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RESEARCH ARTICLE

Bacillus subtilis Ameliorates *Escherichia coli* -Induced Diarrhea in Young Rabbits Through Suppression of Intestinal Inflammation and Remodeling of the Gut Microbiota

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A B S T R A C T

Enterotoxigenic *Escherichia coli* (ETEC) disrupts the intestinal barrier's integrity, leading to severe diarrhea and death in newborns and young animals. *Bacillus subtilis* (*B. subtilis*), a spore-forming probiotic widely distributed in soil and organic substrates, offers multiple physiological benefits, including enhanced nutrient absorption, antioxidant activity, and intestinal mucosal repair. However, its efficacy in treating ETEC-associated diarrhea in rabbits is not well understood. This study explored the protective role of *B. subtilis* and its mechanisms in ETEC-challenged rabbits, focusing on inflammatory responses, intestinal barrier integrity, and microbiota composition. We randomly assigned twenty New Zealand white rabbits to four experimental groups: (1) Control group (CON): saline gavage, (2) Model group (ETEC): ETEC + saline, (3) Norfloxacin group (NOR): ETEC + 0.2 mL/kg/d norfloxacin, and (4) the *B. subtilis* group (BS): ETEC + 1×10^9 CFU/mL *B. subtilis* daily. The results showed that *B. subtilis* intervention alleviated ETEC-induced weight loss and improved colonic pathological damage in rabbits. Moreover, *B. subtilis* was found to impede the induction of the NF-κB signaling pathway and to diminish the concentrations of IL-1, IL-6, and TNF-α. Additionally, it was observed that *B. subtilis* increased the expression of tight junction proteins and reduced the abundance of harmful bacteria, such as Proteobacteria. In summary, *B. subtilis* alleviates ETEC-induced intestinal dysfunction as evidenced by regulating inflammatory responses, maintaining intestinal barrier integrity and modulating the intestinal microbiota.

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INTRODUCTION

Diarrhea is one of the most prevalent diseases affecting the health of livestock and poultry, especially in young animals. Enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of diarrheal disease in humans and animals globally (Hollifield *et al.*, 2023). ETEC pathogenicity stems from two key factors: enterotoxins that disrupt intestinal function and a repertoire of heterogeneous adhesins that facilitate colonization (Upadhyay *et al.*, 2022). The disease has high incidence and mortality rates among children (Khalil *et al.*, 2018), and there are currently few effective, sustainable measures for its prevention or control. Currently, antibiotics remain one of the primary methods for preventing and treating diarrhea caused by ETEC infections (DuPont, 2016). Nevertheless, the extensive and prolonged utilization of antibiotics engenders the potential for bacterial resistance to develop. A survey of ETEC resistance in industrial pig farms in Spain demonstrated that 87.1% of the isolates exhibited resistance to multiple antibiotics, and more than 50% of the isolates demonstrated insensitivity to antimicrobials such as gentamicin, doxycycline, and ciprofloxacin (García-Meniño *et al.*, 2021). In recent years, developing *Escherichia coli* (*E. coli*) vaccines and bacteriophages has emerged as another approach to preventing and controlling ETEC infections. Currently, however, there are no approved ETEC vaccines, and only candidate vaccines are in the clinical trial stage (Salvador-Erro *et al.*, 2025). Today, finding a safe, effective, and low-cost method of preventing and controlling ETEC infection has become a hot topic.

Recent studies have explored novel strategies using natural probiotics to alleviate the pathological consequences of bacterial infections, highlighting their therapeutic potential. Probiotics, in particular, have attracted widespread attention as a potentially effective means of preventing and treating *E. coli* infections. Probiotics are defined as live bacteria or active substances that are beneficial to the host (Klaenhammer *et al.*, 2012). Current research suggests that probiotics play a pivotal role in preventing and treating infectious diseases by maintaining intestinal microecological balance and enhancing host immunity (Kim *et al.*, 2019). *Bacillus subtilis* (*B. subtilis*) is a safe and effective probiotic for applications in both human and animal health. Its association with biological activity suggests a promising alternative to antimicrobial drugs. The *B. subtilis* WS-1 strain, isolated by Du *et al*., has shown protective efficacy against pathogenic *E. coli*-induced diarrhea and mortality in newborn piglets (Du *et al.*, 2019). In another study, *B. subtilis* was found to have the capacity to elicit anti-inflammatory effects in a living organism by constraining the nuclear translocation of the NF-κB-P65 protein and by diminishing the IL-1β and TNF-α levels (Chung *et al.*, 2021). Treatment with *B. subtilis* was observed to reduce ETEC infection and diarrhea in weaned piglets and affect mucosal transcriptomic characteristics (Luise *et al.*, 2019). The mechanisms by which *B. subtilis* mitigates bacterial diarrhea encompass competitive inhibition (Podnar *et al.*, 2022), the production of antimicrobial substances (Taggar *et al.*, 2021), immune response modulation (Mourey *et al.*, 2024), intestinal mucosal repair (Zhang *et al.*, 2016), the enhancement of bacterial homeostasis, antioxidant and anti-inflammatory effects (Xue *et al.*, 2020). Through these pathways, *B. subtilis* can reduce the number of harmful bacteria, modulate the immune system and restore intestinal health, thereby effectively relieving the symptoms caused by bacterial diarrhea and facilitating the healing process of the organism. Nevertheless, research on the therapeutic potential of *B. subtilis* against ETEC-induced diarrhea in rabbits remains limited, particularly regarding its roles in immune regulation and gut microbiota, despite the rabbit's established value as a model for bacterial infections. To fully comprehend the mechanism by which it acts, further in-depth research is necessary.

