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Potential application of *Lactobacillus plantarum* in the prevention of inflammatory bowel diseases in Balb/c mice

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A dysfunctional interaction between the intestinal microbiota and the host immune system is a very important factor responsible for the development of different inflammatory conditions of the gastrointestinal tract. Some lactobacilli have been characterized as probiotics that can modify the gut microbiota and may be beneficial for the prevention of inflammatory bowel disease. To investigate the protective effects of *Lactobacillus plantarum* on dextran sulphate sodium (DSS) induced colitis mouse model. Thirty female Balb/c mice are divided into 3 equal groups. Control group was given saline solution for 10 days, and treated groups were given for 5 days saline solution and 10^9 CFU/mL of *Lactobacillus plantarum* DF68 respectively. At the end of the first period, the second group and third group received 3% DSS in the drinking water during 5 days. Anthropometric measurements are performed on d0 and d10. At the end of the experiment, the animals are sacrificed, the jejunal and colon fragments are removed for histological study and the contents of there are freshly collected under sterile conditions for microbiological study. Oral administration of studied *Lactobacillus plantarum* DF68 resulted in significant amelioration in disease activity index correlated with attenuation of macroscopic colonic damage, histopathological changes and high significant decrease (p< 0.001) of Enterobacteriaceae in both colon and jejunum. This study demonstrates that the protective effect of *Lactobacillus plantarum* DF68 may at least in part be due to its anti-inflammatory activity.

Keywords: Inflammatory bowel disease; *Lactobacillus plantarum*; DSS; intestinal microbiota; mice

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic immune mediated disease affecting the gastrointestinal tract. A variety of defense mechanisms are aimed at minimizing infections caused by IBD, and include physical barriers such as tightly adherent epithelial cells, antimicrobial peptides secreted by intestinal epithelial cells, and complex mucosal innate and adaptive immune arms aimed at eliminating invasive infections (Swidsinski et al. 2002).

At present, the acknowledged pathogenetic mechanisms are featured by immune dysregulation, altered intestinal microflora, oxidative stress, defects in the gastrointestinal mucosal barrier and increased permeability, whose interplay leads to the onset of a state of chronic mucosal inflammation (Kucharzik et al.

2006).

Among the effects claimed for probiotics are beneficial immunomodulation, reduction of serum cholesterol, improved lactose digestion and protection against colon cancer (Holzapfel et al. 1998; Gorbach, 1990). Probiotics have also been studied in infectious diarrhoea, inflammatory bowel disease and pouchitis (Gorbach, 1990; Campieri et al. 1999).

A lot of mechanisms have been proposed to explain the beneficial role of probiotics in IBD, focusing on their ability to colonize the colon and inhibit the growth of pathogenic species (Faubion and Sandborn, 2000). Moreover, probiotics are known to interact with epithelial and immune cells resident in the intestinal mucosa, reinforcing the barrier function and modulating the immune response (García-Lafuente et al. 2001).

Modifications of the quality and quantity of microbial communities may in turn contribute to intestinal inflammation, promoting IBD in genetically predisposed individuals (Jostins et al. 2012). On the other hand, intestinal microbiota can be modulated using probiotics, with attenuation of intestinal mucosal inflammation (Yan et al. 2011; Atarashi et al. 2013).

Lactobacillus plantarum is frequently used in the food and pharmaceutical industries as starter cultures or probiotics because of its health benefit to the host. *L. plantarum* has health-promoting effects including management of the fecal flora composition (Goossens et al. 2003), prevention and treatment of irritable bowel syndrome (Waugh et al. 2009), IBD (Schultz et al. 2002), coronary heart disease (Jones et al., 2004) and certain gastrointestinal symptoms (Lönnermark et al. 2010).

There is considerable public, media, and scientific interest in ‘‘natural’’ products, including probiotics, in modulating intestinal inflammation and health. Here, we used *L. plantarum* DF68 to evaluate the impact of this bacterium on gut infammation and gut microbiota.

