

PAPER

[View Article Online](#)
[View Journal](#) | [View Issue](#)
Cite this: *Food Funct.*, 2020, **11**, 9272

Prophylactic effects of oral administration of *Lactobacillus casei* on house dust mite-induced asthma in mice†

 Lingzhi Li,^{a,b} Zhifeng Fang,^{a,b} Yuan-Kun Lee,^c Jianxin Zhao,^{a,b,d} Hao Zhang,^{a,b,d} Wenwei Lu^{a,b,d} and Wei Chen^{a,b,d,e}

This study investigated the prophylactic effects of five *Lactobacillus casei* strains on house dust mite (HDM)-induced asthma in mice. BALB/c mice were orally administered with *L. casei* strains for one week before HDM treatment. Ketotifen and *Lactobacillus rhamnosus* GG were used as positive controls. All *L. casei* strains decreased the number of granulocytes and the levels of Th2 and Th17 inflammatory cytokines in the lungs, *L. casei*3 significantly decreased the airway inflammation score. Further studies showed that *L. casei*3, *L. casei*4, and *L. casei*5 decreased the chemokine levels, *L. casei*2, *L. casei*4, and *L. casei*5 promoted the secretion of secretory immunoglobulin A (sIgA), *L. casei*2 upregulated the interleukin (IL)-10 levels, and *L. casei*1 had no effect on these immune indices. *L. casei*1 and *L. casei*4 decreased the serum levels of total IgE and HDM-specific IgG1, respectively. *L. casei*3 and *L. casei*5 decreased both HDM-specific IgG1 and total IgE levels. *L. casei*2 did not affect the levels of these immunoglobulins. The gut microbiota analysis revealed that all five *L. casei* strains enhanced the richness of the gut microbiota mainly by increasing the abundance of Firmicutes, while there were differences at the genus level. Thus, the prophylactic effects of *L. casei* on HDM-induced mixed chronic airway inflammatory asthma exerted as they differentially affected the immune responses and gut microbiota composition. *L. casei*3, which exhibited the highest prophylactic effect, increased the acetate and propionate contents in a strain-dependent manner.

 Received 26th May 2020,
 Accepted 9th September 2020

DOI: 10.1039/d0fo01363c

rsc.li/food-function

1. Introduction

Asthma is a complex disease that results from various host–environment interactions.¹ Previous studies have reported that innate and adaptive immune responses contribute to the complexity of asthma.^{2,3} The main characteristics of asthma include abnormal epithelial barrier function⁴ and mixed granulocytic inflammation, which is induced when eosinophilic and non-eosinophilic asthma occur simultaneously.¹ In patients with eosinophilic asthma, the adaptive T helper 2 (Th2) cells secrete interleukin (IL)-5, IL-4, IL-13, and IL-9. IL-5 promotes the survival and maturation of eosinophils. The

chemokines of eosinophils promote the recruitment of eosinophils to the lung mucosa. These eosinophils secrete oxygen free radicals and toxic granules that lead to chronic airway inflammation. In the B-cells, IL-4 promotes the synthesis of immunoglobulin E (IgE), which binds to the IgE receptors located on the mast cells. The cross-linking of allergen and IgE promotes the secretion of biologically active mediators, such as histamine, from the activated mast cells and promote airway inflammation. However, there is limited understanding of non-eosinophilic asthma.^{4,5} In addition to enhanced activation of macrophages and secretion of neutrophil chemokines,⁶ patients with neutrophilic asthma exhibit enhanced secretion of Th17 or Th1 cell inflammatory cytokines.⁷ Currently, the therapeutic strategies for asthma are limited and mainly provide symptomatic relief.¹

The gut microbiota can influence the development of the host immune system. Germ-free (GF) mice, which do not have a well-developed immune system, exhibit mucosal changes, specific dysplasia of intestinal and mesenteric lymphoid tissues, skewed T-cell populations in intestinal tissue, decreased IgA production, and impaired resistance to infection.^{8,9} The intestinal and respiratory tracts have structural similarities as they have the same embryonic origin. The

^aState Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China. E-mail: luwenwei@jiangnan.edu.cn

^bSchool of Food Science and Technology, Jiangnan University, Wuxi 214122, China

^cDepartment of Microbiology & Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

^dNational Engineering Research Center for Functional Food, Jiangnan University, Wuxi 214122, China

^eBeijing Innovation Centre of Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, China

†Electronic supplementary information (ESI) available. See DOI: 10.1039/d0fo01363c

microbiota from the intestinal tract can enter the respiratory tract *via* aspiration.¹ Hence, the gut microbiota plays an important role in the pathogenesis of asthma. Recently, asthma was reported to be associated with alterations in the gut microbiota, such as decreased microbial diversity,¹⁰ decreased abundance of *Lachnospira* spp., *Faecalibacterium* spp., and *Rothia* spp.,¹¹ and increased abundance of total anaerobes in the intestinal tract.¹² Moreover, one study on 957 Dutch infants revealed that the presence of *Clostridium difficile* in stool samples was associated with an increased risk for developing eosinophilic asthma.¹³ Previous studies have demonstrated that restoring the gut microbiota composition can prevent immune dysfunctions in mice.¹⁴ Therefore, the modulation of the gut microbiota can be a potential prophylactic strategy for asthma.

Probiotics (mainly *Lactobacillus* and *Bifidobacterium*) can colonize the intestine and stimulate host immunity and affect the gut microbiota composition.¹⁵ Probiotic stimulation has been shown to be beneficial in intestinal diseases, such as inflammatory bowel disease,¹⁶ because probiotics could enhance the intestinal barrier, increase the host's antioxidant status, modulate the gut microbial composition, and enhance microbial metabolite levels (mainly short chain fatty acids (SCFA)).^{17,18} Probiotic stimulation is also a potential therapeutic strategy for lung diseases. One study reported that the supplementation of *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* decreased the susceptibility to asthma.¹¹ Subsequently, some bacterial strains of probiotics are reported to exert prophylactic effects on asthma by regulating the asthma-related inflammatory responses, host immunity, and gut microbiota.^{19,20} Therefore, it is crucial to identify additional probiotics that exhibit prophylactic effects on asthma.

Lactobacillus casei (*L. casei*) is a common species of *Lactobacillus* and widely used as probiotics, particularly fermented dairy products, with food safety confirmation from the European Food Safety Authority. Some studies in mice have shown that the oral administration of *L. casei* modulated the composition of the gut microbiota and improved metabolic and immune activity.^{21,22} Previously, we had reported that a mixture of five *L. casei* strains exerted a prophylactic effect on asthma.²³ In this study, the prophylactic effect of these five *L. casei* strains isolated from different regions and sources (Table S1†) of China on house dust mite (HDM)-induced asthma was individually evaluated with a special focus on inflammation, immune response, and gut microbiota composition and metabolism. The findings of this study provide a foundation for the application of probiotics as a prophylactic for asthma.

2. Materials and methods

2.1. Propagation of *L. casei* strains

*L. casei*1 (M2-07-F01-L4-2-1), *L. casei*2 (M2-06-F01-L4-2-3), *L. casei*3 (CCFM1073), *L. casei*4 (RS8-5), and *L. casei*5

(FGDLZ41) were obtained from the Culture Collection of Food Microbiology (CCFM) at Jiangnan University. The bacterial strains were grown in MRS medium for 18 h at 37 °C under static conditions. For the study, each candidate strain was adjusted to a total number of 10⁹ CFU for oral administration to each mouse. The concentration of bacterial cells was determined using the gradient dilution method.

2.2. Animal experiments

All animal procedures were performed in accordance with the European Community guidelines (directive 2010/63/EU) and approved by the ethics committee of Jiangnan University (JN. No. 20181030b0800221[231]). To minimize animal suffering, all procedures were performed under anesthesia.

In the HDM-induced asthma model,²⁴ mice were exposed intranasally to 25 µg of purified Dermatophagoides pteronyssinus extract (Greer Laboratories Inc., Lenoir, NC, USA) dissolved in 10 µL sterile saline for 5 days per week from 0 to 5 consecutive weeks; control groups received 10 µL sterile saline. Female Balb/c mice (5–6 weeks old) were obtained from GemPharmatech Co., Ltd (Nanjing, China), housed under specific pathogen-free conditions, and fed with an HDM-free diet. After one week of acclimatization, the mice were randomly divided into control, model, and five *L. casei* groups (six animals per group). Each test mouse was administered 250 µL of *L. casei* strain every day, starting one week before the first sensitization and continued over the whole process; the control and model animals received the same volume of sterile saline. The parameters were analyzed at week 5 after the last challenge (Fig. 1A).

2.3. Bronchoalveolar lavage fluid (BALF) and differential cell counts

BALF was obtained three times using 1 mL PBS containing a protease inhibitor using a tracheal cannula. Total leukocytes were counted using the trypan blue stain (Solarbio, China), and macrophages, lymphocytes, eosinophils, and neutrophils were identified using the Diff-Quick stain kit (Solarbio, China) by standard morphologic criteria, and 200 cells were counted.

2.4. Lung histology

The left lobes of the lung tissues were fixed with 4% paraformaldehyde, embedded in paraffin, cut (5 µm-thick), and stained with hematoxylin and eosin (H&E). A pathologic slice scanner was used. HE-stained sections were viewed and random images were collected. The degree of histological damage to the lung was determined in four individual mice of each group. The qualitative airway inflammation score was scored on a 0–4 scale for each mouse.²⁵ All morphometric tests were performed by trained individuals who were blinded to the sample treatments.