MATERIALS AND METHODS

Study design and methods: 20 male SPF New Zealand White rabbits, aged one month, were purchased from Liaoning Changsheng. The experiment was conducted at the Laboratory Animal Center of the College of Animal Medicine of Northeast Agricultural University after seven days of adaptive feeding. 20 rabbits were randomly assigned to four experimental groups: CON, ETEC, NOR, and BS. The specific experimental procedure is shown in Fig. 1A. During the experiment, changes in rabbit body weight were recorded, and diarrheal condition scores were calculated by two qualified veterinary practitioners according to established standard (Table 1). Subsequent to the conclusion of the experiment, colon tissue, faecal matter, and blood samples were collected.

Table 1: Scale of diarrhea scores

Fecal Appearance	Diarrhea Score
Formed or granular	0
Soft feces, can be shaped	1
The substance is characterized by its substantial thickness, unformed state, and absence of fecal and water separation.	2
Thick, unformed, with separation of feces and water	3

Strain information: ETEC O25:K19 (BNCC195618) and *B. subtilis* (BNCC 109047) were purchased from Beijing BeNa Culture Collection Co., Ltd., China. For preparation of the bacterial suspension, the frozen strains were inoculated, activated, amplified, and finally adjusted to 1×10^9 CFU/mL.

H&E staining: After the colon tissue underwent dehydration, clarification, embedding, and dewaxing, sections were H&E-stained and observed by microscopy.

ELISA: The serum concentrations of IL-1, IL-6, and TNF-α were quantified using commercially available ELISA kits (Nanjing Jiancheng, China).

Real-time PCR: We isolated total RNA from colon tissue with Trizol reagent. After determining the concentration, cDNA was synthesized with the Prime Script RT kit (TaKaRa, Japan). The mRNA abundance was then quantified by qRT-PCR with ChamQ SYBR qPCR Master Mix (Vazyme, China). Gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH, with primer sequences provided (Table 2).

Table 2: PCR primer sequences

Gene	No.	Primer sequence
IL-1β	NM_001082201.1	Forward: TGTCAGTCGTTGTGGCTCTG
		Reverse: AGTCATCCCAGGTGTTGCAG
IL-6	NM_001082064.2	Forward: GCCGGCGGTGAATAATGAGA
		Reverse: TCGTCACTCCTGAACTTGGC
TNF-α	NM_001082263.1	Forward: ACCTGGACCATGGTGGTGTA
		Reverse: TGGCCTGAACACGGCTACG
GAPDH	NM_001082253.1	Forward: AGTATGATTCCACCCACGGC
		Reverse: GATGGCCTTCCCGTTGATGA

Western blotting: RIPA lysis buffer was utilized to homogenize colon tissue. Protein concentration was determined by BCA assay kit (Beyotime Biotechnology, China). Following electrophoresis, membrane transfer, and blocking of equal protein loads, the membranes were incubated with primary antibodies against P-IκB, IκB, P-P65 (1:1000, Wanlei, China), P65 (1:1000, Santa, USA), and GAPDH (1:1000, Wanlei, China). The membrane was then washed with TBST and incubated with the secondary antibody (1:5000, Zhongshan Jinqiao, China) for 1 h. Target protein bands were visualized using ECL reagents (Tanon, China), and quantitative analysis was conducted with ImageJ software (USA).

Immunofluorescence: Following antigen retrieval and blocking, tissue sections were incubated overnight at 4℃ with the primary antibody against P65 (1:200, Santa, USA). After the completion of the washing steps, the sections were then subjected to an incubation with the fluorescent secondary antibody. The cell nuclei were labeled with 4,9,6-trimethyl-2-phenylpyridine and observed under a fluorescence microscope.

