



Oral administration of *Lactobacillus plantarum* JC7 alleviates OVA-induced murine food allergy through immunoregulation and restoring disordered intestinal microbiota

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Abstract

Purpose The incidence and prevalence of food allergy have sharply risen over the past several decades. Oral administration of probiotic strains has been proven as a safe and effective method to control food allergy. In this study, it aims to comprehensively investigate the anti-allergic effect of *Lactobacillus plantarum* JC7.

Methods Balb/c mice were randomly divided into three groups and received OVA (20 µg/mouse, intraperitoneal injection), *L. plantarum* JC7 (2×10^8 CFU/mouse, intragastric administration) + OVA (20 µg/mouse, intraperitoneal injection) or 0.9% saline (intragastric administration) for 3 weeks. Body weight was monitored weekly, and allergic reactions were evaluated after challenge of OVA. Serum levels of OVA-specific immunoglobulins and various cytokines were tested using ELISA, and the cecum microbiota was analysed by 16S rRNA sequencing to explore the relationships between these indicators and OVA-induced food allergy. Western blotting was used to identify the expression levels of phosphorylated IκBα and nuclear factor kappa B p65.

Results OVA-sensitised mice showed mitigation of respiratory manifestations, alleviation of lung inflammation and congestion, and the presence of an intact intestinal villus structure. Furthermore, OVA-specific immunoglobulin E (IgE), OVA-specific-IgG1, and plasma histamine levels were declined in mice treated with *L. plantarum* JC7 than in OVA-sensitised mice. In addition, interferon-γ (IFN-γ) and interleukin 10 (IL-10) levels were significantly increased, while IL-4 and IL-17A levels were clearly decreased in mice that had undergone oral administration of *L. plantarum* JC7, compared with OVA-sensitised mice. These findings indicated imbalances of T helper cell type 1 (Th1)/Th2 and regulatory T cells (Treg)/Th17, which were confirmed by quantitative polymerase chain reaction (PCR). Western blotting demonstrated that the expression levels of phosphorylated IκBα and nuclear factor kappa B p65 were significantly increased in OVA-sensitised mice, but these changes were partly reversed after treatment with *L. plantarum* JC7. Oral administration of *L. plantarum* JC7 increased the richness, diversity, and evenness of cecum microbiota, characterised by higher Bacteroidetes abundance and lower Firmicutes abundance. Additionally, the intestinal microbial community composition was significantly altered in the OVA-sensitised group, indicating a disordered intestinal microbiota that was restored by the oral administration of *L. plantarum* JC7.

Conclusion Overall, *L. plantarum* JC7 can prevent food allergy by rectifying Th1/Th2 and Treg/Th17 imbalances, combined with modifications of disordered intestinal microbiota.

Keywords Food allergy · Th1/Th2 imbalance · Treg/Th17 imbalance · *Lactobacillus plantarum* · Gut microbiota

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Introduction

With an incidence of about 5–8% for adults and 7–10% for children ≤ 3 years of age [1], food allergy has become a major public health issue all around the world [2]. Although some individuals outgrow their food allergy by 3–5 years of age, food allergy predisposes them to an extensive array of allergies (e.g. allergic asthma, atopic eczema, and egg allergy) [1]. The current solution involves avoiding exposure

to allergens; however, it is difficult to completely avoid the presence of food allergens in the human diet, particularly among children (e.g. cow's milk allergy). In susceptible individuals, food allergy generally results from a disordered immune response characterised by a T helper cell type 1 (Th1)/Th2 imbalance towards a Th2 profile, which is accompanied by the production of immunoglobulin E (IgE) [3]. In non-susceptible individuals, the Th1 response is accompanied by production of interferon gamma (IFN- γ) and interleukin 2 (IL-2), along with reduction of the IgE level compared to susceptible individuals [4]. The Th17/regulatory T cell (Treg) imbalance is also participated in the ineffective suppression of inappropriate immune responses [5]. Therefore, an effective strategy for controlling allergic disorders may be to rectify the Th1/Th2 or Treg/Th17 imbalance.

It has been reported that moderate microbial stimulation plays an important role in establishing immune system maturation and oral tolerance [6]. Commercial probiotic products that can promote maturation of the intestinal immune system and regulation of the disordered intestinal immune system have been widely used. The mechanism of action includes maintenance of the balance of intestinal microbiota, acquisition of oral tolerance, and protection of the integrity of intestinal epithelial cells [7]. Lactic acid bacteria have beneficial health effects [6]; there is increasing evidence that some lactic acid bacteria shift the Th1/Th2 balance towards a Th1-dominant state, while living in the gastrointestinal tract and regulating intestinal microbiota [8–10]. Furthermore, the intestinal microbiota has a crucial role in the development and regulation of immune system. Infants with a significant imbalance of microorganisms in their gut microbiota may be at risk of developing atopic dermatitis and allergic sensitisation [11]. The diet, gut microbiota, and their interactions are presumably involved in alterations of mucosal immune tolerance. It has been reported that gut microbiota dysbiosis leads to changes in intestinal epithelial function that result in aberrant Th2 responses towards allergic responses [12, 13]. IgE production can be suppressed by microbiota, highlighting the importance of immune regulation exerted by the intestinal microbiota [14].

Probiotics have demonstrated health effects on the host when consumed in adequate numbers, either in a viable form or a killed form. Some lactobacilli, bifidobacteria, and their metabolites can modulate the intestinal barrier function, absorption of nutrients, and cytotoxicity; the specific effects depend on the species and specific strains, as well as the host's health status [15–17]. The safety and biological activities of probiotics for human health have received attention regarding the use of probiotics for preventing allergic diseases [18]. Although some studies have indicated reduced symptom severity in cow's milk allergy patients after administration of selected probiotic strains [19, 20], the effects of lactic acid bacteria and probiotics

on protection against allergy-related outcomes are not fully elucidated. Furthermore, the effects of probiotics on intestinal microbiota dysbiosis remain controversial.

Lactobacillus plantarum JC7 exhibited immunomodulatory effects in our previous in vitro study. In the present study, an ovalbumin (OVA)-induced food allergy animal model was established using BALB/c mice; *L. plantarum* JC7 was orally administered to mice after treatment with OVA. Serum levels of OVA-specific immunoglobulins and various cytokines were tested, and the cecum microbiota was analysed to explore the relationships between these indicators and OVA-induced food allergy.

Materials and methods

Bacterial strains and culture conditions

L. plantarum JC7 was isolated from naturally fermented pickles purchased from the Yanbian area of Jilin Province. *L. plantarum* JC7 was cultured at 37 °C for 16–18 h. Cell pellets were washed twice and adjusted to 1.0×10^9 colony-forming units/mL. They were then stored at 4 °C until oral administration to mice.