2.5. Lung cytokine and chemokine analyses

Cell-free supernatant from BALF was frozen at –20 °C for cytokine and chemokine measurements. The protein expression of BALF cytokines was measured using commercial ELISA kits:

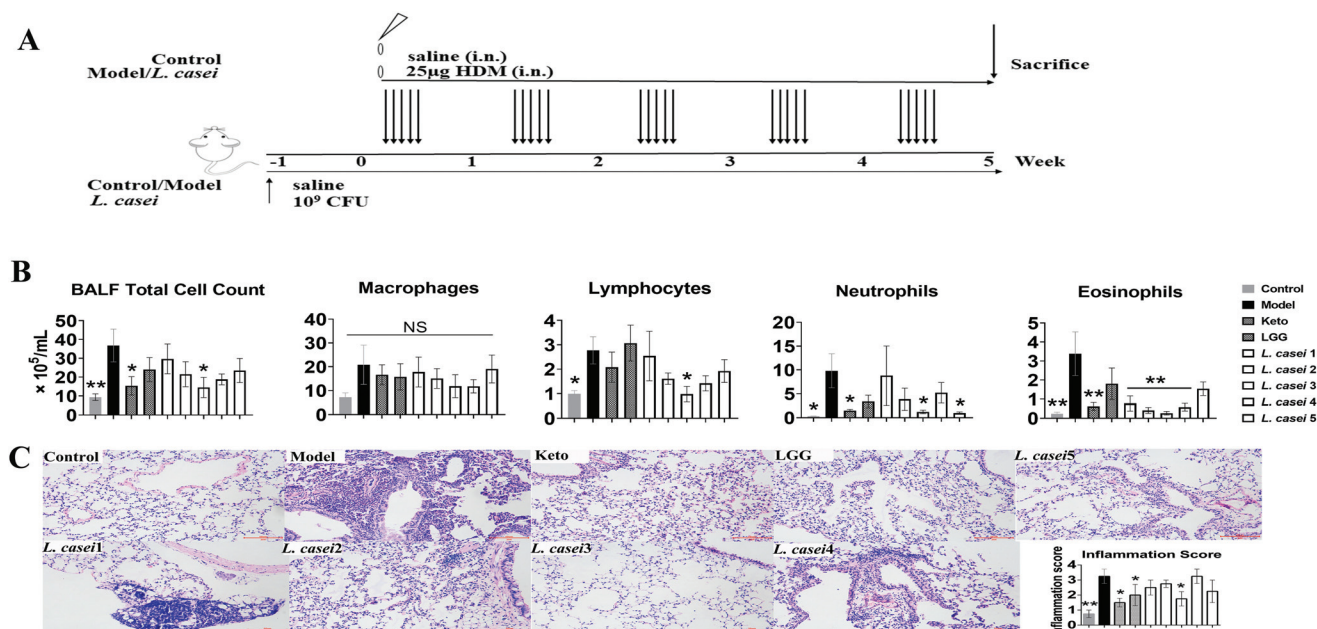


Fig. 1 The ability of five *Lactobacillus casei* strains to decrease airway inflammation in house dust mite (HDM)-induced asthma mouse model. (A) The timeline of HDM sensitization and challenge and the administration of five *L. casei* strains to the model. (B) Quantification of the total and differential cell counts in the bronchoalveolar fluid (BALF). (C) Airway inflammation in histologic lung sections subjected to hematoxylin and eosin staining. Scale bars, 50 μm . Inflammation score in the lung tissues. $n = 4-6$, $*p < 0.05$, $**p < 0.01$ vs. model. Data are represented as mean $\pm$ standard error of mean.

IL-13 and IFN- γ (Senbeijia, China); IL-4 and IL-5 (Mlbio, China); IL-9 (FCMACS, China); and IL-10 and IL-17A (R&D Systems, Minneapolis, USA). The levels of chemokines, CXCL1, and CCL11 (Senbeijia, China) were measured by ELISA. Measurements were performed according to the manufacturers' instructions.

2.6. Lung histamine and secretory immunoglobulin A (sIgA) analyses

The weight of the lungs was determined during sample preparation to ensure that an equal amount of weight was applied for histamine and sIgA measurements. The lung tissues were homogenized in saline solution (1:9, w/v) using a Polytron homogenizer (Scientz-48, China). The levels of histamine and sIgA were measured using Mouse Histamine and sIgA ELISA kits (Senbeijia Biotechnology Co., Ltd, China) according to the manufacturer's protocol.

2.7. Serum immunoglobulin analysis

The serum was frozen at -20°C . Immunoglobulins were measured using commercial ELISA kits. Total IgE was measured using Mouse IgE ELISA kits (Senbeijia, China), HDM-specific IgE, HDM-specific IgG1 and HDM-specific IgG2a were measured using Mouse Serum Anti-HDM IgG1 and Anti-HDM IgG2a Antibody Assay ELISA kits (Chondrex, Inc., USA). Measurements were performed according to the manufacturer's instructions.

2.8. Short-chain fatty acid (SCFA) analysis

SCFA levels in caecum contents (50 mg) were measured using the external standard method by gas chromatography-mass spectrometry (GCMS-QP2010 Ultra, Shimadzu) using a previously described method.²⁶

2.9. Sampling, DNA extraction, amplicon sequencing, and high-flux sequencing

Before the mice were sacrificed, the fecal samples were collected in a sterile tube, snap-frozen, and stored at -80°C . DNA was extracted from the fecal samples using the Fast DNA SPIN Kit for Feces (MP Biomedicals; Carlsbad, CA, USA), following the manufacturer's instructions. The 16S rRNA gene was subjected to PCR amplification using specific primers for the V3-V4 region of bacterial rRNA (341F: 5'-CCTAYGGGRBGCASCAG-3' and 806R: 5'-GGACTACNNGGTA-TCTAAT-3'). The PCR products were purified using the TIANGel Mini Purification Kit (Tiangen, Beijing, China). The purified PCR products were quantified using the Qubit dsDNA HS Assay Kit (Life Technologies Corporation, Carlsbad, CA, USA) and QuantiFluorTM-ST (Promega, USA). The amplicon libraries were pooled in equimolar amounts and subjected to sequencing on an Illumina MiSeq high-flux sequencing platform (Illumina, San Diego, USA). The sequencing data of 16S rRNA were processed as described previously.²⁷ Briefly, the sequencing data were filtered, merged, and demultiplexed for quality control. The raw sequence reads were clustered into operational taxonomic units (OTUs) at 97% similarity. The taxon-

omy of the obtained OTUs was analyzed using the Ribosomal Database Project (RDP) Classifier Bayesian Algorithm (<http://rdp.cme.msu.edu/>) against the SILVA 16S reference database.

2.10. Bioinformatic analyses

The dataset comprising 1 102 685 high-quality (21 621 counts per sample on average) classifiable read counts were generated after Illumina MiSeq sequencing of 51 samples. The number of counts in the samples ranged from 8238 to 45 973. All data were subjected to total sum scaling (TSS) normalization. The R phyloseq²⁸ and vegan packages²⁹ were used to analyze the community diversity profile. The data used for alpha diversity analysis were not standardized. The differences between the groups were analyzed by the Mann–Whitney/Kruskal–Wallis (non-parametric) tests. Linear discriminant analysis (LDA) effect size (LEfSe) was used to analyze the taxonomic differences between microbial communities.³⁰ The LDA score was computed for differentially abundant taxa between the two groups. The statistical significance was determined based on the following criteria: $|\log_{10} [\text{LDA score}]| \geq 2.0$ and $p < 0.1$. The microbial function was predicted using PICRUST (<http://huttenhower.sph.harvard.edu/galaxy>).³¹

2.11. Statistical analysis

The animal experiment data were analyzed using GraphPad Prism version 8 (La Jolla, CA, USA) and SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The differences were compared using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, and were considered statistically significant at $*p < 0.05$ or $**p < 0.01$. The data are expressed as mean $\pm$ standard error of mean (SEM). The analysis of the gut microbiota was performed using QIIME2 software.

3. Results

3.1. *L. casei* strains exert differential inhibitory effects on airway inflammatory cells

The effect of five *L. casei* strains on asthma was individually evaluated using an HDM-induced asthma mouse model (Fig. 1A). The mice were treated with *L. casei* both before and during exposure to HDM. Ketotifen and *Lactobacillus rhamnosus* GG (LGG) were used as positive controls. The *L. casei*-supplemented groups exhibited decreased total bronchioalveolar fluid (BALF) cell counts and decreased number of lymphocytes, eosinophils, and neutrophils, which are the major hallmarks of airway inflammation, when compared with the model group (Fig. 1B). However, the number of macrophages was similar in *L. casei*-supplemented and model groups. *L. casei*1, *L. casei*2, and *L. casei*4 significantly decreased the number of eosinophils ($p < 0.01$), while *L. casei*5 significantly decreased the number of neutrophils ($p < 0.05$). The *L. casei*3-supplemented group exhibited significantly decreased number of lymphocytes ($p < 0.05$) when compared with the model group. Furthermore, the inflammation score of the *L. casei*3-supplemented group (evaluation of HE-stained lung sections)

was lower than that of other *L. casei* strain-supplemented groups (Fig. 1C). These results indicate that all five *L. casei* strains exerted prophylactic effects on asthma by decreasing the number of inflammatory granulocytes. The highest inhibition of granulocytic inflammation was exhibited by *L. casei*3.

3.2. Differential effects of *L. casei* strains on the immune response in BALF

Medical consensus suggests that asthma can be prevented by suppressing Th2 and Th17 cell responses. The levels of Th2 cytokines (IL-4, IL-5, IL-13, and IL-9) and Th17 cytokine (IL-17A) were analyzed in the BALF. The model group exhibited increased levels of Th2 and Th17 cytokines. *L. casei*2, *L. casei*3, *L. casei*4 and *L. casei*5 significantly decreased the levels of IL-13 and IL-5 ($p < 0.01$ or $p < 0.05$). *L. casei*5 significantly decreased ($p < 0.01$) the levels of IL-4. *L. casei*1 and *L. casei*4 significantly decreased ($p < 0.05$) the levels of IL-9. *L. casei*3 significantly decreased the levels of both IL-4 ($p < 0.01$) and IL-9 ($p < 0.05$) (Fig. 2A). All five *L. casei* strains significantly decreased the levels of IL-17A ($p < 0.01$ or $p < 0.05$).

The decreased number of eosinophils and neutrophils induced by *L. casei* strains may be caused by decreased production of CCL11 (eotaxin-1) and CXCL1 (KC). Compared to the model group, the protein levels of CCL11 were significantly downregulated ($p < 0.05$) upon supplementation of *L. casei*3 and *L. casei*4, while those of CXCL1 were significantly downregulated upon supplementation of *L. casei*3 ($p < 0.05$) and *L. casei*5 ($p < 0.01$). Supplementation of *L. casei*1 and *L. casei*2 did not affect the levels of these chemokines (Fig. 2C).

The levels of major asthmatic mediators in lung homogenates were also analyzed. All *L. casei* strains, except *L. casei*1, significantly decreased ($p < 0.05$) the levels of histamine (Fig. 2D). The levels of sIgA were significantly upregulated upon supplementation of *L. casei*2 ($p < 0.01$), *L. casei*4 ($p < 0.01$), and *L. casei*5 ($p < 0.05$) (Fig. 2E).

