DOI: 10.26599/FSHW.2026.9251015

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Lipoteichoic acid derived from *Bifidobacterium bifidum* contributes to the remission of TNBS-induced acute colitis in mice

Dingwu Qu[abc], Saisai Feng[d], Shunhe Wang[ac], Leilei Yu[ac], Fengwei Tian[ac], Hao Zhang[ac], Wei Chen[ace], Qixiao Zhai[ac*]

[a] *State Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi, Jiangsu, 214122, P.R China*

[b] *Hubei Provincial Engineering and Technology Research Center for Food Ingredients, Hubei University of Arts and Science, Xiangyang, Hubei, China*

[c] *School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China*

[d] *Institute of Food Sciences, Shanxi Normal University, Taiyuan 030031, China*

[e] *National Engineering Research Center for Functional Food, Jiangnan University, Wuxi, Jiangsu, 214122, China*

ABSTRACT: *Bifidobacterium bifidum* is recognized for its immunomodulatory potential, yet the specific components mediating its anti-inflammatory effects remain poorly defined. Here, we systematically dissected the role of *B. bifidum*-derived lipoteichoic acid (LTA) in modulating mucosal immunity and alleviating 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced acute colitis. Initial in vitro assays revealed that the IL-10-inducing activity of *B. bifidum* in mesenteric lymph node cells resides mainly in heat-resistant cell wall fractions, particularly LTA, acting through a TLR2-associated (and pharmacologically TLR2-sensitive) pathway. Comparative analyses among multiple *B. bifidum* strains demonstrated that LTA immunostimulatory potency strongly predicted protective efficacy against colitis, with FJSWX19M5 strain LTA exerting the most pronounced anti-inflammatory effects in both conventional and germ-free mouse models. In vivo, mice received LTA by oral gavage (0.2 mg/mouse, 200 μL) prior to TNBS challenge. LTA from FJSWX19M5 significantly improved survival, ameliorated colonic injury and inflammation, reduced IL-1β production, and modulated Treg cell distribution, outperforming other strain LTAs. Transcriptomic profiling showed that FJSWX19M5 LTA intervention prominently altered colonic gene expression, activating multiple immune- and inflammation-related signaling pathways, such as Jak-STAT, PI3K-Akt, and IL-17. Furthermore, FJSWX19M5 LTA enhanced both T and B lymphocyte proliferation and cellular immunity in healthy mice. Together, these results establish that the anti-colitis and immunoregulatory benefits of *B. bifidum* are largely attributable to strain-specific LTA, highlighting its mechanistic link to TLR2-involved mucosal immune modulation and supporting its potential as a defined immunomodulatory molecule for IBD-relevant intervention.

1. Introduction

The gut microbiota, as a vital component of the human immune system, plays a central role in maintaining intestinal homeostasis and modulating inflammatory responses. *Bifidobacterium*, one of the predominant probiotic genera in the gut, is closely associated with various health conditions. Numerous clinical and basic studies have shown that a decrease in the abundance of *Bifidobacterium* is a common feature

***Corresponding author**
zhaiqixiao@jiangnan.edu.cn

Received 10 November 2025
Received in revised from 21 December 2025
Accepted 23 January 2026

in immune-related diseases such as inflammatory bowel disease (IBD), indicating its potential significance in maintaining intestinal immune function and anti-inflammatory responses[1, 2]. Moreover, the composition and metabolic activity of the gut microbiota have emerged as promising novel targets for modulating host immunity and preventing chronic inflammatory disorders[3, 4].

Nevertheless, the molecular basis underlying the immunomodulatory and anti-inflammatory effects mediated by *Bifidobacterium* remains incompletely understood. Recent studies suggest that interactions between probiotic surface components and host Toll-like receptors (TLRs) play a crucial role in regulating immune responses. Multiple classes of bacterial effector molecules have been implicated in host immunomodulation, including extracellular polysaccharides/exopolysaccharides, peptidoglycan (PG), and extracellular metabolites, as well as other cell-envelope-associated molecules. Among these components, cell wall constituents such as lipoteichoic acid (LTA)，exopolysaccharides (EPS) and PG are not only essential structural components of gram-positive bacteria but may also directly mediate the immunomodulatory activities of probiotics[5]. Notably, LTAs derived from different bacterial sources exhibit significant diversity in their biological functions, yet the specific role and underlying mechanisms of LTA from *Bifidobacterium* have not been systematically elucidated. Meanwhile, understanding whether the immunoregulatory effects of *Bifidobacterium* display strain-specificity could be important for the development of targeted microbial therapies[6, 7].

Based on this background, the present study systematically explores the material basis and mechanisms by which *Bifidobacterium* modulates host immunity and alleviates intestinal inflammation. Utilizing both in vivo and in vitro models, we focus on the ability of *B. bifidum* cell wall LTA to induce the secretion of the anti-inflammatory cytokine IL-10 from key immune cells, such as mesenteric lymph node (MLN) cells and bone marrow-derived dendritic cells (BMDCs), as well as the dependence of this effect on TLR signaling pathways. Furthermore, we analyze the anti-inflammatory and immune-enhancing effects of this active component in both conventional and germ-free (GF) mouse models of colitis. These studies will help elucidate the core material basis and molecular mechanisms underlying *B. bifidum* -mediated immunoregulation, providing a theoretical foundation for targeted prevention and treatment of IBD using probiotics and their functional molecules[8].

2. Materials and Methods

2.1 Strains and growth conditions

The required *Bifidobacterium* strains for the experiment were activated in MRS medium (de Man, Rogosa, and Sharpe, MRSc) containing 0.1% cysteine. After reaching the stationary phase, the bacterial cultures were centrifuged and resuspended in PBS. The bacterial suspension was then serially diluted with PBS, and the optical density at 600 nm (OD600) was measured for each dilution to establish a standard curve between dilution factors and absorbance values. According to the standard curve, the bacterial suspension was adjusted to an OD600 of 0.5, corresponding to a concentration of approximately $1\sim5 \times 10^8$ CFU/mL. For

subsequent cell experiments, all strains were standardized to OD600 = 0.5, and the suspensions were stored at 4 °C until use.

2.2 LTA, EPS, PG, and membrane protein preparation

For LTA extraction, bacterial cells were washed three times with PBS and resuspended in 10 mL of 0.1 M citrate buffer (pH 4.7), then sonicated on ice (400 W, 30 min; Vibracell VC 750 Ultrasonic Processor, Hielscher, Germany). The lysate was mixed with an equal volume of water-saturated n-butanol and incubated at 37 °C with agitation (220 rpm) for 1 hour. After centrifugation (12,000 × g, 30 min, 4 °C), the aqueous phase was collected, dialyzed (3.5 kDa cutoff) for 3 days, and lyophilized. LTA purification was performed as described by Matsuzaki et al.: crude extracts were solubilized in 15% n-propanol (in 0.1 M sodium citrate buffer, pH 4.7), applied to an Octyl-Sepharose FF hydrophobic column (Sigma-Aldrich, St. Louis, MO), rinsed with 15% n-propanol in 0.1 M sodium acetate buffer (200 mL, pH 4.7), and eluted with 35% n-propanol in 0.1 M sodium citrate buffer (300 mL). LTA-containing fractions were identified by inorganic phosphate assay, dialyzed against endotoxin-free water, lyophilized, and weighed[9, 10].

For EPS extraction, crude EPS was prepared from freshly collected bacterial cells. After cell removal, the supernatant was obtained by sonication (81 W, 9 min; Vibracell VC 750 Ultrasonic Processor). Proteins were precipitated by adding TCA to a final concentration of 4% (w/v) and incubating overnight at 4 °C. The supernatant was then mixed with three volumes of cold ethanol (4 °C) to precipitate EPS, which was collected by centrifugation, redissolved in double-distilled water, dialyzed using a 10 kDa cutoff membrane for 3 days, and lyophilized[11]. Total carbohydrate content in EPS was determined using the phenol–sulfuric acid method.

For PG isolation, the method of Wang et al[12]. was followed: heat-killed bacterial cells were boiled in 10% TCA for 30 min, washed with water and dehydrated alcohol, then proteins were digested enzymatically in 100 mM Tris-HCl buffer (pH 7.2) at 37 °C for 14 h. Lipids were removed by sequential extraction with methanol, methanol/chloroform (1:1), and chloroform. After acidification with 0.01 M H_2SO_4, the crude PG was lyophilized and stored at −20 °C.

Membrane proteins were extracted using a commercial membrane protein extraction kit (Babe), following the manufacturer's instructions. Protein concentration was determined by the BCA assay (Blue Skies, Shanghai), according to the kit protocol.

2.3 MLN cell model

The MLN co-culture experiment was performed according to our previous methods[13], and was described as follows: MLN were collected from male C57BL/6J mice. After grinding and filtration through a mesh, the tissues were resuspended in an appropriate volume of complete RPMI 1640 medium to obtain a single-cell MLN suspension, which was then adjusted to a concentration of about 3×10^6/mL. Gentamicin (1%), HEPES (10 mmol/L), β-mercaptoethanol (0.05 mmol/L), L-glutamine (3 mmol/L), anti-CD3 antibody (1 mmol/L), and anti-CD28 antibody (1 mmol/L) were added to the cell suspension and thoroughly mixed. The MLN cell suspension was then mixed with the bacterial suspension at a volume ratio of 10:1, and co-cultured at 37 °C with 5% CO_2 for 3 days. The mixture of PBS and MLN cell suspension was used as the

blank control. After incubation, the samples were centrifuged at 12,000 rpm for 5 minutes at 4 °C, and the supernatant was collected. The levels of IL-10, IL-12, IL-17, and IL-22 in the supernatant were determined using mouse ELISA kits (R&D Systems) according to the manufacturer's instructions. The entire experiment was independently repeated three times.

