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Lacticaseibacillus rhamnosus G7 alleviates DSS-induced ulcerative colitis by regulating the intestinal microbiota

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Abstract

Ulcerative colitis (UC) is an intestinal disease caused by many factors that seriously harms the health of humans and animals. Probiotics are currently widely used to treat intestinal inflammation; however, different strains are specific, and the functions and effects of different strains are still unclear. In this study, *Lacticaseibacillus rhamnosus* G7 isolated from herdsmen yogurt was used. The results of the in vitro evaluation revealed that it had good tolerance and safety. In mice with colitis, G7 alleviated weight loss and colon shortening and reduced the DAI score. After G7 treatment, the levels of proinflammatory factors (IL-1β, IL-6 and TNF-α) and histopathological scores decreased, whereas the level of IL-10 increased. In addition, G7 rebalanced the intestinal microbial composition of colitis model mice by increasing the abundance of *Faecalibaculum* and decreasing the abundance of *Bacteroides* and *Escherichia_Shigella*. In summary, G7 has great potential in the prevention of colitis.

Clinical trial number

Not applicable.

Keywords Probiotics, Inflammatory, *Lacticaseibacillus rhamnosus* G7, Gut microbiota

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease of unknown etiology that involves mainly the rectal and colonic mucosa and submucosa [1]. Abdominal pain, diarrhea and hematochezia are the most common symptoms of UC [2]. Its pathogenesis may be related to many factors, such as intestinal flora imbalance, immune disorders, heredity and the environment [3]. Currently, the clinical treatment of UC commonly involves the use of 5-aminosalicylic acid, glucocorticoids, sulfasalazine, biologics, and immunosuppressants [4, 5]. However, prolonged use of these medications can lead to certain side effects in both humans and animals. Therefore, the identification of green, safe and nontoxic drugs to treat UC is urgently needed.

The intestinal tract of humans and animals possesses a large number of microbiota, the occurrence of various diseases is often associated with disorders of the intestinal microbiota, and there is a complex correlation between microbiota and host interactions. An imbalance in the homeostasis of the intestinal flora often leads to an increase in the number of pathogens, resulting in a subhealthy state of the organism [6–8]. Studies have shown that probiotics play important roles in the healthy development of humans [9] and animals [10] and can regulate the diversity and richness of the intestinal flora [11]. The most commonly used probiotics are *lactic acid bacteria* and *Bifidobacterium*. Numerous studies have shown that the main probiotics that can be used to treat and prevent ulcerative colitis are *lacticaseibacillus rhamnosus* [12, 13], *Lactobacillus acidophilus* [14], *lacticaseibacillus paracasei* [15], *Lactobacillus plantarum* [16] and *Bifidobacterium* [17]. Good probiotics should have the following characteristics: they are safe and nontoxic, they should enter the gastrointestinal tract, they should be able to survive, and they should inhibit the growth and adhesion of harmful bacteria.

Qinghai is a gathering place for ethnic minorities in northwest China. Dairy products are a part of traditional food culture and are indispensable in the daily life of herdsmen. Compared with modern dairy products, traditional dairy products are more ecological and natural, and the content of *lactic acid bacteria* is greater. *Lactic acid bacteria* derived from traditional dairy products are safer. *Lacticaseibacillus rhamnosus* G7 was isolated from the yogurt of herdsmen in Maduo County, Qinghai Province (high-altitude area, 4650 m). However, probiotics have strain specificity, and the mechanisms of action of different strains are still different. Therefore, we conducted a series of safety and probiotic potential evaluations of the G7 strain, including gastric juice and bile salt tests, drug sensitivity tests, antimicrobial activity tests, and hemolysis tests. A mouse colitis model was constructed with 3% DSS to further evaluate the improvement effect of the G7 strain on DSS-induced colitis in mice.

Materials and methods

G7 separation and growth conditions

The specific methods of G7 separation and purification are as follows: 10 mL of yak yogurt is added into a centrifuge tube containing 90 mL of PBS, shaken evenly, and diluted 10 times and 100 times in gradient. 100 μL of diluted liquid was placed on MRS solid culture medium containing 1% calcium carbonate, coated on a flat plate, and cultured anaerobically at 37℃ for 24–48 h. The colonies were selected according to the colony morphology of lactic acid bacteria and placed in a centrifuge tube containing 500μL PBS for mixing, and a certain amount of liquid was taken for plate streaking. According to the above steps, more than three generations were purified, and then Gram staining was performed to confirm the morphology of the strain and 16 S sequencing was performed to confirm the genus.

Bacterial strain and growth conditions

The G7 strain was grown anaerobically on MRS broth and MRS agar and stored in glycerol at -80 °C. The pathogenic bacteria required for the test, including *enterotoxigenic Escherichia coli* ETEC K88, *Escherichia coli* ATCC 25,922, *Escherichia coli* (O157:H7) ATCC 43,888, *Staphylococcus aureus* ATCC 6538, *Proteus vulgaris* ATCC 29,905, *Shigella boydii* ATCC 9207, *Yersinia enterocolitica* ATCC 23,715, *Almonella typhimurium* ATCC 14,028, *Pseudomonas aeruginosa* ATCC 27,853 and *Escherichia coli* ZJTP6, were preserved in the laboratory. They were aerobically grown on NA broth and NA agar and stored in glycerol at -80 °C.

Molecular identification and morphology

Molecular identification of G7 was performed via amplification and sequencing of the 16 S rDNA gene. The amplification procedure was as follows: 95 °C for 5 min; 35 cycles of 95 °C for 45 s, 58 °C for 45 s, and 72 °C for 60 s. The PCR products were subjected to 1% agarose gel electrophoresis to obtain a 1.5 kb molecular band, which was sequenced by Guangzhou Tsingke Biotechnology Co., Ltd. The sequencing results were compared with the sequences in the NCBI database via the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Morphological observation of G7 cells was performed via Gram staining (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and scanning electron microscopy (SEM) (TESCAN MIRA LMS, Czech Republic). Gram staining was performed according to the manufacturer's instructions, and scanning electron microscope samples were prepared as follows: The G7 strain was fixed with 2.5 glutaraldehyde overnight at 4 °C,

and then subjected to gradient dehydration with 30%, 50%, 70%, 80% and 90% ethanol, and finally stored in anhydrous ethanol for later use. The resuspended droplets were placed on a silicon wafer and placed on a Petri dish for total drying. The samples were subsequently directly adhered to the conductive adhesive, sprayed with gold with a 10 mA coating device (Quorum SC7620), and then the morphology of the strain was photographed with a 20 kV SEM.

Bile salt and gastric acid tolerance test

As described by Amal et al., bile salt and gastric acid tolerance tests were performed on G7 strains, and some modifications were made [18]. After the samples were cultured at 37 °C for 24 h, the absorbance of each mixture was measured at $OD_{600\ nm}$ to determine the growth of the strain. MRS-THIO broth without strains was used as a control. The growth rate is expressed by the following formula:

$$\text{Growth rate}(\%) = \left(\frac{\text{OD}_{600\text{nm}}\text{of experimental group}}{\text{OD}_{600\text{nm}}\text{of control group}}\right) \times 100$$

The overnight culture of the G7 strain was inoculated into artificial gastric juice (pH = 3; Dongguan Chuangfeng Automation Technology Co., Ltd., Dongguan, China) at a ratio of 1:9 and cultured at 37 °C. The samples were taken at 0 h and 3 h for plate counting. The survival rate of G7 in artificial gastric juice was expressed via the following formula:

$$\text{Survival rate}(\%) = \left(\frac{3\,\text{hlive bacteria}(\text{CFU/mL})}{0\,\text{h live bacteria}(\text{CFU/mL}}\right) \times 100$$

Antibiotic sensitivity test

The sensitivity of G7 to common antibiotics was determined by Kirby-Bauer disc agar diffusion method. The 12 antibiotics used in this study were ampicillin (10 μg), kanamycin (30 μg), gentamicin (10 μg), chloramphenicol (30 μg), streptomycin (10 μg), tetracyclinel (30 μg), nalidixic axid (30 μg), amoxicillin (20 μg), cefotaxime (30 μg), erythromycin (15 μg), vancomycin (30 μg) and clindamycin (2 μg). Each antibiotic tablet was placed on MRS agar medium containing 10^7 CFU/mL of the G7 strain and incubated at 37 °C for 24 h. The diameter of the disc inhibition zone of each antibiotic was measured. In accordance with Hu et al. [19], the strains were classified as sensitive (≥ 21 mm), intermediate (16–20 mm) or resistant (≤ 15 mm).

