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Semi-rational screening of the probiotics from the fecal flora of healthy adults against DSS-induced colitis mice by enhancing anti-inflammatory activity and modulating the gut microbiota

Running title: L. sakei–B. bifidum against DSS-induced colitis

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Abstract:
Ulcerative colitis (UC), an chronic inflammatory bowel disease, substantially impacts patients' health-related quality of life. In this study, an efficient strategy for discovering high-efficient probiotics has been developed. Firstly, in order to survive in the conditions of the stomach and intestine, high bile salt-resistant and strong acid-resistant strains were screened out from the fecal flora of healthy adults. Secondly, the probiotic candidates were rescreened by examining the induction ability of IL-10 (anti-inflammatory factor) production in dextran sodium sulfate (DSS)-induced colitis mice, and Lactobacillus sakei 07 (L07) was identified and selected as the probiotic P. In the end, fourteen bifidobacterium strains isolated from healthy man stools were examined for their antimicrobial activity. Bifidobacterium bifidum B10 (73.75 % inhibition rate) has been selected as the probiotic B. Moreover, the colonic IL-6 and TNF-α expression of the DSS-induced colitis mice treated with L. sakei 07 (L07) – B. bifidum B10 combination (PB) significantly decreased and the IL-10 expression were up-regulated by PB compared to the DSS group. Furthermore, Bacteroidetes and Actinobacteria decreased and Firmicutes increased in DSS group mice, significantly. More interesting, intestinal flora biodiversity of DSS colitis mice was increased by PB. Of those, the level of B. bifidum increased significantly. The Bacteriodetes/Firmicutes (B/F) ratio has increased, the concentration of homocysteine and LPS in plasma has been down-regulated by PB in DSS-induced colitis mice. Upon administration of PB, the intestinal permeability of the DSS-induced colitis mice was decreased by approximately 2.01-fold. This method is expected to be used in high-throughput screening of the probiotics against colitis. In addition, the L. sakei 07 – B. bifidum B10 combination hold the potential in UC remitting by immunomodulatory and gut microbiota modulation.

Keywords: Probiotics; Ulcerative colitis; Anti-inflammatory; Gut microbiota; Immunomodulatory; Homocysteine; Intestinal permeability
Introduction

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn’s disease (CD) [1]. Ulcerative colitis (UC), a chronic or long lasting IBD, is difficult to be cure due to its rising incidence in recent decades [2]. To our best knowledge, UC causes many sporadic symptoms, including abdominal pain, diarrhea, and bloody mucopurulent stool [3], which is accompanied by the increased in disorder intestinal tract[3] and inflammatory mediators [4].

Gut microbiota can be divided into three categories: (A) Symbiotic bacteria, including Bacteroides, Bifidobacterium bifidum, Lactobacillus, etc.; (B) Opportunistic pathogen, including Enterococcus, Enterobacter, etc.; (C) Pathogenic bacteria. The imbalance of gut microbiota is associated with an interaction among inflammation mechanism, host defense modulation, oxidative stress, and alteration in bacterial-derived metabolism [5]. Colitis can be ameliorated by precision editing of the gut microbiota [6]. Moreover, the generation of inflammation was promoted by a large number of pro-inflammatory factors (such as IL-6, TNF-α). Intestinal permeability can be enhanced by intestinal pathogens, arising immune response and resulting in disrupted epithelial barrier function. Due to the destruction of the integrity of the intestinal mucosa, the barrier function of the prosthetic layer weakened, resulting intestinal immune system to lose tolerance to intestinal bacteria.

Inducing remission and preventing recurrence were the goal of UC treatment [7]. Nowadays, the major drugs used in UC management are corticosteroids, 5-aminosalicylates, immunomodulators and biologics. However, the severe adverse side effects is also very obvious, such as nephrotoxicity, fever, rash, drug hypersensitivity, hepatitis, pancreatitis, lymphadenopathy, abdominal pain, nausea, vomiting, diarrhea exacerbation, myalgia [8]. Due to dissatisfaction with these medications, probiotics as a potential complementary and alternative therapies for the UC treatment has been discovered in recent years.

Probiotics can enhance the intestinal barrier, regulate the immune system and improve health performance [9]. Probiotics has been used in UC remission via barrier function modulation, which involves the competition of nutrients and the production of bacteriocin or antibacterial proteins. It has reported that probiotics, such as Lactococcus lactis subsp. lactis [10], L. curvatus [11] and L. plantarum [12] ameliorate colitis by regulating the expression of IL-10, TNF-α and IL-6. Strategies for increasing the abundance of gut bacteria have targeted producing lactic acid bacteria, especially lactobacilli and bifidobacteria [13, 14].

