**Bacillus polyfermenticus** Ameliorates Colonic Inflammation by Promoting Cytoprotective Effects in Colitic Mice

Eunok Im, Yoon Jeong Choi, Charalabos Pothoulakis, and Sang Hoon Rhee

Abstract

Although human consumption of *Bacillus polyfermenticus* provides several health benefits, the probiotic effect of this bacterium against colonic inflammation has not yet, to our knowledge, been studied. Therefore, we induced colitis in mice by oral or intrarectal administration of dextran sodium sulfate (DSS) or trinitrobenzenosulfonic acid (TNBS), respectively, and investigated the effect of *B. polyfermenticus* on colitis. We found that mice treated with DSS or TNBS along with *B. polyfermenticus* had reduced mortality and severity of colitis (weight loss, diarrhea, and mucosal damages) than mice treated with DSS or TNBS alone. *B. polyfermenticus* also reduced the expression of inflammatory molecules, including chemokine (C-X-C motif) ligand 1, intercellular adhesion molecule, and tumor necrosis factor-α, but enhanced the expression of the antiinflammatory cytokine interleukin-10 in the inflamed mouse colon. Moreover, *B. polyfermenticus* suppressed apoptosis both in vivo in inflamed colonic mucosa and in vitro in colonic epithelial cells stimulated with apoptosis-inducing agents (FasL or *Clostridium difficile* Toxin A) when the apoptotic response was determined by a terminal deoxynucleotidyl transferase dUTP nick end labeling assay and cleavage of poly(ADP-ribose) polymerase or caspase-3, respectively. Treating colonic epithelial cells with *B. polyfermenticus*-conditioned medium (BPCM) enhanced cell proliferation and induced the phosphoinositide 3-kinases/Akt signaling pathway, suggesting that this bacterium can promote epithelial cell proliferation. BPCM also promoted the migration of colonic epithelial cells. These data suggest that *B. polyfermenticus* ameliorates colonic inflammation by suppressing apoptosis and promoting epithelial cell proliferation and migration. J. Nutr. 139: 1848–1854, 2009.

Introduction

The intestinal epithelium provides an efficient physical barrier against microbes and dietary factors from the lumen (1,2). The disintegrated epithelium is predisposed to provoking excessive immune responses by allowing nonregulated penetration of toxic and immunomodulatory factors into the submucosa (1). In a damaged epithelium, rapid resealing is accomplished by epithelial restitution and wound healing. The epithelial wound healing process includes differentiation of stem cells in the crypt region into enterocytes, goblet, or enteroendocrine cells that are the main constituents of an intestinal epithelium (3,4). The differentiated cells rapidly proliferate and simultaneously migrate from the crypt area to the tip of villi (3,4). Intestinal restitution that does not require cell proliferation involves epithelial sheet movement into the damaged area using a driving force from cytoskeleton and integrin rearrangement (5).

Probiotics are live microorganisms that provide health effects to the host when consumed in an adequate amount (6–8). Evidence indicates that probiotics ameliorate symptoms of irritable bowel syndrome or inflammatory bowel disease, enhance immunity against pathogenic bacterial or viral infection, and relieve antibiotic-associated diarrhea (8,9). However, the molecular mechanism by which probiotics provoke beneficial effects is not clear. In addition, probiotics cooperate with nutritional components to exert distinct effects on the intestinal physiology. For instance, *L. rhamnosus* GG regulates the mucosal transport of macromolecules (10) and induces fermentation of nutrients to exert its antioxidant capacity (11). Although probiotic bacteria are mainly referred to the genera of lactobacilli and bifidobacteria, *Saccharomyces boulardii* is also considered as probiotics (12).

