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Recombinant *Lactiplantibacillus plantarum* modulate gut microbial diversity and function

Quntao Huang^{1†}, Junhong Xing^{2†}, Fang Tang¹, Jianluan Ren¹, Chunfeng Wang^{2*} and Feng Xue^{1*}

Abstract

Background Gut microbes are important regulators of host health and can also function as disease indicators. *Lactiplantibacillus plantarum* (*L. plantarum*) used as express and delivery vaccines for mucosal immunity have been shown to activate specific immune responses in numerous studies.

Results The interaction between recombinant *L. plantarum* and the gut microbiota was investigated in this study. The results indicated a change in the amount of gut OTU by recombinant *L. plantarum*. Recombinant *L. plantarum* dramatically boosted the species diversity of gut bacteria based on the Shannon-Wiener index. Beta diversity analysis showed that microbial structure was changed by recombinant *L. plantarum*. Furthermore, recombinant NC8 *L. plantarum* expressing a fusion between the P14.5 protein of the African swine fever virus and IL-33 enhanced the functions of gut bacteria in metabolism and immune regulation. Increased levels of IgG and IgG1 in serum and sIgA in feces, as well as enrichment of CD4⁺ T cells and IgA⁺ B cells, indicated that the gut microbiota exerted an immunomodulatory role when mediated by recombinant *L. plantarum*.

Conclusions These results revealed that recombinant *L. plantarum* exerted its potential role in the gut microbiota and gut immunity. These findings contribute to a broader understanding and utilization of *L. plantarum* bacteria in various therapeutic applications.

Keywords Recombinant *L. Plantarum*, Gut microbes, IgG, sIgA, CD4⁺ T cell

Background

Lactiplantibacillus plantarum (*L. plantarum*) are widely recognized as safe organisms suitable for use in biological therapies [1]. The primary health benefits of administering recombinant *L. plantarum* via mucosa have been successfully demonstrated in animal models, underscoring the potential of recombinant *L. plantarum* for therapeutic applications, such as antibody or vaccine production [2]. For instance, recombinant probiotics expressing the P14.5 protein of the African swine fever virus, when combined with an adjuvant, have been shown to generate specific antibodies against the P14.5 protein in mice and enhance the adaptive immune response in mice [3]. In a previous study, we demonstrated that recombinant *L. plantarum* expressing the HA1 protein of the H₇N₉ virus

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can trigger a specific immune response [4]. However, it remains unclear whether colonization by recombinant *L. plantarum* affects the intrinsic gut microbiota.

The close relationship between the gut microbiome and human health has led to a significant expansion in microbiome research in recent years [5]. The gut microbiota influences health through various pathways, including metabolism, immunological defense, and nervous system modulation [6]. The taxonomic composition of the gut microbiome is shaped by both intrinsic and external microbiome variables. Extrinsic factors encompass numerous environmental layers that interact with or influence the gut microbiome, whereas intrinsic factors pertain to the microbiome's own contributions [7]. Moreover, an abnormal state of the body can affect the composition of the gut microbiome [8]. Conditions such as obesity [9] or inflammatory bowel disease [10] result in a decrease in gut microbial diversity or richness. Another study found significant changes in the fecal microbiota of COVID-19 patients, including an increase in opportunistic pathogens and a decrease in beneficial symbionts [11]. Additionally, resident and transient microbes, along with their metabolites, may facilitate cancer formation and progression through the circulatory system [12].

The gut microbiota can influence host intestinal immune function through derivatives and metabolites, affecting various types of cells, such as intestinal epithelial cells and B- and T-lymphocytes [13]. Moreover, gut microbes serve as barriers by interacting extensively with immune cells [14]. To prevent the invasion of foreign pathogenic bacteria, homeostasis in the gut requires interactions between gut microbes and the immune system [15]. -chain fatty acids produced by gut microbes increase IL-22 synthesis by innate lymphocytes (ILCs) and CD4⁺ T cells through G protein receptor 41 (GPR41) and histone deacetylase inhibition (HDAC) [16]. In the gut, a group of 11 bacterial strains isolated from feces of healthy human donors was found to strongly stimulate IFN-producing CD8⁺T cells. Their presence enhances the host's resistance to *Listeria monocytogenes* [17]. Secreted immunoglobulin A (sIgA) can bind to commensal microbes in the gut and prevent invading pathogens, thereby maintaining intestinal mucosal immunity and barrier function [18]. Studies have suggested that transferring probiotic-mediated gut bacteria can correct gut microbiota imbalances induced by external stimuli or antibiotics, thereby improving the gut immune function [19, 20].