Animals and experimental design

Six-week-old female BALB/c mice were housed in a controlled environment and had free access to standard feed and sterilised tap water. Mice were randomly divided into three groups ($n = 8$ per group) after 1 week of acclimatisation to the laboratory environment. Mice received *L. plantarum* JC7 (200 μ L/mouse with concentration of 1.0×10^9 CFU/mL) by oral gavage three times per week from day 0 for 3 consecutive weeks; they underwent intraperitoneal injection of 20 μ g OVA (Sigma, St. Louis, MO, USA) containing Complete Freund's adjuvant (1:1, v/v) on days 0, 7, 14, and 21. Mice from OVA group were sensitised to OVA containing Complete Freund's adjuvant once per week for 3 weeks by intraperitoneal injection, simultaneously they received saline by oral gavage three times per week for 3 weeks. In parallel, the control group received 200 μ L saline (0.9%) by oral gavage three times per week, followed by intraperitoneal injection of saline. From day 21 to day 28, the mice were orally challenged twice by OVA (50 mg/mL). See Fig. 1 for the experimental schedule. On day 27, all mice were fasted overnight and on day 28, the blood samples were collected by removing the eyeball and mice were killed by dislocation 30 min after the second oral challenge.

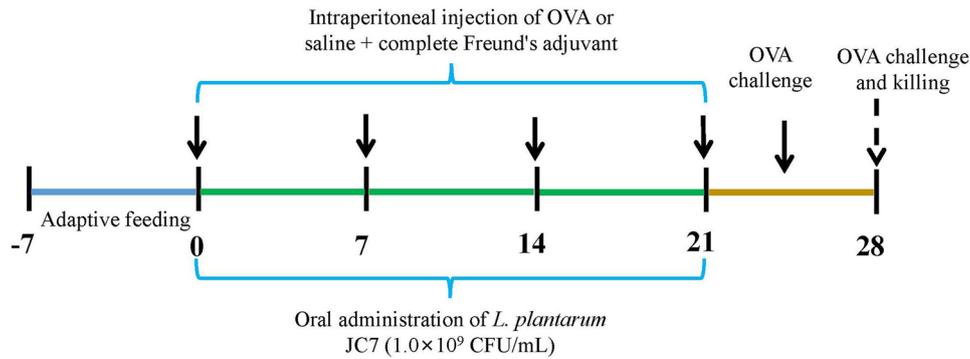


Fig. 1 Experimental schedule. Six-week-old female BALB/c mice were randomly divided into three groups ($n=8$ per group). Mice received *L. plantarum* JC7 ($200 \mu\text{L}/\text{mouse}$ with concentration of 1.0×10^9 CFU/mL) by oral gavage three times per week. In parallel, they underwent intraperitoneal injection of $20 \mu\text{g}$ OVA containing Complete Freund's adjuvant. Mice from OVA group were sensitised

to OVA once per week by intraperitoneal injection, simultaneously they received saline by oral gavage. The control group received $200 \mu\text{L}$ saline (0.9%) by oral gavage, followed by intraperitoneal injection of saline. From day 21 to day 28, the mice were orally challenged twice by OVA (50 mg/mL)

Culture of spleen cells

After killing by dislocation, the mice were soaked in 75% alcohol for 5 min and then under sterile environment, the spleens were removed from BALB/c mice and washed repeatedly by 1 mL syringe until they were pale red in colour using RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin, and 10% foetal bovine serum (HyClone Laboratories, Inc., Omaha, NE, USA). Erythrocytes were lysed using lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The cells were adjusted to a final concentration of 2.5×10^6 cells/mL and cultured in 6-well tissue culture plates in a total of 2.5 mL medium containing OVA ($5 \mu\text{g/mL}$) at 37°C for 48 h in the presence or absence of *L. plantarum* JC7 (2.5×10^6 CFU/mL).

Evaluation of OVA-induced allergic reaction

Hypersensitivity was scored immediately after challenge, using the following scoring standard: 0 = no manifestations; 1 = scratching and rubbing around the nose, head, and ear; 2 = puffiness around the eyes, mouth, and pilar erection; 3 = decreased activity with increased respiratory rate; 4 = wheezing and laboured respiration accompanied with diarrhoea; 5 = cyanosis around the mouth and tail; and 6 = death [21].

Measurement of plasma histamine level

Blood was collected into a chilled tube containing heparin sodium as an anticoagulant by removing eyeball. The plasma was collected and frozen at -80°C . An ELISA kit (Usinlife Science & Technology Co., Ltd., Wuhan, Hubei,

China) was used to measure histamine levels in accordance with the manufacturer's instructions. The minimum detection concentration of the ELISA kit was 0.5 ng/mL .

Detection of OVA-specific IgE, IgG2a and IgG1 levels

Serum was collected and frozen at -80°C . OVA-specific IgE level was detected as previously described [22]. Subsequently, 96-well high binding microtiter plates were coated with $10 \mu\text{g mL}^{-1}$ OVA. The plates were assessed at 450 nm. Serial dilution of a pool of hyperimmune serum samples was used as a standard for IgE, IgG1, or IgG2a on each assay plate. The detection values were expressed in arbitrary units, deduced from the optical densities of the reference serum curve with a high level of antibodies after the subtraction of blank wells. All tests were performed in triplicate.

Histopathological examination

At the end of the experiment, the distal colon tissues and lung tissues (coming from the same lobe) of the mice were removed and firstly fixed in 10% phosphate-buffered formalin, then embedded in paraffin, followed by cutting into $3 \mu\text{m}$ cross sections, and stained with haematoxylin and eosin (H&E) for histopathological examination.

Measurement of cytokine production

The IL-4, IL-10, IL-17A, IFN- γ in serum samples and culture supernatants were determined using commercially available ELISA kits (BioLegend, San Diego, CA, USA), in accordance with the manufacturer's instructions. Briefly, 96-well plates were coated with Capture Antibody solution (provided in ELISA kits) to incubate overnight (16–18 h)

at 4 °C. After blocking by Assay Diluent A and washing plates, add standards, serum samples or culture supernatants into appropriate microwells, and then incubate for 2 h at room temperature with shaking. After washing plates, add Detection Antibody solutions against different cytokines to microwells to incubate for 2 h, followed by adding Avidin-HRP solution for 30 min. Then, TMB substrate was added to occur colour reaction and 2 M H₂SO₄ was used to stop reaction. The absorbance was read at 450 nm within 15 min.

Measurement of the mRNA expression levels of cytokines and transcription factors

Total RNA was extracted from cultured splenocytes that had been stimulated with OVA, in the presence or absence of *L. plantarum* JC7, using the quantitative polymerase chain reaction (qPCR) as previously described [22]. All tests were performed in triplicate. Then, the results were calculated using CFX Manager Software. Data were counted using the 2^{-ΔΔCt} method; normalisation was performed relative to GAPDH expression. All the primer sequences referred to Duan et al. [22]. The primer sequences are listed in Table 1.