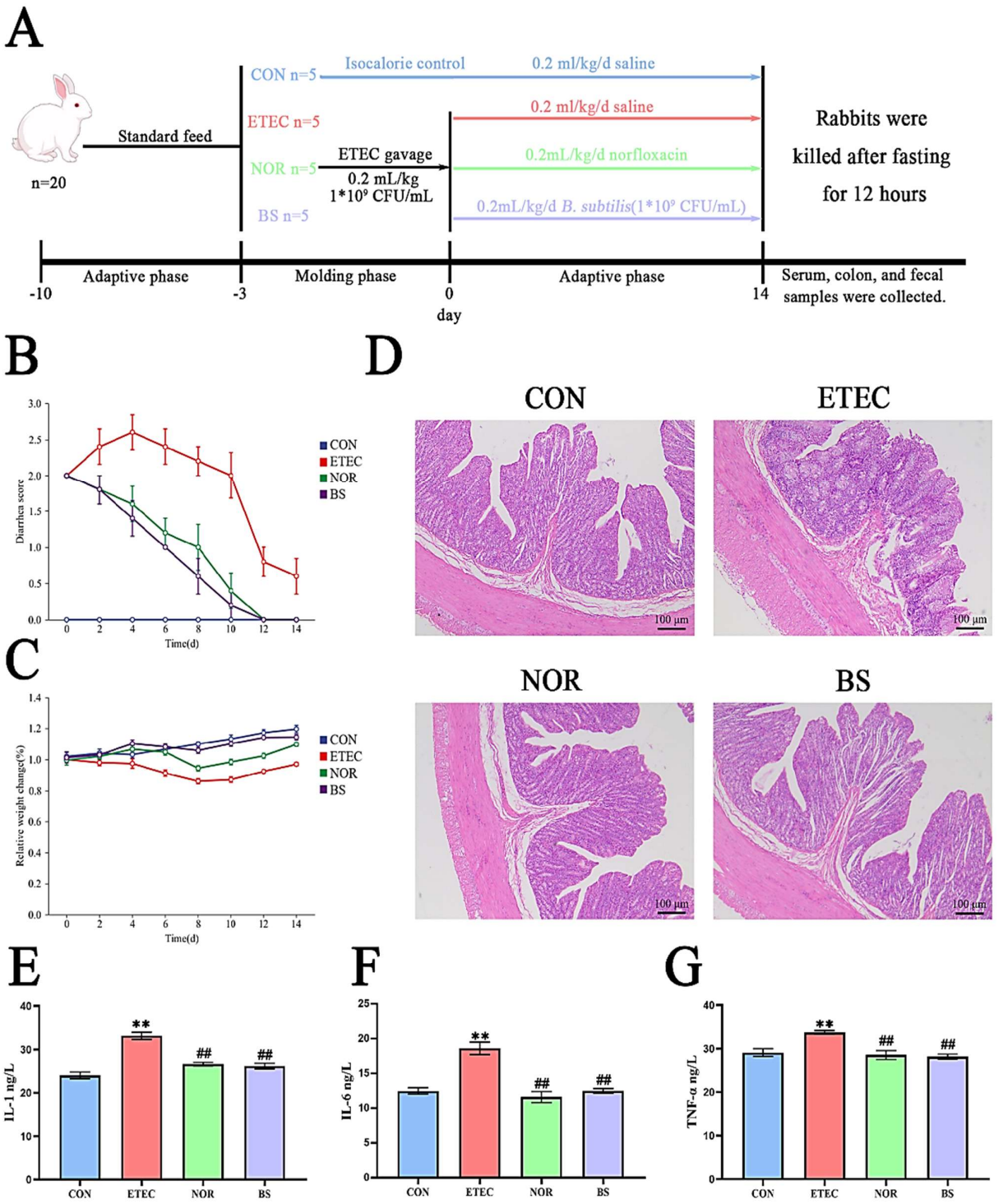

Fig. 1: *B. subtilis* attenuates pathological symptoms and reduces systemic inflammatory response in ETEC-infected rabbits. (A) Diarrhea Score (B) Relative change in body weight (C) Pathologic section of colon (D, E, F) Serologic inflammatory factor expression. Compared to the CON group, **P<0.01, *P<0.05. Compared to the ETEC group, ##P<0.01.

Immunohistochemistry: Following blocking, paraffin sections were incubated overnight at 4°C with the respective primary antibodies: Claudin-3, Occludin, and ZO-1 (1:200, Wanlei, China). Following this, sections were treated with HRP-conjugated goat anti-rabbit IgG (1:300, Servicebio, China). Subsequent to the completion of the washing process, the sections were subjected to staining with DAB substrate solution (Servicebio, China).

Gut microbiota analysis: Total DNA extracted from rabbit fecal samples was used to amplify the hypervariable V4-V5 regions of the bacterial 16S rRNA gene with primers 338F/806R, followed by sequencing on the Illumina NovaSeq platform (USA).

Statistical analysis: The bioinformatics analysis of the microbiome was conducted using QIIME 2 (version

2019.4). Statistical analysis was performed with GraphPad Prism 8.0 (USA). All data were expressed as mean ± SD. Intergroup differences were assessed by one-way ANOVA, with statistical significance defined as $P<0.05$.

RESULTS

***B. subtilis* alleviates pathological symptoms in rabbits with diarrhea:** During the experimental period, no rabbit fatalities were observed. A comparison of CON and ETEC revealed that diarrhea symptoms persisted throughout the experimental period in the former group, while the feces were predominantly thick and unformed, devoid of fecal-water separation. The diarrhea scores of BS and NOR exhibited a gradual decrease, leading to the complete dissolution of diarrhea symptoms and a return to normal fecal status on the 12th day. This was characterized by an evolution from unformed thick feces to pellet-shaped feces (Fig. 1B). Final body weights in the ETEC group were significantly reduced relative to CON. Although NOR and BS treatment increased body weight compared to ETEC, values in these groups remained lower than in CON (Fig. 1C).

Colonic H&E staining revealed a substantial amelioration of ETEC-induced colonic damage in rabbits by *B. subtilis* (Fig. 1D). The CON group showed intact colonic tissue architecture. In contrast, ETEC challenge induced marked inflammatory cell infiltration and evident damage to the intestinal mucosa. Histological samples from BS and NOR exhibited milder colonic lesions in comparison to ETEC. ETEC challenge induced significant inflammatory cell infiltration in the colonic tissue. The intervention of *B. subtilis* was found to be effective in mitigating the histopathological damage induced by ETEC in the colonic tissues of rabbits.

***B. subtilis* alleviates the inflammatory responses induced by ETEC in rabbits:** ETEC-challenged rabbits exhibited a marked increase in serum IL-1, IL-6, and TNF-α levels compared to CON ($P<0.01$). Subsequent to the intervention of *B. subtilis*, these levels demonstrated a significant decrease ($P<0.01$) (Fig. 1E-G). Moreover, a lack of statistical significance was observed in the comparison of BS and CON, suggesting that *B. subtilis* can mitigate the ETEC-induced systemic inflammatory response in rabbits.

***B. subtilis* alleviates ETEC-induced intestinal barrier damage in rabbits:** We found that *B. subtilis* supplementation significantly ameliorated the clinical symptoms of diarrhea in ETEC-challenged rabbits. To further investigate the mechanism, we examined intestinal barrier-related proteins. The immunohistological results (Fig. 2A-D) of the colon showed that the expression of ZO-1, Claudin-3, and Occludin proteins was significantly downregulated in ETEC ($P<0.05$), which was reversed by *B. subtilis* intervention. The findings indicated that *B. subtilis* treatment has the potential to impede the disruption of the mechanical barrier of rabbit colon induced by ETEC and enhance the function of the colonic barrier.

