Prev. Nutr. Food Sci. 2024;29(3):270-278
https://doi.org/10.3746/pnf.2024.29.3.270
ISSN 2287-8602

Probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 Alleviates 2,4,6-Trinitrobenzene Sulfonic Acid-Induced Colitis Through Short-Chain Fatty Acid Production and Inflammatory Cytokine Regulation

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ABSTRACT: The development of inflammatory bowel disease (IBD) is closely linked to inflammatory damage and dysbiosis. Recently, probiotics are being increasingly used to improve intestinal health. Probiotic-based therapies can prevent IBD by restoring the balance of gastrointestinal microbiota, reducing gut inflammation, and increasing the concentration of short-chain fatty acids (SCFAs). The present study aimed to investigate the protective effects of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13, a novel probiotic strain derived from dadih (Indonesian curd from buffalo milk), on 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in BALB/c mice. The results showed that probiotic Dad-13 supplementation at a dose of 10^7 or 10^9 CFU/mL improved the clinical symptoms of IBD and enhanced the production of SCFAs, particularly propionate and butyrate. Moreover, probiotic Dad-13 supplementation significantly decreased the levels of pro-inflammatory cytokines [tumor necrosis factor-α, interleukin (IL)-6, and IL-1β] and significantly increased the levels of anti-inflammatory cytokines (IL-10). These findings show that *L. plantarum* Dad-13 can effectively prevent TNBS-induced colitis by modulating SCFA production and inflammatory cytokines.

Keywords: cytokines, inflammatory bowel diseases, *Lactobacillus plantarum*, probiotics, short-chain fatty acid

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammation within the gastrointestinal tract. Its symptoms include diarrhea, abdominal pain, rectal bleeding, fatigue, and weight loss. Generally, IBD can be categorized into two main types: Crohn's disease (CD) and ulcerative colitis (UC). CD can affect various parts of the intestine in a discontinuous pattern, whereas UC begins in the rectum and spreads throughout the entire colon in a continuous pattern (Spiller and Major, 2016). IBD has a multifactorial pathogenesis, including genetics, immune dysregulation, and gut microbiota (Loubet Filho et al., 2022). The prevalence of IBD worldwide continues to increase annually and is predicted to be a socioeconomic burden (Bopanna et al., 2017).

Intestinal microbiota imbalance or dysbiosis has been reported in patients with IBD. Compared to individuals without IBD, patients with IBD show increased levels of Bacteroidetes and Proteobacteria and decreased levels of Firmicutes. Moreover, individuals with IBD show decreased levels of *Faecalibacterium prausnitzii* (Santana et al., 2022). A reduced abundance of bacteria that produce short-chain fatty acids (SCFAs) lead to decreased levels of SCFA, an essential metabolite of bacteria. This reduction leads to impaired B cell maturation and differentiation and decreased quantities of regulatory T cells (Tregs), further weakening the mucosal defense (Huang et al., 2023). Alterations in microbiota composition can induce gastrointestinal tract inflammation, disrupting the intestinal balance, which is a characteristic feature of IBD. The inflammatory cells of innate and adaptive immunity that infiltrate the lamina propria can generate pro-inflammatory cytokines [e.g., interferon-γ, interleukin (IL)-17, tumor necrosis factor (TNF)-α, or IL-1β], exacerbating inflammation and causing damage to the epithelial tissue

Received 4 April 2024; Revised 3 May 2024; Accepted 10 May 2024; Published online 30 September 2024
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(Parada Venegas et al., 2019).

Recently, probiotics ("living microorganisms, which when administered in adequate amounts, confer a health benefit on the host") are being increasingly used to improve intestinal health (Hill et al., 2014). Previous studies have demonstrated that probiotic-based therapies can restore the balance of gastrointestinal microbiota, reduce inflammation, and increase SCFA concentrations (Selvamani et al., 2022; Thananimit et al., 2022). Probiotics enhance intestinal barrier integrity by metabolizing SCFAs and other compounds to increase the production of mucin and tight junction (TJ) proteins in intestinal epithelial cells (Huang et al., 2023). SCFAs counteract tissue inflammation by reducing NOD-like receptor family pyrin domain containing three inflammasome activation (Zhang et al., 2022), increasing zonula occludens-1 (ZO-1) expression, and stimulating antimicrobial peptide production, thereby increasing intestinal barrier integrity and preventing the invasion of pathogenic bacteria to improve IBD (Loubet Filho et al., 2022). Recent evidence suggests that probiotics can regulate intestinal immune responses. They can help control the overactivation of immune cells in the intestines, decrease the levels of pro-inflammatory cytokines (e.g., IL-6, TNF-α, and IL-1β), increase the levels of anti-inflammatory cytokines (e.g., IL-10 and transforming growth factor-β), and inhibit the activity of the NF-κB signaling pathway, thereby ameliorating intestinal inflammation (Popov et al., 2021; Huang et al., 2023).