2.4 BMDCs model

The induction of BMDCs was performed as follows: Bone marrow was extracted from the femurs and tibias of 6～8-week-old male C57BL/6J mice. After centrifugation, the supernatant was discarded and red blood cell lysis buffer was added. Following another centrifugation and removal of the supernatant, the cell pellet was resuspended in complete medium containing 20 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, PeproTech). The cells were seeded at a density of 2×10^6 cells per 100 mm bacterial culture dish and incubated at 37 °C with 5% CO_2. On day 3, half of the medium was replaced; thereafter, the medium was changed every other day until day 9. The suspended and loosely adherent cells collected on day 9 were considered BMDCs and identified using $CD11c^+$ expression. BMDCs were co-cultured with the bacterial suspension at a cell-to-bacteria ratio of 1:10 for 24 hours. After co-culture, the supernatant was collected by centrifugation and cytokine levels were measured[14].

2.5 Induction and evaluation of colitis

Six-week-old male BALB/c mice purchased from Charles River Company (Shanghai, China) were raised at the animal facility of Jiangnan University. The animal experimental designs for the two experimental settings are shown in Table 1 and Table 2, respectively. After flexible feeding, acute colitis in mice was induced by rectal perfusion with haptenic TNBS[15]. The mice were sacrificed by inhalation of 100% CO_2. All national and institutional guidelines for the care and use of mice were followed in this study. This study was approved by the Ethics Committee of Jiangnan University (JN.No 20220615b1200920[229] and 20221015c0320130[436]).

Table 1. Effect of lipoteichoic acid on acute colitis in conventional mice

Group	Time (days)		
	1~7	8	9-13
Control	Control reagent+ 200 μL PBS	35% ethanol +200 μL PBS	200 μL PBS
TNBS	Presensitization solution+200 μL PBS	TNBS+200 μL PBS	200 μL PBS
CJ238	Presensitization solution+200 μL LTA	TNBS+200 μL LTA	200 μL LTA
FJSWX19M5	Presensitization solution+200 μL LTA	TNBS+200 μL LTA	200 μL LTA
FXJWS17M4	Presensitization solution+200 μL LTA	TNBS+200 μL LTA	200 μL LTA

Note: The sensitization solution consisted of 1% (w/v) TNBS dissolved in an acetone/olive oil mixture (v/v = 4:1), while the control solution was an acetone/olive oil mixture (v/v = 4:1) without TNBS. A total of 150 μL of sensitization or control solution was evenly applied to a bare area of skin (1.5 × 1.5 cm) on the dorsal surface of each mouse for sensitization. The concentration of TNBS for rectal administration was 1000 μg/mouse, and the TNBS ethanol solution contained 35% ethanol. The rectal administration volume was 50 μL/mouse. The concentration of the lipoteichoic acid solution was 1 mg/mL, and the gavage volume was 200 μL/mouse.

Table 2. Effect of lipoteichoic acid on acute colitis in germ-free mice

Group	Time (days)		
	1~7	8	9~13
Control	200 μL PBS	35% ethanol +200 μL PBS	200 μL PBS

TNBS	200 μL PBS	TNBS+200 μL PBS	200 μL PBS
LTA	200 μL LTA	TNBS+200 μL LTA	200 μL LTA

Note: The dosage of TNBS was 2.5 mg per mouse, dissolved in 35% ethanol, with 100 μL administered rectally to each mouse. For the control group, 100 μL of 35% ethanol solution was administered rectally. After administration, mice were held in an inverted position for 60 seconds to prevent leakage of the solution, and cotton was used to maintain body temperature. The oral administration dose of LTA derived from FJSWX19M5 was 0.2 mg per mouse, with a gavage volume of 200 μL per mouse.

After induction of colitis with TNBS, the body weight of mice was recorded daily. The general condition, including mental status, fur appearance, food intake, hematochezia, diarrhea, and mortality, was closely monitored. The severity of colitis was assessed using the Disease activity index (DAI), with scoring criteria summarized in Table 3. At the end of the experiment, mice were euthanized by CO_2 asphyxiation. The colon was carefully excised for accurate measurement of colon length and spleen weight. Distal colon samples were collected for further analysis. Cytokine levels were determined using ELISA kits (R&D Systems) according to the manufacturer's instructions. For histological evaluation, distal colon tissues were fixed, embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological scoring of colitis severity was performed as described in Table 4. The proportion of $Foxp3^+CD25^+CD4^+$ T cells in the spleen was analyzed by flow cytometry and the gating strategy was performed according to our previously described method[15, 16].

Table 3. Assessment criteria for disease activity index (DAI)

Weight loss (%)	Stool consistency	Occult/Gross bleeding	Score
<2	**Normal**	**None**	**0**
2~5	**Soft**	**Occult blood positive**	**1**
5~10	**Soft**		**2**
10~15	**Loose**	**Gross blood**	**3**
>15	**Loose**		**4**

Note：Normal = well-formed stool; Soft = loose stool not adhering to the perianal area; Loose = watery, unformed stool adhering to the perianal area.

Table 4. Scoring criteria for colon pathology

Degree of inflammation	Inflammatory cell infiltration	Crypt damage	Goblet cell loss	Score
None	None	None	None	0
Mucosal layer	Localized, mild	Localized	Localized	1
Serosal layer	Diffuse, severe	Diffuse	Diffuse	2

2.6 Evaluation of immune enhancement activity in vivo

A total of 20 male BALB/c mice aged 6～8 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal procedures were reviewed and approved by the Ethics Committee of Jiangnan University (ethical approval number: JN.No20220615b1200920[229]). For the lymphocyte proliferation assay, mice were randomly divided into Control group and LTA group, with 5 mice in each group. Mice in the LTA group were orally administered 200 μL of LTA solution (0.2 mg/mouse) daily, while mice in the Control group were orally administered 200 μL of PBS daily. The treatments were continued for 10 days. After 10 days, all mice were sacrificed, and the spleen and colon tissues were collected. The procedure for the ConA-induced splenic lymphocyte proliferation assay in mice was performed as described by Yu[17], with the stimulation index (SI) used to assess the proliferation rate of splenic lymphocytes. For the delayed-type hypersensitivity (DTH) assay, mice were similarly divided into a control group and an LTA

group, with 5 mice per group. Mice in the LTA group received 200 μL of LTA solution (0.2 mg/mouse) orally once daily, and those in the control group received 200 μL of PBS orally once daily, for a total of 14 days. The DTH assay followed the method of Wei[18], using ear swelling as an indicator of the DTH response.

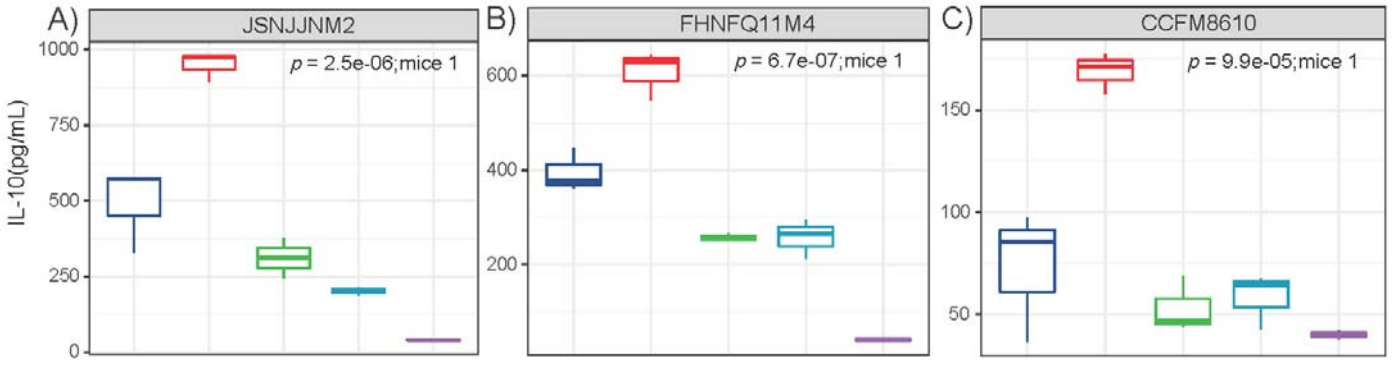
2.7 Statistical analysis

Statistical analyses and data visualization were performed using GraphPad Prism 8.4.3 and RStudio (version 4.0.4). Data are presented as mean ± standard error of the mean. Differences between group means were assessed using one-way analysis of variance (ANOVA) for data with normal distribution and homogeneity of variance, followed by Tukey's post hoc test for multiple comparisons. For data that did not meet these conditions, nonparametric rank-sum tests were applied. *p*-value adjustment was performed for multiple testing corrections. p-value < 0.05 was considered statistically significant.

3. Results

3.1 B. bifidum may induce IL-10 production in MLN cells through heat-resistant components in the deeper layers of their cell wall

Previous experiments have shown that the efficacy of *B. bifidum* in alleviating TNBS colitis does not depend on its metabolic activity but may be related to the immune stimulating ability of the bacteria themselves. Therefore, in this study, the ability of MLN cells to produce IL-10 in vitro was used as the evaluation method. Firstly, the ability of different bacterial suspensions' post-ultrasound supernatants, equal volume ultrasound precipitates, and boiled bacterial suspensions to induce MLN to produce IL-10 was compared. The results indicated that the IL-10 stimulating ability of most *B. bifidum* to MLN was enhanced after ultrasound, and the substances with this inducing activity mainly existed in the bacterial suspension precipitate after ultrasound. It was insensitive to heat treatment and was not easily exfoliated by ultrasound. In contrast, *Enterococcus faecalis* had almost no stimulating activity before ultrasound (Fig. 1). The substances suggesting that *B. bifidum* may induce IL-10 production through polysaccharides or glycolipids (such as PG or phosphatidic acid) in the deeper layers of its cell wall.