*Bacillus polyfermenticus* was first isolated from an air sample in 1933 and more recently reported to produce an antimicrobial...
agent bacteriocin (13). Given the fact that *B. polyfermenticus* can be delivered to the intestine in a form of endospore that is relatively resistant to stomach acidity, digestive enzymes, and bile salts contributing to its longer presence in the gastrointestinal tract, it has been used for human consumption to help maintain the gut physiology (13–15). Oral consumption of *B. polyfermenticus* in humans stimulates IgG production and modulates the number of CD4+, CD8+, or natural killer cells (16). These studies suggest that *B. polyfermenticus* could be a possible probiotic strain possessing beneficial effects in human gastrointestinal tract. Moreover, like other probiotics, this bacterium interacts with nutritional factors in the intestine to help maintain the gut physiology. For instance, the intake of *B. polyfermenticus* as a nutritional supplement improves lactose metabolism in the intestine (15). Nevertheless, the probiotic effect of *B. polyfermenticus* in intestinal inflammation has not been investigated yet. In this study, we evaluated the role of *B. polyfermenticus* in colonic inflammation.

**Materials and Methods**

**Mice and materials.** CD-1 mice (8-wk-old males) were purchased from Charles River Laboratory. Mice were housed under specific pathogen-free conditions in an isolator (4 mice per cage) at a constant temperature (22°C) and in 12-h-light/dark cycle. Mice were given free access to water and unenriched mouse food (Harlan Teklad Laboratory diet no. 8604). This diet contains (g/kg): 466.4 carbohydrate, 244.8 protein, 44.0 fat, 36.9 fiber, and 78.4 ash (17). The Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center and Harvard Medical School approved all procedures. Antibodies against cleaved caspase-3, cleaved PARP, and Erk1/2 were purchased from Cell Signaling Technology. Monoclonal antibody recognizing heat shock protein (HSP) 72/18 (HSP90) was from Stressgen. Glucose-regulated endoplasmic reticulum stress response protein 78 (GRP78) antibody (H-129) was from Santa Cruz Biotechnology. Human colonic epithelial cells (NCM460) were cultured in M3D medium supplemented with 10% fetal bovine serum, 1% i-glutamine, and 10 K/U penicillin and 100 mg/L streptomycin as previously described (19,20).

**Dextran sulfate sodium-induced colitis.** Mice consumed dextran sulfate sodium (DSS; 4%; MP Biomedicals) dissolved in regular tap water during the entire experimental period, as previously described (21,22).

**Oral administration of *B. polyfermenticus*.** Freeze-dried *B. polyfermenticus* was provided by BINCx Co. *B. polyfermenticus* was dissolved in sterile PBS (101 colony-forming units/mL) and orally administered in a 100-μL total volume daily by gastric gavage for 14 d to determine survival rate or for 10 d to obtain colon tissues. Control mice were treated with the same volume of sterile PBS.

**Survival rate and clinical assessment of colitis.** CD-1 mice were administered with *B. polyfermenticus* for 1 wk by gastric gavage and then provided with DSS (4%) and *B. polyfermenticus* for another 14 d. Mice were monitored for mortality, diarrhea (scaled 0–4), rectal bleeding (scaled 0–4), and body weight change, as previously described (21,23).

**Histology.** The transverse colon segments (1 cm) were fixed in 10% buffered formalin, paraffin-embedded, and stained with hematoxylin and eosin. The histologic severity of colitis was graded on a scale of 0–4, as described (21).

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6 Abbreviations used: BP, Bacillus polyfermenticus; BPCM, *B. polyfermenticus*-conditioned medium; CXCL-1, chemokine (C-X-C motif) ligand 1; DSS, dextran sodium sulfate; GRP78, glucose-regulated endoplasmic reticulum stress response protein 78; HSP, heat shock protein; IL, interleukin; P38, phospho-inositide 3 kinase; TNBS, trinitrobenzenesulfonic acid; TNFa, tumor necrosis factor-a; TLR, toll-like receptor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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**2,4,6-Trinitrobenzenesulfonic acid-induced colitis.** Mice were intracolonically (~3.5 cm from the anal verge) infused with 2,4,6-trinitrobenzenesulfonic acid (TNBS; 150 mg/kg in 100 μL of 40% ethanol) enema via a 1-mL syringe fitted with polyethylene canula (Intramedic PE-20 tubing, Becton Dickinson). Control mice were administered with 40% ethanol-PBS solution. All mice were killed 3 d later to harvest colon tissues, as described previously (22).