Lactiplantibacillus plantarum subsp. *plantarum* Dad-13 is a local Indonesian probiotic derived from "dadih," fermented buffalo milk. *L. plantarum* Dad-13 strains can colonize the gastrointestinal tract and do not cause organ and blood translocation (Rahayu et al., 2019). In addition, *L. plantarum* Dad-13 can adhere to the colon and survive in the gastrointestinal tract (Rahayu et al., 2016; Darmastuti et al., 2021). *L. plantarum* Dad-13 is also predicted to produce bacteriocin, which inhibits pathogenic bacteria including *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi* (Suroto et al., 2021). A previous study showed that Dad-13 can promote the growth of butyric acid-producing bacteria, thereby contributing to the increase in overall SCFAs, including propionic and butyric acid (Kamil et al., 2022). This finding indicates that the consumption of *L. plantarum* Dad-13 can control dysbiosis and prevent IBD.

The present study aimed to investigate the protective effects of orally administered *L. plantarum* Dad-13 on 2,4, 6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in BALB/c mice.

MATERIALS AND METHODS

Preparation of probiotic sample

L. plantarum subsp. *plantarum* Dad-13 was obtained from the Food and Nutrition Culture Collection, Center of Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. The isolates were cultured in de Man, Rogosa, and Sharpe broth (Merck KGaA) and incubated at 37°C for 24 h. Then, they were harvested through centrifugation (2,000 *g*, 10 min, 4°C), rinsed with sterile Aquadest, and resuspended to a 1:1 mixture of 10% skim milk (PT. Mirota KSM) and 1% sucrose (PhytoTech Labs) to a final concentration of 10^7 and 10^9 CFU/mL. The bacteria were stored at −20°C until use.

Animals and grouping

Male BALB/c mice (age: seven weeks) were purchased from the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada. They were housed at the Laboratory Animal Center, Center of Food and Nutrition Studies, Universitas Gadjah Mada under standard conditions (12 h light/dark cycle, 22°C, and 50%-60% humidity) with free access to standard diet (AIN 93M) and clean water. After one week of adaptation, the animals were randomly divided into four groups, each comprising six animals: control, TNBS (TNBS-induced colitis group), TNBS+DAD7 (TNBS+*L. plantarum* Dad-13 10^7 CFU/mL), and TNBS+DAD9 (TNBS+*L. plantarum* Dad-13 10^9 CFU/mL).

From the first to the 24th day of the experiment, a 200 μL suspension of 10% milk and 1% sucrose containing 10^7 CFU/mL of *L. plantarum* DAD-13 for the TNBS+DAD7 group and 10^9 CFU/mL of *L. plantarum* DAD-13 for the TNBS+DAD9 group was administered intragastrically. Meanwhile, the control and TNBS groups received no intragastric treatment. On the 15th day, TNBS (Sigma-Aldrich) was injected intrarectally to induce inflammation in the TNBS, TNBS+DAD7, and TNBS+DAD9 groups. The stool consistency, body weight, and fecal bleeding were monitored daily for determining the disease activity index (DAI). After 24 days, the mice were sacrificed, and cecum and colon samples were collected.

All procedures involving animals were evaluated and approved by the Animal Management Committee of Universitas Gadjah Mada (LPPT) (approval number: 00034/04/LPPT/VIII/2023, approval date: August 10, 2023).

TNBS-induced colitis experiment

A mouse model of colitis was induced with 5% TNBS (Sigma-Aldrich) at a dose of 100 mg/kg body weight, in accordance with a previously described technique (Morris et al., 1989). The mice were anesthetized with ketamine, and a catheter was inserted 4 cm through the anus. The mice were maintained head down for 30 s before they

were returned to their cages.

DAI

During TNBS induction, the stool consistency, body weight, and fecal bleeding symptoms were observed daily from the first day to the ninth day after administration. The DAI score was determined by combining the scores of body weight loss (% of initial) (0: none, 1: 1%–5%, 2: 6%–10%, 3: 11%–20%, 4: >20%), stool consistency (0: normal, 1: mildly soft, 2: very soft, 3: watery, 4: more watery), and bloody stool (0: normal color, 1: brown color, 2: reddish color, 3: bloody stool, 4: more bloody) in accordance with a previous study (Mayangsari and Suzuki, 2018).