***B. subtilis* inhibits NF-κB pathway activation and alleviates intestinal inflammation:** Western blotting analysis demonstrated that the P-P65/P65 and P-IκBα/IκBα ratios were significantly higher in colonic tissues of ETEC compared with CON ($P<0.05$). The findings suggest that ETEC invasion triggers the NF-κB signaling pathway, and the P-P65/P65 and P-IκBα/IκBα ratios were down-regulated after *B. subtilis* intervention ($P<0.05$) (Fig. 3A-C). To further elucidate the underlying mechanisms, immunofluorescence detection of NF-κB-P65 was performed. The results demonstrated that the fluorescence intensity in ETEC was significantly higher than that in CON (Fig. 3D, E). Following the intervention of *B. subtilis*, there was a significant attenuation of the fluorescence intensity when compared to ETEC ($P<0.01$). A marked upregulation of IL-1β, IL-6, and TNF-α mRNA was observed in ETEC-challenged rabbits compared to CON ($P<0.05$). BS and NOR groups showed similarly reduced expression levels, with no significant difference from the CON group. However, these levels showed a significant reduction compared to the ETEC (Fig. 3F). This study investigated the role of *B. subtilis* in mediating the NF-κB pathway in a rabbit model of ETEC-induced colon injury. The aforementioned results suggest that *B. subtilis* reduces colonic inflammation in ETEC-challenged rabbits by blocking P65 nuclear translocation as well as diminishing the expression of pro-inflammatory cytokines at the mRNA and protein levels.

***B. subtilis* intervention modulates the structure of the rabbit gut microbiota:** In order to further investigate the protective effects of *B. subtilis*, the microbial communities present in rabbit feces were analyzed. A total of 28,652 OTUs were obtained from 20 samples. The Venn diagram shows a core microbiome of 845 ASVs shared across all four experimental groups (Fig. 4B). Fecal microbiota showed comparable α-diversity across groups, with no significant differences in the Chao1, Shannon, or Simpson indices (Fig. 4A). Principal coordinate analysis (PCoA) showed distinct clustering of microbial communities among the groups, indicating a clear separation (Fig. 4C).

A total of 23 bacterial phyla were detected across all gut microbiota samples. Firmicutes was identified as the predominant phylum, constituting 75.83, 68.05, 74.74, and 75.73% of the communities in the CON, ETEC, NOR, and BS groups, respectively. In addition to the findings, the prevalence of Bacteroidetes was observed to reach 19.80% in CON, 6.03% in ETEC, 19.17% in NOR, and 17.51% in BS. The prevalence of Proteobacteria in ETEC was 19.80%, while in CON, NOR, and BS, it was 1.18, 3.01, and 3.51%, respectively. The prevalence of Proteobacteria exhibited a marked increase following the infection. At the genus level, the relative abundance of *Ruminococcus* was 5.41% in CON, 7.45% in ETEC, 13.71% in NOR, and 11.78% in BS. The prevalence of *Oscillospira* in CON, ETEC, NOR, and BS was 10.93, 5.41, 6.15, and 8.36%, respectively. The prevalence of *Bacteroides* was 11.25% in CON, 5.39% in ETEC, 5.88% in NOR, and 7.13% in BS. Levels of *Bacteroides* were found to be significantly higher in BS compared to the other 3 groups. *Acinetobacter* was found in high abundance in ETEC (10.81%), while it was not detected in CON and NOR, and its presence was minimal in BS (0.02%). In contrast, *Shigella* exhibited a higher abundance in ETEC (7.49%) compared to CON (0%), NOR (0%), and BS (1.70%).