MATERIALS AND METHODS

Source of bacterial isolates

Algerian goat milk was used to isolate the studied *Lactobacillus plantarum* strain. Thirty-five milk samples were homogenized in sterile PBS pH 7.4 (in 1M: 0.136 M NaCl, 2.68 mM KCl, 1.76 mM KH2PO4 and 10.14 mM Na2HPO4×12 H2O). One mL of each sample was submitted to a 10-fold serial dilution (0.9% NaCl) and spread on the surface of de Man, Rogosa and Sharpe (MRS) agar plates. After incubation at 37 °C for 24 h, colonies were randomly selected and screened for their anti-inflammatory activity.

Amplification and sequencing of 16S rDNA

Total DNA was extracted using the commercial kit Bacterial DNA (Omega bio-tek, USA), according to the manufacturer recommendations and was used as a template for 16S rDNA gene amplification. More details on the 16S rDNA procedure were reported by Dib et al. (2014).

Animals and housing conditions

The experiments described in this study comply with the current Algerian legislation covering the protection of animals. Thirty female Balb/c mice weighed between 25 and 30 g were purchased from Pasteur Institute of Algiers (Algeria). The animals were housed in polypropylene cages in controlled temperature (24 °C) and humidity (40–70%) conditions, and in a 12:12 h light–dark cycle. Mice were offered *ad libitum* tap water and a standard diet. All animal experiments were started after 1 week of acclimation.

Colitis induced by DSS

The first group that received 0.3 mL of a saline solution (0.9% NaCl) for 10 days was used as a negative control (n = 10). This group did not receive any treatment. The second and third group (n = 10 each), corresponding to positive control and the *Lactobacillus plantarum* feeding group received, respectively, orally either 0.3 mL of saline solution or 0.3 mL of a pure culture of *Lactobacillus plantarum* containing 10^9 CFU/mL (group 3) for 5 days (first period). At the end of the first period, the second group and third group received 3% dextran sodium sulfate (DSS) in the drinking water during 5 days. On the 10th day, the jejunal and colon fragments are removed for histological study and the contents of the colon and jejunal are freshly collected under sterile conditions to verify their microbial quality by a microbiological study.

Anthropometric determination

In order to study the effects of *Lactobacillus plantarum* on anthropometric parameters, the following parameters were determined for all mice at the beginning (day 0) and at the end of the experiment (day 10): body weight and untake food.

Colons were dissected out and weighed and the mean weight was calculated. Colonic biopsies were taken for macroscopic scoring and histopathological examination.

Histology

Following sacrifice, the colon was excised up to cecum, the length was determined and then emptied with PBS. For light microscopy examination (Olympus, France), the formalin fixed tissues were dehydrated through ascending grades of alcohol, cleared in three changes of xylene, and were embedded in paraffin. As described in a previous report (Bouderbala et al. 2019), serial sections, each of 4-micron thickness, were cut and stained with hematoxylin and eosin.

Microbiological analysis

The potential protective impact of *Lactobacillus plantarum* was determined by classical microbiology methods as described previously (Bouderbala et al. 2019). Brievely, the jejunum and colon were then opened in aseptic condition. The homogenate was serially diluted and the appropriate dilutions were surface plated on specific media: MRS agar (pH= 5.4 (De Man et al., 1960) and Drigalski agar. Selected isolates were evaluated by catalase test, Gram staining, and cell morphology.

Statistical analysis

All data are expressed as mean ± standard error (SE). Statistical analysis was performed using Student's *t* tests or analysis of variance (ANOVA). p values < 0.05 were considered statistically significant, and n represents the number of independent experiments performed.

RESULTS

Bacterium identification

The 16S rDNA amplification and sequencing indicated that the selected isolate presented 99% identity with the 16S rDNA sequences reported for *Lactobacillus plantarum* strains in GenBank database, our isolate was named *Lactobacillus plantarum* DF68.