Histological evaluation

After the mice were sacrificed, colon samples were obtained and preserved in 10% formalin. The fixed colon was dehydrated with graded alcohol (70%–100%) solutions, embedded in paraffin, sectioned to a thickness of 6 μm, and stained with hematoxylin and eosin. The stained tissue sections were observed with an optical microscope (Olympus BX51, Olympus Corp.) using Optilab Viewer 3, in accordance with a previous study (Xia et al., 2020).

Recovery of probiotic in fecal samples

Fresh fecal samples were collected from the rectum of mice after being sacrificed. The samples were homogenized with a sterile spatula and vortexed in 0.9 mL of sterile saline solution (0.85%). Serial 10-fold dilutions (10^{-1} to 10^{-4}) of homogenates were plated on specific media for *L. plantarum* (LPSM media) (Bujalance et al., 2006) and incubated for 48 h at 37°C. After incubation, the final colony count was reported as colony forming units/mL.

SCFAs

The cecal contents were extracted with centrifugation at 16,100 *g* for 15 min at 25°C. Next, 1 mL of supernatant was analyzed using a Shimadzu 2100 plus gas chromatograph with a flame ionization detector (FID) and an application-specific column (RT-CW20M F&F; 30 m×0.25 μm) (Shimadzu Corp.). The gas chromatography conditions were as follows: helium as carrier gas; pressure flow control mode with pressure of 107.1 kPa; 1.04 mL/min column flow and 45.8 mL/min total flow; 28.8 cm/sec linear velocity; 3 mL/min purge flow; and 40.0 split ratio. The initial temperature of the FID was 220°C, whereas that of the column was 110°C, which was maintained for 1 min before increasing by 15°C/min to 180°C and then held for 1.5 min.

Determination of cytokines

The colon tissues were cut and homogenized in phosphate buffered saline solution. The homogenates were centrifuged at 5,000 *g* for 5 min. The supernatant was analyzed for IL-1β, IL-6, IL-10, and TNF-α cytokines using enzyme-linked immunosorbent assay, in accordance with the manufacturer's instructions (Wuhan Fine Biotech Co., Ltd.). The procedures were as follows: (i) samples and standards were added in 96-well coated plates; (ii) the plates were incubated at 37°C for 90 min and washed with wash buffer; (iii) they were then added with secondary antibodies; (iv) the plates were further incubated at 37°C for 60 min, and the wells were washed with wash buffer; (v) horseradish peroxidase was added; (vi) the plates were incubated at 37°C for 30 min and washed several times, (vii) 3,3',5,5'-tetramethylbenzidine substrates were added and incubated at 37°C for 10 min; (viii) stop solution was added, and the absorbance was recorded at 450 nm using a microplate reader (Thermo Fisher Scientific Inc.).

Statistical analysis

The results are expressed as mean±standard error of the mean. The data were analyzed using IBM SPSS Statistics version 23 (IBM Corp.). The statistical significance of mean differences was assessed using one-way analysis of variance, followed by Tukey's test. Statistical significance was considered at $P<0.05$.

RESULTS

DAI

TNBS administration induced weight loss, diarrhea, and bloody stools in mice. The groups with *L. plantarum* Dad-13 supplementation experienced a reduction in symptom severity. The DAI score is a standard parameter to evaluate colitis severity, with higher scores indicating more severe disease condition. TNBS administration increased the DAI scores, but Dad-13 supplementation effectively decreased the scores. There was a reduction in body weight and very soft stool consistency in the TNBS-treated groups from the first day of TNBS induction to the ninth day post-induction, with increasing DAI scores each day. However, the control, TNBS+DAD7, and TNBS +DAD9 groups recovered from diarrhea. As a result, the DAI scores of Dad-13 supplementation groups were significantly lower than the TNBS group (Fig. 1).

