Oral administration of *Lactobacillus plantarum* attenuates inflammatory damage in mice challenged with two pathogens

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Abstract

This study aimed to determine the immunomodulatory effect of *Lactobacillus plantarum* on *Salmonella typhimurium* and *Staphylococcus aureus* infection. A mouse inflammation model was established using *S. aureus* and *S. typhimurium*. The infected mice were treated with low, medium, and high doses \(2 \times 10^8, 4 \times 10^8,\) and \(8 \times 10^8\) colony forming units (CFU)/mL, respectively of three antibacterial *L. plantarum* strains. The body weight changes, spleen and thymus indexes, cytokines (interleukin (IL)-4 and interferon (IFN)-γ), and secreted immunoglobulin A levels were measured. Compared with the model group, all the *L. plantarum*-treated groups show increased body weight, reduced spleen swelling, decreased IFN-γ content, significantly increased IL-4 content, and significantly decreased ratio of IFN-γ to IL-4. sIgA levels increased at the end of the experiment. The three *L. plantarum* strains can effectively attenuate the symptoms of *S. typhimurium* and *S. aureus* infection by regulating the Th1/Th2 response and enhancing sIgA secretion.

Keywords

immunomodulation, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Salmonella typhimurium*, Th1/Th2 response

Introduction

Food-borne pathogens remain a major factor endangering public health. With the globalization of food trade and diversification in food processing technology, *Staphylococcus aureus* and *Salmonella typhimurium* have become the most common food-borne pathogens. Although *S. aureus* is initially sensitive to multiple antibiotics, the abuse of antibiotics causes difficulty in the management of *S. aureus* infection.1 *S. typhimurium* adheres to intestinal epithelial cells, attacks the body’s immune system, disturbs the ratio of Th1/Th2 (T helper cell type 1/T helper cell type 2) cells, and induces the release of various inflammatory cytokines, such as interferon (IFN)-γ.2 Therefore, improving the body’s immune defenses against infection with *S. aureus* and *S. typhimurium* is very important.

The treatment based on microbial carriers is becoming a potential therapeutic strategy.3 Lactic acid bacteria (LAB) are a normal part of the human intestinal flora, and numerous studies have shown that these probiotics play important roles in immune regulation.4 For example, LAB can regulate the body’s immune system, promote local immunological prevention, induce anti-inflammatory cytokines and secreted immunoglobulin A (sIgA), and inhibit

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bacterial invasion. Previously, we isolated *Lactobacillus plantarum* strains H9, L2, and L12 from homemade fermented foods; in vitro experiments showed that these strains exhibit antimicrobial activity and resistance to gastric acid and bile and other probiotic properties. To further study the important role of LAB in immune regulation in vivo, we established an inflammation model of mice infected with *S. aureus* and *S. typhimurium* and assessed the ability of *L. plantarum* to ameliorate pathogenic bacteria infection and to provide a theoretical basis for the development of new probiotics.

**Materials and methods**

**Materials**

A total of 220 female Kunming mice (20 weeks old, weighing 23 ± 2 g) were purchased from Changchun Institute of Biological Products Co., Ltd (SCXK (JI) 2011-0003).

**Experimental strains.** Three *L. plantarum* strains were isolated from homemade fermented foods in Northeastern China. Strains of *S. typhimurium* (CICC 21484) and *S. aureus* (CICC 21600) were obtained from the toxicology laboratory of the College of Food Science and Engineering, Jilin Agricultural University, China.

**Strain activation**

*Activation of LAB.* *L. plantarum* strains H9, L2, and L12 were inoculated into De Man, Rogosa, and Sharpe liquid medium, cultured at 37°C for 24 h, and stored temporarily at 4°C for later use. Before gavage administration, the *L. plantarum* strain was resuspended in 0.9% sterile saline solution to a final concentration of 1 × 10⁹ colony forming units (CFU)/mL.

*S. typhimurium* and *S. aureus* activation. *S. typhimurium* and *S. aureus* were inoculated separately into Luria–Bertani (LB) liquid medium (1%, V/V) and cultured at 37°C for 24 h and temporarily stored at 4°C for later use. The cells were adjusted to a concentration of 1 × 10⁹ CFU/mL before gavage administration.