Antimicrobial activity

In this study, the antibacterial activity of the G7 strain against the indicator strain was determined via the agar diffusion method. The antibacterial test was carried out with reference to the method described by Zhang et al. [20], with some modifications. The diameter of the inhibition zone was measured after incubation at 37 °C for 24 h. The criteria are as follows: less than or equal to 9 mm is negative (-), 9–12 mm is weak (+), 12–16 mm is strong (++), and more than 16 mm is very strong (+++).

Hemolytic activity

ZJTP2 was used as a positive control strain. The bacterial mixture was streaked on a blood agar plate (Changde Bkman Biotechnology Co., Ltd., Changde, China) and cultured at 37 °C for 24–48 h. The plate was subjected to hemolysis screening, α-hemolysis (grass green, translucent circle), β-hemolysis (transparent circle), and γ-hemolysis (no transparent circle).

Adhesion

The adhesion test was performed via the above method, but with slight modifications [20]. First, the Caco-2 cell monolayer was washed twice with PBS, and 1 mL of RPMI-1640 containing the 10^8 CFU/mL G7 strain was added and incubated at 37 °C and 5% CO_2 for 2 h. The medium was subsequently discarded, and the cells were washed twice with PBS to remove the strains that did not adhere to the cells. One milliliter (containing 1% Triton X-100) of PBS was added, and the mixture was incubated at 37 °C for 10 min. The obtained lysate was diluted with a PBS gradient and counted on an MRS agar plate. The plate was incubated at 37 °C for 24–48 h, and the viable bacteria were counted. The adhesion rate was calculated according to the following formula:

$$\text{Ad}\,(\%) = \left(\frac{\text{G7 concentration after 2 h}}{\text{G7 initial concentration}}\right) \times 100$$

G7 strain Preparation and animal experimental design

Thirty 6-week-old C57BL/6J male mice (16–18 g) were purchased from Guangzhou Youda Biotechnology Co., Ltd. and raised at the Animal Experimental Center of Guangdong Ocean University. During the feeding period, standard light (12 h light–dark cycle), temperature (22 ± 2 °C), and sufficient food and water were provided daily. After 7 days of adaptation, the mice were randomly divided into 5 groups: the control group (CON), DSS group (DSS), DSS + Low group (LOW), DSS + Middle group (MID) and DSS + High group (HIGH). The CON group and DSS group were given 200 μL PBS by gavage every day. LOW, MID and HIGH groups were intragastrically administered with 200 μL of corresponding concentration of G7 every day. The test scheme is shown in Fig. 1. Changes in body weight and fecal occult blood were recorded every day. The DAI score of the mice was calculated according to Liu et al. [21].

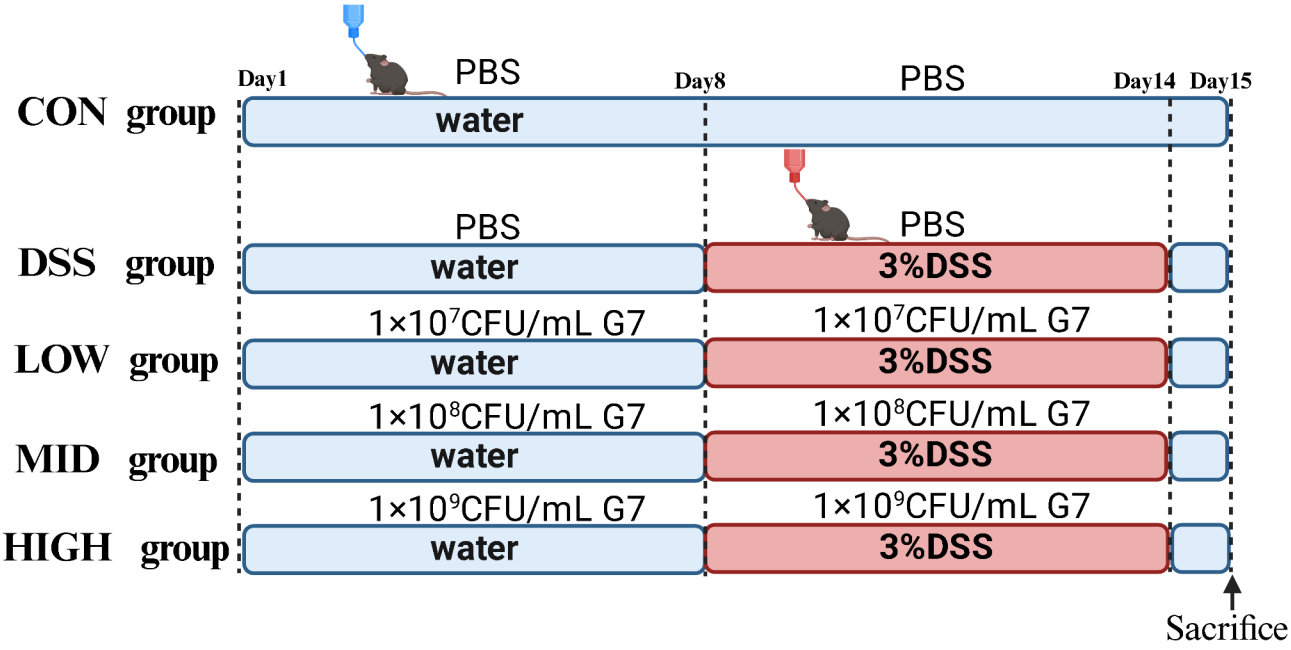

Fig. 1 Animal experimental design scheme

Sample collection

On the 15th day of the experiment, the mice were anesthetized with 3% isoflurane by respiratory pathway for 2 min, and the eyeball blood was taken, centrifuged at 4 °C, 3000 rpm for 15 min, and the serum was collected and stored at -80 °C until use. The mice were then sacrificed by cervical dislocation, and the colon length of each mouse was measured. The colon contents of the mice were collected in a sterile environment for subsequent microbiome sequencing. Part of the colon was placed in 4% paraformaldehyde for subsequent histopathological analysis, and the remaining samples were frozen in liquid nitrogen and stored at -80 °C.

Determination of cytokines in serum

The serum levels of interleukin-1β (IL-1β), interleukin-10 (IL-10), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were determined according to the manufacturer's recommendations (Jiangsu Meimian, China).

Histopathology

Colon tissue fixed with 4% paraformaldehyde was sent to Guangzhou Servicebio Biotechnology Co., Ltd., for hematoxylin and eosin (H&E) staining. HE scoring was performed according to the method described by Liu et al. [21].

Microbiome analysis of the colon

The colon contents (5 from each group) were sent to Beijing Biomarker Biotechnology Co., Ltd., for microbiome sequencing. All microbiome data were analyzed via the BiomarkerCloud platform (https://international.biocloud.net/, accessed on 9 August 2024).

Statistical analyses

All test results were derived from at least three independent experiments. The data are expressed as the means ± standard errors of the means. The chart was created via Prism 8.0 software (GraphPad Software, San Diego, CA, USA). All the data were analyzed via IBM SPSS Statistics 27 via one-way analysis of variance and Student's t test for statistical analysis. A P value < 0.05 was considered statistically significant.