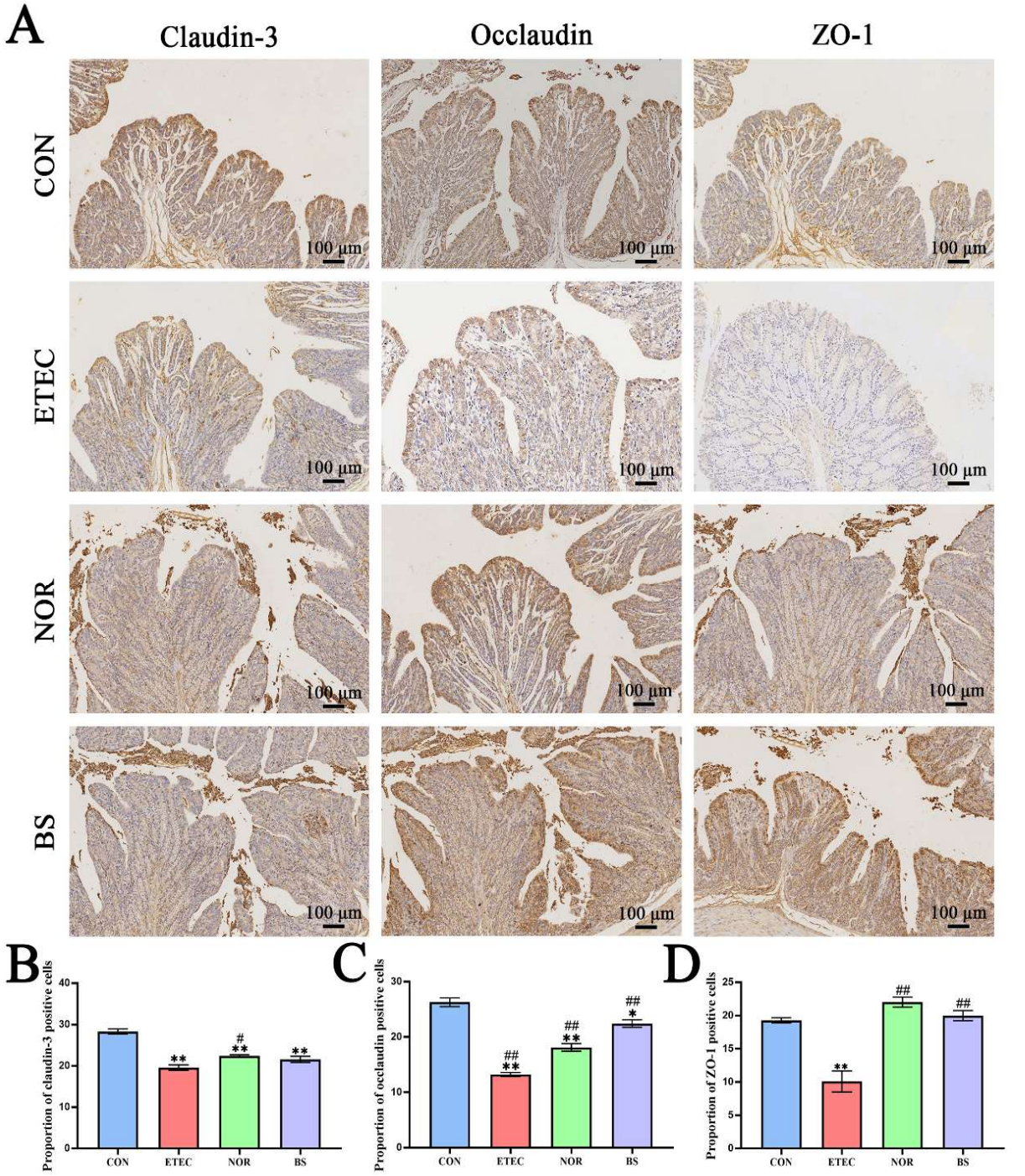

Fig. 2: Effect of *B. subtilis* on the intestinal barrier of rabbits infected with ETEC. (A) Representative image of rabbit colon immunohistochemistry for TJ proteins (B) Claudin-3, (C) Occludin, (D) ZO-1 positive area (%). Compared to the CON group, **P<0.01, *P<0.05. Compared to the ETEC group, ##P<0.01, #P<0.05.

LEfSe analysis and correlation analysis: To identify key microbial biomarkers, we performed LEfSe analysis (Fig. 5A, B). Comparative analysis between groups (CON vs. ETEC; ETEC vs. BS) revealed several genera with significant differential abundance, as indicated by LDA. As shown in Fig. 5A, the abundance of *f_S24_7*, *g_oscillospira*, *g_Bilophil*, and *g_adlercreutzia* was enriched in CON compared to the ETEC (P<0.05), and the intervention of *B. subtilis* increased the abundance of *o_RF32*, *g_Lactobacillus* (P<0.05), while decreasing the abundance of bacteria such as *c_gammaproteobacteria* and *f_Micrococcaceae* (P<0.05). We employed Spearman's correlation analysis to assess associations between differentially abundant genera and other measured parameters (Fig. 5C). Treponema abundance was inversely associated with IL-1 levels (R<-0.5, P<0.05) but favorably linked to Claudin-3 and Occludin (R>0.25, P<0.05). This study found that *Shigella* and *Solibacillus* were positively correlated with inflammatory cytokine levels (R>0.25, P<0.05), and negatively correlated with occludin and ZO-1 levels (R<-0.5, P<0.05).

