

# Preventive Effect of *Lactobacillus plantarum* Isolated from Altay Traditional Fermented Yogurt on DSS-induced Colitis in Mice

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**Abstract:** In this study, Preventive effect of *Lactobacillus plantarum* isolated from Altay traditional fermented yogurt on DSS-induced colitis in mice has been measured. The BALB/c mice were randomly divided into five groups: NC group (normal controls), DSS group (14-day orally administration of saline + 7-day 4% DSS exposure from day 8), low-dose LP-A group (LP-A-L,  $1 \times 10^8$  CFU/kg, 7-day orally administration of LP-A-L + 7-day 4% DSS exposure from day 8), high-dose LP-A group (LP-A-H,  $1 \times 10^9$  CFU/kg, 7-day orally administration of LP-A-H + 7-day 4% DSS exposure from day 8), positive control group (sulfasalazine, 500mg/kg, 14-day orally administration of sulfasalazine + 7-day 4% DSS exposure from day 8). According to the results, the LP-A group, especially the LP-A-H group has significantly increased the length of mice colon, reduced the mice serum levels of pro-inflammatory cytokines, and increased serum levels of anti-inflammatory cytokines. In the histopathological observation of mice colon tissues, congestion and cell infiltration were alleviated, and the goblet cells were increased by the LP-A treatment. Based on the results presented above, this study will lay a certain theoretical foundation for the development of probiotic strain with the effect of preventing colitis.

## 1 INTRODUCTION

In many areas of China, fermented dairy products are an indispensable part of the daily diet. In addition, many ethnic minority areas have greatly reduced the incidence of digestive system diseases due to their special dietary habits (Basso, Camara, Sales-Campos 2019).

The Kazakh people in the Altay region of Xinjiang are very good at using a variety of milk sources to ferment dairy products, such as fermented cow milk, fermented camel milk, fermented horse milk.

Traditional fermented dairy products mostly use natural inoculation, and the fermentation process is a relatively open system. The microorganisms are very diverse and the dynamic changes of microorganisms are also very complicated. In the long-term fermentation and domestication process, a relatively stable flora and ecological environment have gradually formed, at the same time, abundant and excellent microbial resources have been screened and retained. The benefits of lactic acid bacteria in fermented foods to human health have been

extensively studied (He, Zeng, Lv, Mao, Wang 2015).

Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract. In the past ten years, with the increase in the incidence, IBD has become a public health problem of world. IBD is mainly composed of ulcerative colitis (UC), Crohn's disease (CD) and undetermined IBD (Almana, Mohammed, 2019). In recent years, the incidence and prevalence of UC in China have increased year by year. 9% to 11% of UC patients eventually die of colon cancer and rectal cancer. Therefore, UC is listed as a modern refractory disease by the World Health Organization (Feuerstein, Cheifetz 2014).

The pathogenesis of colitis is complicated, and the specific mechanism has not been studied clearly. However, existing studies have shown that the occurrence of colitis are related to immune abnormalities, family inheritance, eating habits and other factors. At the same time, the balance of pro-inflammatory cytokines and anti-inflammatory cytokines, oxidative stress, and impaired intestinal mucosal barrier function are closely related to the pathogenesis of colitis (Patil, Moss, Odze 2016).

At present, the most commonly used treatment methods are aminosalicylate anti-inflammatory drugs (sulfasalazine, mesalazine and derivatives, etc.), corticosteroids (prednisone, prednisone, budesonide, etc.), immunosuppressive drugs (thiopurine, methotrexate), and TNF antagonists (infliximab adalimumab) (Nyambe, Koekemoer, Van de Venter 2019). The newest developments include integrin antagonists that inhibit T cell adhesion and antagonists of pro-inflammatory factors. At the same time, probiotics have been used to improve the clinical symptoms of UC patients (Shen, Zhu, Quan 2018). The use of probiotics to intervene in colitis is a research hotspot in the field of digestion. Therefore, it is very important to develop probiotics with the activity of preventing UC (Grover, Rashmi, Srivastava, Batish. 2012).

In this study, we will research the preventive effect of *Lactobacillus plantarum* isolated from Altay traditional fermented yogurt on DSS-induced colitis in mice. *Lactobacillus plantarum* is very representative of probiotics. In addition, food-derived probiotics are safer and more available. Our study will lay a certain theoretical foundation for the development of probiotic strain with the effect of preventing UC.

## 2 MATERIALS AND METHODS

### 2.1 Experimental Strain

The *Lactobacillus plantarum* strain was isolated from traditional fermented yogurt in the Altay region of Xinjiang, China. We named it LP-A.