Fecal microbiota transplantation (FMT), also called fecal bacteriotherapy, has been used for 50 years for treatment of Clostridium difficile–associated diarrhea and pseudomembranous colitis with great success and few adverse effects [15]. In our present work, probiotics were screened from fecal flora of healthy adults, and the probiotics combinations (L. sakei- B. bifidum) has been designed for remission of the DSS-induced colitis disease. The probiotics holds the potential to apply to handle with the UC via gut microbiota modulation and anti-inflammatory properties.

Materials and methods

Strains screening

Lactic acid bacterial strains were isolated from fecal microbiota of healthy adults. To ensure general health, the vital signs of the healthy adults volunteers should
meet the following standards: 1) be free of known metabolic and gastrointestinal diseases, with no history of metabolic or gastrointestinal diseases; 2) avoid taking medications or alcohol that would impact gut function; 3) be willing to complete all necessary study questionnaires and to donate stool specimens as required; 4) to voluntarily sign a written informed consent form before participation in the study.

Lactic acid bacteria were isolated on MRS agar (pH 5.6) incubated at 30 °C for 72 h. Acid-resistance of lactic acid bacteria colonies isolated from the MRS agar were screened by culturing on MRS agar (pH 3.0). The bile salt tolerance of the colonies were screened by culturing on MRS agar containing 0.30% ox bile salts at 37°C. *Bifidobacteria* were selective cultured anaerobically in MRScm ( MRS medium supplemented with 0.5 g/L L-cysteine and 50 mg /L mupirocin ) at 37°C according to the method of Ferraris L [8]. In order to obtain monoclonal strain, a single colony was repeatedly restreaked on MRScm agar or MRS agar. Chromosomal DNA was isolated and the 16S rRNA gene was amplified by PCR [3]. The strains were identified by DNA sequencing (Sango Biotech, China) according to the method of Tyagi, N. et al [16].

**Animal**

C57BL/6J mice (seven weeks old, male) were purchased from Super-B&K Laboratory Animal Corp., Ltd. (Shanghai, China). C57BL/6J mice were kept at 22°C under a 12-h light/dark cycle supplied with sufficient water and standard rodent diet. C57BL/6J mice were acclimatized to laboratory conditions for 7 days before conducting experiments. Mice were divided into 4 groups (n=12) with different treatments. Control Group: normal chow diet. DSS Group: normal chow diet and drink water with 3% acute dextran sulfate sodium (DSS) for one week (7 days). DSS+P Group: normal chow diet and drink water with 3% DSS for 7 days, 2.0 mL *Lactobacillus sakei* 07 (L07) (1×10^10 CFU/mL) was administered by oral (3 times/day) from the 4 rd to 8th day. DSS+PB Group: normal chow diet and drink water with 3% DSS for 7 days, 2.0 mL the mixture of *Lactobacillus sakei* 07 (L07) (1×10^10 CFU/mL) and *Bifidobacterium bifidum* B10 (2×10^10 CFU/mL) were administered by oral (3 times/day) from the 4 rd to 8th day. Afterwards, the mice were sacrificed on the 9th day. Serum and colons were collected for follow-up experiment. Colonic tissue was homogenized and centrifuged at 4 °C (12000 g × 15 min), and subsequently the supernatant were used to determine cytokines by ELISA kit (R&D system, USA) according to the manufacturer's instructions. Endotoxin levels (Lipopolysaccharides, LPS) were measured by using a limulus amebocyte lysate assay (LAL) endotoxin assay kit (Wilmington, MA). The levels of homocysteine (Hcy) in plasma and colon mucosa were measured by high-performance liquid chromatography-fluorescence detection according to the report of Chen M et al. [17]. This study was approved by the Ethics Committee of Cangzhou Central Hospital (approval No. 2017-063) (Cangzhou, Hebei, China). All animal care and experimental protocols were approved by Animal Care and Research Committee of Cangzhou Central Hospital.

**Analyses of Hemoglobin Concentration in Blood and DAI**

Hemoglobin concentration were determined according to the method of HiCN [18]. The disease activity indices (DAI) composed of three parameters (weight loss, stool consistency, and bloody stools) were calculated to assess severity of colitis according to the report of Sang L X [19]. Briefly, disease activity index consisted of...
a scoring for stool consistency (0–3) and body weight loss (0–3), a visual observation of blood in feces (0–4).