Colon length

After the mice were sacrificed, their colon lengths were measured from the ileocecal junction to the mid colon (the mid colon to the anus was cut for viable cell count and histological analysis). Compared with the control

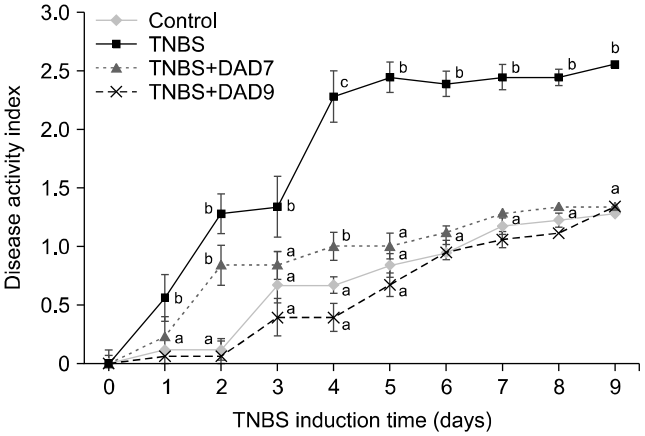

Fig. 1. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on the disease activity index score. A score of 0 represents no disease symptoms, whereas a score of 4 represents the most severe symptoms. Data are presented as the mean±SE (n=6). Different letters (a-c) indicate significant differences (P<0.05). TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

group, the TNBS-treated group showed significantly decreased colon length (P<0.05) (1.62±0.15). However, the TNBS+DAD7 and TNBS+DAD9 groups did not exhibit TNBS-induced colon shortening (3.66±0.17 and 4.17±0.24). The administration of Dad-13 demonstrated significant therapeutic effects against TNBS-induced colitis, leading to a noticeable increase in colon size (Fig. 2).

Histological evaluation

Examination of colon tissue samples taken from the TNBS group showed colon structure disruption. These samples were characterized by abnormal crypt structure and leukocyte infiltration. However, histological analysis of colon samples from the TNBS+DAD7 and TNBS+DAD9 groups showed a more pronounced recovery in intestinal damage compared with the TNBS group. The Dad-13 supplementation groups had moderate damage in crypt structure and less leukocyte infiltration (Fig. 3).

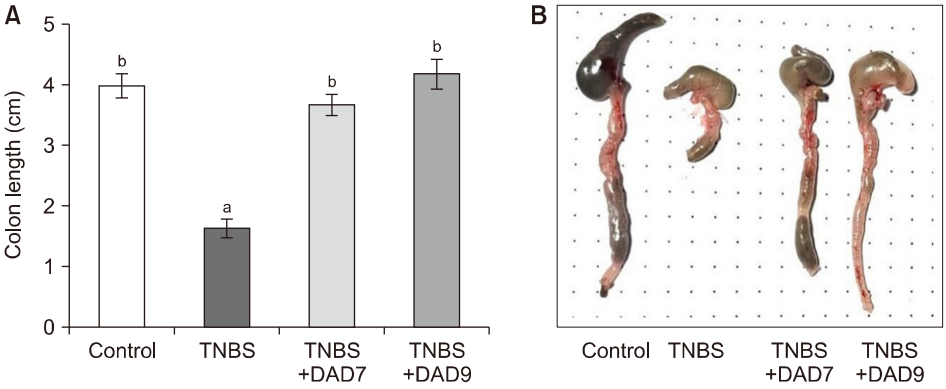

Fig. 2. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on (A) colon length. (B) Photo of the colons in representative mice from each group. Data are presented as the mean±SE (n=6). Different letters (a,b) indicate significant differences (P<0.05). TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