**Preparation of *B. polyfermenticus*-conditioned medium.** *B. polyfermenticus*-conditioned medium (BPCM) was generated as described by Grabig et al. (24). Briefly, *B. polyfermenticus* was incubated for 16 h at 37°C in Luria-Bertani broth. Bacteria were then collected by centrifugation (1000 × g for 1.5 min) and pellets were washed twice in PBS and then resuspended in M3D medium containing 10% fetal calf serum without antibiotics. After 2 h of incubation at 37°C in 5% CO2, culture media were collected and filtered through a 0.22-μm-pore size filter. Conditioned medium was then mixed with the complete M3D medium (1:2 ratio) for a cell treatment.

**Wound-healing model assay.** Confluent NCM460 cells were maintained in the complete M3D medium for 24 h and cell monolayer was scrapped with single-edged razor blade and washed with medium. Wounded monolayer was then incubated with BPCM for 18 h followed by examination under an inverted microscope. Migration was assessed without knowledge of the treatments using the image analysis software ImageJ.

**Quantitative cell migration assay using Boyden chambers.** Cell migration was quantified using quantitative cell migration assay kit (Millipore). Briefly, the Boyden chamber assay is consisted of a hollow plastic chamber sealed at 1 end with a porous membrane. Cells were seeded in this hollow chamber with complete M3D medium. The hollow chamber resided in another chamber filled with BPCM. Cells were allowed to migrate overnight through the pores to the other side of the membrane. Then, the inner tube was removed and carefully washed and any nonmigratory cells on the inside of the membrane were carefully scraped away. Cells migrated on the opposite side of the membrane were quantified and presented as mean ± SD for each group (n = 3 per group).

**Quantitative real-time PCR.** Colon tissues were obtained from mice treated with TNBS with or without oral administration of *B. polyfermenticus* and total RNA was prepared using an RNAeasy Plus Mini kit (Qiagen). An equal amount of RNA (2 μg) was transcribed into cDNA using a High Capacity Reverse Transcription kit (Applied Biosystems) by following the manufacturer’s instruction. To evaluate the cytokine gene expression, quantitative real-time PCR was performed on an Applied Biosystems 7500 Fast Real-Time PCR system with TaqMan Universal Master mix using the standard conditions from Applied Biosystems. After the sample was incubated for 2 min at 50°C followed by AmpliTaq Gold activation for 10 min at 95°C, 40 cycles were run with denaturing temperature 95°C (15 s) and an annealing/extension temperature of 60°C (1 min). The primer pairs and FAM dye-labeled TaqMan minor groove binding probe for cytokine genes or the GAPDH gene for use as the internal control were purchased from Applied Biosystems. The level of expression was calculated based upon the PCR cycle number (Ct) at which the exponential growth in fluorescence from the probe passes a certain threshold value (Ct). Relative gene expression was determined by the difference in the Ct values of the target genes after normalization to RNA input level using the Ct value of GAPDH. Relative quantification was represented by standard 2−ΔΔCt calculations. ΔΔCt = (CtTarget gene − CtGAPDH) (25).

Each reaction was performed in triplicate.

**Terminal deoxynucleotidyl transferase dUTP nick end labeling assays.** Colon tissues from each mouse were snap-frozen in OCT compound (Sakura Finetek). Sections were used for evaluating apoptosis using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Apoptosis Detection kit (Upstate Biotechnology), as previously described (21). Using the appropriate antibodies, immunoblotting analysis was performed as we previously described (21,26).