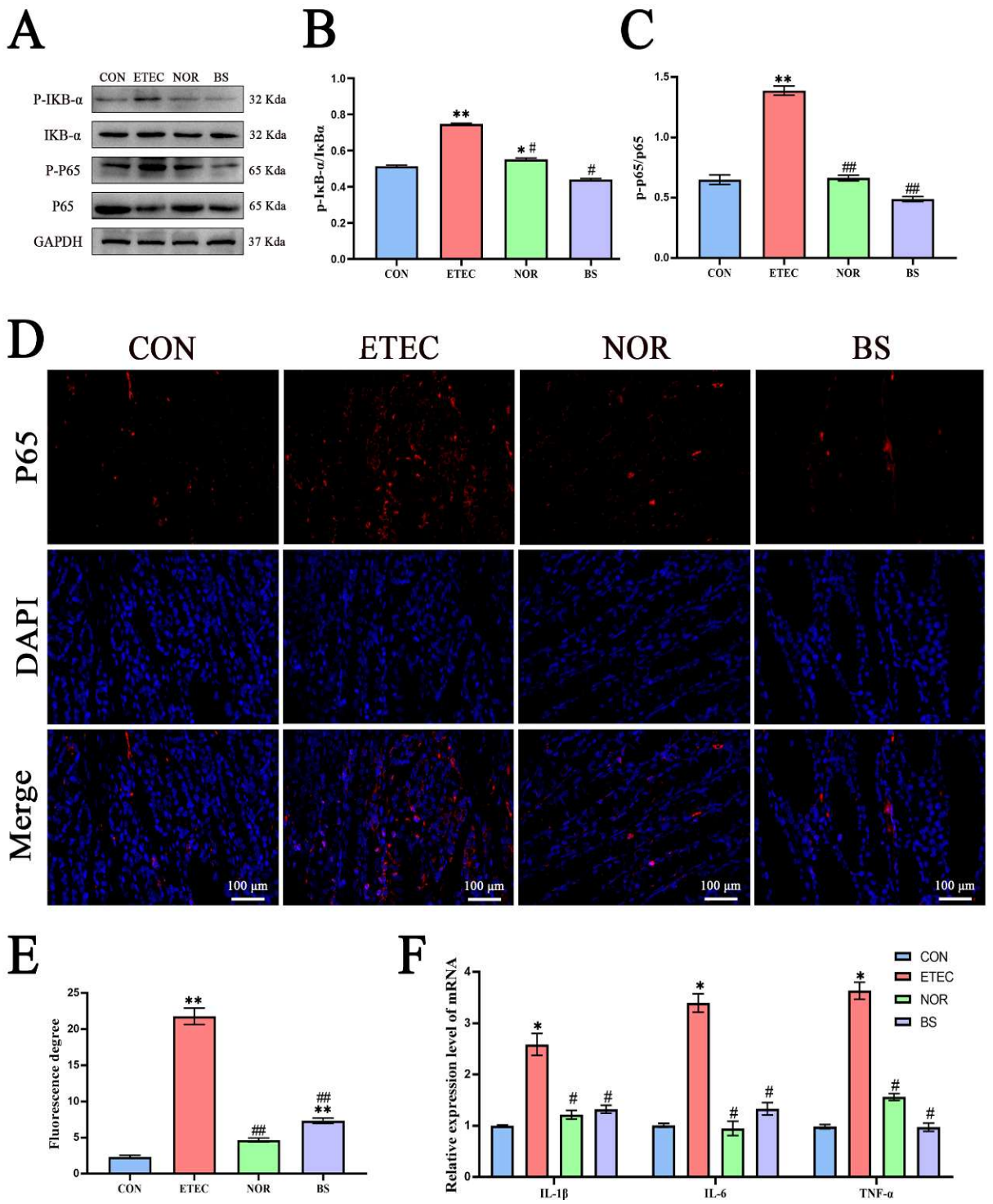

Fig. 3: *B. subtilis* mitigates ETEC-induced intestinal inflammation via suppression of the NF-κB pathway. (A) NF-κB pathway-related protein expression (B) p-IκBα/ IκBα (C) p-p65/p65 (D) P65 immunofluorescence image (E) P65 relative fluorescence intensity (F) Relative inflammatory factor mRNA expression. Compared to the CON group, **P<0.01, *P<0.05. Compared to the ETEC group, ##P<0.01, #P<0.05.

DISCUSSION

ETEC is a common environmental pathogen that can cause severe acute watery diarrhea in newborns and young animals and rapid dehydration or death in newborns (Ledwaba *et al.*, 2020). The production of enterotoxins by ETEC has been demonstrated to cause a disruption in ionic homeostasis within intestinal epithelial cells. This is achieved through the upregulation of the levels of cAMP and cGMP. The elevation of intracellular cGMP impairs intestinal fluid absorption, ultimately triggering net fluid secretion and the onset of diarrhea (Foulke-Abel *et al.*, 2020). Norfloxacin is a fluoroquinolone antibiotic that is widely used due to its broad-spectrum antibacterial activity. It exhibits significant inhibitory effects against Gram-negative bacteria. This study demonstrated the