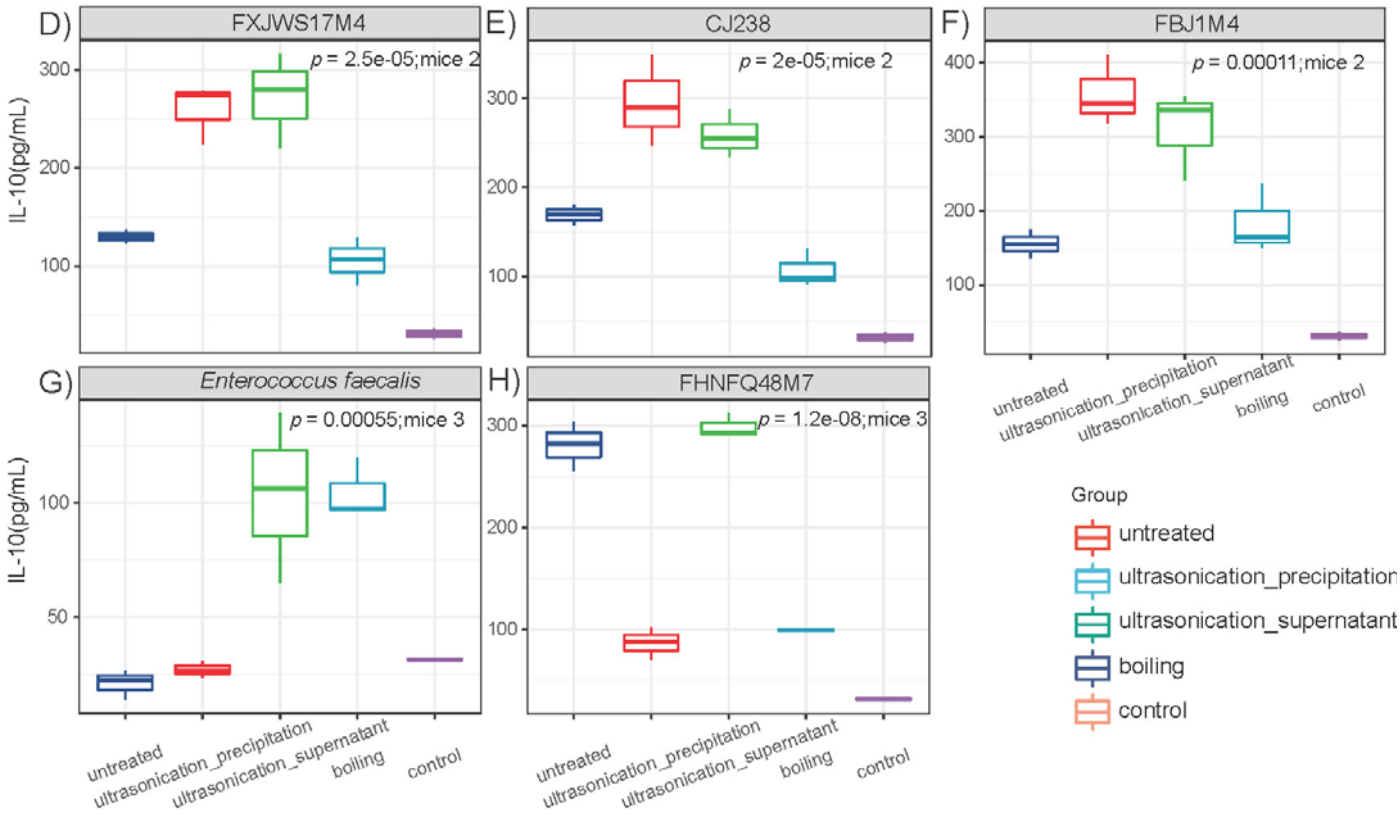


Figure 1. Effects of different bacterial treatments on the ability of *Bifidobacterium bifidum* to induce IL-10 production in MLN cells of mice. A), B), D–F), and H) indicate the ability of different B. bifidum strains to induce IL-10 secretion by MLN cells. C) and G) represent the effects of control strains, *Lactiplantibacillus plantarum* CCFM8610 and *Enterococcus faecium*, respectively. MLN cells were isolated from three normal mice. The boiling condition was 98°C for 20 min, and the ultrasonic treatment was performed at 81 W for 4 min (cycles of 3 s on, 3 s off). *p* values were determined by one-way ANOVA.

3.2 B. bifidum-induced IL-10 production in MLN cells may mainly depend on their LTA and is associated with TLR2 signaling

Guided by the above results, we next performed a comparative screen of major cell-envelope constituents rather than focusing on a single preselected factor. We extracted EPS, PG, LTA, and membrane proteins from a single *B. bifidum* strain. Each of these four components was subjected to fivefold serial dilutions at equivalent concentrations. The results showed that, in the absence of an immune stimulant, the undiluted capsular polysaccharide exhibited the highest ability to induce IL-10 production by MLN cells, with an IL-10 level of 253.63 pg/mL. LTA showed the strongest stimulatory effect at a fivefold dilution, with an IL-10 level reaching 379.61 pg/mL. In comparison, the whole *B. bifidum* cells had nearly no IL-10-inducing activity without immune stimulation. Upon immune activation (with the addition of a stimulant), the IL-10-inducing activity of LTA increased significantly to 1662.73 pg/mL, whereas the maximum induction by capsular polysaccharide increased only to 288.71 pg/mL. Both membrane proteins and peptidoglycan showed no effective IL-10-inducing activity under either condition (Fig. 2A, B).

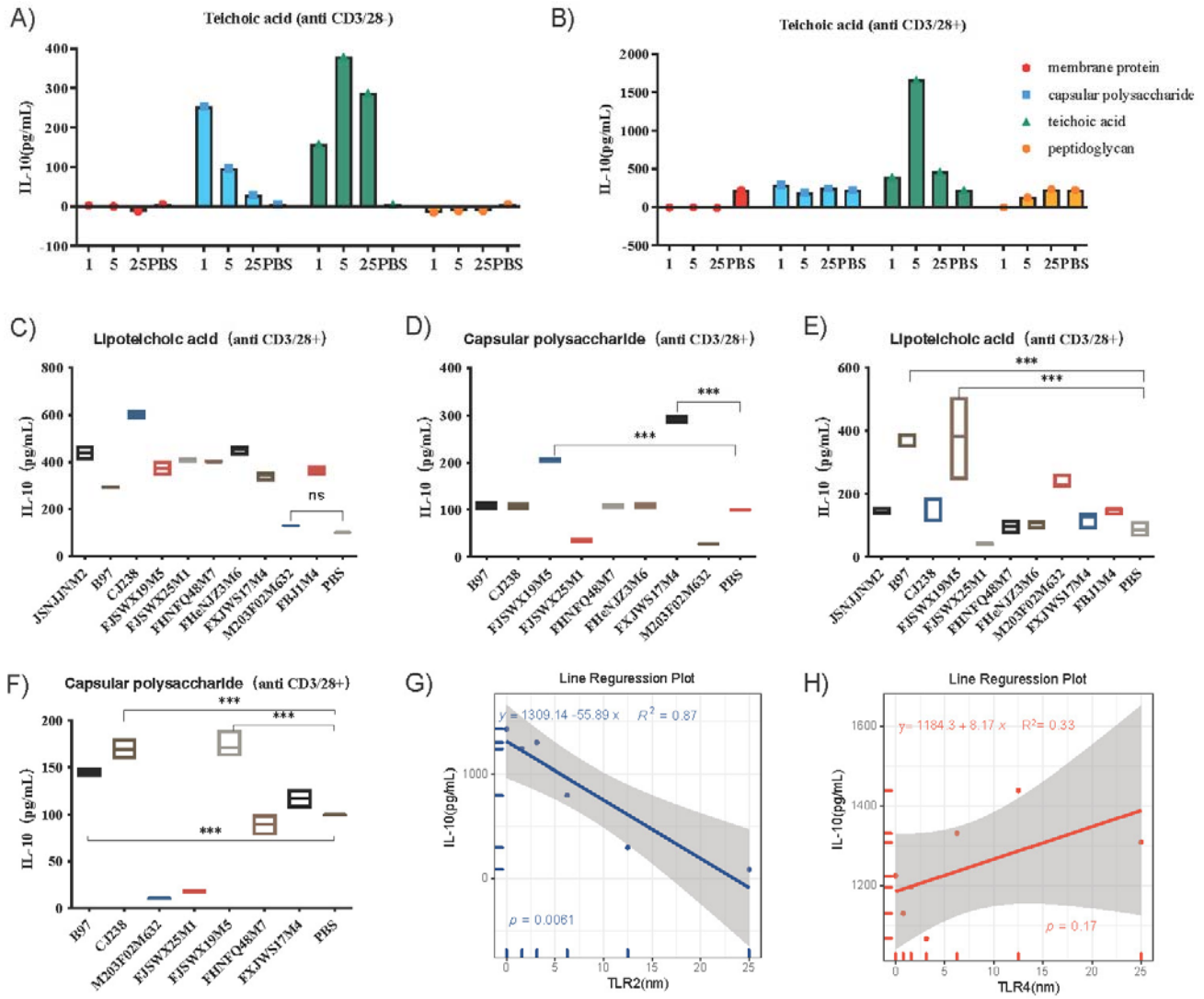


Figure 2. Effects of various active components of *Bifidobacterium bifidum* on the ability to induce IL-10 production in MLN cells of mice. A) and B) indicate the ability of different concentrations of each bacterial component to induce IL-10 secretion by MLN cells from normal mice in the absence or presence of a stimulant (anti-CD3/28 antibody, BD Pharmingen), respectively; D) and E) show the IL-10-inducing ability of LTA and CPS from different bacterial strains on MLN cells from normal mice in the presence of the stimulant, respectively; E) and F) show the IL-10-inducing ability of LTA and CPS from different bacterial strains on MLN cells from GF mice in the presence of the stimulant, respectively; G) and H) show the effects of adding a TLR2 inhibitor (C29, MCE) and a TLR4 inhibitor (TAK242, R&D), respectively, on the ability of LTA to induce IL-10 secretion by MLN cells.