Western blotting

Fresh spleens were removed from experimental mice; total proteins and nuclear proteins were obtained using a membrane and cytosol protein extraction kit. Proteins from spleens were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis, then transferred to polyvinylidene fluoride membranes. After membranes had been blocked in 5% skim milk, they were incubated overnight at 4 °C with antibodies against nuclear factor kappa B (NF-κB) subunit IκBα (1:1000; Proteintech Group, Rosemont, IL, USA), phospho-IκBα (1:1000; Cell Signaling Technology, Danvers, MA, USA), p65 (1:1000, Cell Signaling Technology), nuclear factor erythroid 2-related factor 2 (1:1000; Proteintech), or GAPDH (1:5000; Proteintech). They were subsequently incubated with horseradish-conjugated secondary antibody (1:5000; Proteintech). Signals were visualised using an enhanced chemiluminescence kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). The image was detected on a gel imaging system (Tanon 4500) and the band grey value was measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

Analysis of intestinal microbiota composition

At the end of the experiment, the cecum of the mice was collected in a sterile environment, and the faecal contents were separated immediately into cryotubes and stored at -80 °C. An OMEGA Soil DNA Kit (M5635-02; Omega Bio-Tek, Norcross, GA, USA) was used to extract total genomic DNA

Table 1 Primer sequences for real-time PCR

Gene	Primer sequence (5'-3')	Length of amplified fragment (bp)
IL-4	ACAGGAGAAGGGACGCCAT (forward)	95
	GAAGCCCTACAGACGAGCTCA (reverse)	
GATA-3	GAAGGCATCCAGACCCGAAAC (forward)	255
	ACCCATGGCGGTGACCATGC (reverse)	
IFN-γ	TCAAGTGGCATAGATGTGGAA (forward)	92
	TGGCTCTGCAGGATTTTCATG (reverse)	
T-bet	GCCAGGGAACCGCTTATATG (forward)	137
	GACGATCATCTGGGTCACATT (reverse)	
IL-17A	GCGCTGTGTC AATGCGGAGG (forward)	169
	CTGGCGGACAATCGAGGCCA (reverse)	
RORγt	TACGGCAGCGCACCAACCTC (forward)	136
	AGCCGCTTGGCAA ACTCCACC (reverse)	
IL-10	TGAGGATCAGCAGGGGCCAGT (forward)	104
	CTGGCTGAAGGCAGTCCGCA (reverse)	
Foxp3	GGAAGCCCCGGAGAGGCAGA (forward)	123
	TGCAGGCTCAGGTTGTGGCG (reverse)	
Gapdh	TTCACCACCATGGAGAAGGC (forward)	237
	GGCATGGACTGTGGTCATGA (reverse)	

of samples. Both quality and quantity of extracted DNA were measured by NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. It should be noted that bacterial 16S rRNA genes (V3-V4 region) were amplified using the forward primer 338F (5'-ACTCCTACGGGAGGCAGC A-3') and reverse primer 806R (5'-GGACTACHVGGGTWT CTAAT-3'). Purified and quantified PCR amplicons were sequenced using the Illumina MiSeq Platform with MiSeq Reagent Kit v3 by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). Where, microbiome bioinformatics analyses were conducted using QIIME2 2019.4 [23], with few modifications from the tutorial guidelines (<https://docs.qiime2.org/2019.4/tutorials/>). Sequence data analyses were

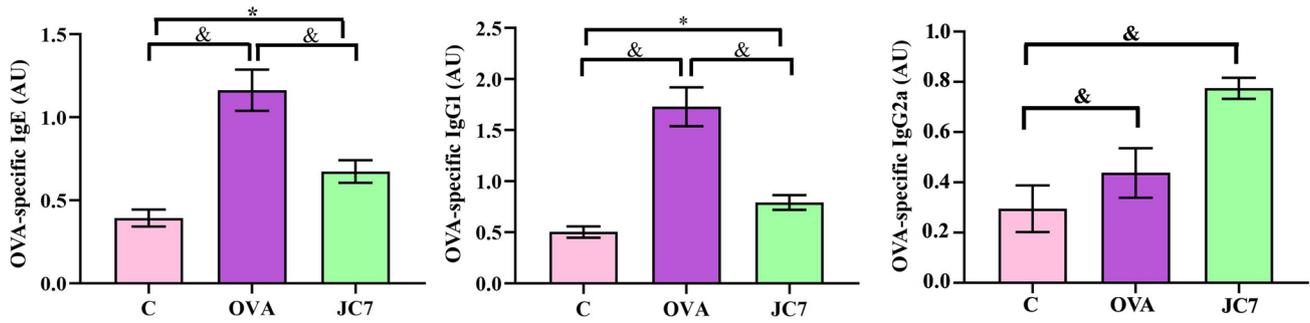
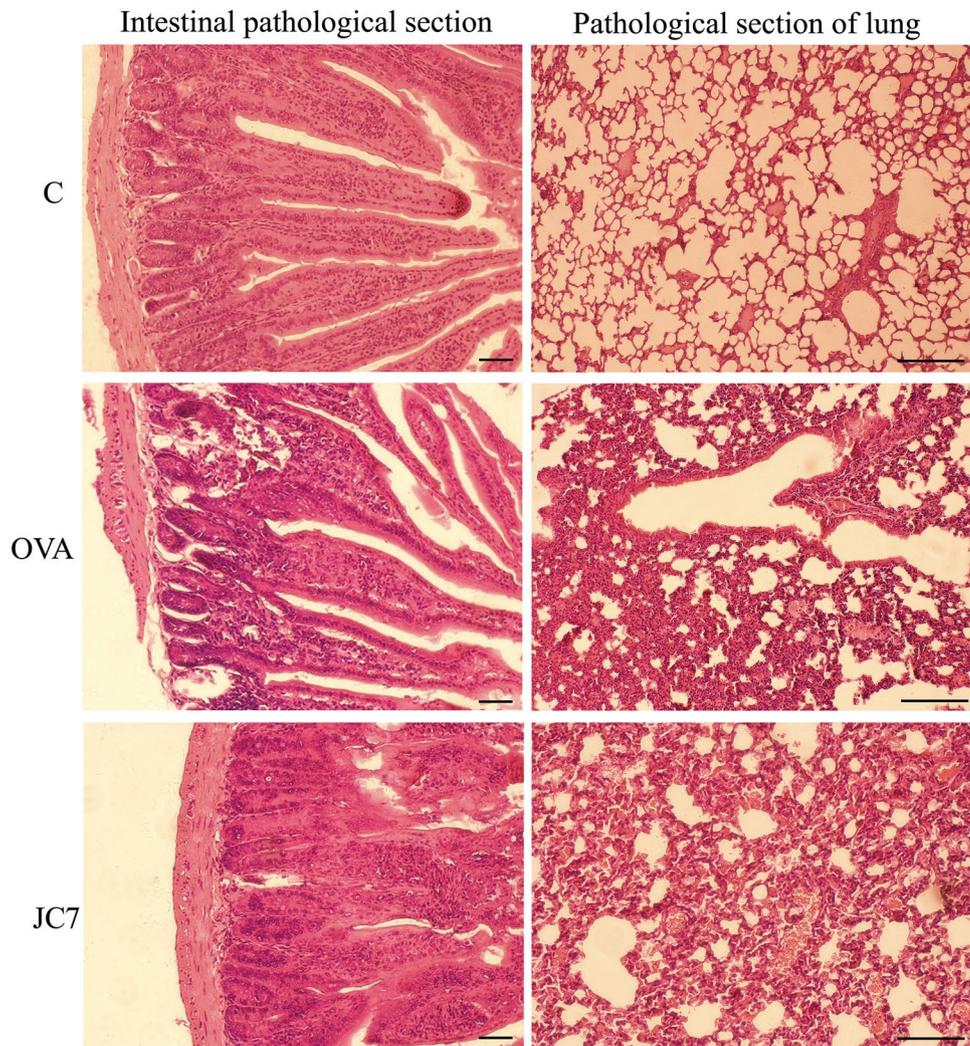