Results

Identification and morphological observation of the G7 strain

In this experiment, as shown in Fig. 2, the G7 colonies were round, convex, smooth, and slightly white, which was consistent with the characteristics of lactic acid bacteria. The results of Gram staining revealed gram-positive rods that were blue–purple (Fig. 2c). The results of 16 S rDNA gene sequencing revealed that G7 had high homology (100%) with *L. rhamnosus* 18,960 (MW674379.1), *L. rhamnosus* 16,097 (MW479249.1) and *L. rhamnosus* 15,041 (MW463572.1). The G7 strain was scanned via SEM, and the bacterial morphology of the G7 strain was smooth, rounded and rod shaped (Fig. 2d).

Tolerance to bile salts and gastric acid

The pH value of the gastric juice is approximately 3, the bile salt concentration is between 0.03 and 0.3%, and the

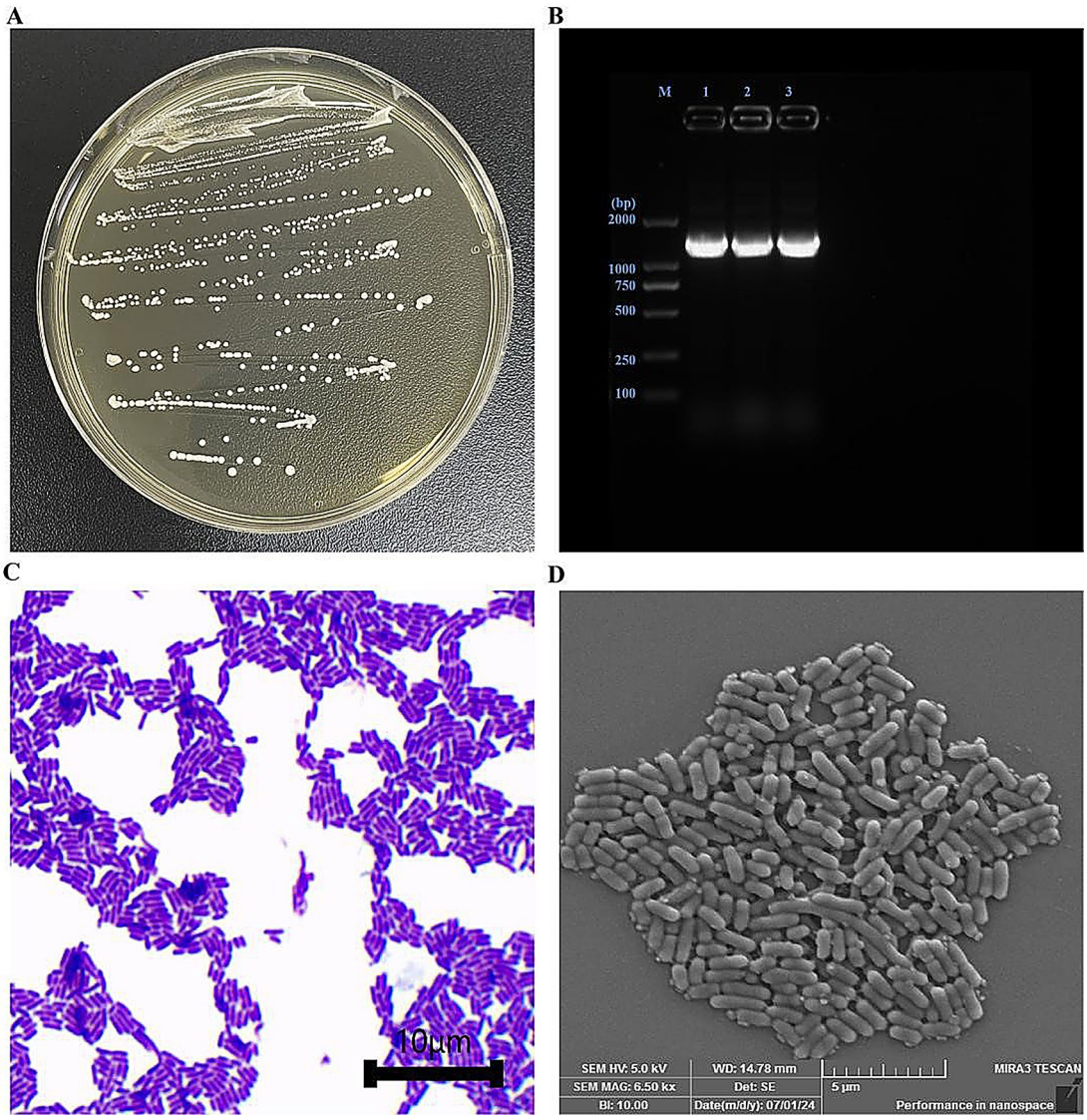

Fig. 2 Molecular identification and morphological observation of the G7 strain. (**A**) Colonial morphology. (**B**) Electrophoretic bands of PCR products (No. 1, No. 2 and No. 3 are PCR products of the G7 strain). (**C**) Gram staining. (**D**) Scanning electron microscopy

food remains in this range for 1–3 h. Therefore, we used artificial gastric juice (pH = 3) and 0.3% bile salt to determine the tolerance of the G7 strain. The results revealed that the survival rate of G7 in artificial gastric juice (pH = 3) was 87.52 ± 0.41. In 0.3% bile salt, the growth rate was 21.23 ± 1.72.

Antibiotic sensitivity analysis

The sensitivity of G7 to 12 antibiotics was detected, and the results are shown in Table 1. G7 was sensitive to six antibiotics, among which tetracycline, nalidixic axid, erythromycin and clindamycin were highly sensitive, and the diameter of the inhibition zone was greater than 21 mm.

Antimicrobial activity analysis

Table 2 shows the antibacterial activity of the G7 strain against 10 pathogens. G7 had a certain effect on 10 pathogens, among which the inhibitory effect on ATCC 27,853 was the strongest, the diameter of the inhibition zone reached 17.78 mm, followed by that on ATCC 23,715, and the diameter of the inhibition zone reached 17.69 mm.

Hemolytic activity analysis

The hemolysis results are shown in Fig. 3. *Bacillus cereus* ZJTP2 produced a clear and transparent hemolysis zone (β-hemolysis), and G7 did not produce a hemolysis zone (γ-hemolysis).

Table 1 Results of the G7 antibiotic susceptibility test

Antibiotic	Antibiotic susceptibility
Ampicillin	S^{21}
Kanamycin	R^{0}
Gentamicin	R^{12}
Chloramphenicol	S^{21}
Streptomycin	R^{11}
Tetracycline	S^{26}
Nalidixic axid	S^{24}
Amoxicillin	I^{19}
Cefotaxime	I^{20}
Erythromycin	S^{29}
Vancomycin	R^{0}
Clindamycin	S^{26}

Note: S represents sensitive (≥ 21 mm), I represents intermediate (16–20 mm), R represents resistant(≤ 15 mm), and the number in the upper right corner represents the corresponding diameter of inhibition zone

Table 2 Antibacterial activity of strain G7

Indicators strains	diameter of inhibition zone (mm)
Enterotoxigenic Escherichia coli ETEC K88	14.70 ± 0.82
Escherichia coli ATCC 25,922	14.88 ± 0.27
Escherichia coli (O157:H7) ATCC 43,888	14.96 ± 0.77
Staphylococcus aureus ATCC 6538	14.21 ± 0.42
Proteus vulgaris ATCC 29,905	17.24 ± 0.43
Shigella boydii ATCC 9207	16.57 ± 0.35
Yersinia enterocolitica ATCC 23,715	17.69 ± 0.62
Almonella typhimurium ATCC 14,028	16.49 ± 0.38
Pseudomonas aeruginosa ATCC 27,853	17.78 ± 0.28
Escherichia coli ZJTP6	17.57 ± 0.21

Note: The values are expressed as the means ± SDs

Adhesion

The adhesion results showed that the G7 strain had good adhesion performance (10.69 ± 0.56).