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Data in analyzed by 1-way ANOVA followed by a Newman-Keuls post hoc test. In addition, DSS-treated mice were compared with DSS + BP-treated mice to test the effect of **B. polyfermenticus**. Difference in the survival is shown by Kaplan-Meier plot. The log-rank test was used to compare significant survival difference between the vehicle and DSS-treated groups, and DSS and DSS + BP-treated group followed by the multiple-comparison Bonferroni method (Fig. 1A). Data in Figures 1B–D and 2B,D were compared by ANOVA [with treatment and time (Fig. 1B–D) or treatment and histological indexes (Fig. 2B,D) as categories] followed by the multiple-comparison Tukey-Kramer test to assess differences between groups. Data in Figures 1E and 3B were analyzed by 1-way ANOVA followed by a Newman-Keuls post hoc test. In Figures 4A and 5B were compared by ANOVA (with treatment and time as categories) followed by the multiple-comparison Tukey-Kramer test to assess differences between groups. Data in Figure 5C and Table 1 were analyzed by paired and 2-tailed t test. P < 0.05 was considered significant. Statistical analyses were conducted with Graphpad Prism software.

**Results**

*B. polyfermenticus* reduces the severity of *DSS*-induced colitis in mice. Administration of *B. polyfermenticus* revealed that this bacterium did not affect the expected increase in body weight or cause any other signs of a disease, including rectal bleeding and diarrhea (data not shown). Moreover, *B. polyfermenticus* did not change the cytokine expression [e.g. tumor necrosis factor-α (TNFα), chemokine (C-X-C motif) ligand 1 (CXCL-1), interleukin (IL)-8Rb, or IL-10] in the colon (data not shown), implying that this bacterium does not disturb the physiological balance in mouse intestine.

We next investigated whether *B. polyfermenticus* provides a protective effect against colonic inflammation. *B. polyfermenticus* dramatically reduced the mortality (Fig. 1A). More than 90% of mice treated with DSS and *B. polyfermenticus* survived, whereas only ~35% of mice treated with DSS alone survived. Whereas mice provided with DSS alone had marked weight loss (>14%) due to colonic inflammation compared with the vehicle-treated mice, mice fed with DSS and *B. polyfermenticus* were resistant to weight loss (DSS + BP group, ~2% vs. DSS group, ~14% weight loss) (Fig. 1B). To determine the severity of colitis, clinical parameters of colitis such as rectal bleeding and diarrhea were assessed (Fig. 1C,D). *B. polyfermenticus* significantly ameliorated clinical signs of colitis caused by DSS. Moreover, shortening of the colon length (one of the macroscopic signs of colitis representing the severity of colitis) was less evident in mice treated with DSS + BP than mice treated with DSS alone (Fig. 1E,F). These data suggest that *B. polyfermenticus* ameliorates the development and progress of DSS-induced colitis.

We next examined whether *B. polyfermenticus* has a protective effect on histological damages in colonic mucosa of colitic mice. The H&E-stained colonic tissues from mice treated with *B. polyfermenticus* and DSS showed histologically intact colonic mucosa and substantially reduced leukocyte infiltration in the colon, whereas mice treated with DSS alone revealed highly inflamed colonic mucosa, represented by the severe erosion and the massive leukocyte infiltration (Fig. 2A). We further evaluated several histopathological parameters of colonic inflammation (ulceration, neutrophil infiltration, and edema). Colonic tissue sections obtained from DSS + BP-treated mice had reduced histopathological scores compared with mice that received DSS alone (Fig. 2B). These data indicate that *B. polyfermenticus* reduced mucosal damages in DSS-induced colitis.

Whereas DSS provides direct damage to intestinal epithelial cells leading to loss of epithelial barrier integrity and consequently acute colitis, TNBS is suggested to haptenize macromolecules in the gut to derive Th-1-driven intestinal inflammation, representing a different pathophysiology of colonic inflammation (22). Therefore, we next examined whether *B. polyfermenticus* affects TNBS-induced colitis. We found that massive leukocyte infiltration was evident in the colonic mucosa of TNBS-treated mice (Fig. 2C). In contrast, coadministration of TNBS and *B. polyfermenticus* resulted in less leukocyte infiltration in the colon (Fig. 2C). The histological integrity of colonic epithelium was more preserved in mice treated with TNBS + BP compared with mice treated with TNBS alone (Fig. 2D), These