Based on these findings, we further evaluated the immunostimulatory activities of LTAs and capsular polysaccharides derived from ten different *Bifidobacterium* strains in MLN cells from both conventional and GF mice. As shown in Fig. 2C, under immune-activated conditions, Except for M203F02M632, the LTA of other strains could significantly induce ordinary mouse MLN cells to produce more IL-10, and the LTA from CJ238 had a better effect. Fig. 2D showed that capsular polysaccharides from different strains could induce only low levels of IL-10 in MLN cells. Further results (Fig. 2E, F) showed that, under immune activation, the LTAs from B97 and FJSWX19M5 had the strongest ability to induce IL-10 production in MLN cells from GF mice, with levels of 384.54 pg/mL and 390.37 pg/mL, respectively. Similarly, the capsular polysaccharides from FJSWX19M5 and CJ238 also demonstrated relatively strong IL-10-inducing activity in GF MLN cells,

at 159.23 pg/mL and 162.49 pg/mL, respectively. Overall, the IL-10 induction levels of capsular polysaccharides from all strains were significantly lower than those of LTAs.

Furthermore, mechanistic analysis revealed that increasing concentrations of a TLR2 antagonist significantly weakened the ability of LTA to induce both IL-10 and IL-17 secretion in MLN cells (Fig. 2G), whereas changes in a TLR4 antagonist concentration had no significant effect (Fig. 2H). These results suggest that the induction of IL-10 production in MLN cells by *B. bifidum* LTA is primarily sensitive to pharmacological inhibition of TLR2 signaling.

3.3 The ability of different B. bifidum strains to relieve colitis is closely related to the immunomodulatory activity of their LTA in vitro

In the TNBS-induced colitis mouse model, we previously screened and identified *B. bifidum* strains with protective effects against colitis (FJSWX19M5, CJ238, FHENJZ3M6, and JSNJJNM2), as well as strains without significant protective effects (FBJ1M4, M203F02M632, FXJWS17M4, and FHNFQ48M7). Multidimensional scaling (MDS) cluster analysis, based on each strain's ability to induce immune factors (IL-10, IL-17, and IL-12) in the MLN in vitro model and their efficacy in alleviating colitis, revealed a clear separation between the protective and non-protective strains (Fig. 3A). This suggests that the protective effect of *B. bifidum* against colitis is closely related to the immunostimulatory activity of its LTA.

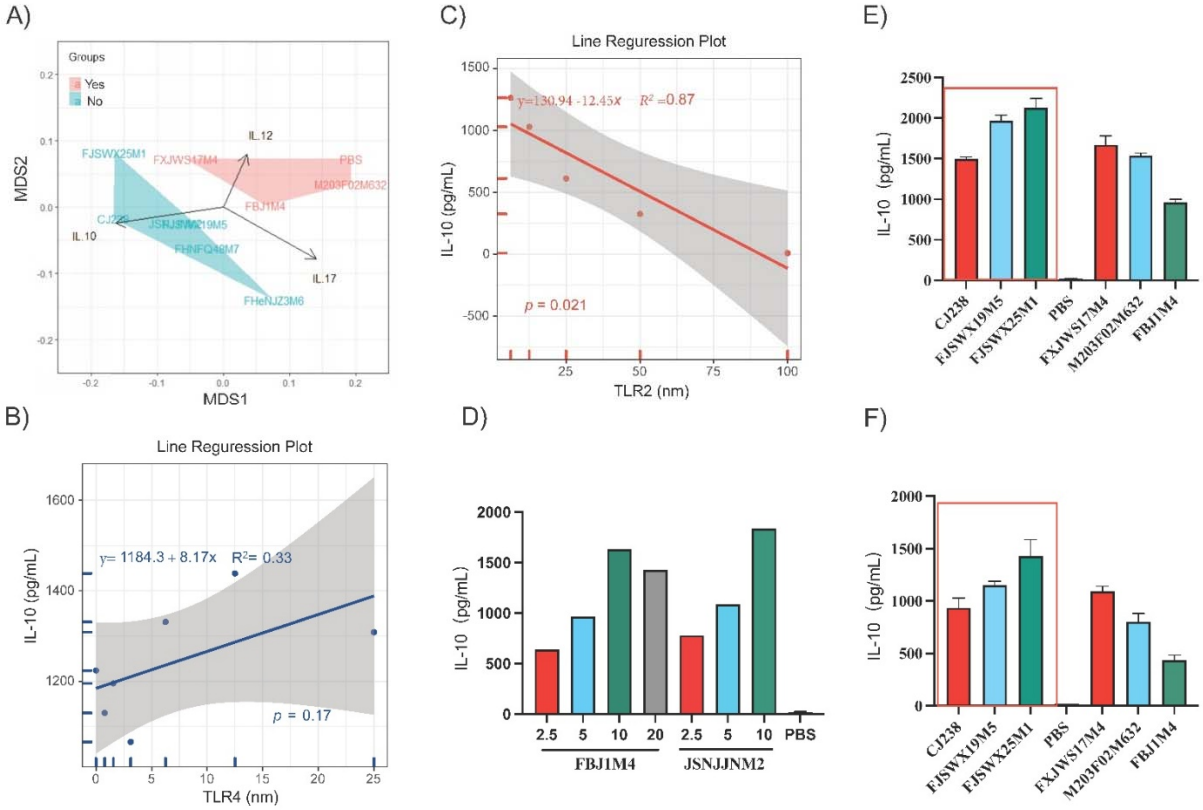


Figure 3. Correlation between the anti-colitis ability of *Bifidobacterium bifidum* strains and the in vitro immunostimulatory activity of LTA. A) MDS analysis of the correlation between the anti-colitis ability of different strains and the MLN-stimulating activity of their corresponding LTAs; B) and C) Effects of adding TLR4 inhibitor (TAK242, R&D) and TLR2 inhibitor (C29, MCE), respectively, on the ability of LTA to induce IL-10 secretion by BMDCs; D) Induction of IL-10 secretion by BMDCs in response to different doses of LTA, with the x-axis values representing the volume added (μL); E) and F) Comparison of the ability of LTAs from different strains to induce IL-10 secretion by BMDCs and BMDMs, respectively; strains marked with red boxes indicate anti-colitis activity, while unmarked strains do not.

Furthermore, the immuno-inducing capacity of LTA was evaluated using a BMDC model. The results showed that the immunoregulatory activity of LTA in BMDCs also depended on the TLR2 signaling pathway rather than TLR4 (Fig. 3B, C). With increasing amounts of LTA from FBJ1M4 and JSNJJNM2, the IL-10-inducing capacity in BMDCs increased accordingly (Fig. 3D). Among the tested strains, LTAs from FJSWX25M1 and FJSWX19M5 exhibited the strongest ability to induce IL-10 production in BMDCs, while FBJ1M4 was the least effective (Fig. 3E). Similarly, the BMDM co-culture system demonstrated that strains with protective effects against colitis showed significantly greater IL-10-inducing capacity.

3.4 LTA of B. bifidum has strain-specific effect against TNBS colitis in normal mice

To clarify the strain-specific effects of *B. bifidum*-derived LTAs on TNBS-induced acute colitis, we administered LTAs from three strains—CJ238, FJSWX19M5, and FXJWS17M4—to mice by gavage for seven days prior to TNBS exposure. All LTA interventions led to improved survival compared to the model group, with the CJ238 group achieving 100% survival and the other two groups having just one death among ten mice (Fig. 4A). The gross appearance and quantitative assessment show that LTA from FJSWX19M5 markedly ameliorated TNBS-induced colon injury and shortening, as reflected by less severe mucosal damage and significantly longer colons relative to the model group. CJ238 conferred partial protection, whereas FXJWS17M4 had only minor effects on colonic morphology and length (Fig. 4B, C). In terms of clinical indicators, both body weight and DAI were substantially improved in LTA-treated groups. Mice receiving FJSWX19M5 exhibited the most pronounced recovery in body weight ($P < 0.01$), while CJ238 also promoted significant weight gain ($P < 0.05$). All three LTA groups showed a significant reduction in DAI compared with the model group, with FJSWX19M5 presenting the strongest improvement (Fig. 4D, E). The spleen index, an indicator of systemic inflammation, was restored by both FJSWX19M5 and CJ238 treatment, with CJ238 demonstrating a more robust effect (Fig. 4F). Assessment of colonic cytokines revealed that only FJSWX19M5 significantly decreased colonic IL-1β levels relative to the model group, while CJ238 and FXJWS17M4 were not effective in this regard (Fig. 4G). To further characterize immunomodulatory effects, we conducted flow cytometry analysis to evaluate Treg cell levels (gating strategy shown in Fig. 4H). Notably, FJSWX19M5 resulted in significant reductions of Treg proportions in both spleen and MLN ($P < 0.05$), while CJ238 only reduced Treg cells in the MLN. No significant difference was observed with FXJWS17M4 (Fig. 4I, J).