Effect of G7 on ulcerative colitis

To evaluate the effect of the G7 strain on inflammatory mice, as shown in Fig. 1, we used 3% DSS to establish ulcerative colitis model mice. After 7 days of treatment with 3% DSS, the body weight of the DSS group was significantly lower than that of the CON group ($p < 0.01$) (Fig. 4). After oral administration of the G7 strain, the weight loss trend of the mice was alleviated to some extent. Compared with the DSS group, the MID group and HIGH group presented the most obvious alleviation of weight loss ($p < 0.01$). The DAI score and colon length differed among the five groups. Compared with those in the CON group, the colon lengths of the mice in the DSS group were significantly shorter ($P < 0.01$). However, after treatment with the G7 strain, the degree of colon shortening in the mice was alleviated compared with that in the DSS group ($P < 0.01$).

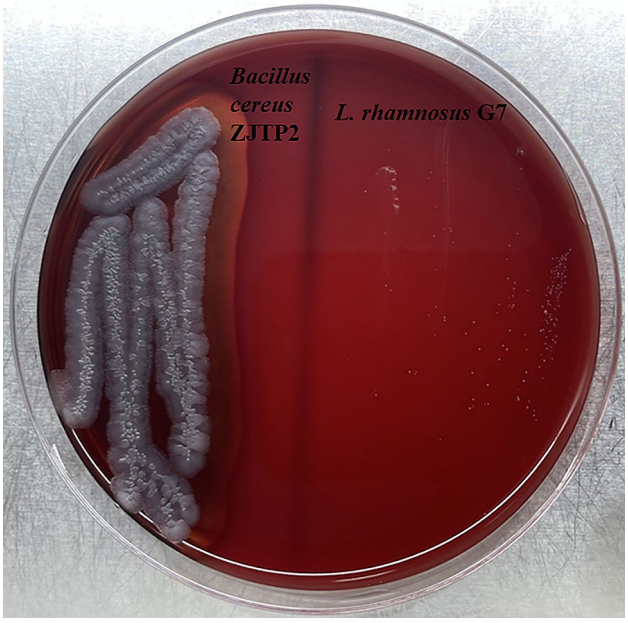

Fig. 3 Hemolysis results for the G7 strain. The left side of the figure shows the hemolysis results for the control bacteria, and the right side shows the hemolysis results for the G7 strain

G7 alleviated histopathological damage in the mice with colitis

To better evaluate the protective effect of G7 in inflammatory bowel disease, we performed histopathological analysis of colon tissue. As shown in Fig. 5, compared with the CON group, the DSS group presented extensive colon damage, including loss of crypt structure, abscess, unclear mucosal structure, and a large amount of inflammatory cell infiltration in the lamina propria and mucosa ($P < 0.01$). Compared with the DSS group, oral administration of the G7 strain improved pathological damage to varying degrees, among which the MID and HIGH groups presented the most significant improvement ($P < 0.05$). In summary, oral administration of a certain dose of the G7 strain can alleviate the symptoms of DSS-induced colitis and has a certain preventive effect.

Effects of G7 on the serum levels of inflammatory cytokines

The concentrations of related cytokines in the serum are shown in Fig. 6. Compared with those in the CON group, the concentrations of all the proinflammatory factors increased significantly after DSS treatment ($P < 0.05$), and the level of the anti-inflammatory factor IL-10 decreased significantly ($P < 0.01$). After oral administration of the G7 strain, the levels of related cytokines were comparable to those in the CON group. Overall, our results show that intervention with a certain dose of the G7 strain can reduce the serum levels of proinflammatory factors and

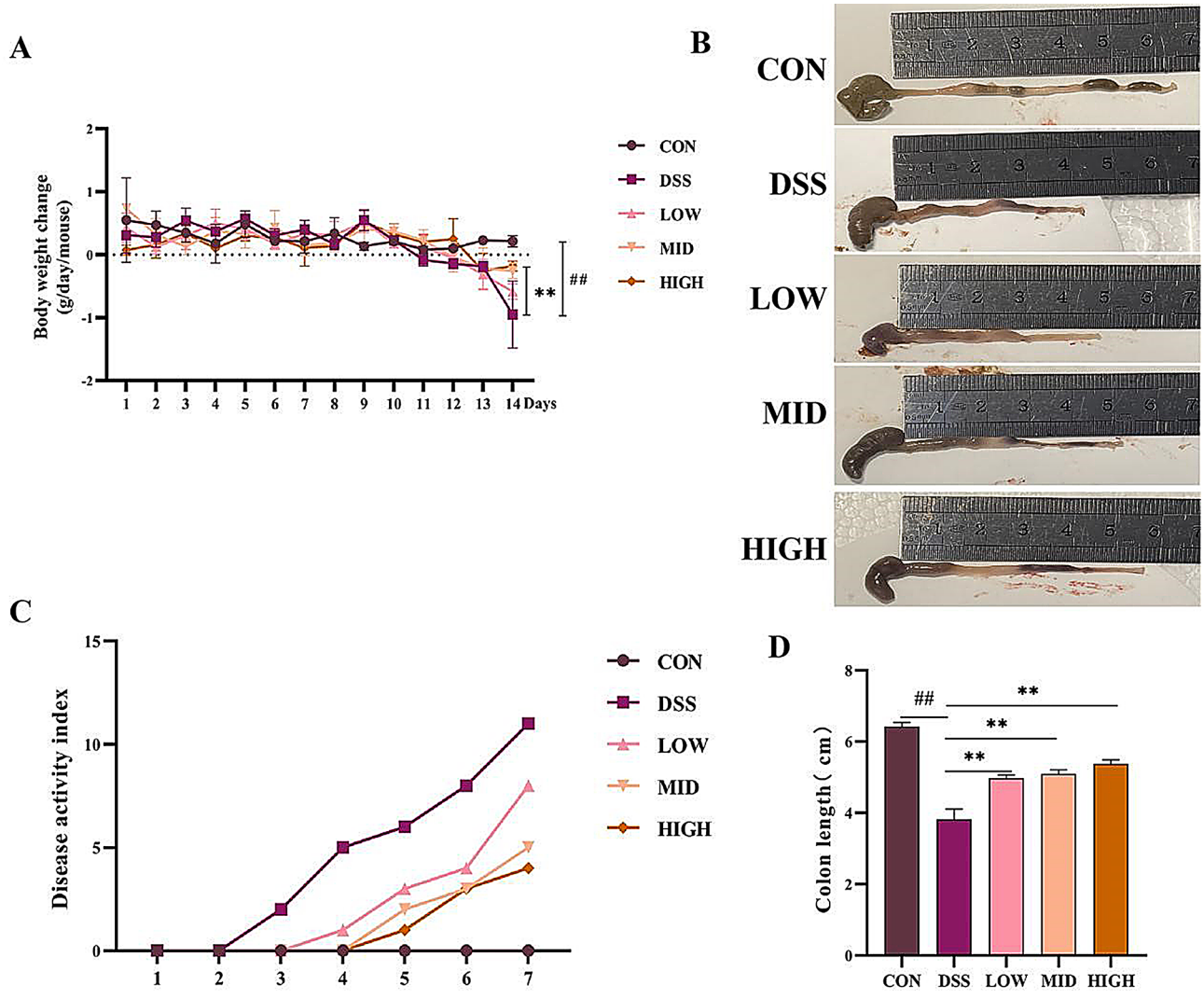

Fig. 4 Effect of the G7 strain on DSS-induced colitis in mice ($n = 3$). (**A**) Body weight change. (**B**) Image of the colon. (**C**) DAI score index. (**D**) Statistical analysis of colon length in the mice. The data are expressed as the means ± SEMs. $^{\#\#}P < 0.01$, compared with the control group. ** $P < 0.01$, compared with the DSS group

increase the levels of anti-inflammatory factors after DSS induction.

