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Lactobacillus plantarum C29 Alleviates TNBS-Induced Memory Impairment in Mice

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In a preliminary study, *Lactobacillus plantarum* C29 was found to suppress 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice. Therefore, to understand whether an anti-colitic probiotic C29 could attenuate memory impairment, we examined the effects of C29 on TNBS-induced memory impairment in mice. Orally administered *Lactobacillus plantarum* C29 attenuated TNBS-induced memory impairment in mice in the Y-maze, noble object, and passive avoidance task tests. C29 treatment increased TNBS-suppressed hippocampal brain-derived neurotrophic factor expression and inhibited TNBS-induced hippocampal NF-κB activation and blood LPS levels. Moreover, C29 restored the TNBS-disturbed gut microbiota composition. These findings suggest that C29 can alleviate memory impairment presumably by restoring the gut microbiota composition.

Keywords: *Lactobacillus plantarum*, memory impairment, colitis, gut microbiota

The bidirectional networks between the central nervous system and gut microbiota are maintained through neural, endocrine, and immune pathways [1, 2]. Stimulation to the brain leads to the secretion of hormones such as corticosteroids, which disturbs the gut microbiota composition and increases their endotoxin production, resulting in gastrointestinal inflammation [2–4]. Gastrointestinal inflammation raises the absorption of gut microbiota endotoxins into the blood and causes systemic inflammation, resulting in cognitive failure and rheumatoid arthritis [5, 6]. The intrarectal injection of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in mice causes colitis through disturbance of the gut microbiota and their LPS overproduction, resulting in memory impairment [5]. Therefore, inhibiting gut inflammation is beneficial to the prevention of memory impairment conditions such as Alzheimer's disease.

Among probiotics, including lactobacilli and bifidobacteria, many lactobacilli exhibit a variety of beneficial effects, such as inhibiting the growth of pathogens, preventing inflammation and memory impairment, and modulation of the host immune system [6, 7]. Of these, lactobacilli including *Lactobacillus sakei*, *Lactobacillus fermentum*, and *Lactobacillus reuteri* are known to mitigate colitis in rodents with TNBS- or dextran sulfate sodium-induced colitis [8–11]. *Lactobacillus plantarum* C29 (formerly *Lactobacillus pentosus* var. *plantarum* C29: 29.1% relatedness to *L. pentosus* DSM 20314^T and 90.4% to *L. plantarum* ATCC 14917^T by a DNA-DNA hybridization experiment) and CLP-0611, which were isolated from kimchi (traditional Korean fermented cabbage), inhibited LPS-induced NF-κB activation in macrophages [8, 12]. C29 also suppressed the innate immune response in aged rats and scopolamine-induced memory impairment in mice [13, 14]. However, whether C29 could simultaneously alleviate memory impairment through the amelioration of colitis remained unclear.

Therefore, we investigated whether *L. plantarum* C29, an anti-inflammatory probiotic, could simultaneously alleviate TNBS-induced colitis and memory impairment in mice.

Male C57BL/6 mice (20–22 g, 6-week-old and SPF) were purchased from RaonBio Inc. (Korea). The animals were housed five per cage in wire cages and acclimatized for 7 days before experiments in an AAALAC International-

approved facility. The mice were housed at 20–22°C and 50 ± 10% humidity and fed with standard laboratory chow and water ad libitum. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Kyung Hee University (IRB No. KHUASP(SE)-16-114) and performed in accordance with the university guidelines on laboratory animal care and use. All behavioral tasks were conducted between 14:00 and 18:00 h.

The mice were divided into seven groups (for colitis) or four groups (for memory impairment) of six mice each to measure the effective dose of C29 against TNBS-induced colitis and memory impairment according to the method of Eun *et al*. [10]. Test agents were orally administered once a day for 3 (to examine the anti-colitic effect) or 5 days (to examine the memory impairment-ameliorating effect). Memory behaviors were measured 2 h after the final administration of C29 in the Y-maze, noble object recognition, and passive avoidance tasks, like previously reported [13, 15]. The mice were sacrificed 2 h after the final behavioral task. Myeloperoxidase activity in colon tissue was measured according to the method of Lim *et al*. [9]. Immunoblotting for hippocampal tissues were performed according to the method of Kim *et al*. [16]. The fecal microbiota composition and LPS levels were analyzed according to the methods of Kim *et al*. [16]. C29 was cultured in MRS broth as previously reported [14].