Overall, these results demonstrate that LTAs from different *B. bifidum* strains have distinct effects in mitigating TNBS-induced colitis, with FJSWX19M5 exhibiting the most promising protective capacity in terms of colon morphology, inflammation, and immunoregulation.

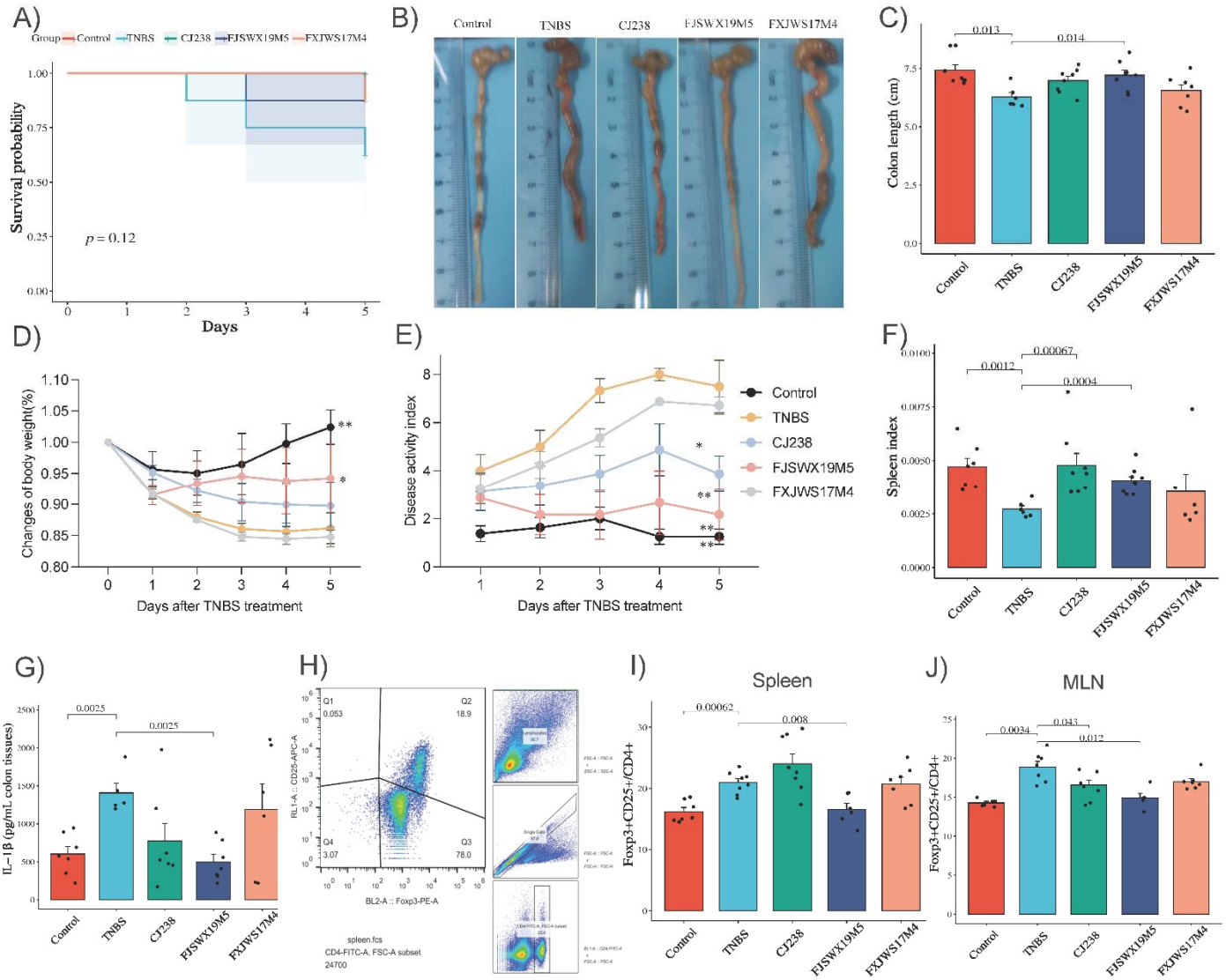


Figure 4. Effects of LTA from various *Bifidobacterium bifidum* strains on TNBS-induced acute colitis in conventional mice. A) Survival analysis of colitis mice in each group; B) and C) Representative images of colons and colon lengths from colitis mice, respectively; D) and E) Changes in body weight and disease activity index of colitis mice during LTA intervention, respectively; F) and G) Spleen index and colonic inflammatory cytokine levels in colitis mice of each group, respectively; H) Gating strategy for Treg determination by flow cytometry; I) and J) Proportion of Treg-positive cells in spleen and MLN from colitis mice in each group, respectively. *p* values between two groups were determined by t-test or Wilcoxon test.

3.5 LTA of B. bifidum FJSWX19M5 has a protective effect on gut inflammation in GF mice

Further experiments were conducted to investigate the effects of LTA from FJSWX19M5 on colitis in GF mice. Due to the influence of strain background and the absence of gut microbiota, the selected germ-free C57BL/6 mice in this study were more tolerant to TNBS. The pre-experiment determined the TNBS dosage at 2500 μg per mouse. On day 3 after TNBS exposure, mice in the model group showed a significant shortening of the colon ($P < 0.05$), thickening and redness of the intestinal wall, and in some cases, bleeding (Fig. 5A). In the LTA intervention group, most mice exhibited a significant recovery in colon length ($P < 0.05$) and improvement in swelling, although one mouse showed no obvious improvement (Fig. 5B).

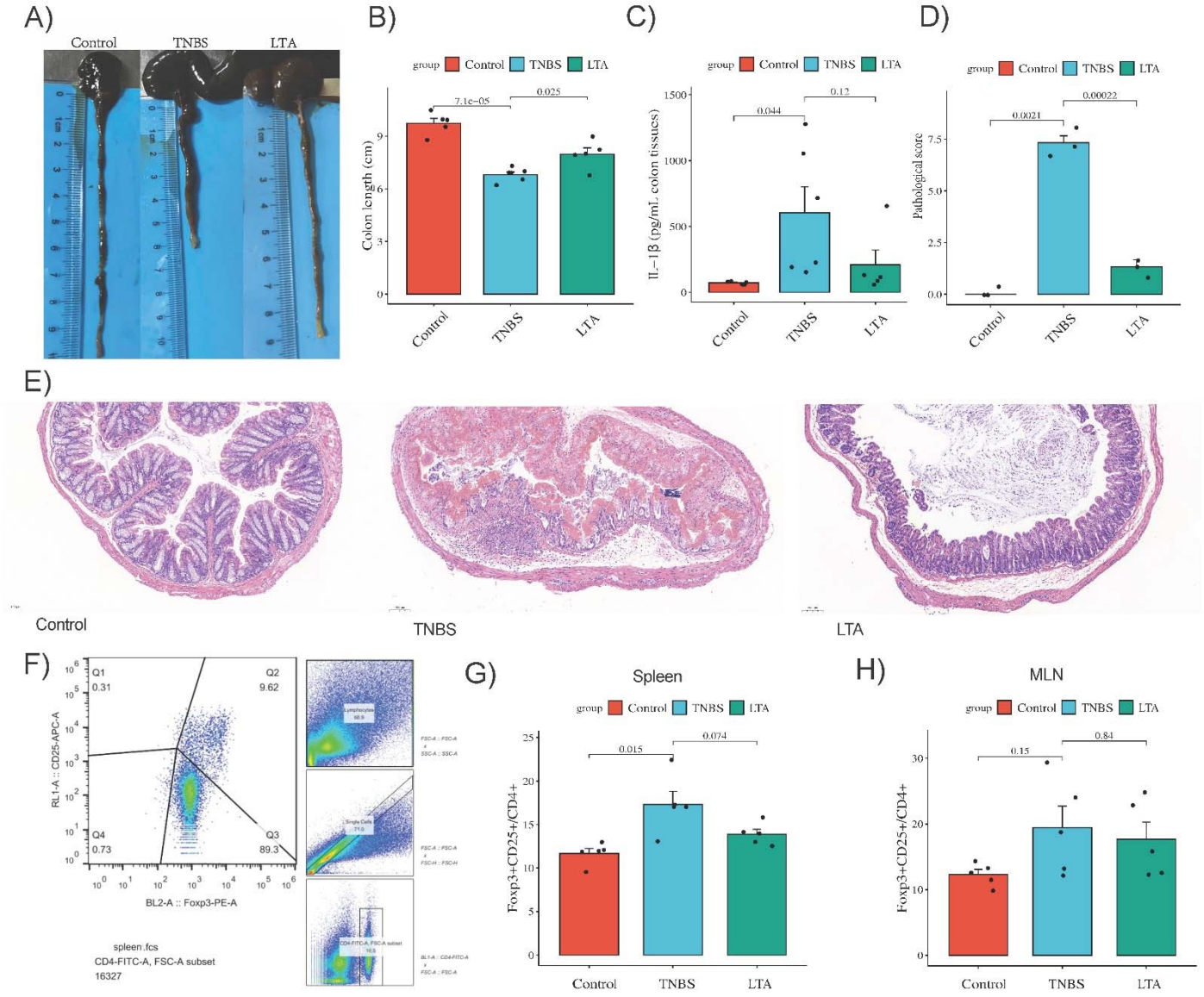


Figure 5. Effects of LTA from *Bifidobacterium bifidum* FJSWX19M5 on TNBS-induced acute colitis in germ-free mice. A) and B) Representative colon images and colon lengths from mice in each group, respectively; C) Colonic inflammatory cytokine levels in colitis mice from each group; D) and E) HE scores and representative staining results of colons in each group, respectively; F) Gating strategy for Treg determination by flow cytometry; G) and H) Proportion of Treg-positive cells in spleen and MLN from colitis mice in each group, respectively. *p* values between two groups were calculated using t-test or Wilcoxon test.