Fig. 3 OVA-specific IgE, IgG1 and IgG2a levels in serum. The results are expressed as means \pm SEM. ANOVA was used to calculate the statistical significance. C: control group; OVA: OVA-sensitised

group; JC7: group sensitised by OVA accompanied by oral administration of *L. plantarum* JC7. * denotes significance $p < 0.05$, & denotes significance $p < 0.001$. $n = 8$ for each group

Fig. 4 Characterisation of intestinal and pulmonary reactions (photographs are obtained from one representative animal in each group). The intestinal and pulmonary reactions in the colon and lung were observed under a microscope. C: control group; OVA: OVA-sensitised group; JC7: group sensitised by OVA accompanied by oral administration of *L. plantarum* JC7. Bar = 100 μ m



JC7, the crypt or mucosal damage was alleviated (Fig. 4). The mice in OVA-sensitised mice were with severe respiratory manifestations, so the pathologic changes of lungs were also detected. As shown in Fig. 4, the lungs from

OVA-sensitised mice were with obvious hyperemia and congestion points as well as cell infiltration in pulmonary tissue interspace compared with mice in control group. After treatment of *L. plantarum* JC7, the number of large

alveolar cavity was obviously decreased and most alveolar walls were complete, but these damages were only partly relieved compared to the control group (Fig. 4).

Evaluation of cytokine levels in serum samples and splenocyte supernatants

The IL-4 level was significantly elevated in OVA-sensitised group in comparison with the control group, but significantly decreased upon the oral administration of *L. plantarum* JC7 (Fig. 5A). Notably, the IFN- γ level showed an opposite trend compared to the IL-4 level. In OVA-sensitised mice, the IFN- γ level was significantly reduced compared to the control group; however, treatment with *L. plantarum* JC7 significantly increased the IFN- γ level in comparison with the OVA group (Fig. 5B). These results suggested that a Th1/Th2 imbalance occurred after OVA sensitisation, and the oral administration of *L. plantarum* JC7 could reverse this imbalance. Moreover, the IL-10 level showed a trend similar to the IFN- γ level; it was significantly reduced after OVA sensitisation compared to the control group but was elevated after oral administration of *L. plantarum* JC7 (Fig. 5C). The IL-17A level was also examined in this study; it was influenced by OVA sensitisation and varied in a manner similar to the IL-4 level. In addition, the secretion of above cytokines in splenocyte supernatants showed similar change compared to that in serum.

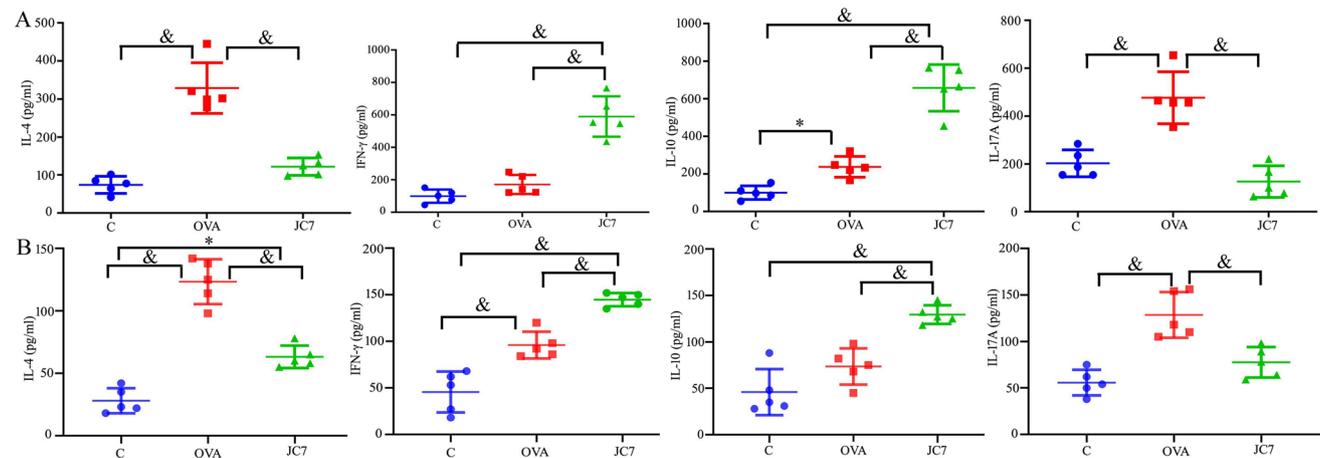


Fig. 5 Effect of *L. plantarum* JC7 on cytokine secretions in vivo (A, serum, $n=8$ for each group) or in vitro (B, splenocyte supernatants, $n=4$ for each group) from mice with OVA-induced food allergy. Serum was collected by taking the eyeball. The secretions of different cytokines in serum or supernatants were determined by ELISA. Mice were orally administrated with 200 μ L of *L. plantarum* JC7

Effects of *L. plantarum* JC7 on the expression levels of cytokines and transcription factors

qPCR was used to analyse the expression levels of IL-4, GATA-3, IL-10, forkhead box P3 (Foxp3), IFN- γ , T-bet, IL-17A, and retinoic acid-related orphan receptor gamma t (ROR γ t) in splenocytes. In comparison with the control group, IL-4, GATA-3, IL-17A, and ROR γ t expression levels were significantly upregulated after OVA administration; they were significantly downregulated after oral administration of *L. plantarum* JC7 (Fig. 6). Notably, the expression levels of IL-10, Foxp3, IFN- γ , and T-bet presented a completely opposite contrast to IL-4, GATA-3, IL-17A, and ROR γ t expression levels. These results were similar to the changes in cytokine levels in the serum samples and splenocyte supernatants and further verified the Th1/Th2 and Treg/Th17 imbalance induced by OVA.