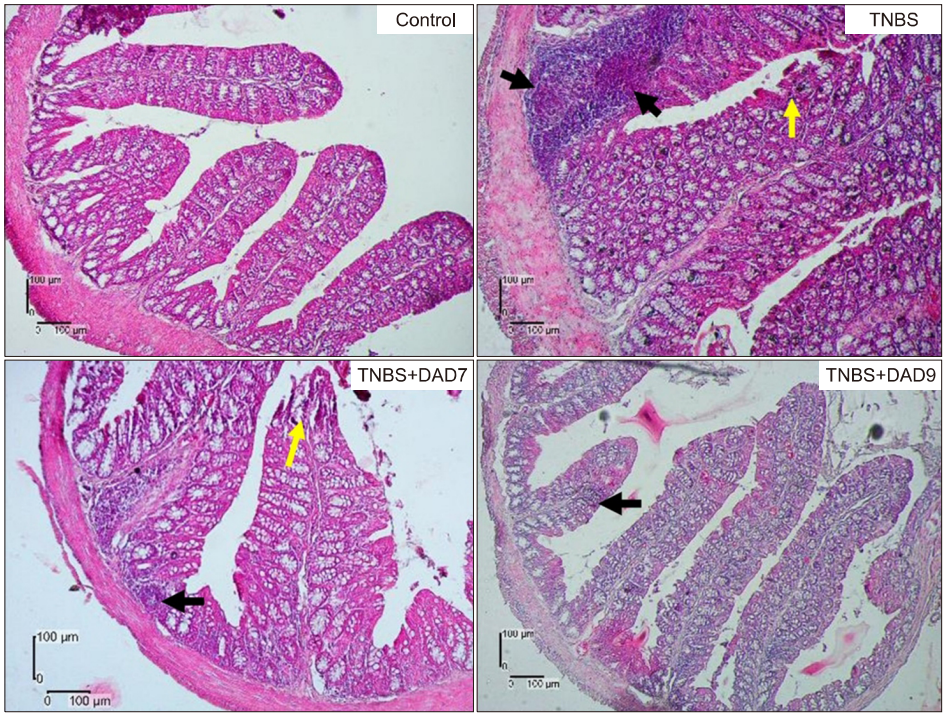

Fig. 3. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on sectioned colon tissues of mice stained with hematoxylin and eosin. The histological images are representatives of each group (×100). Black arrows indicate leukocyte infiltrations. Yellow arrows indicate abnormal crypt structure. TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

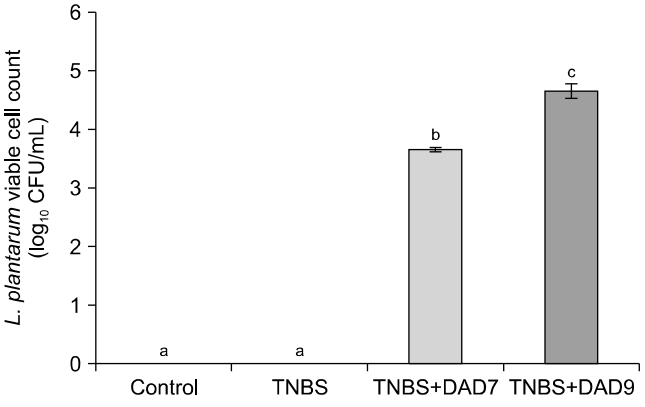

Fig. 4. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on *L. plantarum* viable cell count. Data are presented as the mean±SE. Different letters (a-c) indicate significant differences (P<0.05). TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

Recovery of probiotic in fecal samples

The survival of Dad-13 probiotic in the gastrointestinal tract was assessed by cultivating fecal samples on specific media for *L. plantarum* (LPSM). *L. plantarum* was absent in the healthy control and TNBS groups. However, viable *L. plantarum* cells were detected in the TNBS+DAD7 and TNBS+DAD9 groups (Fig. 4). Notably, *L. plantarum* cell counts were significantly higher in the TNBS+DAD9 group than in the TNBS+DAD7 group (P<0.05).

SCFAs

Compared with the healthy control group, the TNBS group showed a significant decrease in acetic acid, propionic acid, and butyric acid (all P<0.05). This phenomenon has been associated with the development of colitis. In the TNBS+DAD7 and TNBS+DAD9 groups, there was a significant increase in propionic acid and butyric acid (P<0.05), but no significant increase in acetic acid was observed compared with the healthy control group (Fig. 5).

Pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β)

Because of their crucial roles in the development of inflammation, TNF-α and IL-1β are vital pro-inflammatory cytokines involved in the pathogenesis of IBD (Xia et al., 2020). Therefore, the concentrations of these cytokines were assessed in colon tissue. In the healthy control group, the cytokine levels were relatively low (6.11±0.12 pg/mg for TNF-α, 36.5±0.24 pg/mg for IL-1β, and 26.75±0.58 pg/mg for IL-6). However, the cytokine levels were markedly elevated in the TNBS group than in the control group (P<0.05). Compared with the TNBS group, the TNBS+DAD7 and TNBS+DAD9 groups exhibited significantly decreased expression levels of TNF, IL-6, and IL-1β (P<0.05) (Fig. 6).

Anti-inflammatory cytokines (IL-10)

IL-10 primarily reduces inflammation by decreasing the release of pro-inflammatory cytokines (Liu et al., 2021). When TNBS was administered, the expression of IL-10 in the TNBS group decreased to 33.16±1.3 pg/mL. However, the TNBS+DAD7 and TNBS+DAD9 groups showed an increase in IL-10 expression (77.46±2.8 and 90.37±3.76 pg/mL, respectively), which approached the value of the healthy control group (104.43±1.3 pg/mL). Compared with the TNBS+DAD7 group, the TNBS+DAD9 group had significantly higher IL-10 production (Fig. 7).

