial flora balance and immune regulatory functions, among other effects [25]. In this study, the expression levels of five cytokines -IL-4, IL-6, IL-10, IL-1β, and TNF-α- in murine peripheral blood and colonic tissue were measured to comprehensively evaluate the regulatory mechanism of Saccharomycetes on the intestinal immune response in *Salmonella*-infected mice. Compared to the Control, the SM group showed significantly elevated levels of IL-6, IL-1β, and TNF-α, reflecting the intense intestinal inflammatory response triggered by *Salmonella*. Simultaneously, IL-4 and IL-10, as core anti-inflammatory factors, increased following *Salmonella* infection, which caused intestinal damage and enhanced the body's compensatory anti-inflammatory response [26]. However, compared to the SM group, the JM+SM group exhibited decreased levels of IL-6, IL-1β, and TNF-α in peripheral blood cytokine content and mRNA expression in colonic tissue, suggesting that Saccharomycetes may alleviate the pro-inflammatory response by inhibiting the colonization of *Salmonella* [27]. This indicates that Saccharomycetes possess certain preventive and therapeutic effects against colitis induced by *Salmonella*.

The mucosa of the intestinal lining is an important immune tissue within the body. Its secretion and release of CD3, $FOXP_3$, and sIgA play crucial roles in maintaining intestinal homeostasis and immune responses [28]. CD3 is a key surface factor of T-cells, involved in T-cell activation and immunity; sIgA is the core antibody of intestinal mucosal immunity, responsible for neutralizing pathogens and preventing their adhesion to intestinal epithelial cells; $FOXP_3$ is the core transcription factor of regulatory T-cells, responsible for maintaining immune tolerance [29-31]. Previous studies indicate that *S. boulardii* can significantly increase sIgA content in the small intestine of mice and reduce intestinal colonization by pathogens such as *Salmonella* [32]. However, it is important to distinguish between therapeutic and preventive contexts: in the cited study, *S. boulardii* was administered as a treatment after infection

was established, where sIgA upregulation represents a protective compensatory response to an ongoing infection. In our preventive model, a different mechanism is at play. A characteristic of *Salmonella*-induced colitis is damage to the colonic epithelial barrier function and the intestinal lining mucosa. In this study, we further investigated the secretion of CD3, $FOXP_3$, and sIgA in the colons of *Salmonella*-infected mice treated with Saccharomycetes. The experiment revealed that $FOXP_3$ expression did not differ significantly among groups. However, the positive rates of CD3 and sIgA in the colonic tissue of the SM group were significantly higher than those in the Control group. This elevation likely represents a compensatory immune hyper-activation triggered by substantial *Salmonella* colonization and epithelial damage-a secondary reaction to an already breached intestinal barrier, rather than an effective protective response. In the JM+SM group, the positive rates of CD3 and sIgA were significantly lower than those in the SM group. Critically, this reduction does not indicate impaired immunity; rather, it suggests that *S. cerevisiae* pre-gavage effectively prevented or minimized initial *Salmonella* colonization, thereby reducing the need for a massive compensatory immune response [33]. Therefore, Saccharomycetes exert a certain protective effect on the mucosal tissue of the intestinal lining.

In summary, this study isolated a strain of *S. cerevisiae* from a local farm and investigated its effects on *Salmonella*-induced colitis and immune function impairment through pre-gavage with the yeast suspension. The results indicate that this yeast has a certain alleviating effect on Salmonella-induced colitis, demonstrates good safety, exerts antibacterial efficacy, and holds the potential to substitute for antibiotics.

Declarations

Availability of Data and Materials: The datasets generated and analyzed during the current study are available from the corresponding author (X.L.) upon reasonable request.

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