Effects of *L. plantarum* JC7 on the relative abundances of intestinal microbiota at the phylum level

Four of the most abundant bacteria at the phylum level are presented in Fig. 7A. Firmicutes, Bacteroidetes, and Proteobacteria were the dominant microbiota because of high relative abundances at the phylum level. While the relative abundance of Firmicutes was significantly greater in the OVA group than in the control group; however, it sharply decreased after the oral administration of *L. plantarum* JC7. In comparison with the control group, the relative abundance of Bacteroidetes was significantly lessened

(1.0×10^9 CFU/mL). Results are expressed as means \pm SEM for IL-4, IFN- γ , IL-10 and IL-17A. C: control group; OVA: OVA-sensitized group; JC7: group sensitized by OVA accompanied by oral administration of *L. plantarum* JC7. * indicates significance $p < 0.05$, & indicates $p < 0.001$

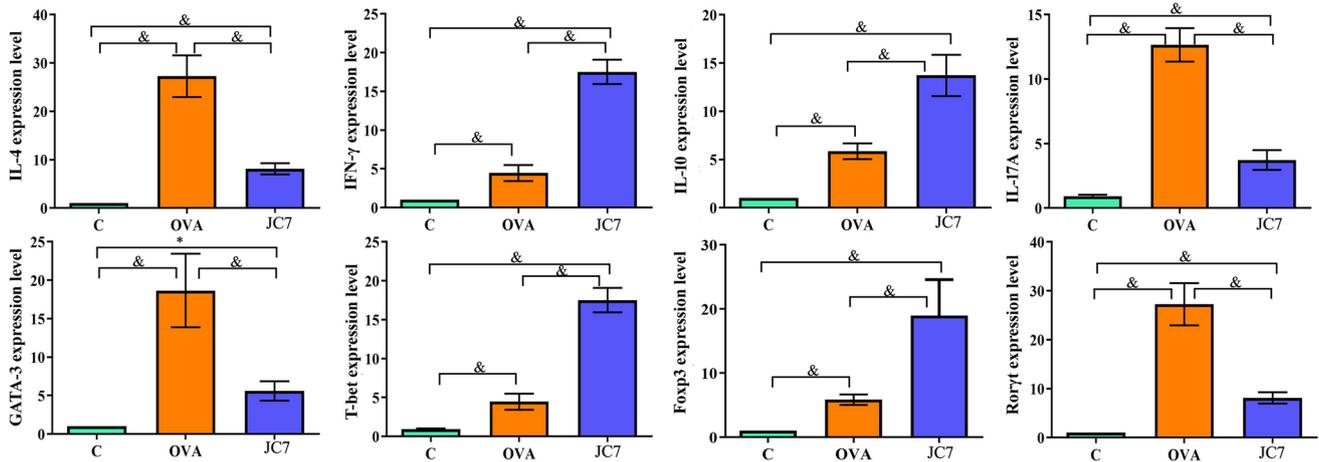


Fig. 6 Effect of *L. plantarum* JC7 on mRNA expression of cytokines and transcription factors in splenocytes. The mRNA expression of cytokines and transcription factors in splenocytes was measured using qPCR. OVA: OVA-sensitized group; JC7: group sensitized by

OVA accompanied by oral administration of *L. plantarum* JC7. The expression of all genes was normalised to GAPDH expression. C: saline-sensitized group. * indicates significance $p < 0.05$, & indicates $p < 0.001$. $n = 4$ for each group

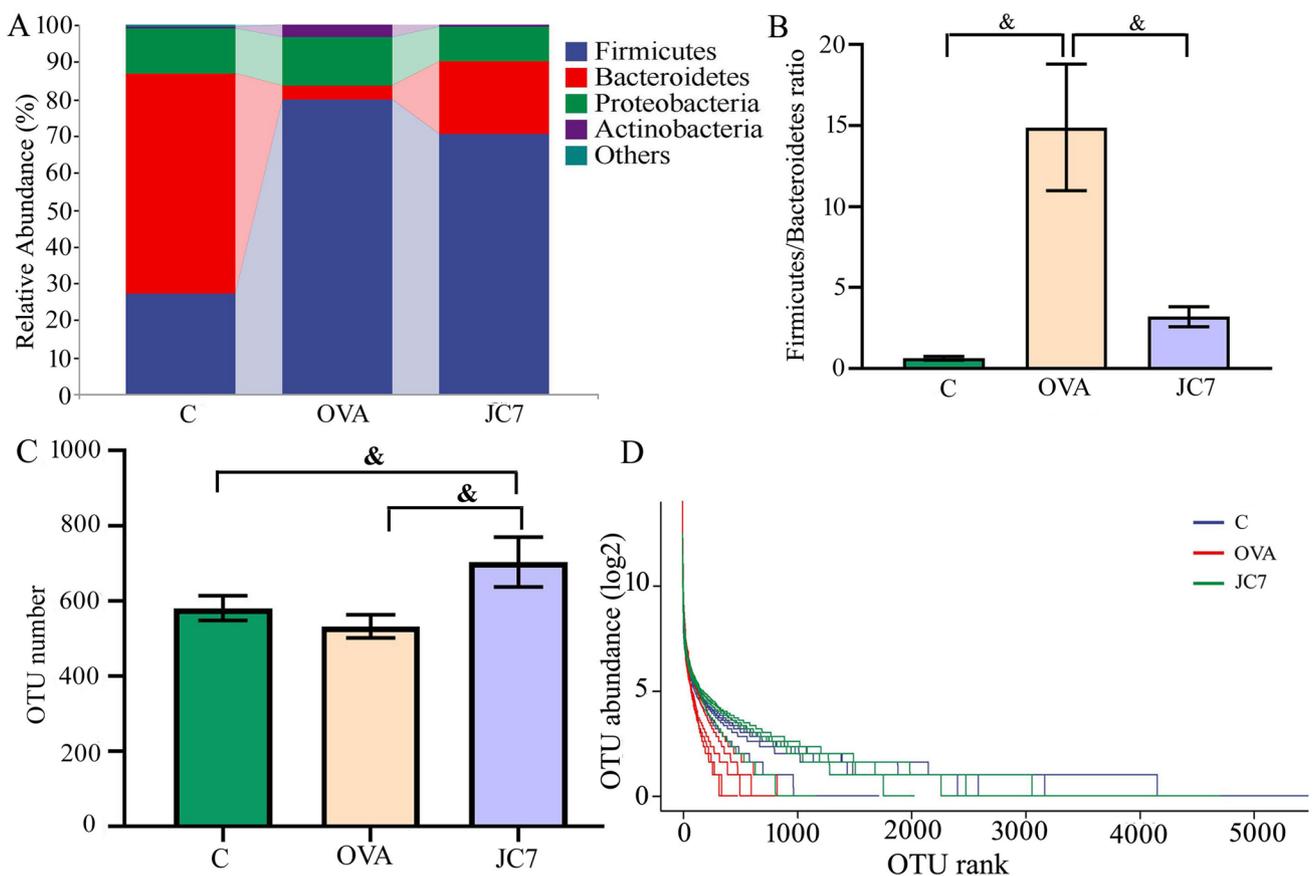


Fig. 7 *L. plantarum* JC7 modulates intestinal microbiota composition in OVA-induced mice at the phylum level. **A** Microbial composition of mice intestinal faeces under different treatments. **B** The ratio of Firmicutes /Bacteroidetes. **C** OTU number among different groups.