**Methods**

**Mouse grouping and processing.** After 6 days of adaptive feeding, the mice were randomly divided into 22 groups according to body weight, with 10 mice in each group. The experimental groups were as follows: *S. typhimurium* group (control group, model group, and LAB intervention group); *S. aureus* group (control group, model group, and LAB intervention group). Following the method of Ren et al. and Wang et al., the pathogen-infected mouse model was established after a modeling period of 7 days and a test period of 30 days. Negative control group was treated with 0.3 mL of 0.9% sterile saline solution gavage for 37 days.

**Model.** The mice in the model group and experimental group were inoculated daily with *S. typhimurium* or *S. aureus* using 0.3 mL of 1 × 10⁹ CFU/mL via oral gavage for 7 days. After 7 days, all the mice in the experimental group were treated with LAB in three doses, high (0.8 mL, 8 × 10⁸ CFU), medium (0.4 mL, 4 × 10⁸ CFU), and low (0.2 mL, 2 × 10⁸ CFU), via oral gavage. The mice in the model group were given 0.3 mL of 0.9% sterile saline solution daily. Each group was gavage-administered once a day. The same groups of mice were housed in the same cage with natural light and ventilated environment. The temperature was (20 ± 1)°C, and the relative humidity was 50% ± 10%. The mice had free access to food and water.

**Measurement of mouse body weight and organ indices**

From the first day of the experiment, the mice body weights were recorded every 5 days to monitor their health status. Half of the mice in each group were sacrificed by cervical dislocation on the 15th day, and the other half were sacrificed at the end of the test. Their thymus and spleen were collected to calculate the organ index according to the following formula:

\[
\text{Organ Index (mg/g)} = \frac{\text{organ mass (mg)}}{\text{body weight (g)}}
\]

**Detection of IFN-γ, interleukin-4, and sIgA contents**

Blood samples were obtained from the eyeball blood in 15 and 30 days of the test. The mice were sacrificed by abdominal injection of anesthesia with 200 mg/kg of amyllobarbitone. The serum was
prepared by centrifugation at 4°C and 3000 g for 10 min. A 1 cm sample of the colon was obtained, washed with sterile saline solution to collect the intestinal contents, and stored at −80°C until use. Cytokines (IFN-γ and interleukin (IL)-4) in serum and sIgA in the intestinal fluid were quantified using enzyme-linked immunosorbent assay (ELISA) kits.

Data analysis
All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 6.0 software. All data were analyzed and compared between groups using one-way analysis of variance. \( P < 0.05 \) was considered statistically significant.

Results
Pathogen infection model
The mice in the experimental and model groups were inoculated with \( S. \) typhimurium or \( S. \) aureus at a concentration of \( 1 \times 10^9 \) CFU/mL. After 1 week, the mice showed a marked reduction in activity and food consumption, more diluted stools, and higher amounts of defecation. The intestinal anatomy appeared rough, with darkening and congestion; the other findings included a black-red liver, enlarged or atrophied immune organs, bleeding, and other symptoms; these results were consistent with the studies of Ren et al.\(^6\) and Wang et al\(^7\). Thus, the pathogen infection model was established successfully.

Effect of Lactobacillus strains on mouse body weight
As shown in Figure 1(a) and (b), the model group exhibited decreased body weight, partial hair loss, and appeared lethargy. The body weight in all experimental groups showed an upward trend on the 15th day. The mice also exhibited increased food intake and activity and excreted normal-colored stools. All three doses of Lactobacillus increased the body weight, with the high-dose H9 strain group showing the most significant increase in body weight (31.89 g) among the mice infected with \( S. \) typhimurium (Figure 1(a)). This increase was close to that of the control group (34.08 g). In the \( S. \) aureus-infected mice (Figure 1(b)), treatment with H9 strain also showed the best weight recovery (31.67 g), the value obtained was close to that of the control group (32.59 g).

Effect of Lactobacillus strains on the organ index in mice
The thymus and spleen are the most important immune organs in mice. The organ index can reflect the growth of spleen and thymus. The thymus and spleen indexes can be used to assess the immune ability of mice. As shown in Table 1, the thymus index of the \( S. \) aureus group and \( S. \) typhimurium group decreased after infection for 30 days (model group: 2.03 and 2.46 vs control group: 3.09). The spleen index increased (model group 9.97 and 10.61 versus control group 4.55), yielding a significantly higher value in the model group in comparison with that of the control group (\( P < 0.01 \)). After 15 days of Lactobacillus treatment, the spleen index decreased (except for that of mice infected with \( S. \) typhimurium and treated with the low dose of L2 and medium dose of L12; in this case, spleen index increased slightly but not significantly). After 30 days, the spleen index of all mice decreased. The high-, medium-, and low-dose H9 strain groups caused the most marked effect on \( S. \) aureus- and \( S. \) typhimurium-infected mice immune organs, achieving levels close to those of the control group.