### 2.2 Animal Experiments

Fifty male BALB/c mice (6 weeks old) were obtained from Chongqing Medical University, Chongqing, China (SCXK (YU) 2017-0001). After 7 days of adaptive feeding, the mice were randomly divided into five groups: NC group (normal controls), DSS group (14-day orally administration of saline + 7-day 4% DSS exposure from day 8), low-dose LP-A group (LP-A-L,  $1 \times 10^8$  CFU/kg, 7-day orally administration of LP-A-L + 7-day 4% DSS exposure from day 8), high-dose LP-A group (LP-A-H,  $1 \times 10^9$  CFU/kg, 7-day orally administration of LP-A-H + 7-day 4% DSS exposure from day 8), positive control group (sulfasalazine, 500mg/kg, 14-day orally administration of sulfasalazine + 7-day 4% DSS exposure from day 8). On the last day of the experiment, the mice were fasted for 12 h. All mice

were sacrificed and the whole blood was taken from the inferior vena cava, centrifuged at 4000 rpm for 10 min, and collected the serum. The mice colon tissues were obtained.

### 2.3 Determination of IL-1B, IL-6, IL-4, IL-10, TNF-A Level in Mice Serum

The serum levels of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-4, IL-10, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were quantified by commercial kits which were purchased from BioLegend Inc., USA.

### 2.4 Histopathological Observation

Colon tissues of mice were put into a pre-prepared fixative (10% formalin) to denature and coagulate the protein of tissues and cells. The water of tissue were gradually removed by alcohol from low-concentration to high-concentration. Then put the tissue block in transparent agent xylene to be transparent, and replace the medium alcohol in the tissue block with xylene. The transparent tissue block were put in the melted paraffin. After the paraffin was completely immersed in the tissue block and embedded, then cut into thin slices. After hematoxylin-eosin (H&E) staining, gum were added to the slices and covered with a cover glass for mounting. The sections were observed under the microscope to analyze the morphological changes of mice colon tissues.

### 2.5 Data Statistical Analysis

The SPSS 19.0 statistical software (SPSS Institute Inc., Chicago, IL, USA) was used to analyze the data of each group using the one-way ANOVA method to detect the significant differences between the groups ( $p < 0.05$ ).

## 3 TEST RESULTS AND DISCUSSIONS

### 3.1 Colon Length of Mice

When UC occurs, colon wall thickening, edema, colon congestion and hypertrophy, and ulcers all shorten the length of the colon. Therefore, the reduced length of the colon can indirectly reflect intestinal inflammation. As shown in Table 1, the length of the colon in the DSS group was significantly shortened. In the LP-A groups, the length of the colon

significantly increased compared with the DSS group ( $p < 0.05$ ).

Table 1: Colon length of mice.

Groups	Colon length (cm)
NC	8.75 ± 1.07 <sup>a</sup>
DSS	6.97 ± 0.22 <sup>e</sup>
LP-A-L	7.18 ± 0.62 <sup>d</sup>
LP-A-H	7.55 ± 0.49 <sup>c</sup>
Sulfasalazine	7.80 ± 0.67 <sup>b</sup>

Significant differences were found between groups with different letter representations ( $p < 0.05$ ).

### 3.2 Serum Levels of Pro-inflammatory Cytokines

The imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines is regarded as an important pathogenesis of UC (Macdonald, Mlonteone, Pender 2000). IL-1 $\beta$  can increase the cytokines produced by macrophages, such as IL-6, TNF- $\alpha$  and IL-8, so that neutrophils can accumulate to the inflammation site and enter the intestinal lesions, thereby causing a series of intestinal lesions, such as colonic epithelium injury, crypt abscess, which eventually cause the onset of UC (Lee, Lee, Choi, Lee, Kim, Ye 2012, Rafa, Benkhelifa, AitYounes, Saoula, Belhadeif, Belkhelifa, Boukercha, Toumi, Soufli, Moralès, de Launoit, Mahfouf, Nakmouche, TouilBoukoffa 2017). In Table 2, the high levels of pro-inflammatory cytokines were shown in the DSS group. After orally administration of LP-A, all the cytokines were significantly lower than the DSS group ( $p < 0.05$ ), especially the LP-A-H group were similar with the sulfasalazine group.

Table 2: Serum levels of pro-inflammatory cytokines.

Groups	IL-1 $\beta$ (ng/L)	IL-6 (pg/mL)	TNF- $\alpha$ (ng/L)
NC	31.47 ± 4.07 <sup>e</sup>	21.32 ± 3.21 <sup>e</sup>	11.22 ± 2.04 <sup>e</sup>
DSS	75.24 ± 5.12 <sup>a</sup>	60.48 ± 6.02 <sup>a</sup>	44.01 ± 3.13 <sup>a</sup>
LP-A-L	62.57 ± 5.25 <sup>b</sup>	39.88 ± 5.11 <sup>b</sup>	30.19 ± 3.08 <sup>b</sup>
LP-A-H	56.64 ± 4.19 <sup>c</sup>	30.42 ± 2.79 <sup>c</sup>	27.35 ± 2.11 <sup>c</sup>
Sulfasalazine	49.86 ± 3.23 <sup>d</sup>	30.02 ± 2.84 <sup>d</sup>	22.27 ± 2.32 <sup>d</sup>

Significant differences were found between groups with different letter representations ( $p < 0.05$ ).