Effect of the G7 strain on the intestinal flora induced by DSS in mice

To investigate the effects of the G7 strains on the DSS-induced intestinal microbiota, we performed 16 S rRNA sequencing of the colon contents. According to the Venn diagram (Fig. 7A), there were 288 microorganisms in the five groups of gut flora, of which 87, 61, 3, 8, and 5 different microorganisms were found in the CON, DSS, LOW, MID and HIGH groups, respectively. Alpha diversity reflects the species richness and species diversity of individual samples. The Chao1 and ACE indices were not significantly different between the groups. As shown in Fig. 7B-E, the Shannon and Simpson indices were not significantly different between the DSS group and the CON group. Interestingly, compared with those of the DSS group, the Shannon and Simpson indices of the MID group and the HIGH group were significantly different ($P < 0.05$). To compare the similarity of different samples in terms of species diversity, we used Weighted_UniFrac for beta diversity analysis. As shown in the NMDS (Fig. 7F) and PCoA (Fig. 7G) results, there was a clear clustering separation between the CON and DSS groups, indicating that the microbial compositions differed markedly between them. However, after oral administration of G7, the microbial composition of the MID and HIGH groups was more similar to that of the CON group. The results showed that DSS significantly altered the intestinal microbiota, and oral administration of a certain amount of the G7 strain could regulate the intestinal microbiota.

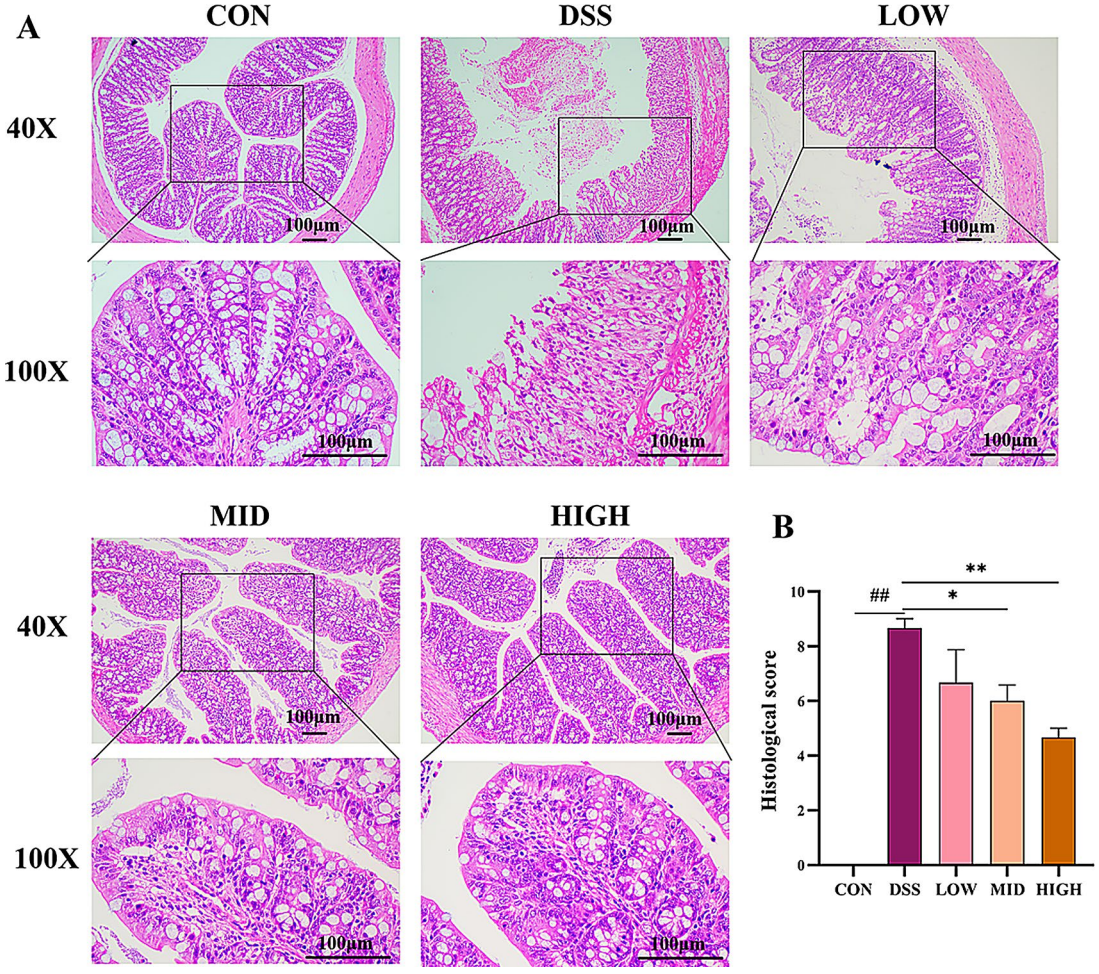

Fig. 5 Evaluation of the effect of G7 on histopathological damage in DSS-induced colitis mice ($n=3$). (**A**) HE-stained colon tissue sections. Scale bar: 100 μm. (**B**) Pathological score of colon tissue. The data are expressed as the means ± SEMs. $^{\#\#}P<0.01$, compared with the control group. * $P<0.05$, ** $P<0.01$, compared with the DSS group

G7 altered the composition of the gut microbiota

As shown in Fig. 8, *Firmicutes* (CON: 56.95%, DSS: 45.98%, LOW: 48.20%, MID: 72.66%, HIGH: 70.36%) and *Bacteroidota* (CON: 35.21%, DSS: 27.45%, LOW: 25.31%, MID: 20.31%, HIGH: 23.05%) were the dominant phyla in the mouse colon microbiota. Compared with those in the CON group, the abundances of *Proteobacteria* and *Verrucomicrobiota* in the DSS group increased by 7.12- and 3.11-fold, respectively. However, after oral administration of G7, the levels of *Proteobacteria* and *Verrucomicrobiota* decreased in both the MID and HIGH groups. At the genus level, the levels of *Escherichia_Shigella*, *Bacteroides* and *Akkermansia* were increased, and the levels of *Faecalibaculum* and *Lactobacillus* were decreased in the DSS group compared with those in the CON group. After oral administration of the G7 strain, the levels of *Escherichia_Shigella*, *Bacteroides* and *Akkermansia* decreased in the MID and HIGH groups, and the levels of *Faecalibaculum* increased, tending toward those in the CON group.

As shown in Fig. 9, LEfSe was used to analyze the significance of differences between groups. There were 17, 4, 6, 9, and 8 significant differences in the CON, DSS, LOW, MID, and HIGH groups, respectively. *Unclassified_Muribaculaceae*, *Lactobacillus* and *Candidatus _Saccharimonas* were more abundant in the CON group, whereas *Escherichia _ Shigella* was more abundant in the DSS group. However, after intervention with the G7 strain, the relative abundance of pathogenic bacteria (*Escherichia_Shigella*) was low, and the relative abundances of *Verrucomicrobiae* at the class level and *Akkermans*i.a. at the genus level in the LOW group were relatively high. In the MID group, the relative abundances of *Firmicutes* at the phylum level and *Erysipelotrichales* and *Clostridiales* at the order level were greater. In the HIGH group, the relative abundances of *Clostridia* at the class level and *Oscillospirales* at the order level were greater. In general, oral administration of a certain amount of the G7 strain can alleviate DSS-induced colitis in mice by stabilizing the balance of the intestinal flora.