Probiotics are reported to suppress asthmatic immune responses by inducing Treg (IL-10) cell responses. In this study, *L. casei*2 markedly increased the levels of IL-10 ($p < 0.01$) (Fig. 2F).

These results indicate that *L. casei* exerted prophylactic effects on asthma through various immunoregulatory mechanisms in the respiratory tract.

3.3. *L. casei* strains differentially suppress asthma-related Igs in the serum

The serum levels of asthma-related Igs were analyzed (Fig. 3). The levels of total IgE and HDM-specific IgG1 in *L. casei*-supplemented groups were downregulated when compared with those in the model group. Supplementation with *L. casei* strains did not affect the levels of HDM-specific IgG2a. HDM-specific IgE was undetectable in any group. *L. casei*3 and *L. casei*5 significantly decreased the levels of both total IgE and HDM-specific IgG1. *L. casei*1 significantly decreased ($p < 0.05$) the levels of total IgE, while *L. casei*4 significantly decreased ($p < 0.01$) the levels of HDM-specific IgG1. *L. casei*2 did not affect

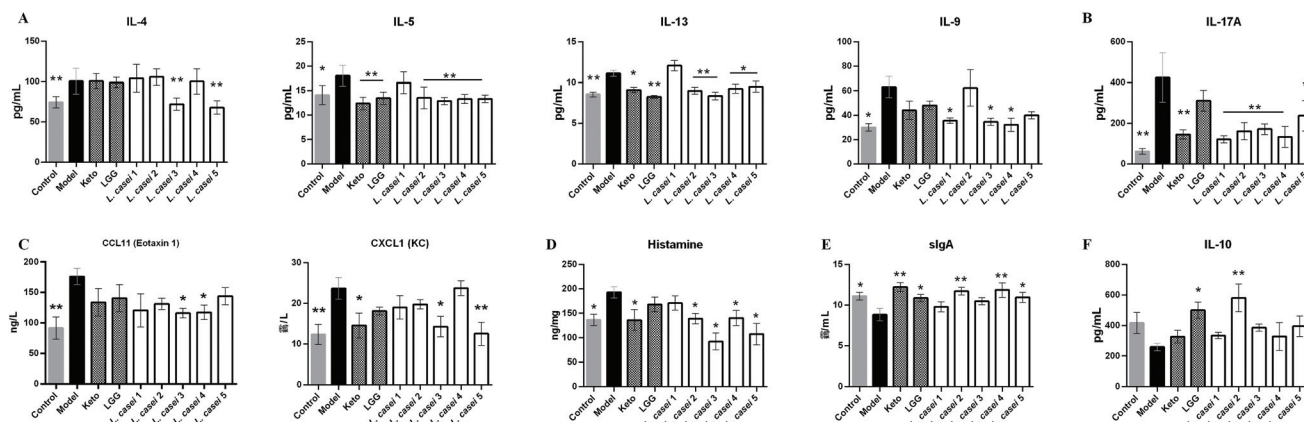


Fig. 2 Effect of five *Lactobacillus casei* strains on the levels of cytokines and immune mediators in the bronchoalveolar fluid (BALF) of house dust mite (HDM)-induced asthma mouse model. (A) The levels of Th2 (interleukin (IL)-4, IL-5, IL-13, and IL-9) cytokines. (B) Th17 (IL-17A). (C) The protein expression levels of CCL11 (eotaxin 1) and CXCL1 (KC). The levels of (D) histamine and (E) secretory immunoglobulin A (sIgA). (F) The levels of T regulatory cell cytokine (IL-10). $n = 6$, * $p < 0.05$, ** $p < 0.01$ vs. model. Data represent mean $\pm$ standard error of mean.

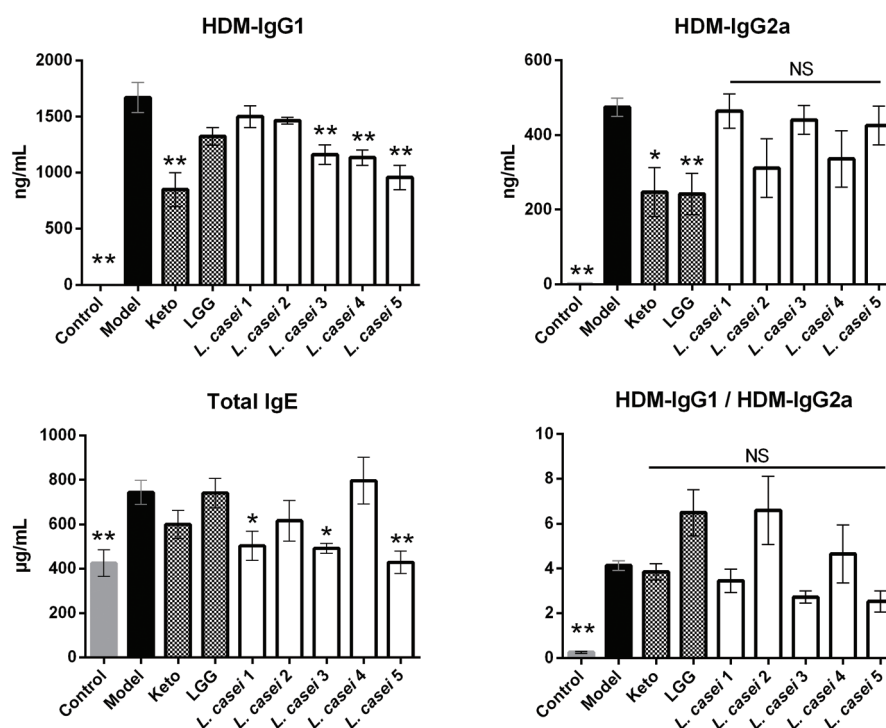


Fig. 3 Effect of five *Lactobacillus casei* strains on the serum levels of total immunoglobulin E (IgE), house dust mite (HDM)-specific IgG1, HDM-specific IgG2a, and HDM-specific IgG1/HDM-specific IgG2a in HDM-induced asthma mouse model. $n = 6$, * $p < 0.05$, ** $p < 0.01$ vs. model. Data are represented as mean $\pm$ standard error of mean.

the levels of these indicators. In addition, HDM-specific IgG1/HDM-specific IgG2a was often used to evaluate the Th2/Th1 immune balance. This balance in *L. casei*1, *L. casei*3 and *L. casei*5 groups were partly restored when compared with those in the model group. These results indicate that the different strains of *L. casei* exert differential prophylactic effects on asthma through various immunoregulatory mechanisms.

3.4. Differential effects of *L. casei* strains on the composition of the gut microbiota

The effects of *L. casei* on the gut microbiota were evaluated by analyzing the overall microbiota diversity, including within-group and across-group microbial diversities. The alpha diversity indices, including Shannon, Chao1, and observed OTU indices, were analyzed to examine the microbial diversity

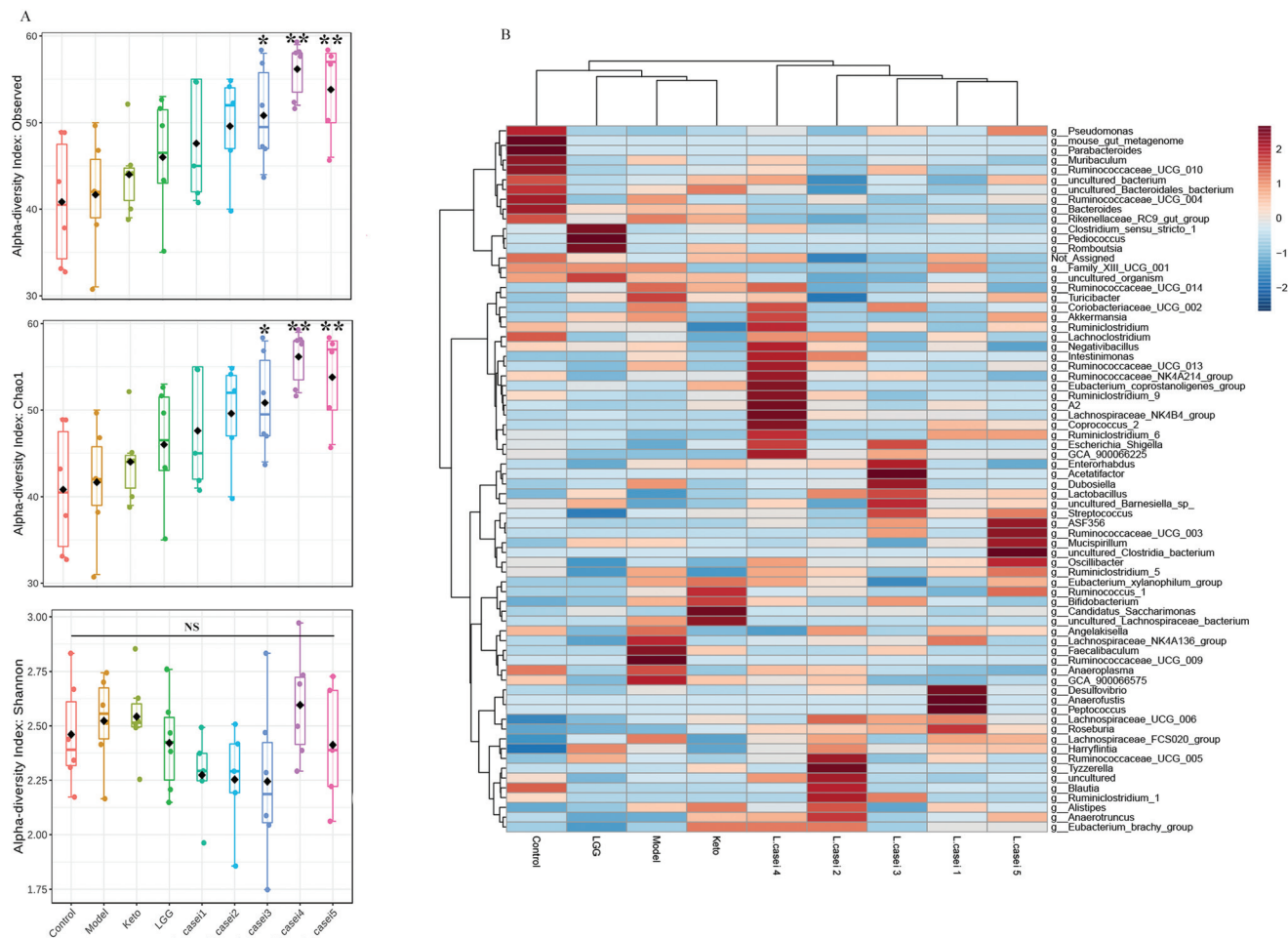


Fig. 4 Effect of five *Lactobacillus casei* strains on the composition of the gut microbiota in the house dust mite (HDM)-induced asthma mouse model. (A) Boxplot for α -diversity measured based on observed, Chao1, and Shannon indices at the genus level. (B) Heatmap analysis of species abundance clustering at the genus level. The heatmap shows the genera ranked based on abundance. Each row in the heatmap represents one genus, while each column represents one group. The red to blue color bar indicates the relative abundance of each genus. $n = 5-6$. * $p < 0.05$, ** $p < 0.01$.