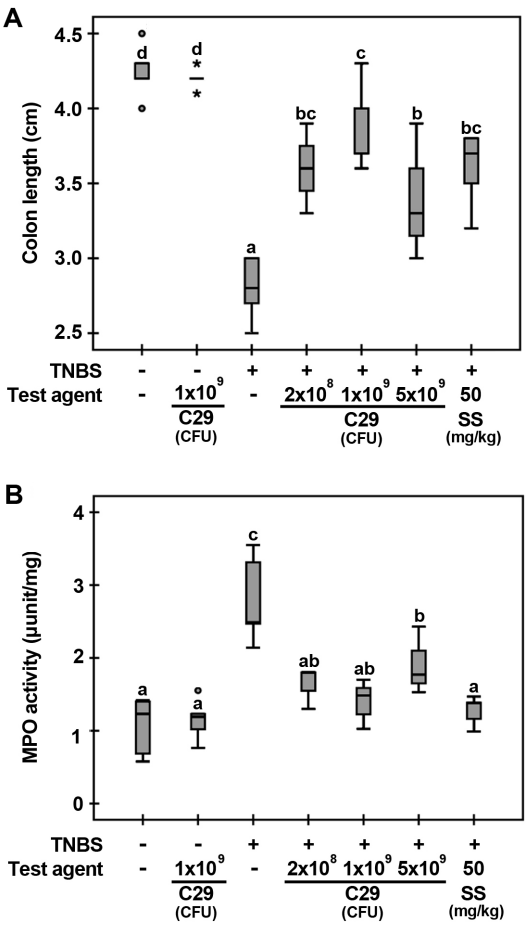

Fig. 1. Effect of C29 on colon length (**A**) and colonic myeloperoxidase (MPO) activity (**B**) in mice with TNBS-induced colitis.

Data are shown as box plots (n = 6). Means with same letters are not significantly different ($p < 0.05$).

All experimental data were indicated as the mean ± standard deviation (SD) and statistical significance was analyzed using one-way ANOVA followed by a Student-Newman-Keuls test ($p < 0.05$).

Treatment with TNBS in mice raised colon shortening and increased the myeloperoxidase activity in the colon (Fig. 1). Treatment with C29 at doses of 1×10^9 and 5×10^9 CFU/mouse showed similar results, whereas C29 at a dose of 0.2×10^9 CFU/mouse showed weak attenuation of colitis. Next, to confirm the ameliorating effect of C29 on memory impairment, we orally treated C29 at a dose of 1×10^9 CFU/mouse and measured colitis markers and memory-related behaviors. TNBS treatment significantly raised myeloperoxidase activity in the colon and LPS levels in the blood (Fig. S1). Treatment with TNBS also reduced spontaneous alterations in Y-maze tasks; TNBS reduced spontaneous alterations to 76.3% of normal control mice (Fig. 2). C29 treatment reversed TNBS-induced decrease in spontaneous alterations, reversing the TNBS-induced memory impairment to 94.2% of normal control mice. Treatment with TNBS also slashed the latency time in the passive avoidance task, whereas C29 treatment restored the TNBS-reduced latency time to 97.1% of normal control mice. In the acquisition trial, no differences in latency were observed among the test groups. Furthermore, C29 restored the TNBS-induced reduction of memory index in the novel object recognition task. C29 raised the TNBS-suppressed expression of the brain-derived neurotrophic factor (BDNF) in the hippocampus, whereas it suppressed TNBS-induced NF-κB activation. Moreover, C29 reduced TNBS-induced blood LPS levels.