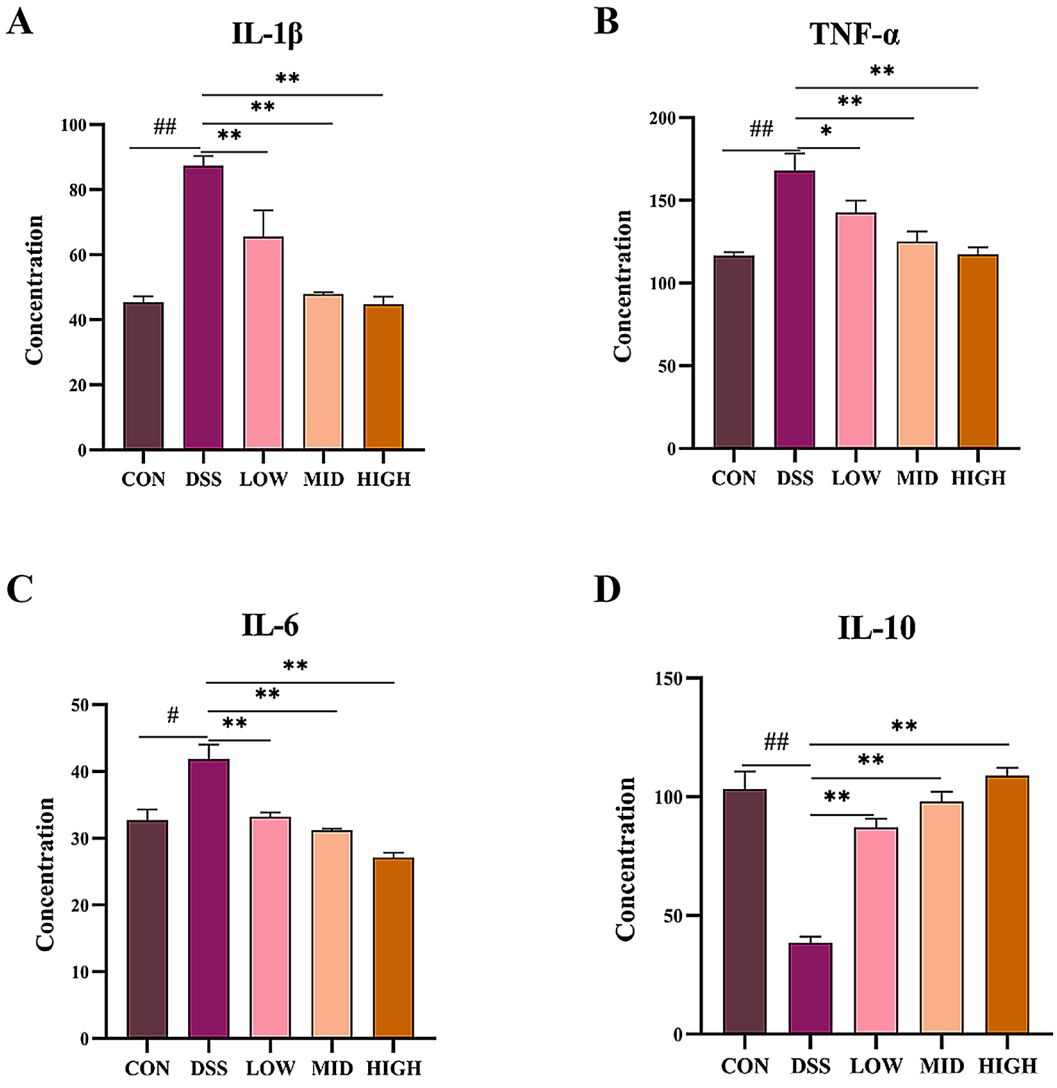

Fig. 6 Effects of the G7 strain on the levels of serum inflammatory factors ($n=3$). (**A**-**D**) Statistical analysis of the levels of serum inflammatory factors. The data are expressed as the means ± SEMs. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the control group. * $P<0.05$, ** $P<0.01$, compared with the DSS group

Discussion

UC is a nonspecific chronic intestinal inflammatory disease, and its pathogenesis is very complex. Many studies have shown that probiotics have certain preventive and palliative effects on ulcerative colitis. In recent years, the application of lactic acid bacteria has been shown to promote health in both humans and animals. Lactic acid bacteria are microorganisms in the intestine. The G7 strain is derived from the yogurt of herdsmen, which is closer to natural ecology and safer. The pH of gastric juice is approximately 3.0, and the bile salt concentration in the small intestine is between 0.03% and 0.3% [22]. Probiotics can only exert probiotic effects in the small intestine after safe passage through the gastrointestinal tract. Therefore, tolerance to bile salts is also one of the criteria for screening probiotics. In this study, the G7 strain had a certain tolerance to artificial gastric juice and bile salts. The second most critical criterion for probiotic selection is that potential probiotics must be safe for humans and animals. Probiotics have inherent and mobile genetic components that can confer resistance to a variety of antibiotics [23, 24]. When strains carrying drug resistance genes enter the host, certain pathogens are also resistant, which can cause severe harm to both humans and animals [25]. The most common characteristic of probiotics is that they can inhibit the growth of pathogenic bacteria [19]. One of the conditions that probiotics should meet is that they can inhibit the growth of bacteria to a certain extent. The adhesion of bacteria to tissues is considered to be the first and key step in microbial colonization [26]. Bacteria can compete with pathogenic bacteria for

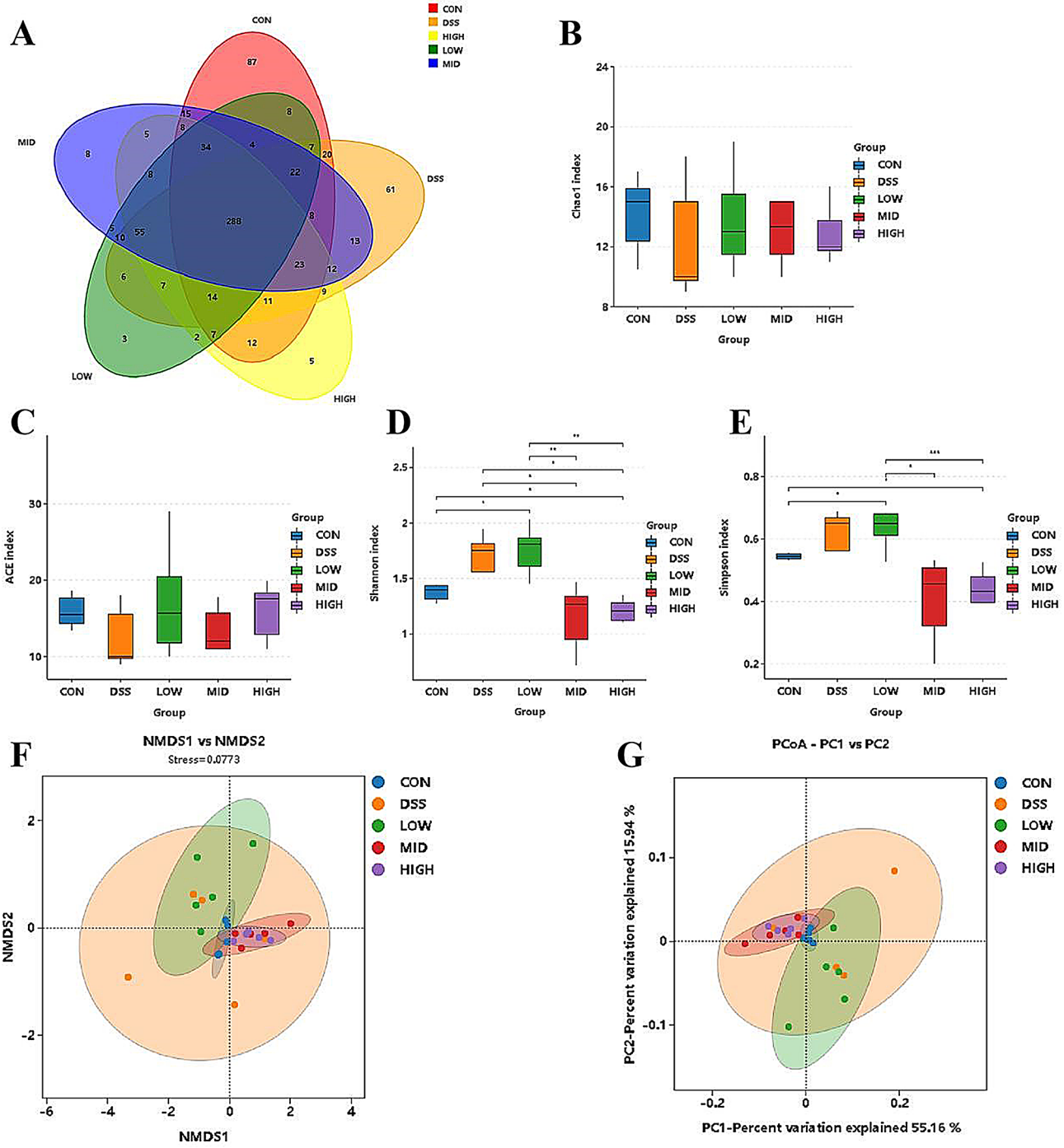

Fig. 7 Effects of the G7 strain on the diversity of the intestinal flora in DSS-induced colitis mice ($n = 5$). (**A**) OTU results. (**B**-**E**) α diversity analysis. (**F**-**G**) β-Diversity analysis of PCoA and NMDS data. The data are expressed as the means ± SEMs. * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$ Comparison within the group