Effect of *Lactobacillus plantarum* on disease activity index (DAI) and colon length

As shown in Figure 1a and b, body weight gain and untake food were high significantly decreased in DSS group ($p < 0.001$) compared to control. This difference was significant in *Lactobacillus plantarum* DF68 groups ($p < 0.05$) compared to the control.

In contrast, mice received DSS showed an increase in diarrhea and bleeding compared with the control group and *Lactobacillus plantarum* DF68 groups ($p < 0.001$).

Also, a significant decrease of colon length was observed in DSS group ($p < 0.001$) compared with the control group and *Lactobacillus plantarum* DF68 groups. The *Lactobacillus plantarum* groups decreased the severity of colitis, as represented in Figure 2.

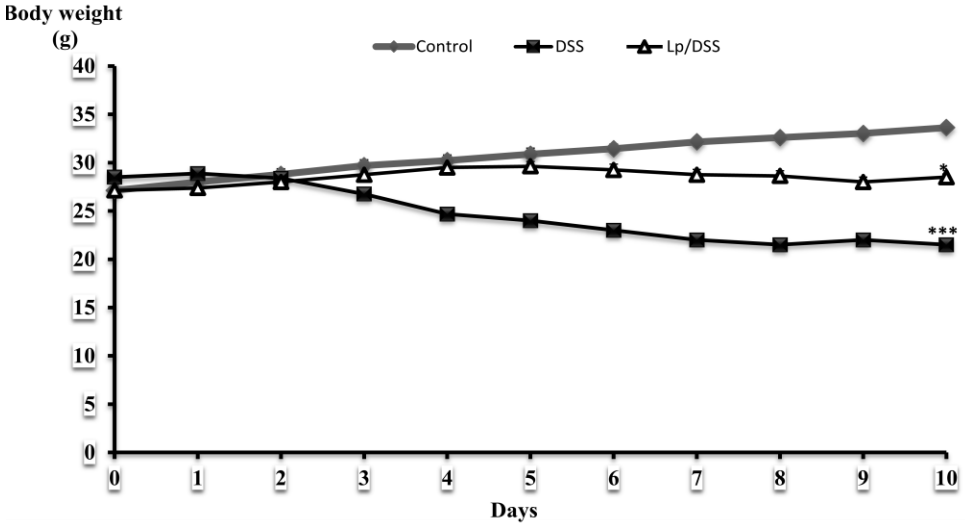

Figure 1: Effect of *Lactobacillus plantarum* on disease activity index (DAI) in control and dextran sodium sulfate (DSS)-induced mice.

Data are mean ± SE, $n = 10$. *** $p < 0.001$ versus DSS. *** $p < 0.001$ versus Control.

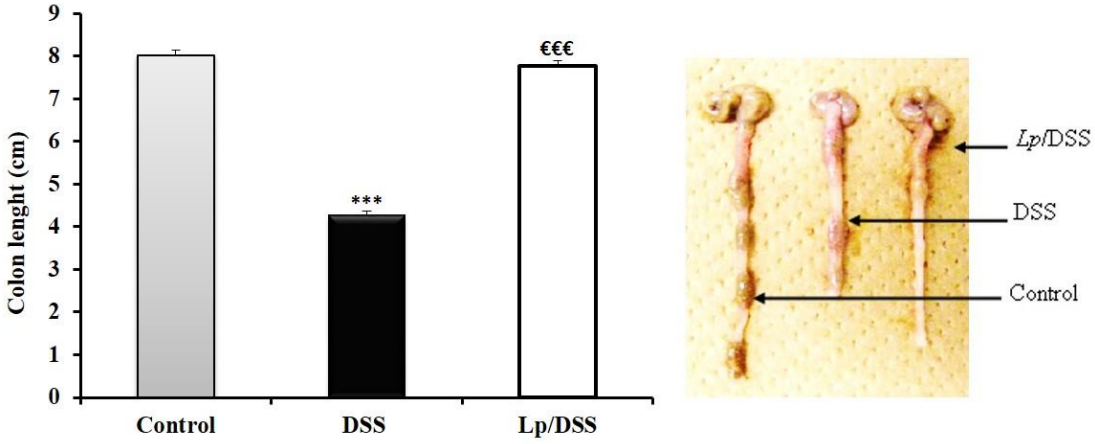

Figure 2: Effect of *Lactobacillus plantarum* on colon length in control and dextran sodium sulfate (DSS)-induced mice.
Data are mean ± SE, *n* = 10. *** *p* < 0.001versus DSS. *** *p* < 0.001 versus Control.