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**FIGURE 1** Protective effect of *B. polyfermenticus* on survival rate (A), weight change (B), rectal bleeding (C), diarrhea (D), and colon length (E) of the mice from vehicle, DSS, or DSS + BP groups. Results are means ± SD, n = 16–25. The difference in survival between vehicle and the DSS group as well as between the DSS and DSS + BP groups was significant, P < 0.001 and P = 0.001, respectively (A). *Different from corresponding DSS-treated mice at that time, P < 0.001; †different from corresponding vehicle control mice at that time, P < 0.001 (B–D). Two-way ANOVA results showed a treatment × time interaction, P = 0.0001 (B–D). Means without a common letter differ, P < 0.05 (E).
data indicate that *B. polyfermenticus* reduced mucosal damages in TNBS-induced colitis.

Moreover, quantitative real-time PCR data showed that *B. polyfermenticus* suppressed the expression of proinflammatory molecules, including CXCL-1, TNFα, and intercellular adhesion molecule-1, whereas it enhanced the expression of the antiinflammatory cytokine IL-10 expression in the mouse colon inflamed by TNBS (Table 1). These data demonstrate that *B. polyfermenticus* confers a protective effect to DSS- or TNBS-induced colitis models.

**B. polyfermenticus suppresses apoptosis in inflamed colonic mucosa.** Because inflammatory responses in the gut cause apoptosis in intestinal mucosa (21), we investigated whether *B. polyfermenticus* suppresses apoptosis in colonic mucosa to exert a protective effect against DSS-induced colitis. Massive apoptotic cells represented by TUNEL positive immunofluorescence staining (overlapped TUNEL with DAPI-stained nuclei) were observed in colonic mucosa of mice treated with DSS alone (Fig. 3A). In contrast, apoptosis-positive cells were substantially reduced in colonic mucosa of mice cotreated with DSS and *B. polyfermenticus* (Fig. 3A). The number of TUNEL-positive cells was substantially decreased from ~70 to ~5% by *B. polyfermenticus* treatment (Fig. 3B), suggesting that *B. polyfermenticus* protects the colonic mucosa from apoptosis in DSS-induced colitis.

Because *B. polyfermenticus* reduces DSS-induced colitis, it is possible that it may also protect colonocytes from apoptotic stimuli. To test this, we treated human colonic epithelial cells (NCM460) with apoptosis-inducing factors FasL or *Clostridium difficile* toxin A (27) in the absence or presence of BPCM. Treating the colonocytes with BPCM dramatically inhibited PARP cleavage induced by FasL or toxin A stimulation (Fig. 3C, D). Furthermore, we found that caspase-3 cleavage induced by toxin A was blocked in the presence of BPCM (Fig. 3E). These data indicate that soluble factors from *B. polyfermenticus* suppress apoptosis in both colonic mucosa and colonic epithelial cells.

**B. polyfermenticus upregulates cytoprotective molecules and enhances intestinal epithelial cell proliferation.** While the onset of inflammatory responses in the gastrointestinal tract induces erosion and ulceration of mucosal tissues leading to impaired bowel function, concurrent activation of protective mechanisms modulates the degree of inflammation. Mucosal wound healing is a critical protective mechanism of the intestinal mucosa to maintain functional integrity.

Because mucosal wound healing is governed by a sophisticated mechanism, including cell proliferation or migration...
(28,29), we first examined whether \textit{B. polyfermenticus} affects intestinal epithelial cell proliferation. To test this, we plated the same number of cells in culture dishes and cultivated for 24 or 48 h with or without BPCM. After a 48-h incubation, the cell number was significantly increased in the culture treated with BPCM (Fig. 4A). In 24 h, however, BPCM did not increase cell proliferation. These results indicate that epithelial cell proliferation was promoted by \textit{B. polyfermenticus} when incubated for 48 h.

Because the phosphoinositide 3 kinase (PI3K)-dependent signaling pathway mediates cell proliferation and cell survival (30), we tested whether \textit{B. polyfermenticus} induces PI3K activation in colonic epithelial cells. PI3K activation was evaluated by measuring Akt phosphorylation, which is an established readout of PI3K activation (20). BPCM strongly induced Akt phosphorylation in a time-dependent manner (Fig. 4B).