According to Fig. 5C, compared with the control group, the model group had significantly increased colonic IL-1β levels ($P < 0.05$), while there were no significant changes in the levels of the other two cytokines (IL-10 and IL-17). Compared with the model group, the LTA intervention group showed a decrease in colonic IL-1β levels, although this reduction was not statistically significant. HE staining revealed that the control group had an intact colonic mucosa with regularly arranged crypts and no obvious inflammatory cell infiltration. In the TNBS model group, the mucosal structure was severely disrupted, with a decrease or disappearance of crypts, accompanied by massive infiltration of inflammatory cells and obvious tissue edema. The LTA intervention group showed a marked decrease in mucosal injury and inflammatory cell infiltration compared with the model group, with partial restoration of tissue structure. Regarding pathological scores, the TNBS group had significantly higher scores than the control group ($P < 0.001$), while scores in the LTA group

were significantly lower than those in the TNBS group ($P < 0.001$) (Fig. 5D, E). Flow cytometry analysis demonstrated that TNBS exposure led to a significant increase in the proportion of Treg cells in the spleen of GF mice ($P < 0.05$), and the LTA-treated group showed partial restoration of splenic Treg cell levels compared to the model group. In contrast, TNBS exposure and LTA intervention had no significant effect on Treg cell levels in the MLN of GF mice (Fig. 5F-H). In summary, LTA from FJSWX19M5 exerts a certain protective effect against colitis in GF mice.

3.6 LTA of B. bifidum FJSWX19M5 stimulation significantly alters the colonic transcriptome profile in GF mice

PCA revealed a clear separation between the control group and the LTA-stimulated group along the PC1 axis, with high intragroup aggregation (Fig. 6A), indicating significant differences in gene expression patterns between the two groups. Volcano plot analysis showed many differentially expressed genes between the LTA group and the control group. Notably, upregulated genes (shown in red) were significantly enriched, while downregulated genes (shown in blue) were relatively fewer (Fig. 6B). Bar plots further illustrated the most representative upregulated and downregulated genes (Fig. 6C). GO enrichment analysis of the DEGs demonstrated that these genes were mainly enriched in terms such as extracellular region, regulation of biological quality, ion channel activity, cell-cell signaling, and plasma membrane region (Fig. 6D). This suggests that LTA stimulation may affect key biological processes such as cellular signaling and cell adhesion. KEGG pathway analysis revealed that significant pathways affected by LTA stimulation included cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, PI3K-Akt signaling pathway, and the IL-17 signaling pathway (Fig. 6E). These pathways are mainly related to immune regulation, inflammatory response, and cell communication. Additionally, metabolic and disease-related pathways, such as the proteasome pathway and AGE-RAGE signaling pathway in diabetes, were also found to be activated. In summary, FJSWX19M5 LTA stimulation markedly alters the gene expression profiles of colonic tissues in GF mice and activates multiple inflammatory and immune-related signaling pathways, suggesting that FJSWX19M5 LTA may significantly affect mucosal immune response and barrier function in the colon.

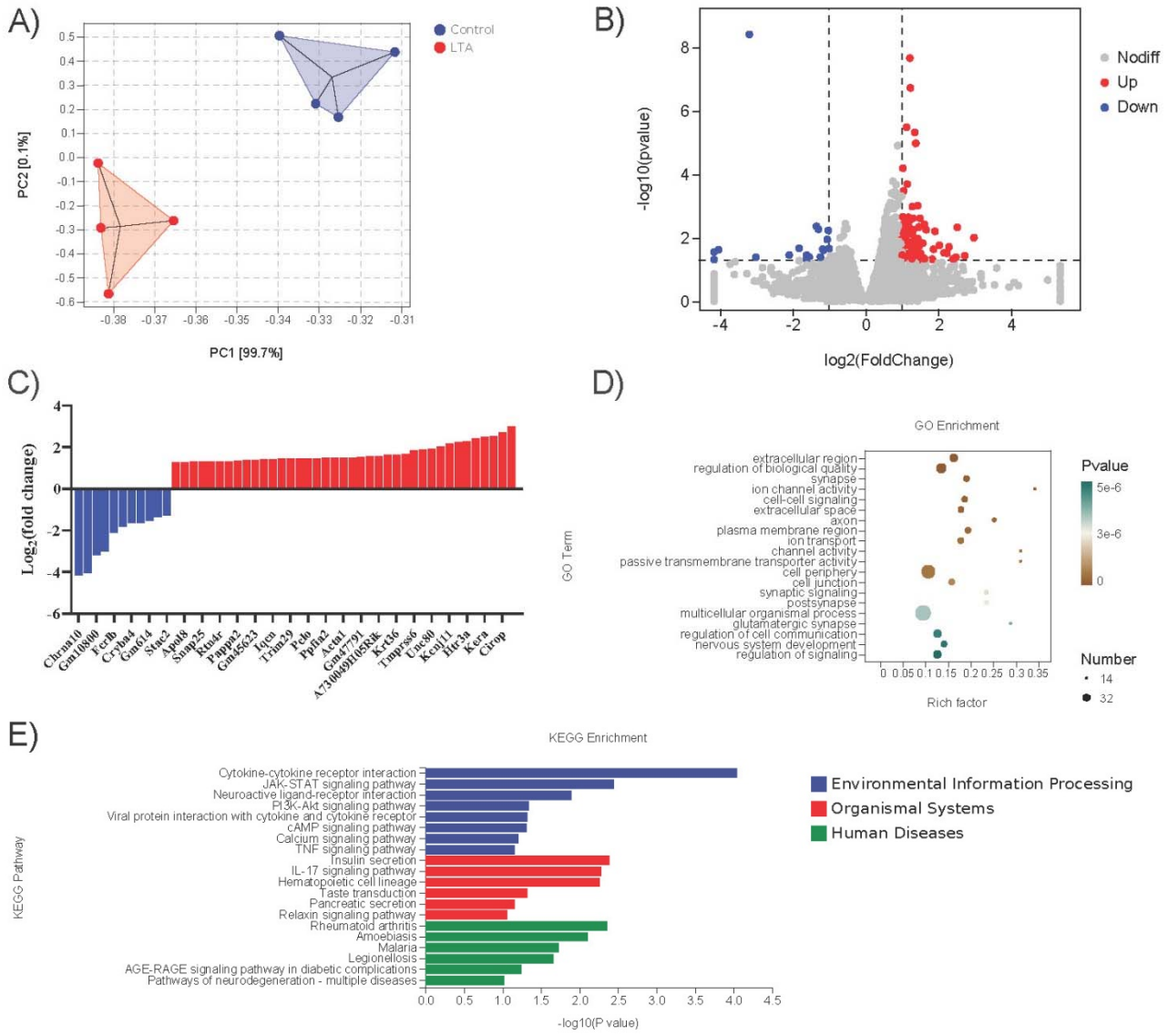


Figure 6. Effects of LTA from *Bifidobacterium bifidum* FJSWX19M5 stimulation on the colonic transcriptome profile of GF mice. A) Principal component analysis (PCA) of gene expression in colonic tissue samples from the control and LTA-treated groups; B) Volcano plot of differentially expressed genes (DEGs); red represents upregulated genes, blue represents downregulated genes, and grey indicates genes with no significant difference; C) Bar graph of significantly up- and down-regulated genes among the DEGs, with blue indicating downregulated and red indicating upregulated genes; D) GO enrichment analysis of DEGs, where bubble size indicates the number of genes and color represents the *p*-value; E) KEGG pathway enrichment analysis of DEGs, with different colors representing different categories of functional pathways.

3.7 LTA of B. bifidum FJSWX19M5 can enhance immune function in normal mice

The *in-vivo* immunomodulatory activity of FJSWX19M5 LTA was further evaluated in normal steady-state mice. In the lymphocyte proliferation assay, the stimulation index of the LTA group was significantly higher than that of the control group ($P < 0.05$) (Fig. 7A), indicating that LTA significantly promoted the proliferation of both T and B lymphocytes in mice. In the DTH experiment, although LTA treatment had no significant effect on the spleen and thymus indices of mice, it significantly increased the degree of ear swelling ($P < 0.05$) (Fig. 7B-D), suggesting that LTA enhances the function of T lymphocytes.

These results demonstrate that *B. bifidum* FJSWX19M5 LTA markedly promotes cellular immunity in mice and has the potential to enhance immune function.