### 3.3 Serum Levels of Anti-inflammatory Cytokines

IL-4 can inhibit the production of IL-1, TNF- $\alpha$  by human monocytes and the formation of superoxide anions derived from monocytes. IL-10 is mainly produced by mononuclear macrophages, which can inhibit NK cell, Th1 cell response and macrophage

cytokine synthesis (Lopetuso, Chowdhry, Pizarro 2013). Studies have shown that IL-10 synthesis is reduced in UC patients, and IL-10 knockout mice can develop spontaneous colitis (Keubler, Buettner, Häger, Bleich 2015). As shown in Table 3, the LP-A groups, especially the LP-A-H group has significantly increased serum levels of anti-inflammatory cytokines compared to the DSS group ( $p < 0.05$ ).

Table 3: Serum levels of anti-inflammatory cytokines.

Groups	IL-4 (pg/mL)	IL-10 (ng/L)
NC	63.38 ± 8.43 <sup>a</sup>	181.10 ± 33.62 <sup>a</sup>
DSS	34.42 ± 4.19 <sup>e</sup>	94.55 ± 25.15 <sup>e</sup>
LP-A-L	43.91 ± 4.08 <sup>d</sup>	112.61 ± 30.28 <sup>d</sup>
LP-A-H	54.16 ± 3.98 <sup>c</sup>	149.49 ± 29.13 <sup>c</sup>
Sulfasalazine	56.22 ± 4.15 <sup>b</sup>	166.67 ± 32.27 <sup>b</sup>

Significant differences were found between groups with different letter representations ( $p < 0.05$ ).

### 3.4 Histopathological Observation of Mice Colon Tissues

According to Figure.1, the histopathological section of the mice colon tissues showed that the epithelial cells of the colon tissue in the normal group were intact, the mucosal layer was tightly connected, and there was no obvious inflammatory cell infiltration. At the same time, the goblet cells and crypt structures

were clearly visible. While in the DSS group, a large number of inflammatory cells infiltrate were observed, the glands were arranged disorderly, and goblet cells were greatly reduced. After treatment with LP-A, congestion, edema, and cell infiltration were alleviated, and the goblet cells were increased compared with the DSS group. In the LP-A groups, LP-A-H had the more obvious effect on improving colon tissue.

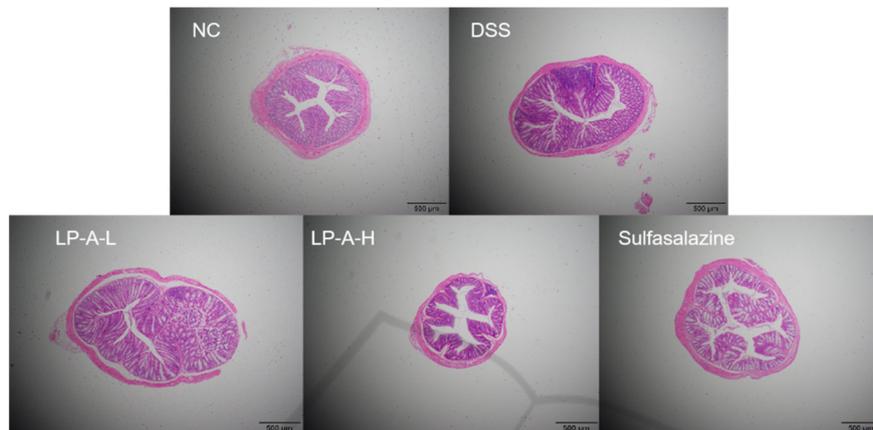


Figure 1: Histopathological observation of mice colon tissues (H&E staining, 500×).

## 4 CONCLUSION

This study confirmed the preventive effect of *Lactobacillus plantarum* isolated from Altay traditional fermented yogurt on DSS-induced colitis in mice. *Lactobacillus plantarum* is very representative of probiotics. In addition, food-derived probiotics are safer and more available. According to the results, the LP-A group, especially the LP-A-H group has significantly increased the length of mice colon, reduced the mice serum levels of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ), and increased serum levels of anti-inflammatory cytokines (IL-4, IL-10). In the histopathological observation of mice colon tissues, DSS induced congestion and cell infiltration were alleviated, and the goblet cells were increased by the LP-A treatment. Based on the results presented above, this study will lay a certain theoretical foundation for the development of probiotic strain with the effect of preventing colitis.

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