indispensable nutrients and niches after colonizing the intestine. The main immune benefit of lactic acid bacteria is their ability to regulate the host immune response through interactions with the gastrointestinal mucosa [27]. During growth and metabolism, some antibacterial substances, including bacteriocin, lactic acid, organic acid and hydrogen peroxide, are produced. The results of this study revealed that the metabolites of the G7 strain had certain inhibitory effects on the growth of gram-negative and gram-positive bacteria. In this study, G7 strain had good adhesion, and the adhesion rate was similar to that of S. P et al. [28]. In addition, the hemolysis result of the G7 strain was γ-hemolysis, and the strain was safe after it entered the body. In summary,

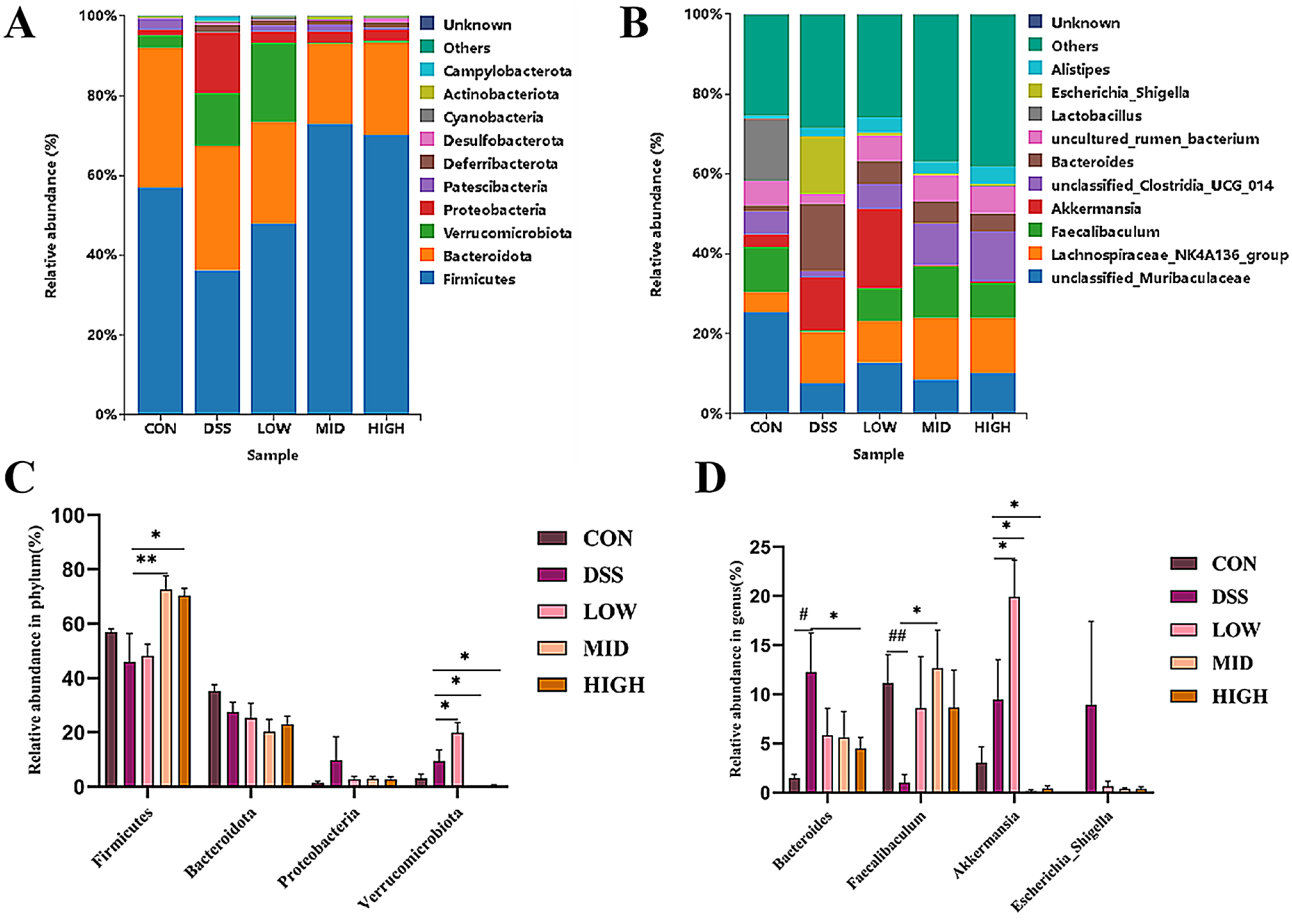

Fig. 8 Effects of the G7 strain on the composition of the intestinal microflora in DSS-induced colitis mice ($n=5$). (**A**-**B**) Abundance of each group at the phylum and genus levels (%). (C-D) Statistical analysis of representative bacteria at the phylum and genus levels. The data are expressed as the means ± SEMs. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the control group. * $P<0.05$, ** $P<0.01$, compared with the DSS group

the G7 strain meets the screening criteria for probiotics and has certain probiotic potential characteristics.

The method of establishing UCs via the DSS is simple and has good reproducibility. Therefore, DSS is commonly used to induce ulcerative colitis, study its pathogenesis and evaluate the effects of drug treatment [29]. In this study, 3% DSS was used to construct a mouse model of ulcerative colitis to observe the effect of the G7 strain on DSS-induced ulcerative colitis in mice. The goal of 3% DSS is to destroy the colonic mucosa, change the permeability of the intestinal mucosa, and allow macromolecular substances to enter the intestinal mucosa, causing damage to the colonic tissue [30]. After 7 days of induction with 3% DSS, the body weights of the mice in the model group tended to decrease, and the DAI scores increased. The colon of the model group was shortened, accompanied by edema, hemorrhage and ulceration. HE staining revealed that the crypt disappeared and that local ulcers, inflammatory cell infiltration and goblet cells decreased in the damaged part of the colon, which suggested that the mouse colitis model established in this study was successful. In this study, compared with the DSS group, the use of the G7 strain obviously alleviated the shortening of the colon and the development of inflammatory lesions related to colon tissue.