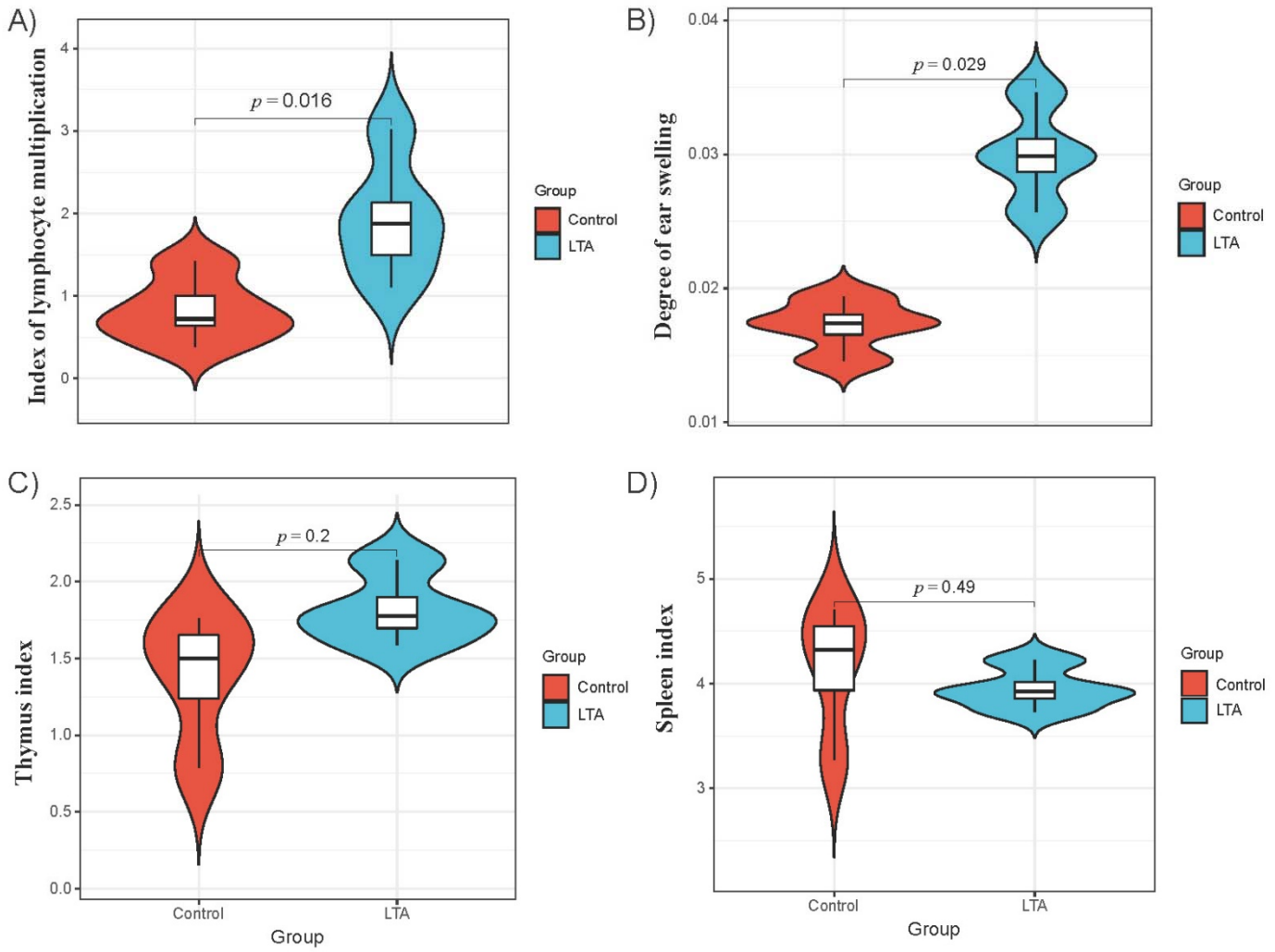


Figure 7. Effects of *Bifidobacterium bifidum* FJSWX19M5 LTA on the immunoregulatory capacity of normal mice. A) Comparison of lymphocyte proliferation index in mice; B) Comparison of ear swelling in mice; C) Comparison of spleen index; D) Comparison of thymus index. *p* values between two groups were calculated using t-test or Wilcoxon test.

4. Discussion

This study set out to elucidate the immunomodulatory mechanisms of *B. bifidum* strains in ameliorating experimental colitis, with a particular focus on the role of strain-specific LTA. Our results reveal that LTA from select *B. bifidum* strains, particularly FJSWX19M5, robustly induces IL-10 production in mucosal immune cells through TLR2-sensitive signaling. In both conventional and GF mice, FJSWX19M5 LTA alleviated colonic inflammation. It also modulated key immune cell populations, including Tregs, and reprogrammed colonic gene expression related to host immunity. These findings establish strain-specific LTA as a pivotal mediator of host-microbe interactions, offering new insights into next-generation probiotic design.

The enhanced IL-10 induction by bacterial cell wall components, particularly heat-resistant and ultrasound-insensitive fractions, pinpoints the unique immunostimulatory architecture of *B. bifidum* (Fig. 1). The identification of LTA, rather than capsular polysaccharides or membrane proteins, as the most potent

IL-10–inducing component aligns with prior studies implicating Gram-positive cell wall polymers in mucosal tolerance[19]. Mechanistically, TLR2 antagonist–sensitive, but not TLR4 antagonist–sensitive, IL-10 and IL-17 responses to LTA support the involvement of TLR2 in LTA sensing (Fig. 2), consistent with reports highlighting TLR2 as a dominant receptor for bacterial LTAs[20, 21]. Our data further suggest that LTA accessibility may influence the magnitude of host responses. This accessibility may be affected by bacterial structural integrity, linking bacterial processing to innate sensing and downstream immune modulation.

Notably, only LTAs from specific *B. bifidum* strains induced strong IL-10 responses and conferred protection against TNBS-induced colitis (Fig. 3). This strain specificity is supported by multi-parameter cluster analyses revealing robust correspondence between in vitro IL-10–inducing activity and in vivo anti-inflammatory efficacy. These observations extend previous findings of functional heterogeneity among probiotic strains[22, 23]. Importantly, our work pinpoints FJSWX19M5 LTA as a uniquely efficacious strain-derived molecule. Comparisons with high-impact literature further underscore our innovation: while several groups have reported metabolite-based immune modulation[24], and others have linked bacterial surface molecules to host phenotypes[25], our study provides functional and mechanistic evidence that specific LTA chemotypes can be rationally selected for targeted immune modulation.

Functionally, LTA-FJSWX19M5 outperformed other strains' LTAs in restoring colonic morphology, reducing pro-inflammatory cytokines, and regulating Treg populations (Fig. 4). Together, these effects are consistent with improved mucosal immune homeostasis. Our GF mouse model further revealed that this protection is not microbiota-dependent, but a direct effect of the LTA-host immune interface (Fig. 5). These findings resonate with recent reports asserting the microbiota-independent efficacy of select microbial molecules[26, 27]. They also support LTA as a defined "postbiotic" molecule for IBD-relevant preclinical investigation.

Transcriptomic analyses illuminate the breadth of LTA-mediated host modulation. LTA treatment in GF mice activated gene sets related to cytokine signaling, the Jak-STAT and PI3K-Akt pathways, and downstream immune-metabolic crosstalk (Fig. 6). Intriguingly, pathway enrichments for cell adhesion and inflammatory signaling echo patterns noted in studies of mucosal healing and barrier restoration[28, 29]. These molecular signatures suggest that FJSWX19M5 LTA orchestrates a program of mucosal tolerance, epithelial repair, and immune homeostasis, supporting its translational interest while remaining within a preclinical scope.

The academic and clinical implications of these results are substantial. At the theoretical level, our findings advance the emerging paradigm that probiotic benefits can stem from defined immunomodulatory molecules with structure–function specificity, rather than from whole organisms alone[25, 30]. By linking LTA sequence specificity with efficacy, our work opens the door to rational screening and engineering of probiotic strains for personalized therapies. Clinically, strain- and molecule-level precision could mitigate the variable outcomes observed in conventional probiotic trials and enable safer, more standardized interventions for IBD

and related disorders. However, we emphasize that our data support mechanistic and preclinical potential rather than clinical readiness.

Nevertheless, several limitations merit discussion. While our in vitro and in vivo data are consistent, the sample size of animal experiments was modest; larger, multi-institutional cohorts are needed to bolster translational confidence. LTAs from only a subset of strains were characterized in molecular detail, and the precise structural motifs conferring immunoregulatory activity remain to be mapped by advanced glycomics[31]. In addition, the LTA used in this study represents a chromatography-enriched preparation tracked by inorganic phosphate during purification. Full structural confirmation by NMR/MS and detailed lipid moiety profiling were not performed due to limited material availability. Therefore, we primarily interpret our findings at the level of TLR2-sensitive immunophenotypes and their concordance with in vivo anti-inflammatory effects and will prioritize comprehensive structural elucidation to establish a stricter structure–activity relationship in future work. Furthermore, the use of TNBS-induced colitis models, although standard, may not fully recapitulate the chronic, multifactorial pathology of human IBD[32]. Importantly, LTA immune modulation can be dose-dependent: a recent study on LTA from *Lactobacillus reuteri* L1 reported divergent effects across doses, with low-dose LTA showing protective immunomodulation whereas higher doses could aggravate intestinal injury and dysbiosis, underscoring the need for cautious dose selection and interpretation[31]. Accordingly, the LTA concentrations used here were chosen to elicit robust, reproducible readouts (i.e., pharmacological exposure for mechanistic testing) and may not directly reflect steady-state physiological levels; therefore, we avoid over-extrapolation and will prioritize quantifying intestinal/mucosal LTA and defining exposure–response relationships in future work. Future studies should assess LTA efficacy in spontaneous colitis settings, dissect host genetic contributions, and integrate high-dimensional spatial analyses of mucosal immune-microbe dynamics.