within the group (Fig. 4A). The Shannon index metrics, including the evenness and richness parameters, revealed that there was no significant difference in the microbial diversity between the *L. casei*-supplemented and model groups. However, *L. casei* increased the Chao1 and observed OTU (richness) indices. In the across-group microbial diversity analysis, hierarchical clustering of the heatmap revealed significant alterations in the abundance of genera, which resulted in distinct clustering of bacterial genera (Fig. 4B). Interestingly, the microbial cluster of *L. casei*-supplemented groups was distinct from that of the control and model groups. These results indicate that the supplementation of *L. casei* increased the species population of the gut microbiota in the model group, which might further drive the formation of a new gut microbial structure.

To directly determine the increased abundance of species in the *L. casei*-supplemented groups, the composition of the gut microbiota was analyzed. The analysis revealed that >80%

of OTUs were assigned to Firmicutes and Bacteroidetes, which concurred with the results of previous studies.³² Consistent with the results of alpha diversity analysis, *L. casei* increased the total number of gut microbiota (Fig. 5A and Fig. S1†). The most abundant phylum in the *L. casei*-supplemented groups was Firmicutes. *L. casei*2, *L. casei*3, *L. casei*4, and *L. casei*5 significantly increased (all $p < 0.05$) the abundance of Proteobacteria, Actinobacteria ($p < 0.01$), Bacteroidetes ($p < 0.01$) and Proteobacteria, and Deferribacteres ($p < 0.01$) (Fig. 5B–F), respectively.

Compared to the model group, the *L. casei*-supplemented groups exhibited alterations in 28 genera (Fig. S2†). Of these 28 genera, 5 were differentially abundant in the control and model groups. *L. casei*2 increased the abundance of *Turicibacter* and decreased the abundance of *uncultured_bacterium*. *L. casei*3 and *L. casei*4 increased the abundance of *Ruminococcaceae_UCG_010*. *L. casei*5 increased the abundance of *Ruminococcaceae_UCG_014*. All five *L. casei* strains decreased

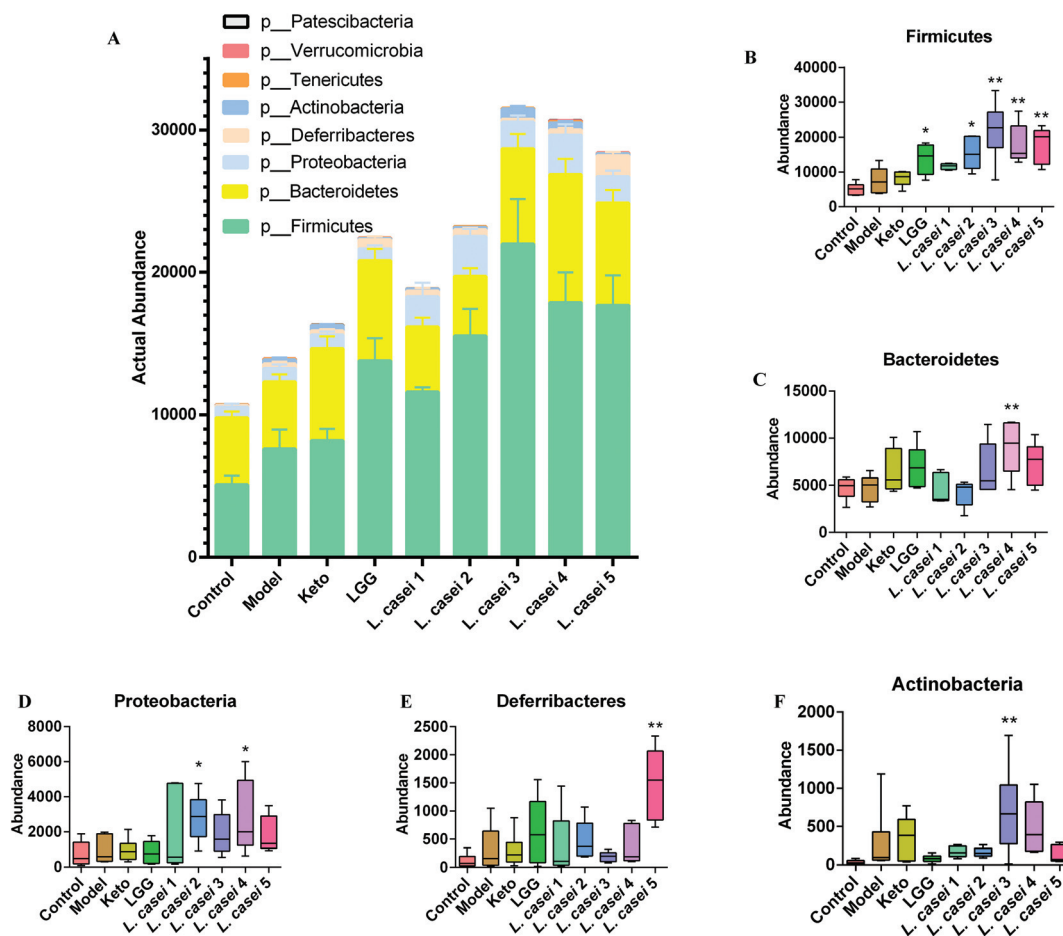


Fig. 5 Effect of five *Lactobacillus casei* strains on the abundance of bacterial phyla in the house dust mite (HDM)-induced asthma mouse model. (A) The abundance of bacterial phyla in the control, model, Ketotifen, *Lactobacillus rhamnosus* GG (LGG), and five *L. casei* supplementation groups. (B–F) The abundance of (B) Firmicutes, (C) Bacteroidetes, (D) Proteobacteria, (E) Deferribacteres, and (F) Actinobacteria in each group. $n = 5–6$, * $p < 0.05$, ** $p < 0.01$ vs. model. Data represent mean $\pm$ standard deviation of mean.

the abundance of *Bacteroides* (Fig. 6A). The abundance of the following four genera were markedly decreased in the *L. casei*-supplemented groups: *uncultured_Bacteroidales_bacterium*, *Ruminococcus_1*, *Anaeroplasm*, and *uncultured_organism* (Fig. 6B). The abundances of 19 genera were markedly increased in the *L. casei*-supplemented groups. Of these 19 genera, the abundances of 8 genera were increased in all *L. casei*-supplemented groups, while those of 11 genera were increased in some *L. casei*-supplemented groups (Fig. 6C). Among the genera exhibiting increased abundance, 80% belonged to the phylum Firmicutes (Fig. 6D). *ASF356*, *Pediococcus*, *Lachnospiraceae_NK4B4_group*, *Clostridium_sensu_stricto_1*, and *Coproccoccus_2* were not detected in the control or model groups. These results suggest that *L. casei* similarly modulates the gut microbiota composition at the phylum level but differentially modulates the gut microbiota at the genus level.

3.5. Differential effects of *L. casei* strains on gut microbiota metabolism

Intestinal microbiota metabolism is closely related to the development of asthma. Therefore, the gut microbial functions

were predicted using the 16S rRNA sequencing data in PICRUST. In total, 328 biological pathways were identified. The heatmap and cluster analysis revealed distinct patterns of metabolism in the control, model, and *L. casei*-supplemented groups (Fig. 7A). This indicated that the alterations in the gut microbiota composition affect the microbial metabolic patterns. As shown in Fig. S3,† compared to the model group, *L. casei*1, *L. casei*2, *L. casei*3, *L. casei*4, and *L. casei*5 significantly affected (all $p < 0.05$) 12, 53, 19, 1, and 12 biological pathways, respectively. After filtering out the pathways associated with drug development, human diseases, plant functions, and organismal systems,³³ the altered pathways enriched in the *L. casei*-supplemented groups were primarily associated with carbohydrate (fructose and mannose metabolism and pentose phosphate pathway), amino acid (valine, leucine, and isoleucine degradation), and lipid metabolisms (alpha-linolenic acid metabolism, ether lipid metabolism, biosynthesis of unsaturated fatty acids, and synthesis and degradation of ketone bodies) and biosynthesis of other secondary metabolites (betalain biosynthesis and indole alkaloid biosynthesis)