Next, the study moved on to the effect of C29 on the gut microbiota composition of mice with TNBS-induced colitis using selective culture media (Fig. 3, Table S1). Treatment with TNBS boosted the population of Enterobacteriaceae and enterococci, but decreased that of bifidobacteria, lactobacilli, and clostridia. In comparison with untreated mice, C29-treated mice showed a significant reduction in the number of Enterobacteriaceae and a rise in the number

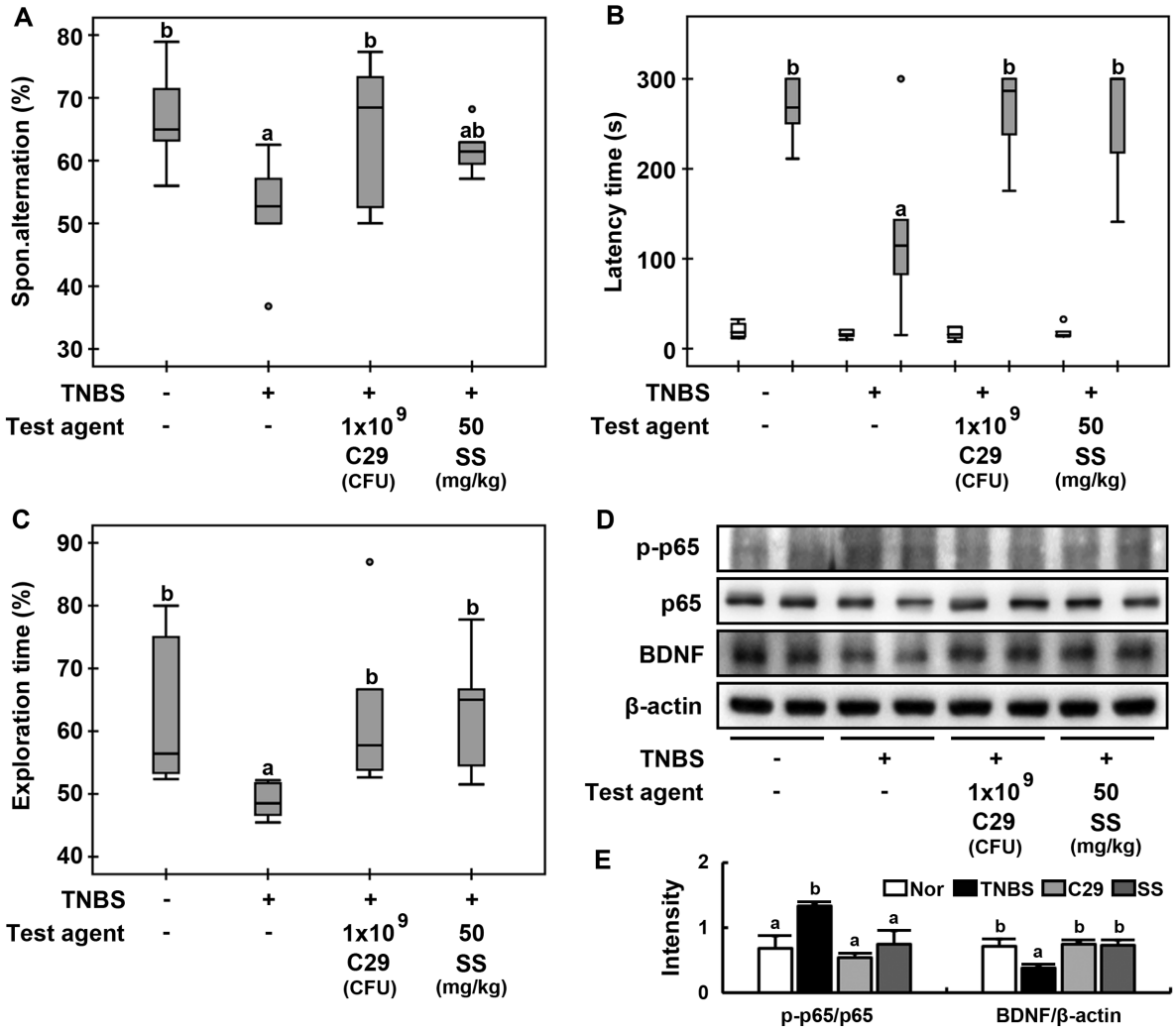

Fig. 2. Effect of C29 on TNBS-induced memory impairment in mice.