(D) OTU abundance among different groups. C: control group; OVA: OVA-sensitized group; JC7: group sensitized by OVA accompanied by oral administration of *L. plantarum* JC7. * denotes significance $p < 0.05$, & denotes significance $p < 0.001$. $n = 5$ for each group

in the OVA group; it was significantly increased after oral administration of *L. plantarum* JC7. Furthermore, after OVA sensitisation, the relative abundance of unknown bacteria sharply declined in comparison with the control group; this change was reversed after oral administration of *L. plantarum* JC7. In addition, the Firmicutes/Bacteroidetes ratio was calculated to compare their abundance change among three groups (Fig. 7B), it was significantly higher in OVA-sensitised mice in comparison with the control group, but after treatment by *L. plantarum* JC7, this ratio was obviously declined. In addition, OTU number was analysed and the results demonstrated that oral administration of *L. plantarum* JC7 could markedly increase OTU number compared to OVA-sensitised group (Fig. 7C). The OTU rank abundance curve among different groups showed consistent changes with OTU number (Fig. 7D).

Effects of *L. plantarum* JC7 on the alpha diversity analysis of the intestinal microbiota

Alpha diversity analysis is used to determine the species richness, diversity, and evenness. In the present study, the Chao1 and Observed species indexes represented species richness. As shown in Fig. 8, either Chao1 or Observed species indexes were significantly lower in the OVA-sensitised group than in the control group, indicating obviously reduced species richness in the OVA-sensitised group. However, Chao1 and Observed species indexes were significantly upregulated after the oral administration of *L. plantarum* JC7, suggesting that *L. plantarum* JC7 could increase species richness in the mouse intestinal microbiota. Shannon and Simpson indexes represent the species diversity, and they were generally similar among the three groups, although the Simpson index significantly differed between the OVA-sensitised and *L. plantarum* JC7-treated groups, indicating that the effects of the treatments on species diversity were weaker than the effects on species richness. In addition, the Pielou_e index, which represents species evenness, was significantly greater in the *L. plantarum* JC7-treated group than in the OVA-sensitised group.

Effects of *L. plantarum* JC7 on the intestinal microbial community composition

The beta diversity analysis results are shown in Fig. 9A. In this study, beta diversity analysis was completed using the weighted_UniFrac distance algorithm at a confidence level of 0.95. The contributions of PCo1 and PCo2 to the overall variation were 49.3% and 20.3%, respectively. Generally, a closer distance between two points was indicative of a smaller difference in intestinal microbiota. In this study, the intestinal microbial communities were clearly distinguishable. OVA stimulation resulted in significant differences in

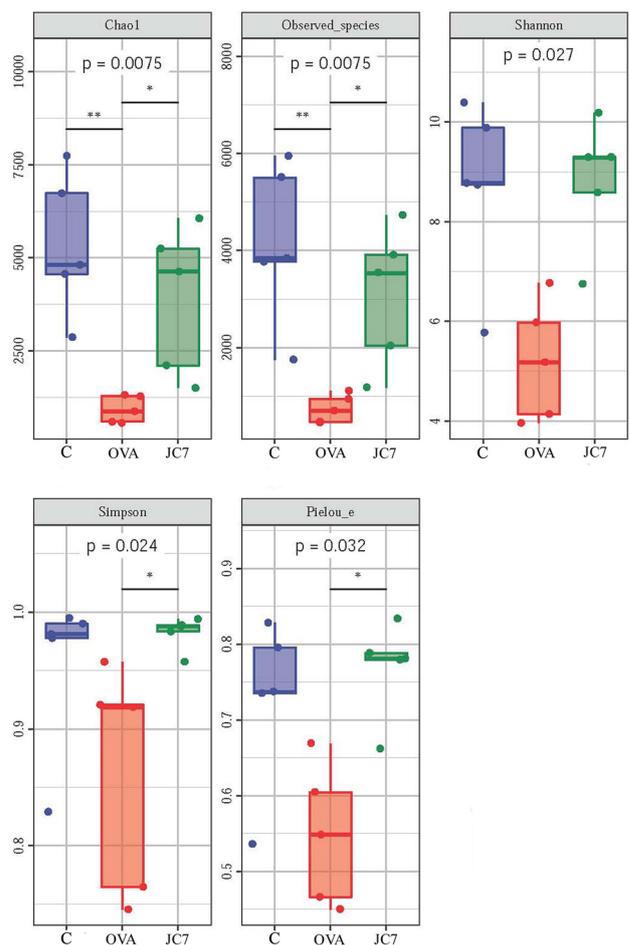
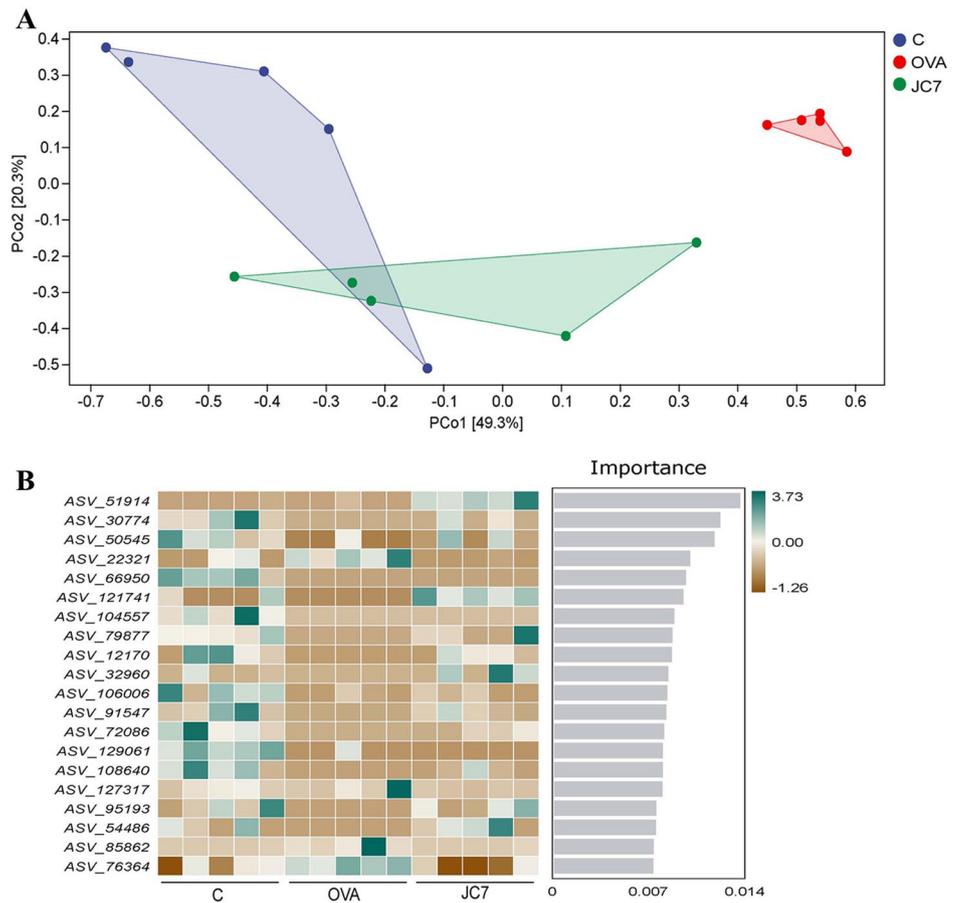


Fig. 8 Effects of *L. plantarum* JC7 on Alpha diversity of intestinal microbiota. Chao1 and Observed species indexes represent species richness; Shannon and Simpson indexes represent the species diversity; Pielou_e index represents species evenness. C (blue): control group; OVA (red): OVA-sensitised group; JC7 (green): group sensitised by OVA accompanied by oral administration of *L. plantarum* JC7. * denotes significance $p < 0.05$, ** denotes significance $p < 0.01$. $n = 5$ for each group

intestinal microbial community structure, compared with the control and *L. plantarum* JC7-treated groups. However, the projector distance between the control and *L. plantarum* JC7-treated group partly overlapped, indicating greater similarity between these two groups.