Moreover, the family of heat-shock proteins (e.g. HSP72) is known to have a cytoprotective effect in intestinal epithelial cells (18). Kojima et al. (31) showed that microbial molecules from enteric microbiota upregulated these proteins in intestinal epithelial cells, rendering the intestinal mucosa more sustainable to an intestinal injury. In addition, GRP78 also confers cytoprotective effects in many different types of cells (32). In these contexts, we found that BPCM substantially enhanced the protein expression of HSP72 and GRP78 in colonic epithelial cells (Fig. 4C,D). Taken together, these data show that \textit{B. polyfermenticus} enhances epithelial cell proliferation and up-regulates cytoprotective factors, which are related to cell growth and survival.

\textbf{B. polyfermenticus promotes intestinal epithelial cell migration.} Because epithelial cell migration is also an essential element in mucosal wound healing and maintenance of mucosal integrity in the gut, we investigated whether \textit{B. polyfermenticus} increases epithelial cell migration. Our above data demonstrated that after 24 h, cell proliferation did not differ between the control and BPCM-treated group (Fig. 4A). Thus, to avoid a possibility that the changed cell proliferation rate may contribute to a difference in cell migration, we investigated the epithelial cell migration within 24 h after BPCM was applied. The wound healing assay showed that the intestinal epithelial cells treated with BPCM markedly enhanced cell migration into the denuded area (Fig. 5A,B) compared with the vehicle-treated cells.

Moreover, we evaluated BPCM-induced cell migration using a Boyden chamber assay. The cell migration was increased by ~80% by BPCM compared with the control (Fig. 5C). These data demonstrate that microbial molecules from \textit{B. polyfermenticus} promote the intestinal epithelial cell migration. Because epithelial cell migration is one of the principal processes mediating mucosal wound healing, these data suggest that the enhanced epithelial cell migration by \textit{B. polyfermenticus} possibly contributes to the protective effect of this bacterium against...

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{\textbf{B. polyfermenticus} reduces apoptosis in colitic mice. Representative photographs (A) and apoptosis score (B) in colonic mucosa from vehicle, DSS, or DSS +BP-treated groups. Apoptotic cells and nuclei are shown in green (FITC) and blue (DAPI), respectively. Scale bar: 200 \textmu m. Results are means ± SD, \(n = 4\). Means without a common letter differ, \(P < 0.05\). (C) Inhibitory effects of BPCM on FasL-induced apoptosis in NCM460 cells as shown by cleaved PARP levels. RasGAP served as a loading control. (D,E) Inhibitory effects of BPCM on \textit{C. difficile} toxin A-induced apoptosis in NCM460 cells as shown by cleaved PARP (D) and caspase-3 (E) levels. The blot was reprobed with \(\beta\)-actin as a loading control.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Enhanced cell proliferation and cytoprotection by \textit{B. polyfermenticus}. (A) Effect of BPCM on the proliferation of NCM460 cells. Results are means ± SD, \(n = 3\). Means without a common letter differ, \(P < 0.001\). Two-way ANOVA results showed a treatment \(\times\) time interaction, \(P < 0.001\). Effect of BPCM on the phosphorylation level of Akt (B), HSP72 (C), and GRP78 levels (D) in NCM460 cells. The blot was reprobed with Akt, Erk1/2, or \(\beta\)-actin as a loading control, respectively.}
\end{figure}
intestinal inflammation. Our various data indicate that *B. polyfermenticus* mediates several processes associated with mucosal wound healing, probably rendering the mouse more resistant to colitis.

**Discussion**

In this study, we demonstrated that *B. polyfermenticus* protects mouse colonic mucosa from inflammatory responses by suppressing apoptosis and inducing cytoprotective cellular events. Mice fed with *B. polyfermenticus* dramatically reduced the severity of colonic inflammation in DSS- or TNBS-induced colitis. *B. polyfermenticus* exerted significant antiapoptotic effects in vivo in the inflamed colonic mucosa or in vitro in colonic epithelial cells. Similarly, Yan et al. (33) reported that soluble proteins from *L. rhamnosus GG* or *L. paracasei* was also known to induce the HSP expression in intestinal epithelial cells (36). Similarly, *B. polyfermenticus* increases the expression of HSP72 and GRP78 in colonocytes (Fig. 4C, D), suggesting a clue to propose an important mechanism by which this bacterium may mediate a protective effect against colitis.