Effect in the Y-maze (**A**), passive avoidance (**B**), and novel object recognition tasks (**C**). Effect on NF-κB activation and brain-derived neurotrophic factor (BDNF) expression (**D**) and their protein intensities (**E**). Black and white bars in (**B**) indicate acquisition and retention trails, respectively. Memory impairment was induced with the intrarectal injection of TNBS in mice. Test agents (TNBS, vehicle; C29, C29 (1×10^9 CFU/mouse); or SS, 50 mg/kg sulfasalazine) were orally administered once a day for 5 days from 24 h after the final treatment with TNBS. Normal control group was treated with saline instead of TNBS and test agents. BDNF, p65, p-p65, and β-actin were measured by immunoblotting. Data are shown as box plots ($n = 6$). Means with the same letters are not significantly different ($p < 0.05$).

of bifidobacteria, lactobacilli, and clostridia; no effect was observed in the TNBS-induced growth of enterococci by C29 treatment. C29 restored the ratios of number of Enterobacteriaceae to bifidobacteria, Enterobacteriaceae to lactobacilli, and Enterobacteriaceae to clostridia.

Recent studies have focused on the key role of gut microbiota in the gut-brain axis [1, 17]. Gut microbiota of hosts produce toxic substances such as LPS [16, 18]. Overexpression of gut microbiota LPS in the gastrointestinal tract causes colitis through the activation of innate and adaptive immunity [19].

The present study showed that oral administration of C29 simultaneously attenuated TNBS-induced colitis and memory impairment in mice, inhibited TNBS-induced hippocampal NF-κB activation, and raised TNBS-suppressed BDNF expression. Moreover, C29 restored the ratios of Enterobacteriaceae to bifidobacteria, Enterobacteriaceae to lactobacilli, and Enterobacteriaceae to clostridia. These results suggest that C29 can suppress colitis by restoring the TNBS-mediated disturbance in the gut microbiota