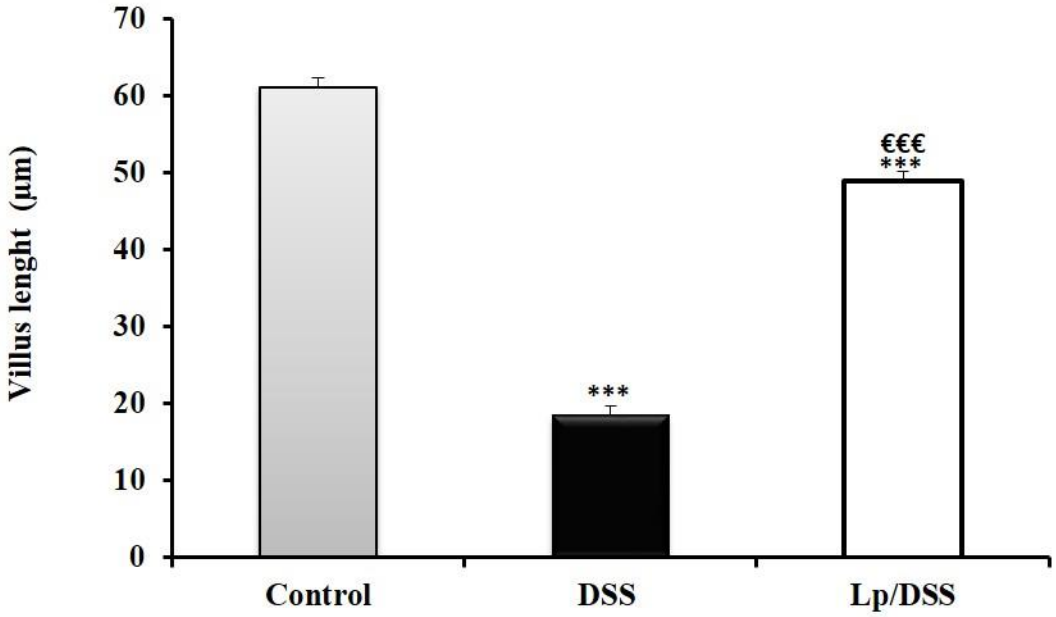

Figure 3: Bacterium ingestion and DSS effect on *villus* length in jejunum fragments in experimental groups compared to negative control.
Values represent mean± SE (standard error) (****p*< 0.001 compared with C-group; *n*=10 mice per group).