Effect of Lactobacillus strains on serum IL-4 and IFN-γ in mice
Figure 2(a) and (b) shows the IL-4 content in the mice sera. Compared with the model group, the IL-4 content in the experimental group increased on the 15th and 30th day. The high-, medium-, and low-dose H9 strain groups showed significant increases in the IL-4 content in mice infected with both pathogenic bacteria (10.3–12.9 pg/mL, \( P < 0.01 \)) but not in a dose-dependent manner. As shown in Figure 2(c) and (d), the IFN-γ content in the mice sera showed no significant difference from that in the model group on the 15th day. With increasing time, the IFN-γ levels decreased until at day 30. The IFN-γ level in \( S. \) typhimurium infected mice was significantly lower in groups L12, H12, and H9 (132–120 pg/mL, \( P < 0.01 \)), but it was close to the level in the blank control group (107 pg/mL). In the H9 strain group, the IFN-γ levels in mice infected
Figure 1. Effects of lactobacilli on the body weight of pathogen-infected mice. (a) Effect of LAB on the body weight of mice infected with *Salmonella typhimurium*. (b) Effect of LAB on the body weight of mice infected with *Staphylococcus aureus*. All groups were fed a normal diet throughout the experimental period.

Table 1. Effects of lactobacilli on the organ indices of mice infected by pathogens.

| Pathogenic bacteria | Grouping | 15 days (mg/10 g) | 30 days (mg/10 g) |
|---------------------|----------|------------------|------------------|
|                     |          | Thymus           | Spleen           | Thymus           | Spleen           |
| *Salmonella typhimurium* | Control  | 3.86 ± 0.45      | 4.98 ± 0.08**   | 3.09 ± 0.84      | 4.55 ± 0.64**    |
|                     | Model    | 3.32 ± 0.67      | 10.73 ± 0.08    | 2.46 ± 0.56      | 10.61 ± 2.71     |
|                     | L2 high  | 5.64 ± 0.71***   | 9.48 ± 1.63     | 4.92 ± 0.24**    | 8.71 ± 0.5       |
|                     | L2 medium| 4.53 ± 0.79**    | 9.57 ± 0.52     | 3.91 ± 0.12**    | 8.57 ± 0.53      |
|                     | L2 low   | 3.41 ± 0.71      | 12.33 ± 1.21    | 3.41 ± 0.03**    | 9.27 ± 0.73      |
|                     | L12 high | 6.75 ± 0.62***   | 8.81 ± 0.81*    | 5.55 ± 0.10***   | 8.67 ± 1.92      |
|                     | L12 medium| 4.08 ± 1.50     | 11.87 ± 0.54    | 3.93 ± 0.48**    | 9.08 ± 1.83      |
|                     | L12 low  | 3.55 ± 0.51      | 9.15 ± 1.50*    | 3.83 ± 0.68**    | 9.02 ± 1.99      |
|                     | H9 high  | 4.13 ± 0.88      | 9.74 ± 0.46     | 2.53 ± 1.15      | 7.58 ± 3.0*      |
|                     | H9 medium| 3.82 ± 0.93      | 9.13 ± 0.36*    | 3.66 ± 1.23*     | 8.26 ± 1.74      |
|                     | H9 low   | 2.76 ± 0.96      | 9.51 ± 2.46     | 3.19 ± 1.13      | 8.33 ± 1.9       |
| *Staphylococcus aureus* | Control  | 3.86 ± 0.45      | 4.98 ± 0.08**   | 3.09 ± 0.84      | 4.55 ± 0.64**    |
|                     | Model    | 2.41 ± 0.62      | 10.08 ± 1.70    | 2.03 ± 1.33      | 9.97 ± 1.68      |
|                     | L2 high  | 3.99 ± 0.93***   | 8.02 ± 0.99*    | 3.38 ± 0.26*     | 7.84 ± 0.95***   |
|                     | L2 medium| 3.88 ± 0.14***   | 8.14 ± 1.65*    | 2.07 ± 1.15      | 8.08 ± 1.87*     |
|                     | L2 low   | 2.91 ± 0.45      | 9.42 ± 0.31     | 3.68 ± 1.34*     | 8.82 ± 1.27      |
|                     | L12 high | 3.08 ± 0.11*     | 10.55 ± 1.22    | 3.88 ± 1.59***   | 8.57 ± 0.55      |
Table 1. (Continued)