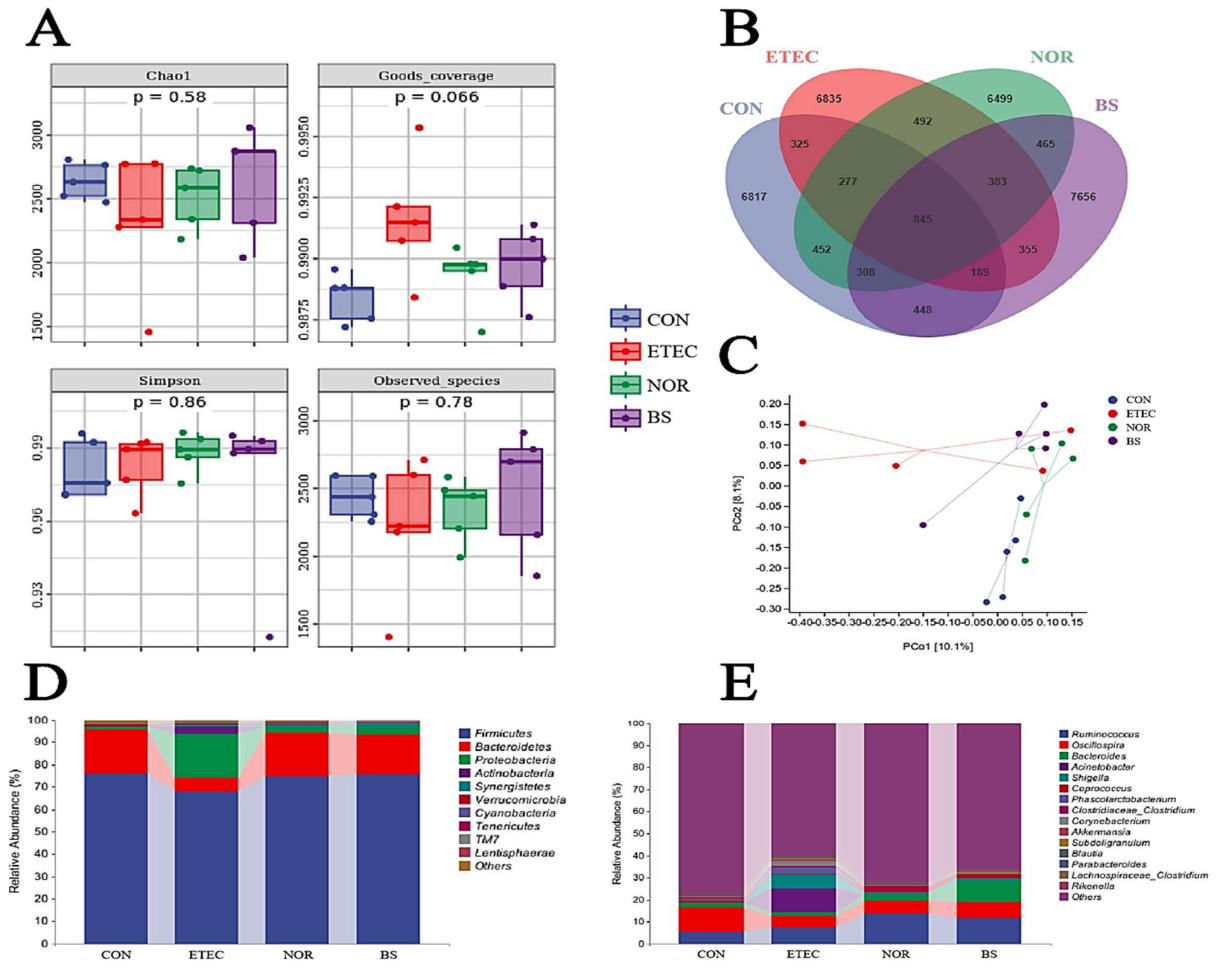

Fig. 4: Effect of *B. subtilis* on the structure of the intestinal flora of rabbits. (A) Alpha diversity index (B) Wayne diagram (C) PCoA analysis (D) Phylum-level hierarchical clustering heatmap of the gut microbiota. (E) Genus-level hierarchical clustering heatmap of the gut microbiota.

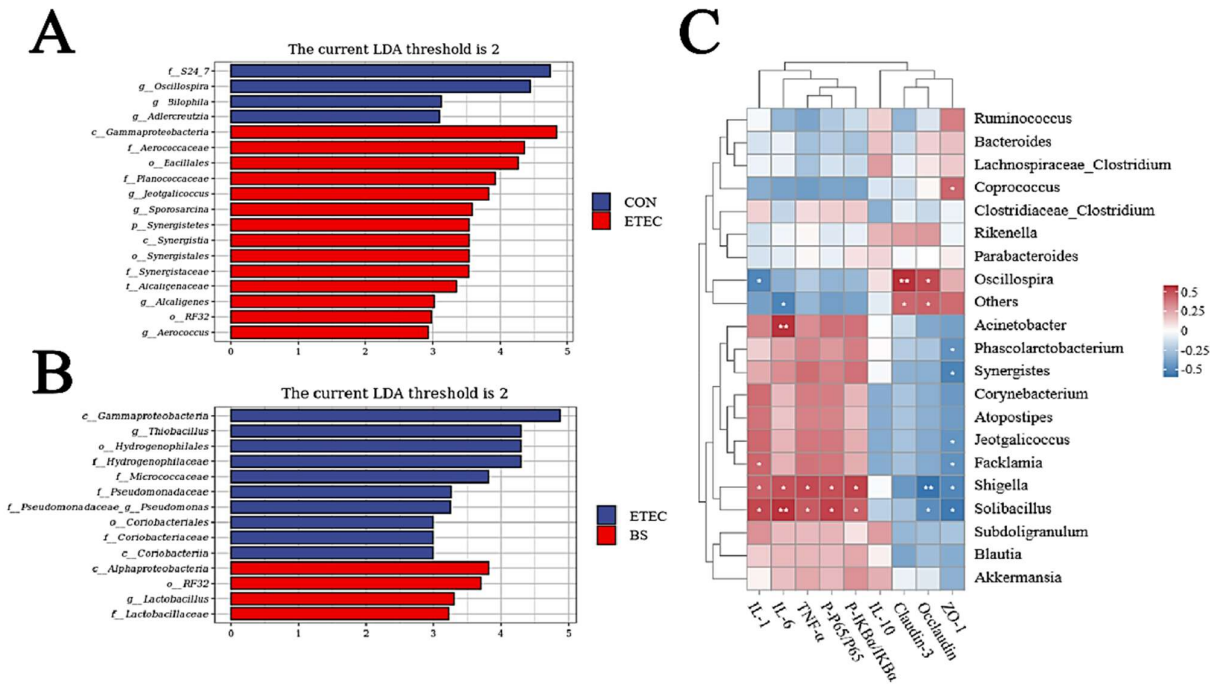

Fig. 5: LEfSe analysis and correlation analysis. (A) Phylum-level LEfSe analysis of gut microbiota across CON and ETEC (B) Phylum-level LEfSe analysis of gut microbiota across ETEC and BS (C) Correlation analysis of the intestinal microbiota with disease-related pathological parameters.

therapeutic efficacy of norfloxacin in treating diarrhea caused by ETEC infection. Probiotics are potential therapeutic agents for diarrhea, according to extensive research. Various probiotics and probiotic preparations have been shown to have a protective effect on the organism (Kopacz *et al.*, 2022). *B. subtilis* can inhibit the proliferation of various pathogenic bacteria. *B. subtilis* prevented Salmonella infection by protecting the intestinal epithelium, ameliorated Salmonella infection-induced intestinal inflammation, and reduced the expression of pro-inflammatory cytokines (Zhang *et al.*, 2022). Diana Luise demonstrated that dietary supplementation with *B. subtilis* alleviates ETEC-induced diarrhea in weaned piglets (Luise *et al.*, 2019). This study aimed to elucidate the protective role of *B. subtilis* against ETEC infection. The results of the study demonstrated that *B. subtilis* alleviated clinical symptoms, reduced ETEC-induced inflammatory responses, increased expression of proteins associated with mechanical barriers, and altered the structure of the intestinal microbiota. These results suggest that *B. subtilis* attenuates ETEC-induced clinical symptoms and pathological changes, positioning it as a promising alternative for future preventive or therapeutic strategies.