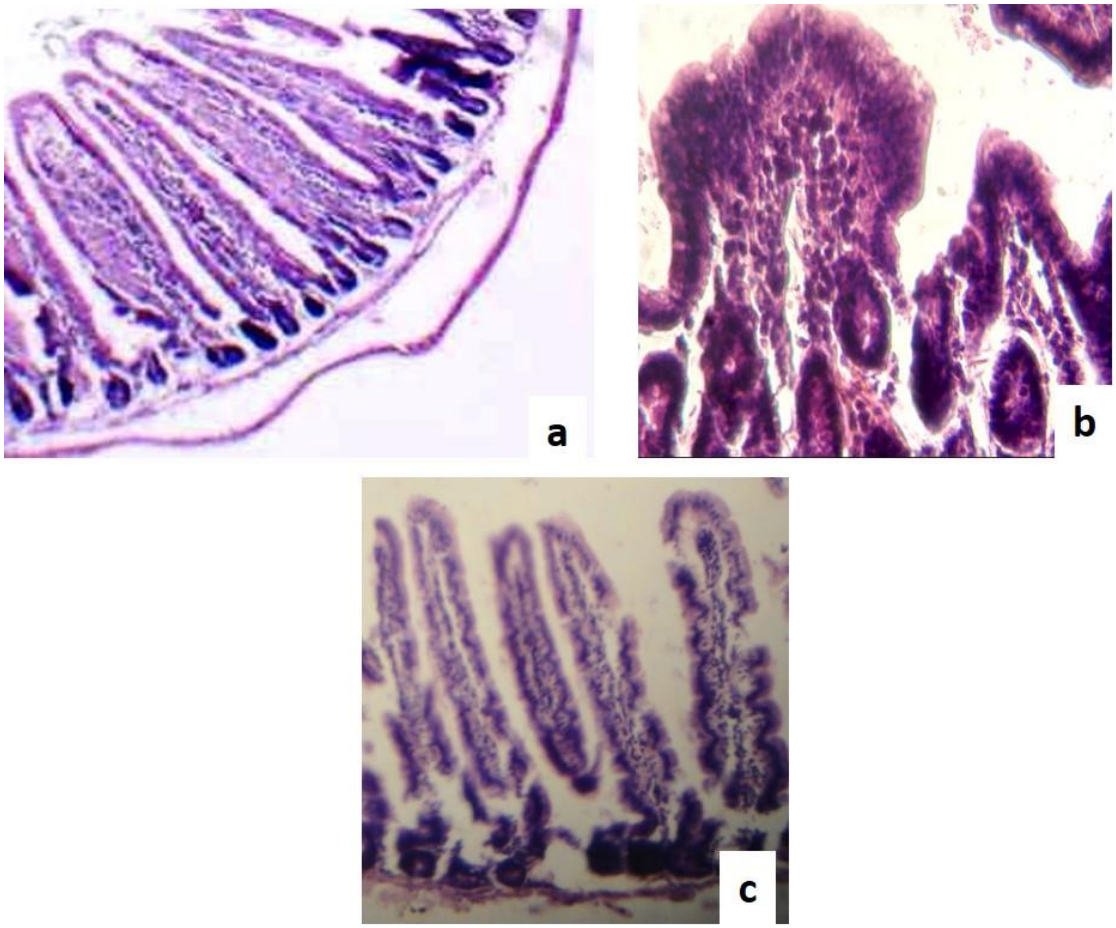

Figure 4: Observation under optical microscope of histological samples of mice jejunum colored to hematoxilin-eosin : control group (a), DSS group (b), Lp/DSS group (c).

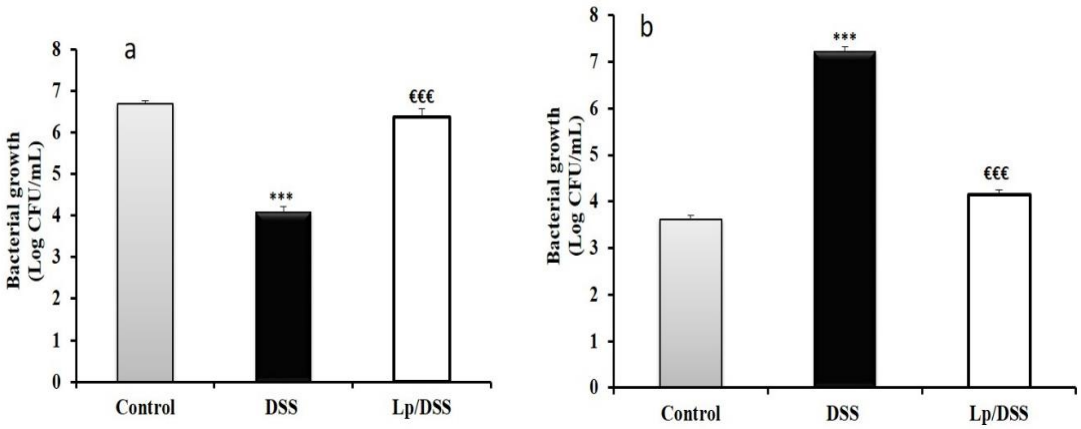

Figure 5: Growth of (a) *Lactobacillus* on MRS agar (pH 5.4), (b) *Enterobacteria* on Drigalski agar in jejunum of different experimental groups.