| Pathogenic bacteria | Grouping  | 15 days (mg/10 g) | 30 days (mg/10 g) |
|---------------------|-----------|-------------------|-------------------|
|                     |           | Thymus            | Spleen            | Thymus            | Spleen            |
| L12 medium          | 2.98 ± 0.33 | 8.79 ± 0.42       | 2.96 ± 0.23       | 7.89 ± 1.02      |
| L12 low             | 2.69 ± 0.48 | 8.05 ± 0.55       | 2.62 ± 0.63       | 7.05 ± 1.35      |
| H9 high             | 3.25 ± 0.34 | 8.30 ± 1.82       | 2.45 ± 0.34       | 6.84 ± 0.42      |
| H9 medium           | 4.08 ± 0.42***| 5.63 ± 0.58***    | 3.44 ± 0.01**     | 5.56 ± 0.58**    |
| H9 low              | 2.91 ± 0.23 | 6.56 ± 2.56**     | 3.88 ± 1.49**     | 5.12 ± 0.43**    |

The data are presented as the organ-to-body weight ratio by mean ± SD. The experimental groups included the S. typhimurium group (control group, model group, and LAB intervention group) and S. aureus group (control group, model group, and LAB intervention group).

*P < 0.05 and **P < 0.01 versus model group.
(Figure 2. Continued)
with *S. aureus* were significantly low (140 pg/mL, $P < 0.01$) and close to that of the control group (110 pg/mL). L2 low-dose group and L12 high-dose group had the best effect on IFN-$\gamma$ in mice infected with *S. typhimurium*. The effect of the two doses of strain on IFN-$\gamma$ was not significant in mice infected with *S. aureus*. The ratio of IFN-$\gamma$ and IL-4 was shown in Figure 2(e) and (f). The IFN-$\gamma$/IL-4 ratio was significantly reduced from the infective phase to the convalescent phase ($P < 0.01$), indicating that all three LAB caused an inhibitory effect on inflammation in the pathogen-infected mice.

**Effect of Lactobacillus strains on sIgA levels**

sIgA is the major immunoglobulin in the intestinal mucosa and is capable of defending against pathogen attack. Compared with the model group, LAB increased the sIgA in the colon of mice after they were infected for 30 days (Figure 2(g)). The high- and middle-dose L2, high-dose L12, and high-dose H9 strain groups exhibited significantly higher sIgA levels than the model group (6.4–9 µg/mL, $P < 0.01$).

**Discussion**

Pathogen infection has become pandemic owing to the overuse of antibiotics and other drugs. Several new treatments are currently being studied to replace antibiotics. LAB can regulate the body’s immune system. The mechanisms by which LAB inhibit pathogen infection are complex. In this study, we demonstrated that lactobacilli can...
modulate the function of Th1/Th2 and antibodies in restoring pathogen-infected mice. This study showed that compared with the control group, the sera of infected mice showed increased IFN-γ and decreased IL-4. By the 30th day, IL-4 had increased in the LAB intervention group, which manifested stimulated Th1 immune response and decreased IFN-γ content. These results may be due to the presence of LAB in the late infection stage, causing an increase in IL-4 (Th2), which is the key to T cell survival and proliferation. Excessive IL-4 level inhibits the production of IFN-γ (Th1). Elevated IFN-γ promotes the proliferation of cytotoxic lymphocytes to eliminate invasive pathogens. As a result, Th2 response increased, continually clearing the surviving pathogens and establishing a memory immune response.

sIgA is an immune barrier that prevents toxins and enteric pathogens from adhering to and penetrating the intestinal epithelium, inhibiting allergens and pathogenic microbes. In this study, the sIgA levels increased with LAB, indicating that sIgA exerted desirable protective effects against *Salmonella typhimurium* and *S. aureus* infection in the intestine.

In conclusion, lactobacilli strains L2, L12, and H9 all have feature potent anti-*S. typhimurium* and *S. aureus* activities. These strains can alleviate the symptoms in pathogen-infected mice by promoting IL-4 secretion and stimulating the secretion of Th1 and sIgA early in the disease development. However, there are other anti-infective mechanisms, such as the production of antibacterial substances, regulation of the intestinal flora, and production of other immune regulatory molecules, remain to be studied.

Declaration of conflicting interests

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