L. plantarum NC8 is an important lactic acid bacterium widely present in various fermented foods and plants. As a probiotic, the NC8 strain plays an important role in the food industry and health sector. It has strong antioxidant capacity and the ability to inhibit the growth of harmful bacteria, which can improve the intestinal

microbiota environment and enhance immunity. In addition, *L. plantarum* NC8 has been studied for the production of functional foods and beverages, with certain health benefits such as antibacterial, antiviral, and lipid-lowering effects. It has strong adaptability, can survive in various environments, and has a wide tolerance range for temperature and pH values, making it widely applicable in food processing and preservation [21].

In a previous study, it was demonstrated that recombinant *L. plantarum* expressing the P14.5 protein of the African swine fever virus can stimulate immune responses targeting virus-specific antigens. We fused and expressed two different adjuvants (CTA1-DD and IL-33) with the p14.5 protein, respectively. CTA1-DD is an artificial adjuvant composed of the enzymatically active CTA1 subunit of cholera toxin and the D domain dimer of the *Staphylococcus aureus* protein A. IL-33 is a multi-functional protein discovered in 2005. It is secreted during cell damage and plays a role in regulating immunity. IL-33 can directly participate in DC responses to stimulate the differentiation of naive T cells into Th2 or helper T cells. During viral infection, IL-33 can help clear a virus by promoting the expansion of NK and NKT cells and enhancing Th1 and CD8⁺T cell responses. The final result showed that the adjuvant effect of CTA1-DD was stronger than that of IL-33 [3]. In this study, we investigated the interactions between *L. plantarum* and the gut microbes. Furthermore, we validated the role of the gut microbiota facilitated by recombinant *L. plantarum*, providing a solid groundwork for research and development of *L. plantarum* vaccines.

Materials and methods

Construction of recombinant *L. Plantarum*

The base sequences of the ASFV E120R gene (gene number: 41901181) and IL-33-Mus gene (gene number: AY905582.1) were obtained from NCBI, and the base sequences of the CTA1 gene and DD gene (gene number: OL547739) were obtained from the literature and optimized and synthesized by Nanjing Genscript synthesis. The p14.5, p14.5-IL-33-Mus (PIM) and CTA1-p14.5-D-D, CPD, genes were cloned into the pLP-S vector. The constructed plasmid was transformed into *L. plantarum* NC8 (CCUG61730). Three recombinant *L. plantarum* including NC8-pLP-S-p14.5, NC8-pLP-S-p14.5-IL-33-Mus and NC8-pLP-S-CTA1-p14.5-D-D were generated. The bacteria were sequenced and identified by Comate Bioscience Co., Ltd. (Changchun, China).

Animals and experimental design

6-week-old C57BL/6 female mice were purchased from Beijing Weitehe Laboratory Animal Technology Co., Ltd. Mice were allowed to eat and drink ad libitum and were acclimated to the environment for one week before the

experiments. Animal experiments followed the requirements of the 113 Animal Management and Ethics Committee 123 of Jilin Agricultural University. Fifty mice were randomly divided into five groups, with ten mice in each group. The mice were orally administered with 200 μ L of NC8-pLP-S, NC8-pLP-S-p14.5, NC8-pLP-Sp14.5-IL-33-Mus or NC8-pLP-S-CTA1-p14.5-D-D with 1×10^9 CFU colony-forming units. The control group was given 0.9% normal saline (200 μ L) by the same method. The mice were first immunized on the 1st, 2nd and 3rd days, then received booster immunization on the 10th, 11th and 12th days, and finally boosted again on the 21st, 22nd and 23rd days.