Each value represents the mean ± SE (Standard error), n = 10 (***p< 0.001 compared with Control group; €€€ p< 0.001 compared with DSS group; n=10 mice per group).

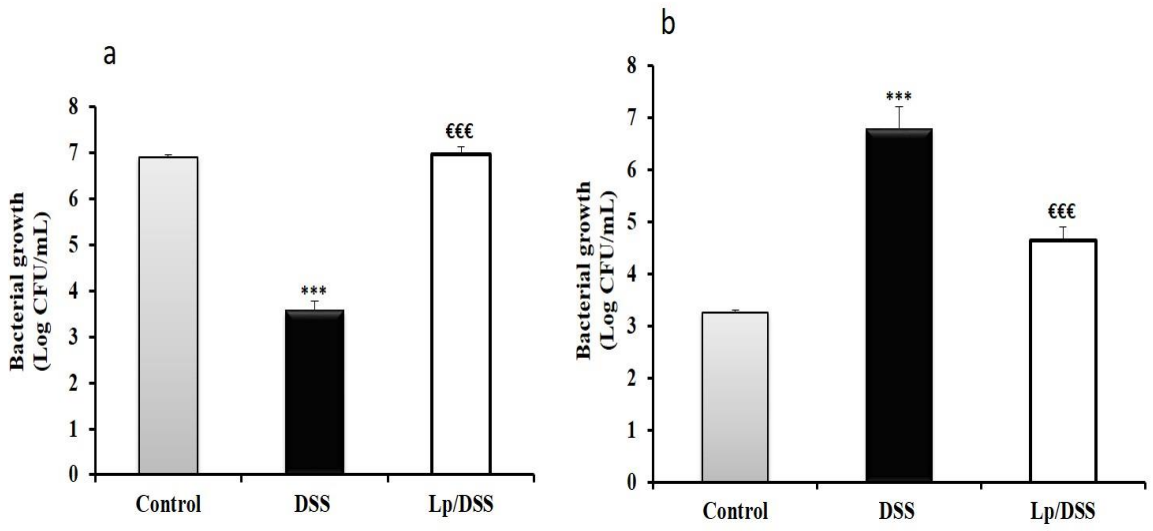

Figure 6: Growth of (a) *Lactobacillus* on MRS agar (pH 5.4), (b) *Enterobacteria* on Drigalski agar in colon of different experimental groups.

Each value represents the mean ± SE (Standard error), n = 10 (***p< 0.001 compared with Control group; €€€ p< 0.001 compared with DSS group; n=10 mice per group)

Histological Study

Histological study jejunum show long *villi* with an unstratified epithelium composed of tall cells with a flat striatum containing regular nuclei at the base and corresponding to the enterocytes (Figure 4a), normal villus, whose average height is 61.18 ±1.2 µm (control group)(Figure 3) .

In contrast, the *villi* of DSS group have hyperplasia of goblet cells, an important inflammatory infiltrate (Figure 4b) and atrophy of villus (18.54± 1.11 µm).

However, the appearance of the intestinal mucosa of *Lactobacillus plantarum* DF68 group (Figure 4c) showed good protective effect on the intestinal *villi* and reduced *villi* damage.

Growth of bacteria

The ingestion of *Lactobacillus plantarum* DF68 minimized pathogenic bacteria and increased benefcial bacteria (Lactobacilli) (Figure 5) in the gut microbiota.