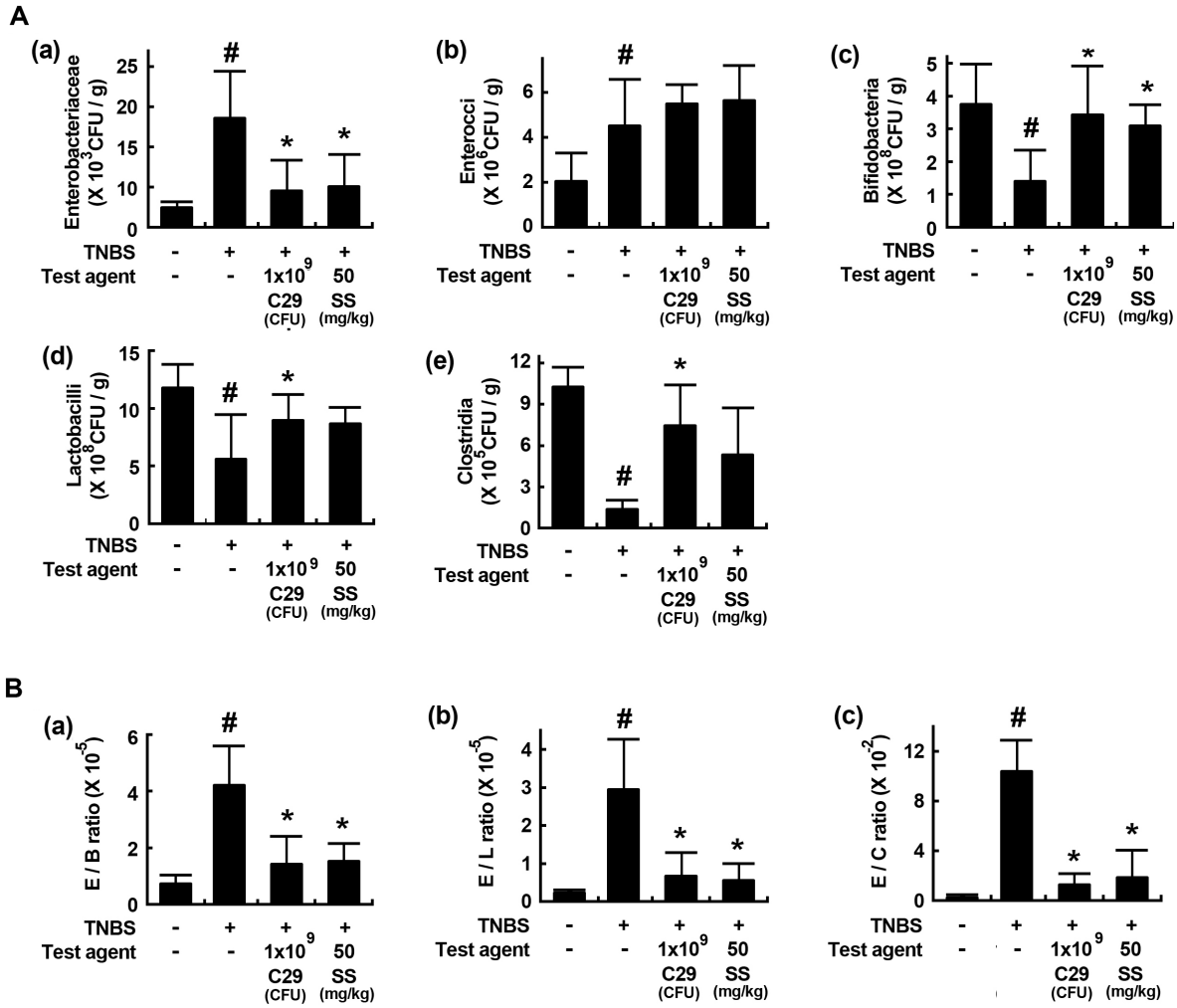

Fig. 3. Effect of C29 on the gut microbiota composition in mice with TNBS-induced colitis.
(**A**) Number of Enterobacteriaceae in DHL (**a**), enterococci in M-Enterococcus (**b**), bifidobacteria in BL (**c**), lactobacilli in MRS (**d**), clostridia in *Clostridium* agar plates (**e**). (**B**) Ratios of number of Enterobacteriaceae to number of bifidobacteria (E/B, **a**), of number of Enterobacteriaceae to number of lactobacilli (E/L, **b**), and of number of Enterobacteriaceae to number of clostridia (E/C, **c**). TNBS, except in the normal control group, was administered intrarectally to mice, which were then orally administered saline, C29 (1×10^9 CFU/mouse), or sulfasalazine (SS50, 50 mg/kg) for 5 days. All values are the mean ± SD ($n = 6$). $^{\#}p < 0.05$ indicates a significant difference vs. the normal control group. $^{*}p < 0.05$ indicates a significant difference vs. the vehicle-treated group.

composition and inhibiting the number of Enterobacteriaceae and the production of gut microbiota LPS. These results were supported by our previous report finding that C29 restored the age-induced disturbance of gut microbiota in aged rats [14]. Additionally, certain probiotics were reported to exhibit stress-induced memory impairment or depression through restoration of the gut microbiota composition [5]. Finally, the present study suggests that anti-colitic C29 can simultaneously alleviate TNBS-induced colitis and memory impairment presumably by correcting the gut microbiota composition.

Supplement

Detailed methods for the colitic and memory-impaired mouse preparation, behavioral tasks, myeloperoxidase activity assay, immunoblotting, ELISA, and determination of gut microbiota composition are described in the Supplementary material.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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