Antibiotic treatment and FMT

Two experimental groups of mice were given combination antibiotics on day -7 for one week in this study. Subsequently, mice received fecal bacterial transplantation in the PBS group and the PIM group on day 0, respectively. Serum, feces, mesenteric lymph nodes and Peyer's patches of mice were collected on day 14 after fecal bacterial transfer for further research. Mice in the control group were orally administered PBS at each stage.

Ampicillin (1 mg/ml), streptomycin (5 mg/ml), vancomycin (0.25 mg/ml), and mucin (1 mg/ml) were added (Solarbio, China) mice were given sterile drinking water for 7 days before FMT to eliminate the mouse gut microbial background as much as possible. On day 7, the PIM group received Group mouse feces and was processed as described previously. Briefly, stool samples were placed in a sterile N2 incubator, mixed well, then inserted into a cell strainer to collect bacteria and centrifuged at 12,000 rpm for 10 min. After that, the samples were placed in 10% glycerol and stored at -80. The glycerol was then replaced with PBS, and the number of viable microorganisms was counted using methylene blue. Mice ($n=5$) were gavaged with a volume of 100 μ L. FMT experiments were independently repeated twice.

The mice were sacrificed by neck dislocation under inhaled isoflurane anesthesia.

Extraction of genome DNA and amplicon generation

The CTAB technique was used to extract whole genomic DNA from samples. On 1% agarose gels, DNA concentration and purity were measured. DNA was diluted to 1ng/L in sterile water according to the concentration. 16 S rRNA/18S rRNA/ITS genes from different locations (16 S V4/16S V3/16S V3-V4/16S V4-V5, 18 S V4/18S V9, ITS1/ITS2, Arc V4) were amplified using particular primers with the barcode (e.g. 16 S V4: 515F806R, 18 S V4: 528 F-706R, 18 S V9: 1380 F-1510R, et al. All PCR procedures used 15 L of New England Biolabs' Phusion® High-Fidelity PCR Master Mix, 2 M of forward and reverse primers, and around 10 ng template DNA. Initial

denaturation at 98 °C for 1 min was followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. Finally, cook for 5 min at 72 °C.

PCR products identification and Library preparation

Combine the PCR products with the same volume of 1X loading buffer (including SYB green) and run electrophoresis on a 2% agarose gel for detection. Equidensity ratios were used to blend PCR products. The PCR products were then purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). Following the manufacturer's instructions, sequencing libraries were created using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), and index codes were added. The Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system were used to evaluate the library's quality. Finally, the library was sequenced on an Illumina NovaSeq device, resulting in 250 bp paired-end reads.

Paired-end reads assembly and quality control

Samples were assigned paired-end readings based on their unique barcode, which were then shortened by removing the barcode and primer sequence. FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) [22] was used to combine paired-end reads when at least portion of the reads overlap the read generated from the opposite end of the same DNA fragment, and the splicing sequences were referred to as raw tags. According to the QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) [23] quality regulated process, quality filtering on the raw tags was done under defined filtering circumstances to generate high-quality clean tags [24]. To detect chimera sequences, the tags were compared to the reference database (Silva database, <https://www.arb-silva.de/>) using the UCHIME algorithm (UCHIME http://www.drive5.com/usearch/manual/uchime_algo.html) [25], and the chimera sequences were removed. Finally, the Effective Tags were obtained.

OTU cluster and species annotation

Uparse software (Uparse v7.0.1001, <http://drive5.com/uparse/>) was used to analyze the sequences [26]. OTUs were allocated to sequences that were 97% similar. For further annotation, a representative sequence for each OTU was screened. The Silva Database (<http://www.arb-silva.de/>) [27] was used to annotate taxonomic information for each representative sequence using the Mothur algorithm. Multiple sequence alignment was performed using the MUSCLE software (Version 3.8.31, <http://www.drive5.com/muscle/>) [28] to investigate the phylogenetic relationship of distinct OTUs and the differences in dominant species in different samples(groups). The abundance of OTUs was normalized using a sequence

number standard that corresponded to the sample with the fewest sequences. On the basis of this output normalized data, subsequent analyses of alpha and beta diversity were conducted.