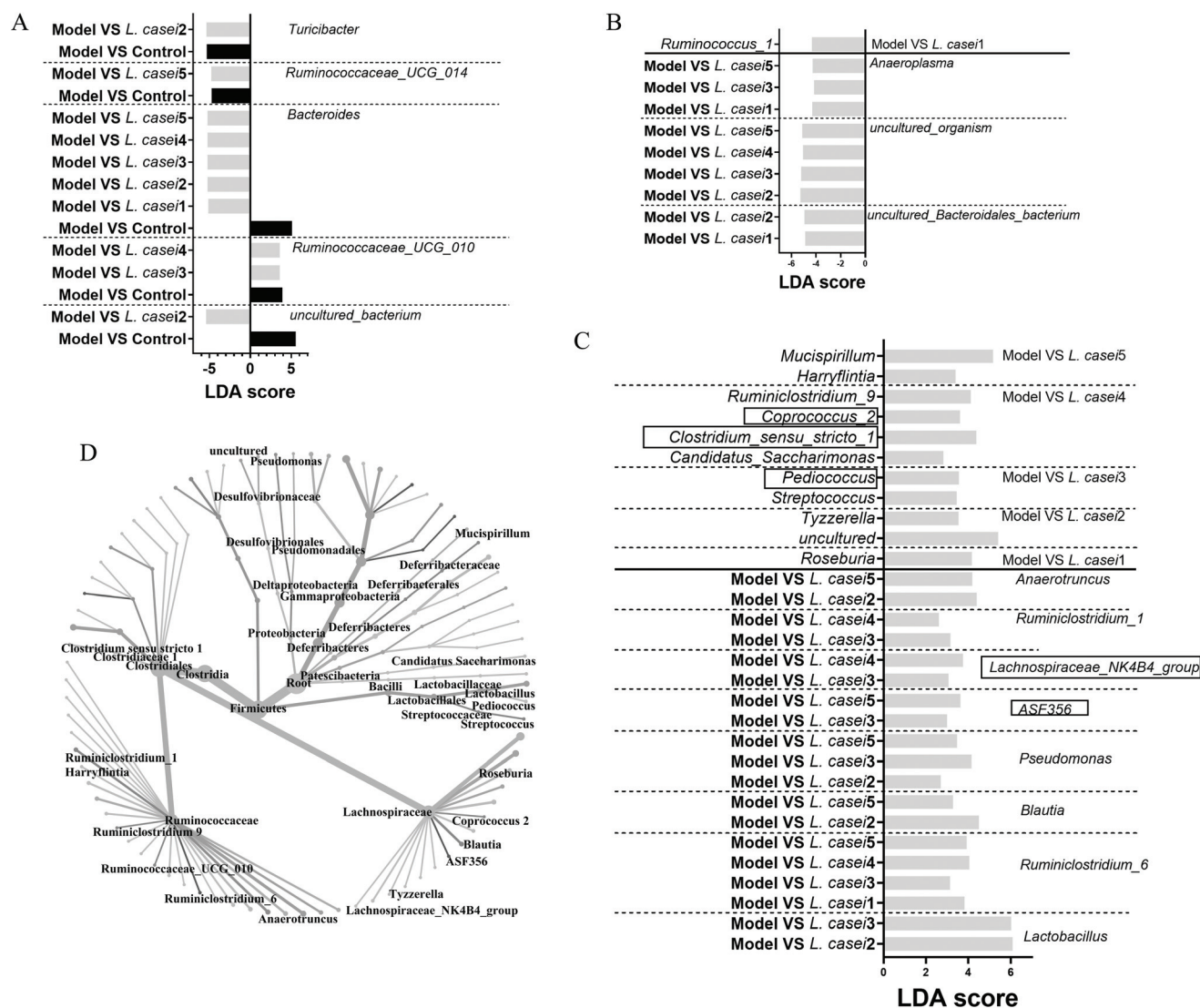


Fig. 6 Effect of five *Lactobacillus casei* on the composition of bacterial genera in the house dust mite (HDM)-induced asthma mouse model. (A) Bar plots reveal that consistent with the control and model groups, the abundance of five genera was altered in the *L. casei*-supplemented groups. (B) Bar plots reveal that compared to the model group, the abundance of 4 genera decreased in the *L. casei*-supplemented groups. The genera above the solid line exhibited individual decreased abundance in the *L. casei*-supplemented groups, while those below the solid line exhibited common decreased abundance in the five *L. casei*-supplemented groups. (C) Bar plots reveal that compared to the model group, the abundance of 19 genera was enriched in the *L. casei*-supplemented groups. The genera in the box were not detected in the control and model groups. The genera above the solid line exhibited individual increased abundance in the *L. casei*-supplemented groups, while those below the solid line exhibited common increased abundance in the five *L. casei*-supplemented groups. (D) Cladogram of enriched bacterial genera in the five *L. casei*-supplemented groups. $n = 5-6$, $|\log_{10} [\text{linear discriminant analysis (LDA) score}]| \geq 2.0$, $p < 0.1$.

(Fig. 7B). Additionally, the analysis of SCFA, which is the most potent intestinal metabolites for alleviation of asthma, revealed that *L. casei*3 increased the contents of acetate and propionate (Fig. 7C).

4. Discussion

Probiotics have been reported to exert prophylactic effects on autoimmune diseases, such as asthma, but the underlying mechanisms are unknown.^{14,15,19,20} *L. casei* is a common pro-

biotic and widely used in fermented dairy products. Some studies have shown that it modulated the composition of the gut microbiota and improved metabolic and immune activity.²¹⁻²² Previously, we had performed a large-scale screening of *Lactobacillus* strains and demonstrated that a mixture of five *L. casei* strains exhibited prophylactic effects on asthma.²³ Here, we investigated the prophylactic effects of each of the five *L. casei* strains on asthma. HDM-induced asthma is a well-established mixed chronic airway inflammatory model in female mice (the female is more sensitive than the male in this model³⁴) that results in simultaneous infiltration of eosi-

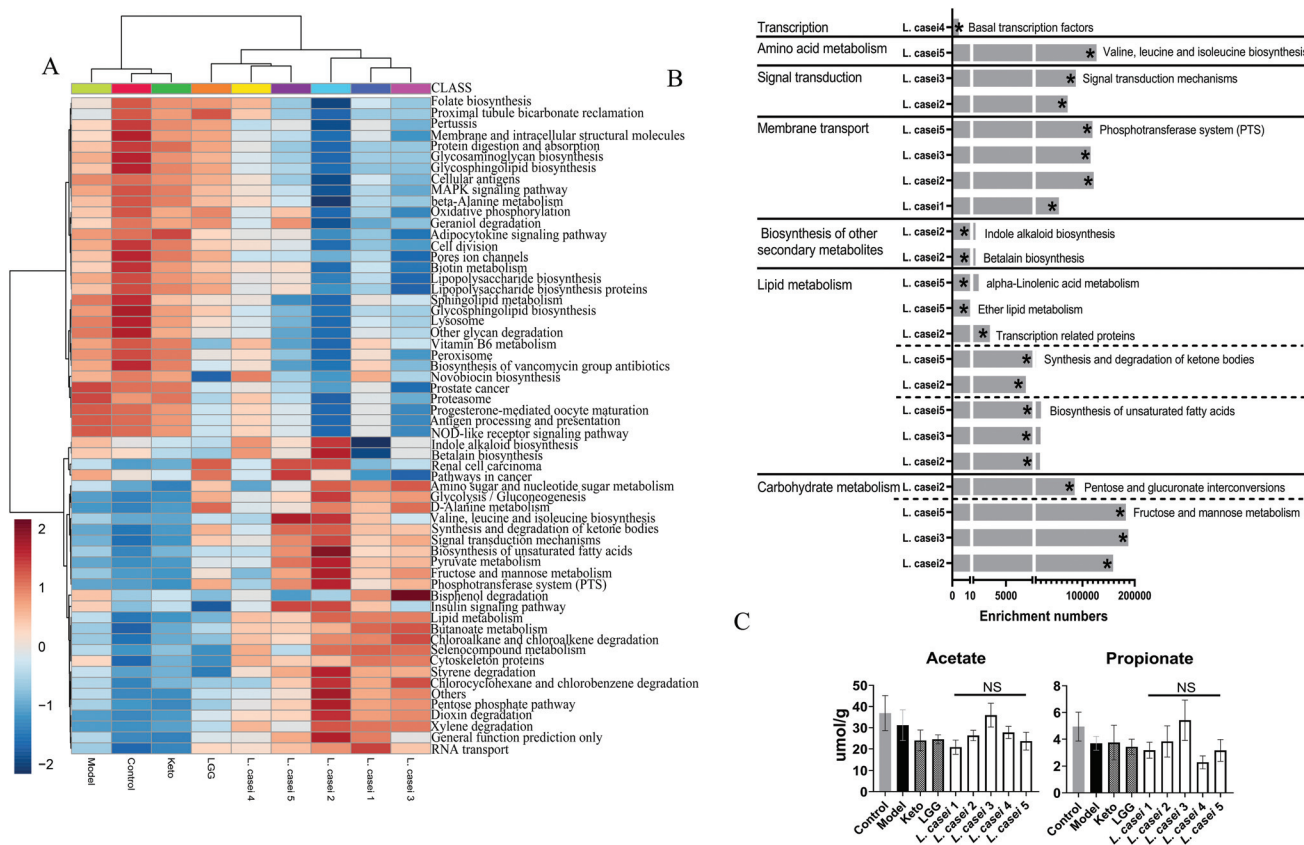


Fig. 7 Effect of five *Lactobacillus casei* strains on the metabolism of the gut microbiota. (A) Heatmap analysis of the fecal metabolite profiles in the control, model, Ketotifen, *Lactobacillus rhamnosus* GG (LGG), and five *L. casei*-supplemented groups. The heatmap shows the top 60 significantly altered metabolites in the groups. Each row in the heatmap represents one metabolite, while each column represents one group. The color bar (blue to red) indicates the relative content of metabolites. (B) Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis based on the individual and common metabolisms enriched in the five *L. casei*-supplemented groups. (C) The levels of acetate, propionate, and butyrate in the cecum of mice. $n = 5-6$, $*p < 0.05$.

nophils and neutrophils in the lungs. This model can comprehensively measure the prophylactic effects of *L. casei* on asthma. The strains used in this study belonged to the same *Lactobacillus* species, were isolated from infants or traditional farming sites, and were orally administered to each mouse at 10^9 CFU (the right concentration to produce benefits³⁵). Thus, analyzing the effects of individual strains on asthma in this study can provide useful insights into the differential prophylactic effects.

Herein, compared with Ketotifen and commercial strain *Lactobacillus rhamnosus* GG, these strains exhibited prophylactic effects on HDM-induced asthma in mice through different modulatory mechanisms. In particular, *L. casei*3 (CCFM1073) strain-dependently improved the composition and metabolite production of the gut microbiota, and regulated the immune response related to infiltration of eosinophils and neutrophils, which might facilitate the highest prophylactic effect on asthma through the gut-lung axis.