**Quantitative real-time polymerase chain reaction (qrtPCR)**

A fresh colon sample was used for total RNA extraction from with TRIZOL reagent (Invitrogen Life Technologies Co Ltd., USA) following the manufacturer's protocol. The relative mRNA expression of cytokines was determined by quantitative real-time polymerase chain reaction (qrt-PCR) with optimal concentrations of primers and probes. Mouse TGF-β primer (forward 5′- ACGAC ATGATAGTCACTGACAACAA -3′ and reverse 5′- TTTGGGTGTCATGGCAAA ACTGTCTC -3′); TNF-α primer (forward 5′-AGA TGTGGAACCTGGCAGAGG-3′; antisense 5′-ACGACGACGAGGAATCTTCATGGAAGAGGGTTGCCTGCTTTCTGGTT 3′); IL-6 primer pair (forward 5′- TAGTCTTCCTCTACCCCAATT TCC -3′; antisense 5′- TGGTCTCTTACGCCACTCTTTC -3′) were used as the primers or probes. Mouse GAPDH primers (forward: 5′-GTATGACTCCA CTCACGCGAAA-3′ and reverse, 5′-GGTCTCCTGCTCTGGAAGATG-3′) were acted as internal controls. The thermal cycler conditions were 30 cycles of 95°C for 15 seconds, 58°C for 20 seconds, and 72°C for 30 seconds. All samples were run in duplicate. Relative quantity or fold change in gene expression of target genes relatively to the house keeping gene 2^ΔΔCt, where ΔΔCt = ΔCt control – ΔCt treated.

**Analysis of intestinal bacteria**

The composition of the bacterial present in colonic contents of the mice was detected by quantitative PCR according to the method of Long T et al. [20]. Briefly, microbial DNA was extracted from fecal contents with the E.Z.N.A.® Stool DNA Kit (Omega Bio- tek, USA). Extracted bacterial DNA was submitted to quantitative PCR. Moreover, the abundance of five representative bacteria in fecal based on the detection of 16S rRNA genes. PCR products were extracted from 2 % agarose gels and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). The primer sequences were as follows: *Escherichia coli* F:(5′-CATGCCCGGCTGTGATGAAAGA-3′) and R: (5′- CGGGTAACGTCATAAGGCA AA-3′), *Enterococcus* spp. F: (5′-GACGAAGTCT GACCAGCA-3′) and R: (5′-TTAGCCCGCTGCTTCTGTT-3′), *Lactobacillus* F: (5′- AGCAGT AGGGATCCTTCCA -3′) and R: (5′- CACCCGCTACACATGGGAG -3′), *Bifidobacteria* F: (5′- GCGTGCTTAAACATGCAAGTC -3′) and R: (5′- CACCCGTTTCCAGGACTATT-3′), *Bacteroides* F: (5′-GAGGAAGGTCC CCCACATGG-3′) and R: (5′-ACCCATAGGGGACGTACTC-3′). *Bacteroidetes* F: (5′- AACAGGATTAGATACCATCC -3′) and R: (5′- GGTTAGGTGCTCTCCGCG TAT -3′), *Firmicutes* F: (5′- TGAACCTYAAAGGAATTGACG -3′) and R: (5′- ACCATGACACACCTGTGTC -3′), *Actinobacteria* F: (5′-TACGGCCGCAAGGCTA GTG -3′) and R: (5′-TCCCCACCTTCTCCT-3′). *Proteobacteria* F: (5′-TCGTCAGCT CGTGYTGTA -3′) and R: (5′- CGTAAGGGCCATGATG -3′).

**Measurement of the colonic mucosal permeability in vitro**

The colonic mucosal permeability was examined by Evans Blue (EB) assay [21]. A small bowel was infused with 0.3 mL EB solution. After washing with normal saline, the small bowel was dried for 24 h, and incubated with 1 mL of formamide.
for 24 h. After centrifuging, the supernatant was collected and examined under an ultraviolet spectrophotometer at a wavelength of 620 nm.