Cytokines are small molecular proteins with a wide range of biological activities that have many functions, such as regulating the immune response, mediating the inflammatory response and repairing damaged tissues [30]. The functions of cytokines can be divided into two types: proinflammatory (IL-1β, IL-6, and TNF-α) and anti-inflammatory (IL-10) [31]. Studies have shown that IL-1β, IL-6, TNF-α and IL-10 are the main immune response factors involved in the inflammatory response [32]. For example, IL-10, produced by T cells and mononuclear macrophages, inhibits the production of proinflammatory cytokines and plays an important role in maintaining intestinal immune homeostasis [33]. Previous studies have shown that DSS-induced ulcerative colitis mice have increased levels of proinflammatory factors and decreased levels of anti-inflammatory factors [34]. In this study, treatment with the G7 strain significantly

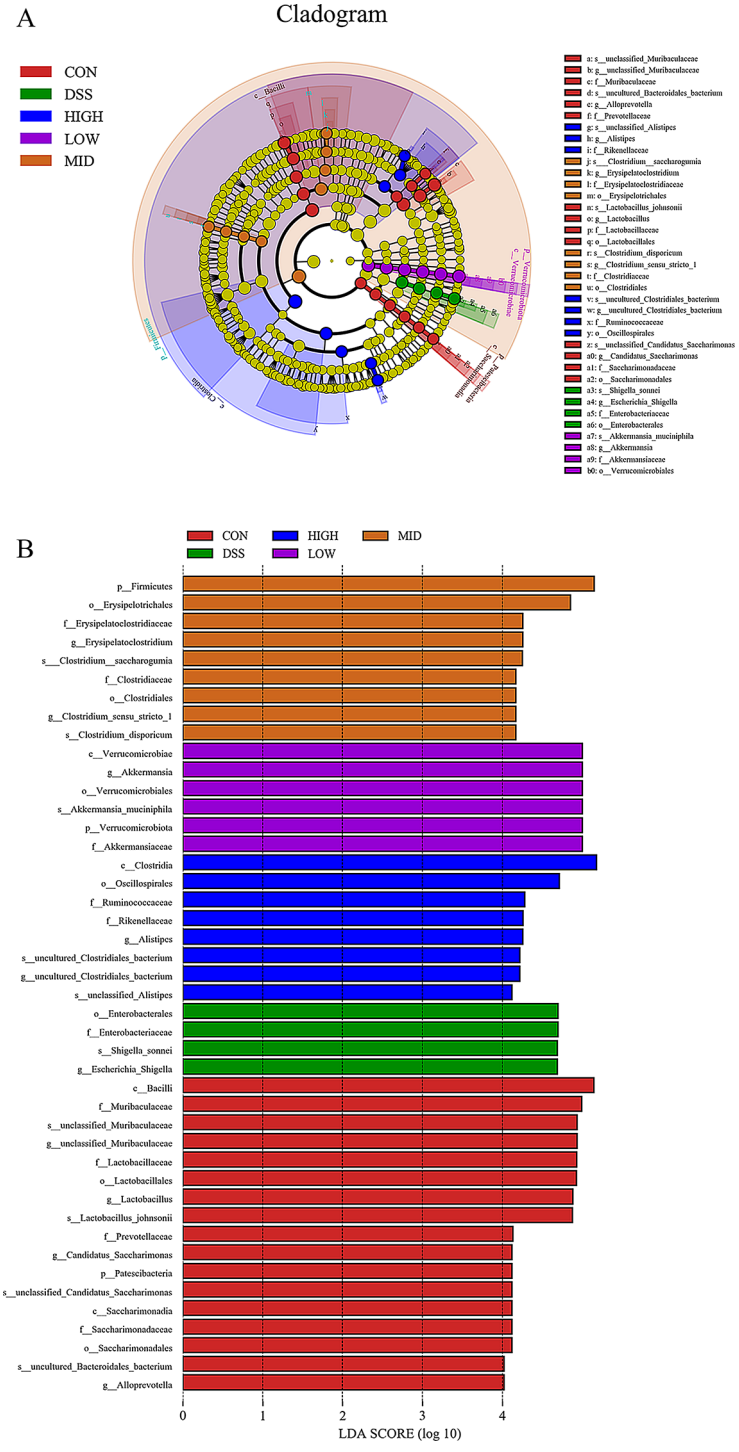

Fig. 9 Effects of the G7 strain treatment on LEfSe. (**A**) LDA analysis. (**B**) LEfSe analysis ($n = 5$)

inhibited the production of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α and promoted the production of the anti-inflammatory cytokine IL-10.

The composition and related abundance of the gut microbiome have a significant effect on the host immune system. The intestinal flora plays an important role in maintaining human health. When an imbalance of intestinal microorganisms occurs, it can cause various diseases [35]. An imbalance of the intestinal microbiota is one of the key factors in the pathogenesis of IBD [36]. Therefore, the use of probiotics to prevent or treat IBD has great potential. The results

of this study revealed that after DSS intervention, the Chao1 index and ACE index decreased, but the Shannon and Simpson indices increased, which may be due to the imbalance of the intestinal microflora in mice and the increase in harmful strains after DSS intervention. In addition, the PCoA results revealed that DSS intervention significantly changed the structure of the intestinal microbiota. However, almost all the groups presented significant differences, indicating that DSS had different effects on the gut microbiota and on the recovery of the G7 strain. Studies have shown that the abundances of *Bacteroides*, *Proteobacteria* and *Escherichia-Shigella* increase and are positively correlated when UC occurs [37, 38]. The presence of *Bacteroides* can induce toxin production, which in turn causes intestinal inflammation in humans [39] and animals, and *Bacteroides* produces succinic acid, which further exacerbates UC [40]. Therefore, a change in the abundance of *Bacteroide*s may be an important indicator of UC. An increase in the abundance of *Proteobacteria* excessively promotes the production of proinflammatory factors by proinflammatory cells, thereby aggravating the degree of UC [41]. The increased abundance of *Escherichia_Shigella* is considered to be a marker of IBD dysregulation and inflammation [40]. It has been reported that the damage caused by DSS to the mucus layer leads to the overgrowth of *Akkermansia* [42, 43]. It is commonly distributed in the colon of mammals, is located within the mucus layer and is capable of degrading mucin. The aberrant number of *Akkermansia* in the DSS group may be due to lesions in the mucus layer caused by DSS, which may further lead to the overproduction of mucin. Interestingly, studies have shown that *Agathobacter*, *Roseburia* and *Faecalibaculum* are the main butyric acid-producing bacteria in the intestine, while butyric acid can significantly inhibit the production of proinflammatory factors by IBD-producing neutrophils. These findings are consistent with our findings [34, 40]. After DSS intervention, the abundances of *Bacteroides*, *Proteobacteria* and *Escherichia_Shigella* in the model group increased. However, after the G7 strain intervention, the abundances of *Bacteroides*, *Proteobacteria* and *Escherichia_Shigella* decreased, and the abundance of *Faecalibaculum* increased. In summary, supplementation with the G7 strain inhibits the harmful microflora in the intestine by increasing the abundance of butyrate-producing bacteria and inhibits the production of related proinflammatory cytokines, which has advantages in alleviating DSS-induced colitis in mice. However, this is limited to the superficial outcomes of G7 on colitis. Future studies are still required to further investigate the mechanism of action and therapeutic targets through which G7 alleviates colitis.

Conclusion

Lacticaseibacillus rhamnosus G7 is a new strain isolated from the yogurt of herdsmen in Maduo County, Qinghai Province. In vitro experiments revealed that G7 had good in vitro resistance and safety. The G7 strain can alleviate weight loss and colon shortening induced by 3% DSS, inhibit the expression of proinflammatory factors, increase the expression of anti-inflammatory factors, promote the growth of beneficial microorganisms in the intestine, and then regulate the homeostasis of the intestinal flora. In summary, the G7 strain can effectively alleviate the symptoms of ulcerative colitis and can be used to prevent colitis.

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Author contributions

JL and MC performed the isolation, probiotic assessment assays and drafted the manuscript. SY, HG, ZW, and YY was involved in data collection, analysis and manuscript revision. DJ, SL, WZ, JL and HT participated in the sample collection and design of the study. JL, MC, XK, ZL, FG, XJ, and YL designed the study and revised the manuscript. All the participating authors read and approved the submitted manuscript.

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Data availability

The raw data presented in this study can be found in the NCBI repository with accession number PRJNA1186119.

Declarations

Ethical approval

All animal experiments were performed in accordance with the guidelines formulated by IACUC-Guangdong Ocean University, ethical approval number 2022-SCUEC-021.

Consent to Publish

Not applicable.

Competing interests

The authors declare no competing interests.

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