During the progression of asthma, enhanced secretion and reciprocal regulation of Th2 and Th17 cell inflammatory cytokines contribute to mixed eosinophilic and neutrophilic

inflammation. Currently, there are no therapeutic agents for multiple phenotypes of asthma.¹ In this study, *L. casei*3, *L. casei*4, and *L. casei*5 inhibited the infiltration of eosinophils and neutrophils into the lung, which was consistent with the suppression of chemokine production. However, *L. casei*1 and *L. casei*2 did not suppress the production of chemokines, which indicated that these *L. casei* strains inhibited eosinophilic and neutrophilic asthma through a mechanism that was not dependent on the suppression of chemokine production in mice. The pathogenic mechanism of asthma indicates that targeting systemic immunity and mucosal immunity can simultaneously affect the production of Th2 and Th17 cell cytokines and related signaling pathways, and *L. casei* is reported to regulate the immune response in humans.^{22,36-38} The Treg cells, which are involved in systemic immunity, regulate multiple immunological processes. In asthma, impairment of Treg cell numbers is associated with increased secretion of T-cell inflammatory cytokines and asthma-related Igs.³⁷ IL-10 is the main cytokine produced by Treg cells. In this study, HDM significantly decreased the levels of IL-10, which was increased upon supplementation of *L. casei*2. This indicated that probio-

tics exert prophylactic effects on asthma by promoting Treg cell response, which is consistent with the previous studies.³⁸ IgA is the major mediator of mucosal immunity.³⁹ Several studies and clinical reports have demonstrated that IgA is associated with innate receptor signaling in T-cells.³⁸ Additionally, the low levels of intestinal and salivary sIgA are reported to be associated with an increased risk for developing asthma.^{40–42} The BALF of the model group exhibited low levels of sIgA in this study. However, the supplementation of *L. casei*2, *L. casei*4, and *L. casei*5 increased the levels of sIgA in the BALF. To our knowledge, we firstly demonstrated the ability of probiotics to enhance sIgA levels under asthmatic conditions, which might be beneficial for asthma prophylaxis. These findings suggest that different strains of *L. casei* exert prophylactic effects on asthma through various immunoregulatory mechanisms.

Some studies have demonstrated that the development of asthma is dependent on the distinct diversity and composition of the gut microbiota during early life.^{10,11,43} Probiotics including *L. casei* are reported to have modulatory effect on the gut microbiota.^{15,19–22} In this study, the diversity of the gut microbiota in *L. casei*-supplemented groups was higher than that in control and model groups. Additionally, the composition of the gut microbiota in *L. casei*-supplemented groups was different from that in the control and model groups. This is inconsistent with the results of previous studies, which reported that probiotics could restore the gut microbial structure of the control group.¹¹ This may be due to the ability of *L. casei* to promote the growth of Firmicutes (>80%). However, Firmicutes exhibiting increased abundance in the *L. casei*-supplemented groups belong to 20 bacterial genera. Recent studies have suggested that the immune dysfunctions are associated with changes in the abundance of specific gut bacterial genera.¹⁴ Hence, the distinct immunoregulatory activities mediated by prophylactic effects of *L. casei* on asthma may be associated with these distinct bacterial genera.

However, the bacteria exerting protective or harmful effects on asthma have not been identified, especially on eosinophilic and neutrophilic asthma. Inflammation is reported to provide a favorable environment for opportunistic pathogens, such as *Turicibacter*,^{44,45} which is reported to be abundant in patients with rheumatoid arthritis.⁴⁶ Moreover, the members of the Erysipelotrichaceae family, including *Turicibacter*, which are enriched in patients with colorectal cancer,^{47,48} can induce immune hyperactivation⁴⁹ resulting in the development of asthma. Here, the abundance of *Turicibacter* was low (only 2%) in the control group. However, the exposure to HDM increased the abundance of this genus, which is consistent with the results of our previous study.²³ This indicated that *Turicibacter* can be a biomarker for the development of asthma in mice. Moreover, *L. casei*2 decreased the abundance of *Turicibacter* in the HDM-induced allergy mouse model, which indicated that specific *L. casei* could inhibit the growth of opportunistic pathogen.

The members of the Gram-positive Lachnospiraceae family, which colonize the inner mucus layer and are in close contact

with the host mucosa, may protect the mucosal barrier by increasing the IgA levels.^{50,51} Additionally, the members of the Lachnospiraceae are reported to be immunogenic, and promote Th1 response and inhibit Th2 response.^{52,53} Thus, the members of Lachnospiraceae could enhance the mucosal barrier function and promote the positive differentiation of T-cells under asthmatic conditions. In this study, the abundance of the members of Lachnospiraceae was markedly high in all *L. casei*-supplemented groups. However, different strains of *L. casei* differentially affected the members. Of note, among these members the *ASF356*, *Lachnospiraceae_NK4B4_group*, and *Coprococcus_2* were not detected in the control or model groups. This suggested that the mechanism underlying the prophylactic effect of *L. casei* on asthma involves enhancing the growth of beneficial bacteria. However, these results remain at the genus level and further metagenomics studies are needed.

The gut microbiota also produces metabolites, which interact with the host. These interactions are observed not only in the intestinal tract but also in surrounding tissues, such as the lungs. This study showed that all *L. casei* altered the metabolic pattern of the gut microbiota, which is consistent with the earlier studies.^{21,22} Previous studies have reported that children with asthma undergoing treatment exhibited altered carbohydrate and lipid metabolisms.⁵⁴ Furthermore, Firmicutes play an important role in modulating carbohydrate and lipid metabolisms.^{55–58} In this study, *L. casei*2, *L. casei*3, and *L. casei*5 promoted the metabolism of carbohydrates and lipids and the growth of Firmicutes. SCFA, as an end-product of carbohydrate and lipid metabolisms, is a potential immunomodulator that decreases the susceptibility to asthma by modulating airway inflammation⁵⁹ and regulating Treg cell expansion.⁶⁰ The decreased levels of acetate and propionate in the model group were increased upon supplementation of *L. casei*3. This indicated that the fermentative efficiency of carbohydrates and lipids was enhanced upon *L. casei* supplementation by promoting the growth of Firmicutes. Subsequently, the corresponding increased production of metabolites, such as SCFA could protect against asthma. Additionally, the induction of biosynthesis of indole alkaloid,⁶¹ valine, leucine, and isoleucine⁶² is reported to protect against asthma. In this study, the levels of these metabolites were upregulated upon supplementation of *L. casei*2 or *L. casei*5. These results suggest that the prophylactic effects of *L. casei* strains on asthma may be mediated by their differential ability to modulate carbohydrate, lipid and amino acid metabolisms.

5. Conclusions

This study demonstrated that five different strains of *L. casei* exhibited prophylactic effects on mixed chronic airway inflammatory asthma in model mice by intraspecific independent regulation of immune responses and the gut microbiota composition and metabolism. Among the five strains, *L. casei*3 (*L.*

casei CCFM1073) exhibited the highest prophylactic effect on asthma. This study provides the potential resources for the application of probiotics as a prophylactic for asthma. Further studies based on these results will help elucidate the mechanism underlying the prophylactic effect of probiotics on asthma and other autoimmune diseases.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [Grant No. 31820103010, 31771953, and 31871774], the Fundamental Research Funds for the Central Universities [JUSRP51903B], and the National First-class Discipline Program of Food Science and Technology [JUFSTR20180102].

References

- 1 A. Papi, C. Brightling, S. E. Pedersen and H. K. Reddel, Asthma, *Lancet*, 2018, **391**, 783–800.
- 2 M. F. Moffatt, M. Kabesch, L. Liang, A. L. Dixon, D. P. Strachan, S. Heath, M. Depner, A. Von Berg, A. Bufer and E. Rietschel, Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma, *Nature*, 2007, **448**, 470–473.
- 3 M. A. Portelli, E. Hodge and I. Sayers, Genetic risk factors for the development of allergic disease identified by genome-wide association, *Clin. Exp. Allergy*, 2015, **45**, 21–31.
- 4 S. R. Del Giacco, A. Bakirtas, E. Bel, A. Custovic, Z. Diamant, E. Hamelmann, E. Heffler, O. Kalayci, S. Saglani, S. Sergejeva, S. Seys, A. Simpson and L. Bjermer, Allergy in severe asthma, *Allergy*, 2017, **72**, 207–220.
- 5 R. H. Green, C. E. Brightling, G. Woltmann, D. Parker, A. J. Wardlaw and I. D. Pavord, Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids, *Thorax*, 2002, **57**, 875–879.
- 6 B. N. Lambrecht and H. Hammad, The immunology of asthma, *Nat. Immunol.*, 2015, **16**, 45.
- 7 C. Doe, M. Bafadhel, S. Siddiqui, D. Desai, V. Mistry, P. Rugman and C. E. Brightling, Expression of the T Helper 17-Associated Cytokines IL-17A and IL-17F in Asthma and COPD, *Chest*, 2010, **138**, 1140–1147.
- 8 J. L. Round and S. K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, *Nat. Rev. Immunol.*, 2009, **9**, 313–323.
- 9 A. J. Macpherson and N. L. Harris, Interactions between commensal intestinal bacteria and the immune system, *Nat. Rev. Immunol.*, 2004, **4**, 478–485.
- 10 T. R. Abrahamsson, H. E. Jakobsson, A. F. Andersson, B. Björkstén, L. Engstrand and M. C. Jenmalm, Low gut microbiota diversity in early infancy precedes asthma at school age, *Clin. Exp. Allergy*, 2014, **44**, 842–850.
- 11 M. C. Arrieta, L. T. Stiemsma, P. A. Dimitriu, L. Thorson, S. Russell, S. Yurist-Doutsch, B. Kuzeljevic, M. J. Gold, H. M. Britton, D. L. Lefebvre, P. Subbarao, P. Mandhane, A. Becker, K. M. McNagny, M. R. Sears, T. Kollmann, W. W. Mohn, S. E. Turvey and B. B. Finlay, Early infancy microbial and metabolic alterations affect risk of childhood asthma, *Sci. Transl. Med.*, 2015, **7**, 307ra152.
- 12 C. Vael, V. Nelen, S. L. Verhulst, H. Goossens and K. N. Desager, Early intestinal *Bacteroides fragilis* colonisation and development of asthma, *BMC Pulm. Med.*, 2008, **8**, 19.
- 13 J. Penders, C. Thijs, P. A. van den Brandt, I. Kummeling, B. Snijders, F. Stelma, H. Adams, R. van Ree and E. E. Stobberingh, Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study, *Gut*, 2007, **56**, 661–667.
- 14 K. Atarashi and K. Honda, Microbiota in autoimmunity and tolerance, *Curr. Opin. Immunol.*, 2011, **23**, 761–768.
- 15 S. P. Borriello, W. P. Hammes, W. Holzapfel, P. Marteau, J. Schrezenmeir, M. Vaara and V. Valtonen, Safety of probiotics that contain lactobacilli or bifidobacteria, *Clin. Infect. Dis.*, 2003, **36**, 775–780.
- 16 M. J. Butel, Probiotics, gut microbiota and health, *Med. Maladies Infect.*, 2014, **44**, 1–8.
- 17 T. D. Nguyen, O. Prykhodko, F. F. Hållénus and M. Nyman, Effects of monobutyrin and tributyrin on liver lipid profile, caecal microbiota composition and SCFA in high-fat diet-fed rats, *J. Nutr. Sci.*, 2017, **6**, e51.
- 18 A. C. Gomes, A. A. Bueno, R. G. M. de Souza and J. F. Mota, Gut microbiota, probiotics and diabetes, *Nutr. J.*, 2014, **13**, 60.
- 19 E. Anatriello, A. Britto, R. Albertini, A. P. De Oliveira, A. Keller and F. Aimbire, *Lactobacillus bulgaricus* reduces the expression of Toll-like 4 receptor on dendritic cells and attenuates the airway inflammation in asthmatic mice, *Eur. Respir. J.*, 2018, **52**, PA1107.
- 20 J. Durack, N. E. Kimes, D. Lin, M. McKean, M. Rauch, M. D. Cabana and S. Lynch, Early-life *Lactobacillus rhamnosus* GG supplementation of high-risk for Asthma infants reprograms gut microbiota development and promotes regulatory T-cells, *J. Allergy Clin. Immunol.*, 2017, **139**, AB15.
- 21 A. Bezkorovainy, Probiotics: determinants of survival and growth in the gut, *Am. J. Clin. Nutr.*, 2001, **73**, 399S.
- 22 S. Spanhaak, R. Havenaar and G. Schaafsma, The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans, *Eur. J. Clin. Nutr.*, 1998, **52**, 899–907.
- 23 L. Li, Z. Fang, X. Liu, W. Hu, W. Lu, Y. K. Lee, J. Zhao, H. Zhang and W. Chen, *Lactobacillus reuteri*, attenuated allergic inflammation induced by HDM in the mouse and modulated gut microbes, *PLoS One*, 2020, **15**, e0231865.