Random forest analysis also revealed differences in microbial community composition. Figure 9B presents the importance of various amplicon sequence variants (ASVs)/OTUs, as well as the abundance distributions of these microbial communities among groups. From top to bottom, the importance of microbial species progressively decreased; species with high importance constituted markers of inter-group differences. Compared with the control group, the numbers of ASV/OTU IDs with a higher degree of importance were markedly decreased in the OVA-sensitised group.

Fig. 9 Beta diversity and random forest distribution of intestinal microbiota under different treatments. **A** Principal coordinate analysis (PCoA) of all samples based on weighted_ UniFrac distance algorithm at a confidence level of 0.95. **B** Random forest distribution of intestinal microbiota of all samples. C: control group; OVA: OVA-sensitised group; JC7: group sensitised by OVA accompanied by oral administration of *L. plantarum* JC7



Moreover, the number of ASV/OTU IDs with a higher degree of importance was increased after the oral administration of *L. plantarum* JC7, compared with the OVA-sensitized group; for example, important differences involved ASV_51914, ASV_22321, and ASV_121741.

Effects of *L. plantarum* JC7 on NF- κ B activation in an OVA-sensitized mouse model

Western blotting was conducted to examine the effects of *L. plantarum* JC7 on OVA-induced activation of the NF- κ B pathway. NF- κ B p65 expression was significantly enhanced in the OVA-sensitized group, but this change was partly reversed after treatment with *L. plantarum* JC7 (Fig. 10A, D). Generally, the activation of NF- κ B p65 was linked with the degradation of phosphorylated I κ B α . Therefore, both I κ B α and p-I κ B α expression levels were investigated by Western blotting. There were no significant differences in I κ B α expression among groups, but p-I κ B α expression was restored in the OVA-sensitized group in comparison with the control group. Notably, there was no significant difference in p-I κ B α expression between the control and *L. plantarum* JC7-treated groups.

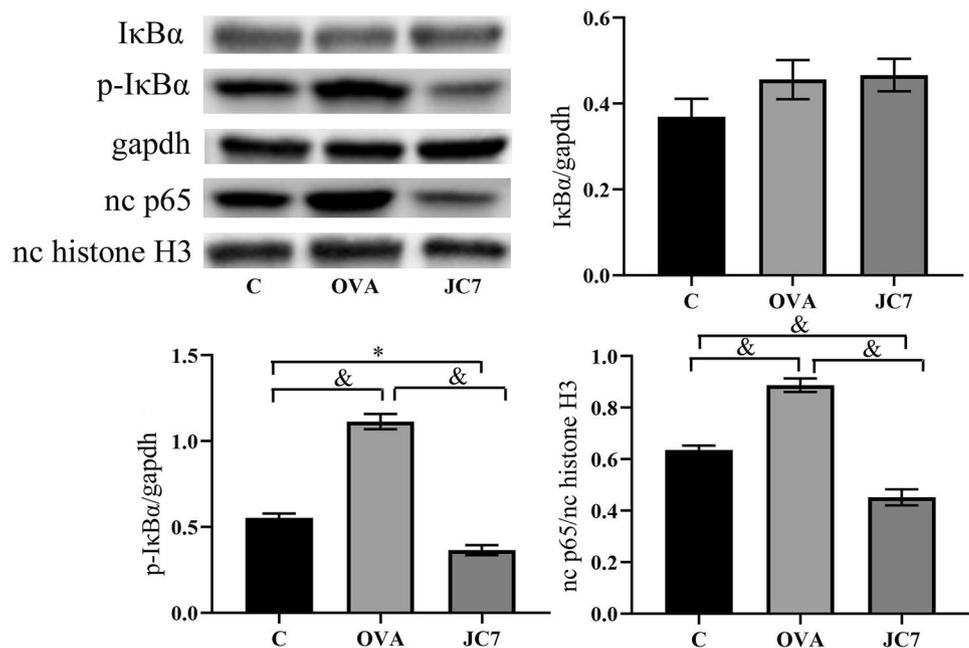
Discussion

A previous study showed that *L. plantarum* JC7 could strongly stimulate spleen cells to secrete IFN- γ but inhibit IL-4 secretion, indicating that *L. plantarum* JC7 has anti-allergic potential. In this study, an OVA-sensitized model was established in BALB/c mice, and *L. plantarum* JC7 was administered to the mice by oral gavage. The results showed that *L. plantarum* JC7 significantly alleviated clinical manifestations; it also decreased plasma histamine and serum Th2 cytokine levels, as well as the humoral response. Furthermore, *L. plantarum* JC7 treatment significantly affected the intestinal microbiota diversity and richness in OVA-sensitized mice.

According to an established scoring standard [22], OVA-sensitized mice had severe allergy manifestations including wheezing and laboured respiration, as well as scratching and rubbing around the nose and ear, which were relieved after the oral administration of *L. plantarum* JC7.

A classic sign of food allergy is an elevated IgE level; effectively controlling the elevation of antigen-specific IgE has been shown to alleviate the food allergy response. In the present study, oral administration of *L. plantarum* JC7 significantly declined OVA-specific IgE and IgG1 levels

Fig. 10 Effect of *L. plantarum* JC7 on NF- κ B signalling pathway-related protein expression. C: control group; OVA: OVA-sensitised group; JC7: group sensitised by OVA accompanied by oral administration of *L. plantarum* JC7. * denotes significance $p < 0.05$, & denotes significance $p < 0.001$



in serum, indicating that *L. plantarum* JC7 may reduce an OVA-sensitised allergic reaction by inhibiting the secretion of an OVA-specific IgE response, but the OVA-specific IgG2a level showed an opposite change to that of IgG1. These results were consistent with a previous study which found that oral administration of *L. plantarum* ZDY2013 and *L. rhamnosus* GG suppressed allergic responses, attenuated serum IgE and relieved allergic symptoms [6]. In addition, a decrease in histamine level in the plasma of mice treated with *L. plantarum* JC7, compared with OVA-sensitised mice, confirmed the classic changes in food allergy (e.g. IgE can mediate mast cell degranulation to release histamine).