**Results**

**Probiotic screening**

FMT has been used for treatment of *Clostridium difficile*–associated diarrhea and pseudomembranous colitis [15], highlighted a disease improvement or resolution rate of 83% -92% [22, 23]. In addition, strains as a probiotic should survive in the conditions of the stomach and bile salts. Therefore, high-bile salt and strong acid-resistant strains were screened from the fecal flora of healthy adults. 76 strains were obtained from fecal flora of healthy adults (the donors). Afterwards, the tolerance of strains in acid conditions (pH 3.0) and 0.3% bile salts were investigated, and the strains marked in red box were selected as the candidates (Fig. 1A). IL-10 as an anti-inflammatory cytokine, mainly secreted by Th2 cells and mononuclear macrophages, which can inhibits the secretion of pro-inflammatory cytokines [24]. Thus, the effect of different candidates on IL-10 production by stimulation of splenic lymphocytes in vitro has been investigated. Of those, strain 07 and strain 34 was showing higher capacity on IL-10 production by stimulating the spleen cells (Fig. 1B). Strain 07 and strain 34 were identified as *Lactobacillus sakei* and *Pediococcus acidilactici* by DNA sequencing. In addition, *Lactobacillus* are widely used as effective and safe probiotics. Therefore, *Lactobacillus sakei* 07 (L07) was selected as the probiotic P.

*Bifidobacterium spp.*, has been used as a preferred drug in UC patients [25], moreover, we found that the abundant of *Bifidobacterium* were lower in DSS-induced colitis mice comparing to the healthy mice (Fig. 4D). Therefore, fourteen *Bifidobacterium* strains isolated from the fecal flora of healthy adults were examined for their antimicrobial activity with *E. coli* as an indicator, which were isolated from bloody stools of DSS-induced colitis mice (Fig. 1C). The inhibition rate of the *E. coli* has been investigated and the result shown that *Bifidobacterium bifidum* B10 with high performance (73.75% inhibition rate) has been select as the probiotic B. In order to assess severity of DSS-induced colitis, disease Activity Index (DAI) score and blood haemoglobin (associated with severity of UC) have been investigated. The result shown that probiotic B (*Bifidobacterium bifidum* B10) alone failed to increase the blood haemoglobin level of DSS-induced colitis, comparing to the probiotic PB and probiotic P group (Table 1). The DAI score of probiotic B is higher than that of probiotic PB and probiotic P group (Fig. 1D). The effect of Probiotic B (*Bifidobacterium bifidum* B10) treated alone on ameliorating DSS-induced colitis is not significant. Therefore, the probiotic PB and probiotic P group were selected for the further study.

**Table 1. Effect of probiotics on Blood haemoglobin**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood haemoglobin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>13.02±0.07</td>
</tr>
<tr>
<td>DSS</td>
<td>9.65±0.28*</td>
</tr>
<tr>
<td>Probiotic P</td>
<td>10.86±0.29</td>
</tr>
<tr>
<td>Probiotic B</td>
<td>9.88±0.26</td>
</tr>
<tr>
<td>Probiotic PB</td>
<td>11.87±0.11</td>
</tr>
<tr>
<td>Mesalazine group</td>
<td>12.74±0.32*</td>
</tr>
</tbody>
</table>

Note: C group: normal diet + 350 μL sterile water (intragastric administration); DSS group: 3% DSS model + 350 μL sterile water; Positive control group: 3% DSS model + 350 μL 20mg/mL mesalazine; Probiotic P group: 3% DSS model + 350 μL 1×10^{10} CFU/mL *Lactobacillus sakei* 07 (L07); Probiotic B group: 3% DSS model + 350 μL 1×10^{10} CFU/mL *Bifidobacterium bifidum* B10; Probiotic PB group: 3% DSS model + 175 μL 1×10^{10} CFU/mL *Lactobacillus sakei* 07.
Effects of Probiotics PB on DSS-induced colitis mice

The function of probiotic PB in DSS-induced colitis mice were examined in vivo. C57BL/6J mice were pre-treated with probiotics P or PB for four days and then administered DSS in drinking water for another four days to induce colitis. Treatment with probiotics PB was continued after DSS administration. The result was shown in Fig. 2. Comparing to the control group, the colon length of DSS group mice was significantly decreased and colon weight/colon length were increased (Fig 2 A). The DSS group had a significantly higher colon weight/length ratio (63 ± 0.92 mg/cm) compared to the other groups. Moreover, it has be observed that the colon weight/length ratio of the DSS-induced mice can be reduced by probiotics PB. And the role of probiotics PB has been enhanced by Bifidobacterium bifidum B10 (probiotics B). In addition, the colon weight/length ratio of the control group (3.8 ± 0.31 mg/cm) is basically the same as that of the DSS-treated group received probiotics PB treatment (4.1 ± 0.25 mg/cm) (Fig. 2 B).