DISCUSSION

The present study evaluated the protective effects of *L. plantarum* subsp. *plantarum* Dad-13, a probiotic strain derived from "dadih" (Indonesian curd from buffalo milk), on mice with TNBS-induced colitis. Our study demonstrated that TNBS successfully mimicked the clinical symptoms of human IBD. The intrarectal administration of TNBS in mice resulted in colonic inflammation with symptoms of diarrhea, bloody stools, weight loss, reduced colorectal length, and impaired intestinal mucosal bar-

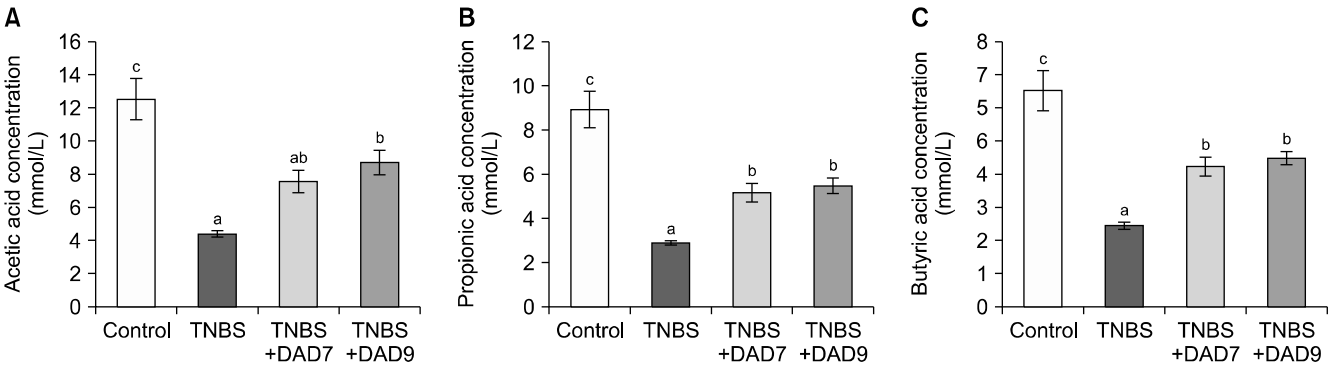

Fig. 5. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on the production of short-chain fatty acids in mice with TNBS-induced colitis: (A) acetic acid, (B) propionic acid, and (C) butyric acid. Data are presented as the mean±SE (n=6). Different letters (a-c) indicate significant differences (P<0.05). TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

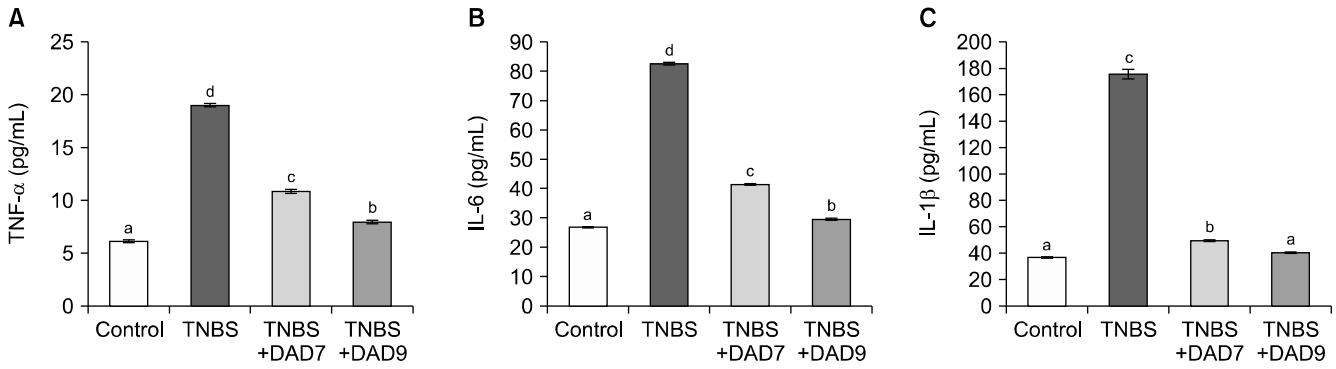

Fig. 6. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on pro-inflammatory cytokines: (A) tumor necrosis factor-α (TNF-α), (B) interleukin (IL)-6, and (C) IL-1β. Data are presented as the mean±SE (n=6). Different letters (a-d) indicate significant differences (P<0.05). TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