However, the enumeration of lactic acid bacteria show a highly significant increase (p <0.001) in both *Lactobacillus plantarum* DF68 group jejunum and colon's (Figure 5a, 6a). In contrast, DSS group induce very significant increase of Enterobacteria in jejunum and colon compared to control group and *Lactobacillus plantarum* DF68 group (Figure 5b, 6b).

L. plantarum DF68 improved the intestinal tract stability as the impact of intestinal microorganisms was less extensive in the *L. plantarum* DF68 group than the DSS group.

DISCUSSION

Microbiota is considered as an essential organ of humans and other animals, which carry out many functions that host cells cannot.

The study of Kim et al. 2019 showed that DSS administration to 8-week-old mice induced severe colitis as assessed by a disease activity index that takes into account body weight loss, stool consistency, and gross bleeding after sacrifice. In contrast, they found that *L. acidophilus* (LA1) prominently improved the survival rates of 6-week old mice with DSS-induced colitis. LA1 administration significantly restored body weight and colon length and reduced disease activity in DSS-treated mice.

Indeed, it was shown that only formulations with a high bacterial load may exert a positive effect like reduction in the duration of diarrhea in children, while those with a low bacterial content have no effect or are even contrary to expectation (Van Niel et al. 2002).

Areas of inflammation may be interspersed with relatively normal mucosa. In Crohn's disease, the predominant symptoms are diarrhoea, abdominal pain and weight loss whereas in ulcerative colitis diarrhoea is the main symptom, often accompanied by rectal bleeding (Jonkers and Stockbrügger, 2003)

The practical application of probiotic strategy has been especially encouraged by the positive results of a trial in its use for the prevention and treatment of pouchitis (Gionchetti et al. 2003; Mimura et al. 2004).

In animal models, several studies used *L. plantarum* to induce spontaneous colitis in mice. The studies showed the beneficial effect of probiotics on gut bacteria by decrease inflammatory scoring and histological injury, increase the numbers of beneficial total bifidobacteria and lactobacilli, decrease the numbers of potential pathogenic enterococci and *Clostridium perfringens* (Xia et al. 2011). Thus, the optimal dose and time of *L. plantarum* exposure is yet to be fully understood. In particular, the protection from visceral pain perception by *L. plantarum* was more evident in normal healthy mice induced with colorectal distension (Duncker et al. 2008), supporting the hypothesis that *L. plantarum* can be protective against inflammation, although the mechanisms remain unknown.

Ismaeil et al. (2020) showed that the colon of *L. plantarum* HK L.137-treated mice was normal mucosa consisted of perpendicular crypts. In contrast, the DSS-induced group revealed severe colitis associated with extensive necrosis of the crypts extended along the whole mucosa to the lamina propria and the muscle layer. The other inflammatory lesions, such as hemorrhage, edema, and neutrophilic infiltration were obviously seen in this group.

It has been reported that the gut microbiota is made more resilient by microbial diversity, contributing significantly to health and wellbeing (Kim and Isaacson, 2015). The condition of the human gut microbiota has been demonstrated to be reflected in the ratio of Firmicutes to Bacteroidetes (Ley et al. 2006). This ratio was decreased in some CD and UC patients, alongside a relative proliferation of proteobacteria (Frank et al. 2007).

CONCLUSION

In summary, we have demonstrated that administration of *Lactobacillus plantarum* to DSS treated mice inhibited inflammation by facilitate the recovery of damage tissue in DSS induced mice inflammation. Additionally, we showed that *L. plantarum* DF68 can induce the intestinal tract stability as the impact of intestinal microorganisms. Therefore, *L. plantarum* DF68 would represent a promising candidate for the prevention of inflammatory disease, pending further investigations to explore the exact mechanisms of action.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

YK, WD and YB designed and performed the experiments, data analysis and also wrote the manuscript. YK, WD, IE and YB performed animal treatments and tissue collection, OK and DS provided scientific advice, DS and OK designed experiments and revised the manuscript. All authors read and approved the final version.

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