Furthermore, the effect of probiotic PB on mRNA expression of cytokines in DSS-induced colitis mice has been investigated. The mRNA expression of IL-10 and TGF-β were down-regulated in DSS induced mice as compared to control group. The TGF-β and IL-10 expression of the DSS group was up-regulated by administration of probiotic P or PB, however, the difference between probiotic P and probiotic PB is not significant (Fig. 3 A, B). The results demonstrated that mRNA levels of IL-6 and TNF-α were elevated in DSS group compared with the control group. Colonic IL-6 and TNF-α expression of DSS-induced mice were significantly reduced after administration of probiotic P or PB (Fig. 3 C,D).

Effect of probiotics PB on the bacterial communities of DSS-induced colitis mice

The relative abundances of different phyla in feces samples were shown in Fig. 4. The high-abundant (>1 % of community) were investigated, including Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria. The abundance of Firmicutes was increased and abundance of Bacteroidetes and Actinobacteria were decreased in DSS group compared with the C group (the control, healthy mice), significantly (Fig. 4 A). In addition, the Bacteroidetes / Firmicutes (B/F) ratio was decreased in DSS-induced mice when compared to healthy mice (C group) (Fig. 4B), the B/F ratio of the DSS-induced colitis mice has been increased after oral administration of probiotics P. More interesting, the ability of probiotics P has been enhanced by probiotics B. The result indicate that oral administration of probiotics (P or PB) was able to restore the abundances of Bacteroidetes and increase the B/F ratio via reshaping of the microbiota composition (Fig. 4B). Biodiversity loss of the intestinal microbial communities in DSS-induced colitis mice has been significantly reduced by probiotics PB treatment.

Furthermore, the relative representative four bacteria (Lactobacillus; Bacteroides, Bifidobacterium bifidum, Escherichia coli) were investigated. The abundance of Escherichia coli and Bacteroides increased significantly in DSS group mice. Of those, the abundance of Escherichia coli increased significantly in DSS group mice, with a 200 times as much as the control group (Fig. 4 C). On the
contrary, the abundances of *Bifidobacterium* and *Lactobacillus* decreased significantly in DSS-induced colitis mice (Fig. 4 D). Although the *Lactobacillus* abundances of DSS-induced colitis mice was not significant recovered, the level of *Bifidobacterium* abundances of DSS-induced colitis mice increased by 95.6% after one week of probiotics PB treatment significantly.

**Effect of probiotics PB on the LPS and homocysteine levels of the DSS-induced colitis mice**

In order to further evaluate the effect of probiotics PB on the intestinal tract, the concentrations of fecal and plasma endotoxin were investigated. The result was shown that the fecal LPS and plasma LPS levels of DSS group were higher than the control group. What’s worse, the plasma LPS levels of DSS group was three times that of control group (Fig. 5 A). The *PLPS/FLPS ratio* of DSS group, which was higher than the control group, have been down-regulated by probiotics (B or PB), however, the difference between probiotic B and probiotic PB was not significant (Fig. 5 B). The plasma endotoxin concentration of DSS+PB group was close to the control group’s.

Furthermore, several studies have found that the endothelial cell barrier can be destroyed by homocysteine (Hcy), resulting increased endothelial cell permeability and promoted inflammation [14, 22]. Therefore, the levels of homocysteine in plasma and colon have been investigated. The result shown that the levels of homocysteine in plasma and colon were increased in DSS-induced colitis mice, comparing to the healthy mice (Fig. 6 A). As assessed by the Evans blue permeability test, we detected a significant increase in intestinal permeability of DSS-induced colitis mice compared with the healthy mice. Upon administration of PB, there was an approximately 2.01-fold decrease in intestinal permeability of DSS-induced colitis mice (Fig. 6 B).

**Discussion**

FMT, also called fecal bacteriotherapy with a resolution rate of 83% -92%, has been used for 50 years for treatment of *Clostridium difficile* -associated diarrhea and pseudomembranous colitis with great success and few adverse effects [15]. Developing human gut microbiota has been regarded as a class of therapeutic methods [21, 26, 27]. Therefore, the fecal microorganism from the fecal flora of healthy adults were select as the source of probiotics. Strains with high acid and bile salts tolerance were screened to handle with the harsh environment of the intestine. *Lactobacillus* were screened by evaluating the induction capacity on IL-10 (an anti-inflammatory cytokine) production. *Bifidobacterium* were screened by examining for their antimicrobial activity. This method is expected to be used in high-throughput screening of the probiotics against DSS-induced colitis or other colitis.