To further explore the signal transduction mechanism involved in the alleviation of food allergy by treatment with *L. plantarum* JC7, NF- κ B signalling-related proteins were detected because they participate in various inflammatory reactions. Moreover, there is increasing evidence that specific probiotic strains can activate the NF- κ B signalling pathway [24]. In the present study, NF- κ B p65 expression was significantly enhanced in the OVA-sensitised group; this change was immediately reversed after treatment with *L. plantarum* JC7. p-IκBα expression was restored in the OVA-sensitised group, but it did not significantly differ relative to the *L. plantarum* JC7-treated group; this implied that treatment with *L. plantarum* JC7 can prevent the degradation of p65-IκBα. Notably, Rocha-Ramírez et al. found that *Lactobacillus* spp. probiotic strains activated NF- κ B signalling to stimulate the inflammatory response [13]. However, in the present study, *L. plantarum* JC7 inhibited the inflammatory response by activating the NF- κ B signalling pathway. Furthermore, *L. plantarum* JC7 treatment increased production

of the NF- κ B downstream cytokine IFN- γ , implying that the NF- κ B signalling pathway may be involved in the alleviation of food allergy.

The interaction between probiotics and intestinal microbiota is vital for immune system maturation and tolerance acquisition [25, 26]. IgE production partly depends on Th2 lymphocytes that produce IL-4. IL-10-secreting Tregs were reported to suppress the generation of Th2 immunity and IgE production. Therefore, microbiota-mediated modulation of any of these pathways presumably contributes to altered susceptibility to food allergy [14]. Moreover, intestinal microbiota have a central role in the development and regulation of the immune system [27]. In humans, most intestinal microbiota are members of four phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [28]. Firmicutes and Bacteroidetes are the two major phyla in the intestinal microbiota of both mice and humans [29]. An early deficiency of Bacteroidetes has been found in young infants with atopic dermatitis and food allergy [30]; Bacteroidales (an order within Bacteroidetes) has also been found to protect against food allergy responses in mice [27]. The relative abundances of Bacteroidetes, Proteobacteria, and Actinobacteria were significantly reduced, whereas the relative abundance of Firmicutes was highly enriched in infants with food allergy [31]. In this study, compared with the control group, the intestinal microbiota was altered after OVA sensitisation and partially restored by treatment with *L. plantarum* JC7. Both OTU abundance and quantities were significantly decreased after OVA sensitisation; these changes were reversed after treatment with *L. plantarum* JC7. Furthermore, the oral administration of *L. plantarum* JC7 promoted

the growth of bacteria from the Bacteroidetes phylum but partly inhibited the growth of bacteria from the Firmicutes phylum, compared with the OVA-sensitised group. These findings were consistent with the results of study in which children with food sensitisation had significantly decreased Bacteroidetes abundance and markedly aggrandised Firmicutes abundance in comparison with healthy children [13]. The Firmicutes/Bacteroidetes ratio is also influenced in patients with food sensitisation, but the degree of change is uncertain [32]. In this study, the Firmicutes/Bacteroidetes ratio was sharply increased in the OVA-sensitised group but reversed after treatment with *L. plantarum* JC7. In a comprehensive perspective, the obviously high Firmicutes abundance and significantly decreased Bacteroidetes abundance might be contributed the elevated anti-OVA IgE level, which finally elicited OVA-induced food allergy. In other words, high anti-OVA IgE level might result in disordered gut microbiota, which further caused the changes both in Firmicutes abundance and Bacteroidetes abundance. In fact, a large observational cohort study in the United States have reported that children with IgE-mediated food allergy had a higher abundance of Bacteroidetes and a lower abundance of Firmicutes than children with resolved food allergy [33].

The alterations observed in this study may be linked to allergic reactions including the humoral response and Th1/Th2 imbalance; previous studies have confirmed that gut microbiota dysbiosis led to altered intestinal epithelial function, resulting in aberrant Th2 responses towards allergic responses, rather than tolerogenic responses [13]. Moreover, the maturation of a healthy gut microbiota in early life is inclined to a change in the Th1/Th2 balance, favouring a Th1 response [34], dysbiosis changes host–microbiota homeostasis, favouring a shift towards a Th2 response [35]. The previous findings were consistent with our results. In OVA-sensitised mice, intestinal microbiota dysbiosis was characterised by changes in the Firmicutes/Bacteroidetes ratio, as well as alterations in the relative abundances of various bacteria. Both the IL-4 level in serum and the expression levels of IL-4 and GATA-3 were significantly decreased, indicating a shift in the Th1/Th2 balance towards a Th2 response. However, the oral administration of *L. plantarum* JC7 increased the serum level of IFN- γ and enhanced the expression levels of IFN- γ and Foxp3, indicating a Th1 response. Furthermore, gut microbes have been reported to induce Treg activation [36], and the results of this study was in line with previous study, both IL-10 expression and secretion level were significantly downregulated in OVA-sensitised mice, while these alterations were alleviated by treatment with *L. plantarum* JC7.

Furthermore, alpha diversity analysis was performed using Chao1, Observed_species, Shannon, Simpson, and Pielou_e indexes, where Chao1 and Observed_species

reflect microbial richness, Shannon and Simpson represent microbial diversity, and the Pielou_e represents microbial evenness. There were significant reductions in the diversity, richness, and evenness of intestinal microbiota in the OVA-sensitised group, compared with the *L. plantarum* JC7-treated group; these results indicated reduced diversity among intestinal bacteria. Importantly, bacterial density and diversity are decreased during diarrhoea; the oral administration of probiotics can restore density and diversity at the family level [37]. In addition, beta diversity analysis showed that the intestinal microbiota community structure significantly differed among groups. Specifically, the intestinal microbiota of *L. plantarum* JC7 group was closer to the control group but farther from the OVA-sensitised group; these findings suggested that OVA sensitisation substantially changed the intestinal microbiota community structure, while oral administration of *L. plantarum* JC7 partly restored the structure. Finally, the changes in cytokines suggested that microbiota might have a key role in shaping the Th1/Th2 and Treg/Th17 balances.

L. plantarum JC7 decreased the OVA-specific IgE level and shifted the Th1/Th2 and Treg/Th17 imbalances by promoting IL-10 and IFN- γ secretion and inhibiting IL-4 and Th17 secretion; the changes might be ascribed to the activation of the NF- κ B signalling pathway. Furthermore, *L. plantarum* JC7 aggrandised the relative abundance of Bacteroidetes but lessened the relative abundance of Firmicutes; it also increased alpha diversity and changed the intestinal microbial community to a structure similar to the control group.

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Author contributions Cuicui Duan: designed and completed most of the experiments, wrote the manuscript and collected the data. Lin Ma, Jie Yu and Yixue: Sun partly participated in the Western blot, ELISA and collected the data. Lifan Liu, Fu Minma and Xiaolei Li: participated in analysis of intestinal microbiota data. Dan Li: helped design the experiment and provided good idea.

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Declarations

Conflict of interest The authors declare no completing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

Ethics approval In this study, all animal experimental procedures were approved by the Committee for Ethics on Animal Care and Use of Changchun Weish Testing Technology Service Co., LTD (20220330-01).

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