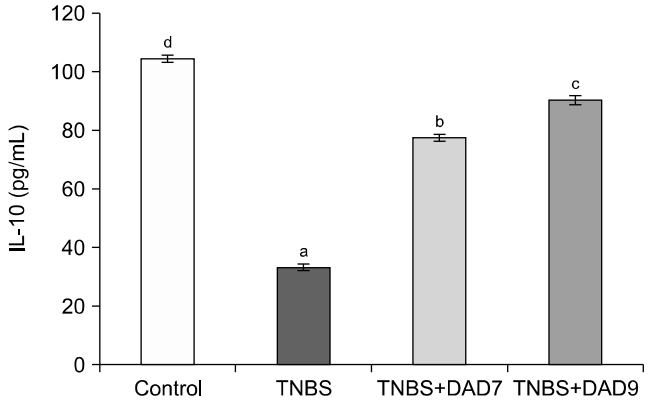

Fig. 7. Effect of probiotic *Lactiplantibacillus plantarum* Dad-13 on anti-inflammatory cytokines. Data are presented as the mean±SE (n=6). Different letters (a-d) indicate significant differences (P<0.05). IL, interleukin; TNBS, 2,4,6-trinitrobenzene sulfonic acid; DAD7, *L. plantarum* Dad-13 at 10^7 CFU/mL; DAD9, *L. plantarum* Dad-13 at 10^9 CFU/mL.

rier function (Luo et al., 2020). TNBS-induced colitis in mice exhibits a typical delayed hypersensitivity reaction driven by T cells. This modification of self-antigens occurs because of the chemical binding of the hapten trinitrophenyl to self-peptides. There is an infiltration of $CD4^+$ helper T cells (Th) in the tissues of mice with TNBS-induced colitis (Neurath et al., 1995).

The colon length and DAI are commonly used to determine IBD severity. A reduction in colon length indicates the emergence of symptoms associated with intestinal edema and mucosal damage, indicating severe colonic inflammation (Chen et al., 2017). In the present study, the TNBS group showed a significant increase in DAI, accompanied by a significant reduction in colon length and tissue damage, after TNBS administration. However, in the probiotic Dad-13 supplementation groups, a lower decrease in DAI was observed, and the colon length was similar to that of the control group (Fig. 1). These findings suggest that *L. plantarum* could alleviate the clinical symptoms of UC in mice, as observed in a previous study (Xia et al., 2020).

To promote their beneficial effects on the host, probiotics must survive through the gastrointestinal tract, resist the gastric environment's acidic conditions, and reach the large intestine in sufficient viable cell quantities to enable colonization and proliferation (Naissinger da Silva et al., 2021). Screening fecal samples is a method for evaluating whether probiotics have survived passage through the gastrointestinal tract (Kristensen et al., 2016). In the present study, viable cells of *L. plantarum* were not found in the healthy control and TNBS groups. Viable cells were detected in the probiotic Dad-13 supplementation groups, but the TNBS+DAD9 group had a higher viable cell count than the TNBS+DAD7 group (Fig. 4). These results demonstrated that *L. plantarum* could survive in the gastrointestinal tract, as observed in previous studies (Rahayu et al., 2016, 2021), and that higher doses of bacterial cells in probiotic formulations may allow a higher recovery of probiotic strains (Taverniti et al., 2019).

Patients with IBD exhibit a significant decrease in the abundance and diversity of beneficial bacteria, including *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, and *Oscillospira* (Alatawi et al., 2022). The reduction in the population of SCFA-producing bacteria leads to lower SCFA levels in the intestine. SCFA, which is derived from the microbial fermentation of dietary fiber by bacteria, serves as the primary energy source for colonic cells and plays a crucial role in maintaining intestinal balance, regulating energy metabolism, and modulating immune response (Shin et al., 2023). Acetate, propionate, and butyrate are the three main SCFAs with the highest abundance in the human gut with concentrations of 3:1:1 (Zhang et al., 2022). SCFAs can enhance the expression levels of ZO-1 and occludin and trigger antimicrobial peptide production, thereby improving the barrier integrity of colon cells and preventing the penetration of pathogenic bacteria, which can ameliorate IBD (Plaza-Diaz et al., 2019). Certain probiotic strains also have the ability to increase the population of SCFA-producing bacteria

in the gastrointestinal tract, resulting in elevated SCFA levels (Mansuy-Aubert and Ravussin, 2023). For example, *L. plantarum* C2, *L. plantarum* C3, and *L. plantarum* P8 have been found to augment SCFA production (Khan et al., 2022). This finding is in line with our result; the production of SCFAs, including acetate, butyrate, and propionate, decreased in the TNBS group, but the production of butyrate and acetate significantly increased in the Dad-13 supplementation groups (Fig. 5).