*Lactobacillus* and *Bifidobacteria* has shown promising anti-inflammatory activity in models of colitis [28, 29], which yielded positive effects in IBD models [30]. Previous studies have shown that numbers of *Lactobacilli* were significantly lower during the active phase of the disease, and *Lactobacillus salivarius*, *Lactobacillus manihotivorans* and *Pediococcus acidilactici* were present in remission, but not during active inflammation [31]. The present study was performed that *Lactobacillus* and *Bifidobacteria* were significantly decreased in the DSS-induced colitis. Moreover, the colonic IL-6 and TNF-α expression significantly
down-regulated and the IL-10 expression were up-regulated in PB-treated mice compared to the DSS group via administration of *L. sakei* 07 (L07) - *B. bifidum* B10 combination (PB), resulting in a significant protective effect.

During the last decade, abundant evidence shown that colonic inflammation is associated with inflammation and gut microbiota[32]. In present study, we found that *Bacteroidetes*, *Actinobacteria* decreased and *Firmicutes* increased in the DSS group mice, significantly. The *Bacteroidetes* / *Firmicutes* (B/F) ratio has been increased after oral administration of probiotics PB to DSS-induced colitis mice. Biodiversity of intestinal flora has been increased after probiotics PB treatment. In addition, the abundant of *Bifidobacterium* increased significantly. This result was consistent with the Munyaka P M et al. report [33]. It has been report that microbiota enriched with *Bacteroidetes* promotes host intestinal immune and redox responses protecting mice from lethal infectious colitis [6].

Lipopolysaccharide (LPS; endotoxin) produced by gram-negative bacteria increases the permeability of the gut mucosal barrier, increasing LPS translocation into the circulation, augmenting endotoxiaemia with consequent systemic inflammation [34]. It has been found that the plasma endotoxin concentrations has been decreased by probiotic PB treatment in this study. It indicated that there are a lot of endotoxin absorbed and accumulated by intestine rather than excreted out of the body in DSS-induced colitis mice, comparing to the healthy mice. Consequently, the *Lactobacillus sakei* 07 (L07), *Bifidobacterium bifidum* B10 and its combination hold the potential in UC rehabilitation via immunomodulatory and gut microbiota modulation. Other report shown that IL-10 plays a novel role in promoting H2S production and homocysteine metabolism [35]. In the present study the levels of homocysteine in plasma were increased in DSS-induced colitis mice comparing to the healthy mice. It implied that the amino acid metabolism is disordered in DSS-induced colitis mice. Therefore, we speculate that the colitis can be relieved by PB via regulation of the amino acid metabolism.

**Conflicts of Interest:**

The authors declare no conflict of interest.

**References**


35. Flannigan KL, Agbor TA, Blackler RW, Kim JJ, Khan WI, Verdu EF, et al. 2014. Impaired hydrogen sulfide synthesis and IL-10 signaling underlie...
Fig. 1. Strains screening from the fecal flora of healthy adults (A). Survival rate % (PBS, pH=3.0)= N4/N0
Fig. 2. PB ameliorated colonic injury in dextran sodium sulfate (DSS)-induced chronic colitis mice. (A) Colon length. (B) Colon weight / Colon length (mg/cm). Abbreviation: C, Control group; DSS, DSS group; DSS+P, DSS + Lactobacillus sakei 07 (L07); DSS + PB, DSS + Lactobacillus sakei 07 (L07) + Bifidobacterium bifidum B10. Values were expressed as the Mean.
Fig. 3. Effects of probiotics on inflammation markers expression. Relative expression level of TGF-
Fig. 4. The abundances of microbial phyla in mice fecal samples (A). In DSS-induced colitis mice and other experimental group, pooled fecal samples were provided separately. The Bacteriodetes / Firmicutes (B/F) ratio of different group (B). Alteration of microbiota composition in C57BL/6J mice (C, D). Relative quantity of four representative bacteria was measured by qPCR. Values were expressed as the Mean.
Fig. 5. Effect of probiotic PB on the fecal LPS and plasma LPS levels (A). LAL assay was used to measure the fecal and plasma endotoxin concentrations. All values are indicated as the mean
Fig. 6. The levels of homocysteine (Hcy) in plasma and colon mucosa in mice with DSS-induced colitis (A). Abbreviation: C, control; DSS: DSS treated group; DSS+PB: Probiotic combination treated group. Effect of probiotics PB on the colonic mucosal permeability of DSS-induced colitis (B). Values were expressed as the Mean.