Because the probiotic bacterium harbors highly complex microbial molecules that may cause various physiological effects, it is very hard to determine a specific molecule responsible for the beneficial effect of the probiotic bacterium. However, recent studies suggested that Toll-like receptors (TRL) recognizing microbe-associated molecule patterns are involved in mediating probiotic outcomes of several commensal bacteria. For instance, TLR2 and TLR4 were proposed to mediate a protective role of *Escherichia coli* strain Nissle 1917 in DSS-induced mouse colitis (24). Rakoff-Nahoum et al. (18) showed that TLR4 engagement with lipopolysaccharide provides a protective effect in DSS-induced mouse colitis. In addition, Rachmilewitz et al. (37) suggested that TLR9 is necessary to mediate an antiinflammatory response of probiotics in DSS-induced colitis model. Moreover, *B. polyfermenticus* did not exert its protective effect in DSS-induced colitis of TLR2−/− mice (E. Im and S. H. Rhee, unpublished observation). Therefore, microbial products stimulating TLR-associated responses appear to play an important role in mediating the beneficial effects of *B. polyfermenticus*, whereas bacteriocin included in such microbial products does not seem to be responsible for such probiotic effects. Given that the recognition of bacterial products by pattern recognition receptors (e.g. TLR) activates innate and subsequently adaptive immunity, it is notable that probiotics may exert an immunomodulatory effect in the gastrointestinal tract.

In summary, our findings directly support a protective role of *B. polyfermenticus* in experimental colitis models, suggesting possible prophylactic or therapeutic applications of *B. polyfermenticus* for inflammatory bowel diseases.

**TABLE 1** Effect of *B. polyfermenticus* on expression of cytokine genes in the colon of colitic mice

<table>
<thead>
<tr>
<th>Gene name</th>
<th>TNBS</th>
<th>TNBS + BP</th>
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<tbody>
<tr>
<td>CXCL-1</td>
<td>19.88 ± 3.38</td>
<td>4.30 ± 1.69*</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.01 ± 0.59</td>
<td>0.22 ± 0.06*</td>
</tr>
<tr>
<td>Inter cellular adhesion</td>
<td>1.53 ± 0.39</td>
<td>0.78 ± 0.34*</td>
</tr>
<tr>
<td>adhesion molecule-1</td>
<td>1.26 ± 0.53</td>
<td>2.94 ± 0.93*</td>
</tr>
</tbody>
</table>

*Values are means ± SD, n = 4. *Different from TNBS-treated mice, P < 0.05.

Moreover, *B. polyfermenticus* promotes the intestinal epithelial cell proliferation and migration (Figs. 4 and 5), which are key elements for mucosal wound healing in the gut. Indeed, mice with damaged intestinal mucosa due to DSS administration were recovered much faster by *B. polyfermenticus* intake compared with the control group (data not shown). These data strongly indicated that *B. polyfermenticus* is also able to promote mucosal wound healing against colonic epithelial injury.

As we previously showed, HSP72 protects intestinal epithelial cells from cellular damage caused by *C. difficile* toxin A (34). Moreover, HSP exert cytoprotective effects in intestinal epithelial cells and represent an important target for probiotic bacteria (18, 31). Secreted molecules from probiotic mixture VSL#3 upregulate HSP72 and HSP25 expression in colonic epithelial cells (35). The conditioned medium from *L. rhamnosus GG* or *L. paracasei* was also shown to induce the HSP expression in intestinal epithelial cells (36). Similarly, *B. polyfermenticus* increases the expression of HSP72 and GRP78 in colonocytes (Fig. 4C, D), suggesting a clue to propose an important mechanism by which this bacterium may mediate a protective effect against colitis.

**Literature Cited**


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