Extensive literature supports a reciprocal relationship between inflammation and the regulation of tight junction (TJ) protein levels. Research findings have indicated a positive correlation between elevated levels of pro-inflammatory cytokines and intestinal damage (Wang *et al.*, 2022), which in turn leads to abnormal intestinal proliferation of harmful microorganisms. In patients diagnosed with enteritis, a decrease in the levels of TJ proteins is frequently observed (Kaczmarczyk *et al.*, 2022). This study examined the inflammatory responses in young rabbits infected with *E. coli*. The rabbits exhibited significantly elevated levels of IL-1, IL-6, and TNF-α in their serum. These elevated levels may contribute to the downregulation of TJ proteins and a compromised epithelial barrier. As fundamental components of the intestinal mucosal barrier, TJ proteins form a selective seal between epithelial cells. By modulating mucosal permeability, they tightly control the passage of luminal contents and establish a critical barrier against harmful agents (Hall *et al.*, 2020). The intervention of *B. subtilis* resulted in increased levels of intestinal TJ proteins and improved intestinal flora composition in obese rats (Sun *et al.*, 2022). The results are consistent with previous studies: TJ levels are reduced in rabbits infected with ETEC. However, after *B. subtilis* intervention, the levels of occludin, ZO-1, and claudin-3 proteins increased significantly. *B. subtilis* preserves the intestinal barrier in ETEC-infected rabbits.

The NF-κB pathway is of pivotal significance in the regulation of innate and adaptive immune responses in inflammatory cells (Liu *et al.*, 2017). Pro-inflammatory cytokines are significant indicators of the ETEC response (Lin *et al.*, 2019). Excessive activation of NF-κB-P65 can lead to inflammation and enhance the inflammatory response. The study found elevated IL-1β, IL-6 and TNF-α levels and activated NF-κB in the ETEC group. Nevertheless, these alterations were ameliorated by *B. subtilis* intervention. Collectively, our data demonstrate that *B. subtilis* modulates the inflammatory response by preventing the activation of NF-κB pathway.

The gut microbiota serves as one of fundamental pillar of gut health, essential for homeostasis, immunity, and defense against pathogens (de Vos *et al.*, 2022). Numerous studies have consistently shown that infections caused by ETEC have the capacity to disrupt the intestinal microbiota. When the intestinal mucosal barrier is compromised, it can allow harmful substances and microorganisms from the intestinal tract to enter the submucosa. Alterations in the intestinal environment can disrupt the dynamic equilibrium of the intestinal microbiota. We examined the effects of ETEC and *B. subtilis* on gut microbiota diversity. The Shannon index has been demonstrated to exhibit heightened sensitivity to species richness, while the Simpson index has been shown to exhibit increased sensitivity to species evenness. The present study revealed that the Chao1, Shannon, and Simpson indices of alpha diversity were not significantly different among the various groups examined. Nonetheless, a divergence in the results of species abundance at the phylum and genus level was observed among the experimental groups. Firmicutes and Bacteroidetes constitute the keystone phyla of the gut microbiota and are pivotal for sustaining intestinal homeostasis. Dysbiosis characterized by altered Firmicutes-to-Bacteroidetes ratios is linked to numerous disease manifestations (Tsai *et al.*, 2025). We observed a clear shift in the microbial community following ETEC infection, characterized by a marked reduction in Firmicutes and Bacteroidetes and a notably expanded Proteobacteria population. An increased abundance of Proteobacteria, a phylum enriched with opportunistic pathogens, is a hallmark of dysbiosis and actively contributes to disease pathogenesis. *B. subtilis* intervention restored a healthier microbial composition by reducing Proteobacteria abundance, suggesting that its protection is partly mediated by suppressing pathobionts. This effect on intestinal microbial composition has been shown to alleviate ETEC-induced intestinal damage. At the genus level, the BS group showed significantly increased abundance of *Bacteroides*, a cornerstone genus in the gut microbiota, compared with the ETEC group. *Bacteroides* benefit the intestine and maintains its homeostasis. The common strain Bv46 of the genus *Bacteroides* has been shown to alleviate symptoms of DSS-induced colitis in mice (Liu *et al.*, 2022). Concurrently, the *B. subtilis* intervention reduced the abundance of *Acinetobacter* species, which are opportunistic pathogens present in the body. When the body's resistance is compromised, they can trigger infections. The alterations in the composition of intestinal microbiota triggered by *B. subtilis* intervention may be associated with its direct or indirect inhibitory effect on pathogenic bacteria.

Conclusions: The study demonstrates that *B. subtilis* inhibits the NF-κB pathway, thereby down-regulating inflammatory mediator. Concurrently, it improves barrier integrity while simultaneously modulating the composition of the gut microbiota to preserve normal physiological function.

Data availability statement: The 16S rRNA datasets prented in this study can be found in online repositories. The link is https://www.ncbi.nlm.nih.gov/sra/PRJNA 1355451.

Ethical approval: The Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University approved the animal experiment and experiment design in this study (#NEAUEC2022 03 23).

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Authors contribution: HF and YZ came up with the design of the study; XC performed experiments; YW, JT, arranged the database; HT, YJ and JW were responsible for the statistical analysis; XY, JH and SZ wrote sections of the manuscript; XC wrote the manuscript. All authors have read, revised, and reviewed the manuscript.

Data availability statement: The dataset in this study can be provided by the corresponding author upon reasonable request.

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