- 24 J. R. Johnson, R. E. Wiley, R. Fattouh, F. K. Swirski, B. U. Gajewska, A. J. Coyle, J. C. Gutierrez-Ramos, R. Ellis, M. D. Inman and M. Jordana, Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling, *Am. J. Respir. Crit. Care Med.*, 2004, **169**, 378–385.
- 25 W. R. Henderson Jr., D. B. Lewis, R. K. Albert, Y. Zhang, W. J. Lamm, G. K. Chiang, F. Jones, P. Eriksen, Y. T. Tien, M. Jonas and E. Y. Chi, The importance of leukotrienes in airway inflammation in a mouse model of asthma, *J. Exp. Med.*, 1996, **184**, 1483–1494.
- 26 N. M. Moreau, S. M. Goupry, J. P. Antignac, F. J. Monteau, B. J. Le Bizec, M. M. Champ, L. J. Martin and H. J. Dumon, Simultaneous measurement of plasma concentrations and ¹³C-enrichment of short-chain fatty acids, lactic acid and ketone bodies by gas chromatography coupled to mass spectrometry, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2003, **784**, 395–403.
- 27 B. Mao, D. Li, J. Zhao, X. Liu, Z. Gu, Y. Q. Chen, H. Zhang and W. Chen, Metagenomic insights into the effects of fructo-oligosaccharides (FOS) on the composition of fecal microbiota in mice, *J. Agric. Food Chem.*, 2015, **63**, 856–863.
- 28 P. J. McMurdie and S. Holmes, phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data, *PLoS One*, 2013, **8**, e61217.
- 29 P. Dixon, VEGAN, a package of R functions for community ecology, *J. Veg. Sci.*, 2003, **14**, 927–930.
- 30 N. Segata, J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W. S. Garrett and C. Huttenhower, Metagenomic biomarker discovery and explanation, *Genome Biol.*, 2011, **12**, 1–18.
- 31 M. G. Langille, J. Zaneveld, J. G. Caporaso, D. McDonald, D. Knights, J. A. Reyes, J. C. Clemente, D. E. Burkepille, R. L. Vega Thurber, R. Knight, R. G. Beiko and C. Huttenhower, Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences, *Nat. Biotechnol.*, 2013, **31**, 814–821.
- 32 P. B. Eckburg, E. M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson and D. A. Relman, Diversity of the human intestinal microbial flora, *Science*, 2005, **308**, 1635–1638.
- 33 O. Wang, T. A. McAllister, G. Plastow, K. Stanford, B. Selinger and L. L. Guan, Interactions of the Hindgut Mucosa-Associated Microbiome with Its Host Regulate Shedding of Escherichia coli O157:H7 by Cattle, *Appl. Environ. Microbiol.*, 2018, **84**, e01738-17.
- 34 C. Carlson, M. Cushman, P. Enright, J. Cauley and A. B. Newman, Hormone replacement therapy is associated with higher FEV1 in elderly women, *Am. J. Respir. Crit. Care Med.*, 2001, **163**, 423.
- 35 G. Wang, X. F. Li, J. X. Zhao, H. Zhang and W. Chen, Lactobacillus casei CCFM419 attenuates type 2 diabetes via a gut microbiota dependent mechanism, *Food Funct.*, 2017, **8**, 3155–3164.
- 36 D. F. Choy, K. M. Hart, L. A. Borthwick, A. Shikotra, D. R. Nagarkar, S. Siddiqui, G. Jia, C. M. Ohri, E. Doran, K. M. Vannella, C. A. Butler, B. Hargadon, J. C. Sciarba, R. L. Gieseck, R. W. Thompson, S. White, A. R. Abbas, J. Jackman, L. C. Wu, J. G. Egen, L. G. Heaney, T. R. Ramalingam, J. R. Arron, T. A. Wynn and P. Bradding, TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma, *Sci. Transl. Med.*, 2015, **7**, 301ra129.
- 37 H. K. Kwon, C. G. Lee, J. S. So, C. S. Chae, J. S. Hwang, A. Sahoo, J. H. Nam, J. H. Rhee, K. C. Hwang and S. H. Im, Generation of regulatory dendritic cells and CD4⁺ Foxp3⁺ T cells by probiotics administration suppresses immune disorders, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 2159–2164.
- 38 J. L. Kubinak, C. Petersen, W. Z. Stephens, R. Soto, E. Bake, R. M. O'Connell and J. L. Round, MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health, *Cell Host Microbe*, 2015, **17**, 153–163.
- 39 B. Corthesy, Multi-faceted functions of secretory IgA at mucosal surfaces, *Front. Immunol.*, 2013, **4**, 185.
- 40 M. Fageras, S. Tomicic, T. Voor, B. Bjorksten and M. C. Jenmalm, Slow salivary secretory IgA maturation may relate to low microbial pressure and allergic symptoms in sensitized children, *Pediatr. Res.*, 2011, **70**, 572–577.
- 41 K. Kukkonen, M. Kuitunen, T. Haahtela, R. Korpela, T. Poussa and E. Savilahti, High intestinal IgA associates with reduced risk of IgE-associated allergic diseases, *Pediatr. Allergy Immunol.*, 2010, **21**, 67–73.
- 42 A. Sandin, B. Bjorksten, M. F. Bottcher, E. Englund, M. C. Jenmalm and L. Braback, High salivary secretory IgA antibody levels are associated with less late-onset wheezing in IgE-sensitized infants, *Pediatr. Allergy Immunol.*, 2011, **22**, 477–481.
- 43 M. Hilty, C. Burke, H. Pedro, P. Cardenas, A. Bush, C. Bossley, J. Davies, A. Ervine, L. Poulter, L. Pachter, M. F. Moffatt and W. O. Cookson, Disordered microbial communities in asthmatic airways, *PLoS One*, 2010, **5**, e8578.
- 44 M. Nguyen, A. Sharma, W. Wu, R. Gomi, B. Sung, D. Hospodsky, L. T. Angenent and S. Worgall, The fermentation product 2,3-butanediol alters P. aeruginosa clearance, cytokine response and the lung microbiome, *ISME J.*, 2016, **10**, 2978–2983.
- 45 P. Rausch, N. Steck, A. Suwandi, J. A. Seidel, S. Kunzel, K. Bhullar, M. Basic, A. Bleich, J. M. Johnsen, B. A. Vallance, J. F. Baines and G. A. Grassl, Expression of the Blood-Group-Related Gene B4galnt2 Alters Susceptibility to Salmonella Infection, *PLoS Pathog.*, 2015, **11**, e1005008.
- 46 J. Chen, K. Wright, J. M. Davis, P. Jeraldo, E. V. Marietta, J. Murray, H. Nelson, E. L. Matteson and V. Taneja, An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis, *Genome Med.*, 2016, **8**, 43.
- 47 W. Chen, F. Liu, Z. Ling, X. Tong and C. Xiang, Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer, *PLoS One*, 2012, **7**, e39743.
- 48 Q. Zhu, Z. Jin, W. Wu, R. Gao, B. Guo, Z. Gao, Y. Yang and H. Qin, Analysis of the intestinal lumen microbiota in an animal model of colorectal cancer, *PLoS One*, 2014, **9**, e90849.