The levels of pro-inflammatory cytokines are elevated in individuals with IBD and animal models of colitis. Inflammatory cytokines play a significant role in regulating the mucosal immune system, wherein neutrophils and macrophages disrupt the epithelium's integrity, resulting in colon damage and elevated levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. During the disease process, macrophages in the colon are activated, triggering the release of TNF-α, IL-1β, and IL-6, which cause inflammatory damage (Luo et al., 2019). Our results demonstrated that the TNBS group had high levels of pro-inflammatory cytokines and low levels of anti-inflammatory cytokines. Probiotic Dad-13 supplementation managed to reduce the upregulation of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines (Fig. 6 and 7). Butyrate is the primary energy source for colonic epithelial cells, and together with other SCFAs, it promotes epithelial homeostasis through IL-18 production via inflammasome activation (Lavelle and Sokol, 2020). Recent reports demonstrate that butyrate reduces macrophage activation and suppresses inflammatory mediators by inhibiting histone deacetylase 3 (HDAC3). SCFAs directly inhibit HDACs through a GPR43-dependent mechanism, which can deactivate inflammatory gene expression in macrophages (Smith et al., 2013; Li et al., 2018). Binding to GPR43, butyrate inhibits inflammatory responses by suppressing NF-κB and inflammatory cytokines (Lee et al., 2017; Ferrer-Picón et al., 2020). Butyrate also signals through the STAT3 pathway in Th1 cells to promote IL-10 expression in colitis models (Lee et al., 2017; Sun et al., 2018). The levels of anti-inflammatory cytokines (e.g., IL-10) are crucial in controlling intestinal inflammation. Our findings indicated that probiotic *L. plantarum* Dad-13 significantly increased IL-10 levels in the Dad-13 supplementation groups (Fig. 7). Increased IL-10 synthesis has been found to significantly alleviate inflammation in a colitis model (Lee et al., 2017). IL-10 can be produced by specific T cell subsets (Tregs) and innate cells (macrophages) to modulate inflammatory reaction by decreasing IL-6 and TNF-α production (Park et al., 2017; Yang et al., 2018). The lower levels of pro-inflammatory cytokines and high levels of anti-inflammatory cytokines in the probiotic Dad-13 supplementation groups suggested that *L. plantarum* Dad-13 can ameliorate the inflammation of TNBS-induced colitis by reducing the production of pro-inflammatory mediators and enhancing the production of anti-inflammatory cytokines.

In conclusion, *L. plantarum* Dad-13 treatment demonstrated a protective effect against TNBS-induced colitis in BALB/c mice by enhancing SCFA production and modulating inflammatory cytokines. *L. plantarum* Dad-13 reduced the impact of damage caused by TNBS, which improved clinical symptoms. Additionally, it reduced the activity of pro-inflammatory cytokines, enhanced the expression of anti-inflammatory cytokines, increased SCFA levels, and increased the population of live *L. plantarum* bacteria. The findings from this research provide evidence that *L. plantarum* Dad-13 has promising potential as an alternative treatment for IBD prevention. However, this study has some limitations. The gut microbiota and the expression of TJ proteins were not determined to evaluate gut microbiota diversity and intestinal barrier function. Further studies on these factors are required to achieve a better understanding of the preventive mechanism of *L. plantarum* Dad-13 in IBD.

ACKNOWLEDGEMENTS

The authors thank the Indonesia Endowment Fund for Education Agency (LPDP) for financial support.

FUNDING

This research was supported by The Indonesia Endowment Fund for Education Agency (LPDP) scholarship for master's degree to RBP - Grant number PD735202309 1300359.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: RBP, YCS, YM, ESR. Analysis and interpretation: RBP, YCS. Sample Production: RBP, YCS. Data collection: RBP, YCS. Writing the article: RBP, YCS. Critical revision of the article: YM, DAS. Final approval of the article: all authors. Statistical analysis: RBP, YCS. Obtained funding: RBP. Overall responsibility: RBP, YCS.

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