In summary, this study demonstrates that strain-specific LTAs from *B. bifidum*, particularly FJSWX19M5, mediate potent, predominantly TLR2-associated (pharmacologically TLR2-sensitive) immune modulation and confer significant protection against murine colitis, independent of the resident microbiota. These discoveries underscore the promise of structurally optimized postbiotic molecules for next-generation immunotherapies. Further research should focus on in-depth molecular characterization and preclinical validation, laying the groundwork for clinical application. Collectively, our findings position *B. bifidum* LTA as a mechanistic lead and a candidate for further preclinical development in IBD-relevant contexts, rather than a definitive therapeutic for IBD at this stage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by National Natural Science Foundation of China (Grant Nos. 32502215 and 32394051); Scientific Research Foundation of Hubei University of Arts and Sciences (Grant No. kyqdf2023046).

Reference

[1] S. Bosselaar, L. Dhelin, E. Dautel, et al., Taxonomic and phenotypic analysis of bifidobacteria isolated from IBD patients as potential probiotic strains, BMC Microbiology. 24 (2024) 233. https://doi.org/10.1186/s12866-024-03368-4.

[2] P.S. Prabhu, R. Kalita, V. Sharma, et al., Understanding inflammatory bowel disease: An integrative framework of microbiome, metabolome, and immunological biomarkers, Journal of Translational Gastroenterology. 3 (2025) 24-38. https://doi.org/10.14218/jtg.2024.00030.

[3] H. Ullah, S. Arbab, Y. Tian, et al., Crosstalk between gut microbiota and host immune system and its response to traumatic injury, Frontiers in immunology. 15 (2024) 1413485. https://doi.org/10.3389/fimmu.2024.1413485.

[4] Y. Hao, Z. Hao, X. Zeng, et al., Gut microbiota and metabolites of cirrhotic portal hypertension: a novel target on the therapeutic regulation, Journal of Gastroenterology. 59 (2024) 788-797. https://doi.org/10.1007/s00535-024-02134-7.

[5] R. Yin, T. Wang, H. Dai, et al., Immunogenic molecules associated with gut bacterial cell walls: chemical structures, immune-modulating functions, and mechanisms, Protein & Cell. 14 (2023) 776-785. https://doi.org/10.1093/procel/pwad016.

[6] R. Preet, M.A. Islam, J. Shim, et al., Gut commensal Bifidobacterium-derived extracellular vesicles modulate the therapeutic effects of anti-PD-1 in lung cancer, Nat Commun. 16 (2025) 3500. https://doi.org/10.1038/s41467-025-58553-4.

[7] B. Pei, S. Peng, C. Huang, et al., Bifidobacterium modulation of tumor immunotherapy and its mechanism, Cancer immunology, immunotherapy. 73 (2024) 94. https://doi.org/10.1007/s00262-024-03665-x.

[8] X. Nie, Q. Li, H. Ji, et al., Bifidobacterium longum NSP001-derived extracellular vesicles ameliorate ulcerative colitis by modulating T cell responses in gut microbiota-(in)dependent manners, npj Biofilms and Microbiomes. 11 (2025) 27. https://doi.org/10.1038/s41522-025-00663-4.

[9] S. Yamasaki-Yashiki, T. Shiraishi, M. Gyobu, et al., Immunostimulatory activity of lipoteichoic acid with three fatty acid residues derived from Limosilactobacillus antri JCM 15950T, Applied and Environmental Microbiology. 90 (2024) e01197-01124. https://doi.org/10.1128/aem.01197-24.

[10] C. Matsuzaki, T. Shiraishi, T.-Y. Chiou, et al., Role of lipoteichoic acid from the genus Apilactobacillus in Inducing a strong IgA response, Applied and Environmental Microbiology. 88 (2022) e00190-00122. https://doi.org/10.1128/aem.00190-22.

[11] H.M. Copeland, P. Cannon, S. Maye, et al., Characterisation and bioactivity of an exopolysaccharide isolated from Lactobacillus rhamnosus LRH30 cultivations, Carbohydrate Polymer Technologies and Applications. (2025) 100857. https://doi.org/10.1016/j.carpta.2025.100857.

[12] X. Wang, S. Li, A. Zheng, et al., Structural characterization and immune activation capacity of peptidoglycan from Corynebacterium glutamicum in RAW264. 7 Cells, International Journal of Molecular Sciences. 26 (2024) 237. https://doi.org/10.3390/ijms26010237.

[13] D. Qu, S. Feng, M. Li, et al., Effects of Bifidobacteria bifidum strains on 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced acute colitis and its potential mechanism, Food Bioscience. 52 (2023) 102387. https://doi.org/10.1016/j.fbio.2023.102387.

[14] M. Sauter, R.J. Sauter, H. Nording, et al., Protocol to isolate and analyze mouse bone marrow derived dendritic cells (BMDC), STAR protocols. 3 (2022) 101664. https://doi.org/10.1016/j.xpro.2022.101664.

[15] D. Qu, L. Yu, F. Tian, et al., Bifidobacterium bifidum FJSWX19M5 alleviated 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced chronic colitis by mitigating gut barrier injury and increasing regulatory T cells, Food & Function. 14 (2023) 181-194. https://doi.org/10.1039/D2FO02659G.

[16] S. Feng, S. Wang, D. Qu, et al., Species-or genus-dependent immunostimulatory effects of gut-derived potential probiotics, Journal of Genetics and Genomics. 50 (2023) 786-794. https://doi.org/10.1016/j.jgg.2022.11.001.

[17] Y. Yu, Q. Song, L. Huang, et al., Immunomodulatory activities of sulfated Cyclocarya paliurus polysaccharides with different degrees of substitution on mouse spleen lymphocytes, Journal of Functional Foods. 64 (2020) 103706. https://doi.org/10.1016/j.jff.2019.103706.

[18] J. Wei, S. Wang, G. Liu, et al., Polysaccharides from Enteromorpha prolifera enhance the immunity of normal mice, International journal of biological macromolecules. 64 (2014) 1-5. https://doi.org/10.1016/j.ijbiomac.2013.11.013.

[19] M. Ventura, G. Longhi, L.M. Vergna, et al., The role of teichoic acids of bifidobacteria in driving the interaction with the human host, Frontiers in Microbiology. 16 (2025) 1616397. https://doi.org/10.3389/fmicb.2025.1616397.

[20] I. Ahmad, T. Xuan, Q. Wang, et al., Bacterial lipoteichoic acid induces capsular contracture by activating innate immune response, Plastic and Reconstructive Surgery. 154 (2024) 333-342. https://doi.org/10.1097/PRS.0000000000011054.

[21] J. Han, L. Ding, X. Zhao, et al., Direct interaction between Lactiplantibacillus plantarum ZJ316-derived lipoteichoic acid and TLR2 mediates anti-inflammatory and barrier-protective effects in intestinal cells, Food & Function. 16 (2025) 6560-6575. https://doi.org/10.1039/D5FO01925G.

[22] M. Heimer, M. Teschler, B. Schmitz, et al., Health benefits of probiotics in sport and exercise-non-existent or a matter of heterogeneity? A systematic review, Frontiers in Nutrition. 9 (2022) 804046. https://doi.org/10.3389/fnut.2022.804046.

[23] M. Rahmannia, M. Poudineh, R. Mirzaei, et al., Strain-specific effects of probiotics on depression and anxiety: a meta-analysis, Gut Pathogens. 16 (2024) 46. https://doi.org/10.1186/s13099-024-00634-8.

[24] J. Suez, N. Zmora, G. Zilberman-Schapira, et al., Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT, Cell. 174 (2018) 1406-1423.e1416. https://doi.org/10.1016/j.cell.2018.08.047.

[25] C.-G. Lee, K.H. Cha, G.-C. Kim, et al., Exploring probiotic effector molecules and their mode of action in gut–immune interactions, FEMS Microbiology Reviews. 47 (2023) fuad046. https://doi.org/10.1093/femsre/fuad046.

[26] H. Om, U. Chand, P.K. Kushawaha, Postbiotics: An alternative and innovative intervention for the therapy of inflammatory bowel disease, Microbiological Research. 279 (2024) 127550. https://doi.org/10.1016/j.micres.2023.127550.

[27] T. Ma, Y. Li, N. Yang, et al., Efficacy of a postbiotic and its components in promoting colonic transit and alleviating chronic constipation in humans and mice, Cell Reports Medicine. 6 (2025) https://doi.org/10.1016/j.xcrm.2025.102093.

[28] Y. Fu, X. Ding, M. Zhang, et al., Intestinal mucosal barrier repair and immune regulation with an AI-developed gut-restricted PHD inhibitor, Nature Biotechnology. (2024) 1-6. https://doi.org/10.1038/s41587-024-02503-w.

[29] Z. Zhao, X. Liu, R. Zhang, et al., Intestinal barrier in inflammatory bowel disease: Mechanisms and treatment, Journal of Translational Gastroenterology. (2025) https://doi.org/10.14218/jtg.2024.00038.

[30] H. Shao, F. Min, M. Huang, et al., Novel perspective on the regulation of food allergy by probiotic: The potential of its structural components, Critical Reviews in Food Science and Nutrition. 64 (2024) 172-186. https://doi.org/10.1080/10408398.2022.2105304.

[31] Y. Liu, L. Mei, L. Wang, et al., The immunomodulatory effects of lipoteichoic acid from Lactobacillus reuteri L1 on RAW264.7 cells and mice vary with dose, Journal of Agricultural and Food Chemistry. 72 (2024) 20930-20943. https://doi.org/10.1021/acs.jafc.4c03408.

[32] M. Gonzalez-Acera, J.V. Patankar, L. Erkert, et al., Integrated multimodel analysis of intestinal inflammation exposes key molecular features of preclinical and clinical IBD, Gut. 74 (2025) 1602-1615. https://doi.org/10.1136/gutjnl-2024-333729.