- 49 M. Maruya, S. Kawamoto, L. M. Kato and S. Fagarasan, Impaired selection of IgA and intestinal dysbiosis associated with PD-1-deficiency, *Gut Microbes*, 2013, **4**, 165–171.
- 50 G. E. Kaiko and T. S. Stappenbeck, Host-microbe interactions shaping the gastrointestinal environment, *Trends Immunol.*, 2014, **35**, 538–548.
- 51 G. M. Nava, H. J. Friedrichsen and T. S. Stappenbeck, Spatial organization of intestinal microbiota in the mouse ascending colon, *ISME J.*, 2011, **5**, 627–638.
- 52 W. Wu, H. P. Liu, F. Chen, H. Liu, A. T. Cao, S. Yao, M. Sun, H. L. Evans-Marin, Y. Zhao, Q. Zhao, L. W. Duck, C. O. Elson, Z. Liu and Y. Cong, Commensal A4 bacteria inhibit intestinal Th2-cell responses through induction of dendritic cell TGF-beta production, *Eur. J. Immunol.*, 2016, **46**, 1162–1167.
- 53 H. Liu, S. Yao, Y. Cong and C. Elson, Commensal flagellated A4 bacteria promote intestinal Th1/Th17 cell development but inhibit Th2 responses, *J. Immunol.*, 2012, **188**, 49.3.
- 54 M. Turpeinen, R. Sorva and K. Juntunen-Backman, Changes in carbohydrate and lipid metabolism in children with asthma inhaling budesonide, *J. Allergy Clin. Immunol.*, 1991, **88**, 384–389.
- 55 R. E. Ley, F. Backhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight and J. I. Gordon, Obesity alters gut microbial ecology, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 11070–11075.
- 56 A. Everard, V. Lazarevic, M. Derrien, M. Girard, G. G. Muccioli, A. M. Neyrinck, S. Possemiers, A. Van Holle, P. Francois, W. M. de Vos, N. M. Delzenne, J. Schrenzel and P. D. Cani, Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice, *Diabetes*, 2011, **60**, 2775–2786.
- 57 J. P. Furet, L. C. Kong, J. Tap, C. Poitou, A. Basdevant, J. L. Bouillot, D. Mariat, G. Corthier, J. Dore, C. Henegar, S. Rizkalla and K. Clement, Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers, *Diabetes*, 2010, **59**, 3049–3057.
- 58 P. J. Turnbaugh, R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis and J. I. Gordon, An obesity-associated gut microbiome with increased capacity for energy harvest, *Nature*, 2006, **444**, 1027–1031.
- 59 A. Trompette, E. S. Gollwitzer, K. Yadava, A. K. Sichelstiel, N. Sprenger, C. Ngom-Bru, C. Blanchard, T. Junt, L. P. Nicod, N. L. Harris and B. J. Marsland, Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis, *Nat. Med.*, 2014, **20**, 159–166.
- 60 P. M. Smith, M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, M. Bohlooly-Y, J. N. Glickman and W. S. Garrett, The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis, *Science*, 2013, **341**, 569–573.
- 61 A. Baxter, J. Steele and S. Teague, Use of indole-3-acetic acids in the treatment of asthma, COPD and other diseases, *U.S. Patent Application* 10/503823, 2005.
- 62 J. Jung, S. H. Kim, H. S. Lee, G. S. Choi, Y. S. Jung, D. H. Ryu, H. S. Park and G. S. Hwang, Serum metabolomics reveals pathways and biomarkers associated with asthma pathogenesis, *Clin. Exp. Allergy*, 2013, **43**, 425–433.

File Name : fig.s1.tif

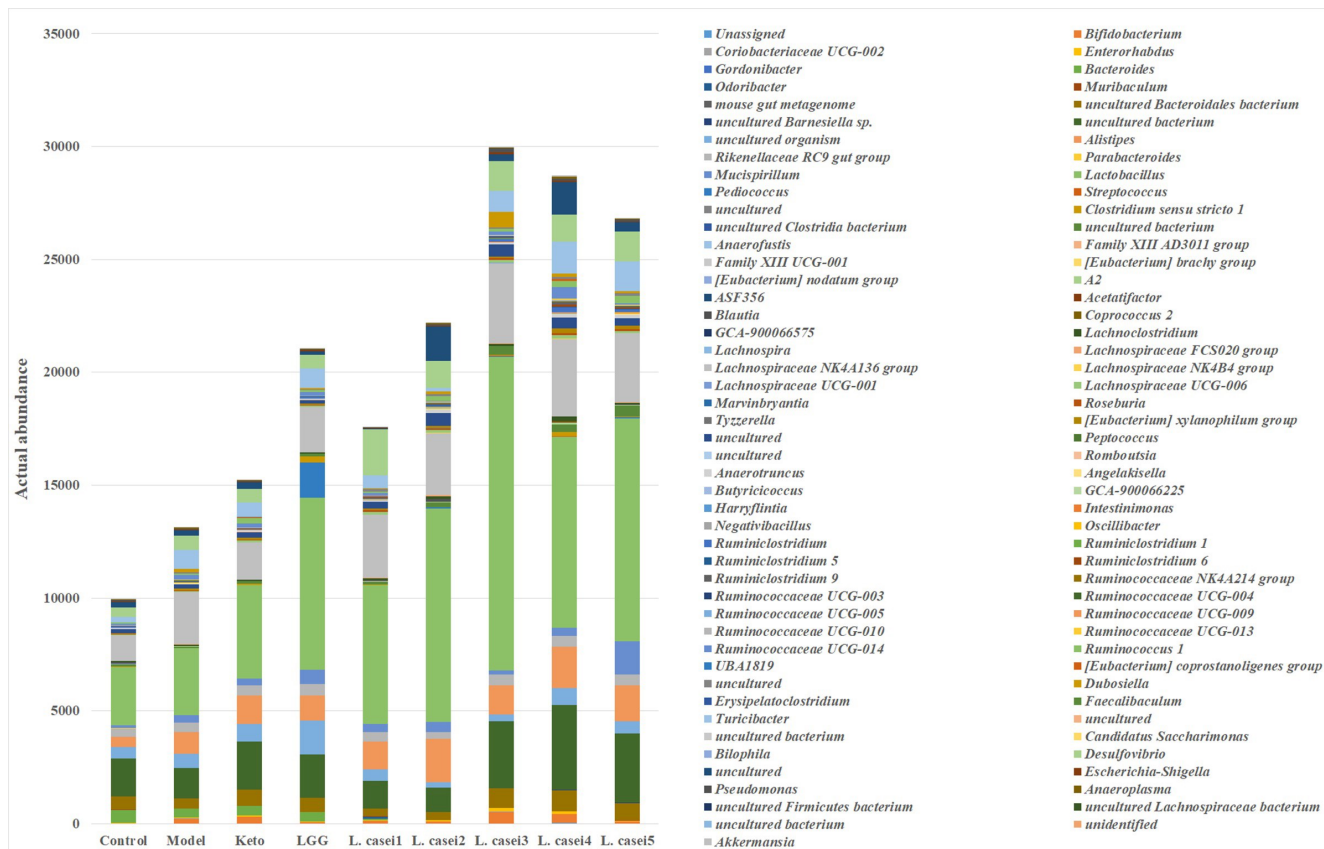
Caption : figure s1. effect of five lactobacillus casei strains on the abundance of bacterial genera in house dust mite (hdm)-induced allergy mouse model. the abundance of bacterial genera in the control, model, ketotifen, lactobacillus rhamnosus gg (lgg), and five l. casei-supplemented groups. n=5-6.

File Name : fig.s2.tif

Caption : figure s2. comparative analysis of bacterial genera from the control, model, ketotifen, lactobacillus rhamnosus gg (lgg), and five lactobacillus casei-supplemented groups. the red box represents significant enrichment, while the blue box represents a significant decrease in the group when compared in pairs. n=5-6, $|\log_{10} [\text{linear discriminant analysis (lda) score}]| \geq 2.0$, $p < 0.1$.

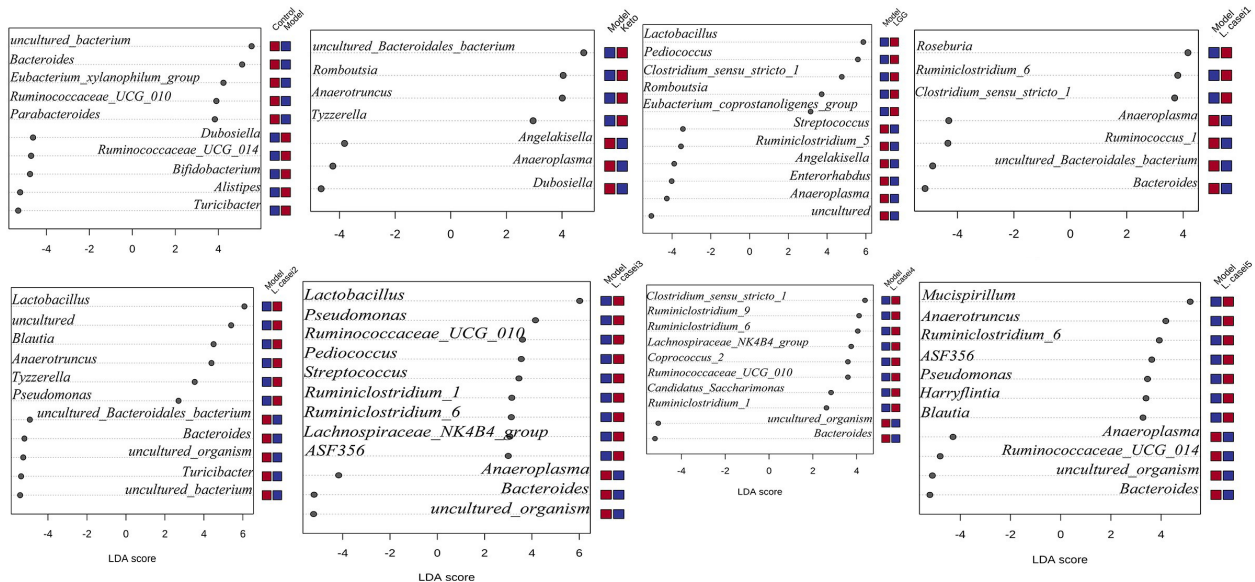
File Name : fig.s3.tif

Caption : figure s3. comparative kyoto encyclopedia of genes and genomes (kegg) pathway analysis of the bacterial metabolism in the control, model, ketotifen, lactobacillus rhamnosus gg (lgg), and five lactobacillus casei-supplemented groups. n=5-6, $p < 0.05$.



Electronic Supplementary Material (ESI) for Food & Function.

This journal is © The Royal Society of Chemistry 2020



Electronic Supplementary Material (ESI) for Food & Function.

This journal is © The Royal Society of Chemistry 2020



Table S1. Strains used in animal experiments

Group	Strain	Source
<i>L. casei</i> 1	M2-07-F01-L4-2-1	Human feces
<i>L. casei</i> 2	M2-06-F01-L4-2-3	Cow dung
<i>L. casei</i> 3	CCFM1073 (JS-WX-3-L-3)	Infant feces
<i>L. casei</i> 4	RS8-5	Fermented milk
<i>L. casei</i> 5	FGDLZ41	Infant feces