Extract the complete 16 S rRNA gene sequences of prokaryotes from the KEGG database and align them to the SILVA SSU Ref NR database using the BLASTN algorithm (BLAST bitscore>1500) to establish a correlation matrix. Map the functional information of prokaryotic whole genomes annotated by both UProC and PAUDA methods from the KEGG database to the SILVA database, achieving functional annotation in the SILVA database. Sequence samples use SILVA database sequences as reference sequences to cluster OTUs, thereby obtaining functional annotation information.

Alpha diversity

The Observed-species, Chao1, Shannon, and Good-coverage indices are used to analyze the complexity of species diversity for a sample. All the indices in our samples were calculated with QIIME (Version 1.7.0) and shown using R. (Version 2.15.3). Two indices were selected to identify Community richness: Chao - the Chao1 estimator (<http://www.mothur.org/wiki/Chao>); Two indices were used to identify Community diversity: Shannon - the Shannon index (<http://www.mothur.org/wiki/Shannon>); One index to characterized Sequencing depth: Coverage - the Good's coverage (<http://www.mothur.org/wiki/Coverage>).

Beta diversity

Beta diversity analysis was performed to assess differences in sample species complexity. QIIME software (Version 1.9.1) estimated beta diversity on both weighted and unweighted unifracs. Prior to cluster analysis, the FactoMineR and ggplot2 packages in R software (Version 2.15.3) were used to reduce the dimension of the original variables using principal component analysis (PCA). To obtain primary coordinates and show complicated, multidimensional data, Principal Coordinate Analysis (PCoA) was used. A distance matrix of weighted or unweighted unifracs between previously collected samples was changed to a new set of orthogonal axes, with the first primary coordinate displaying the largest variation factor, the second principal coordinate displaying the second maximum factor, and so on. PCoA analysis was displayed by WGCNA package, stat packages and ggplot2 package in R software (Version 2.15.3). Unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage and was conducted by QIIME software (Version 1.9.1).

Flow cytometry analysis

The mouse mesenteric lymph nodes and spleen were collected to prepare a single-cell suspension, and cells were examined using Alexa Fluor® 700-labeled anti-mouse CD₃ (BD, USA), BB700-labeled anti-mouse CD4 (BD, USA), APC-labeled anti-mouse CD₈ (BD, USA), APC-labeled anti-mouse B220 (BD, USA) and FITC-labeled anti-mouse IgA (BD, USA). Detected by FLOW cytometry with LM Fortessa. The data were analyzed by FlowJo7.6.1 software.

ELISA

On the 14th day after FMT, serum and feces were collected from 3 mice in each group, and 300 µL of PBS solution containing 1% PMSF was added to each 0.1 g of feces, mixed and placed in a 4 °C refrigerator overnight. Aspirate the supernatant the next day (gently aspirate with a pipette to avoid aspirating the precipitate), aliquot, and store in a -80 °C refrigerator. Following the ELISA KIT manufacturer's instructions, serum samples were tested for IgG (Beyotime, China), IgG1 (MEIMIAN, China) and IgG2a (MEIMIAN, China), and stool samples were tested for sIgA (MEIMIAN, China).

Statistical analysis

All data were analysed with Graphpad Prism 8.0 software. Student's t test was used for pairwise comparisons, and one-way ANOVA was used for comparisons of more than two groups. The values are shown as the mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001.

Results

Recombinant *L. Plantarum* affect the number of intestinal OTUs

The OTUs were clustered based on the effective tags of all samples with 97% identity (Identity) to study the species composition of each sample. Subsequently, species annotation was performed on the out sequences. The number of OTUs per sample is shown in Fig. 1A. The diversity of the microbial communities was assessed using Shannon–Wiener analysis (Fig. 1B). Species accumulation analysis (Fig. 1C), and quantitative analysis of species annotation (Fig. 1D) was conducted for all samples, demonstrating sufficient data sampling and sequencing depth. The 16 S rRNA gene sequence database covered nearly all microbial communities. A total of 884 OTUs were shared by all groups. Within the PBS, NC8, P14.5, CPD, and PIM groups, 243, 182, 138, 93, and 137 distinct OTUs were observed, respectively (Fig. 1E). Additionally, there were 467 and 948 OTUs unique to PBS and NC8, respectively, with 1610 OTUs in common (Fig. 1F).

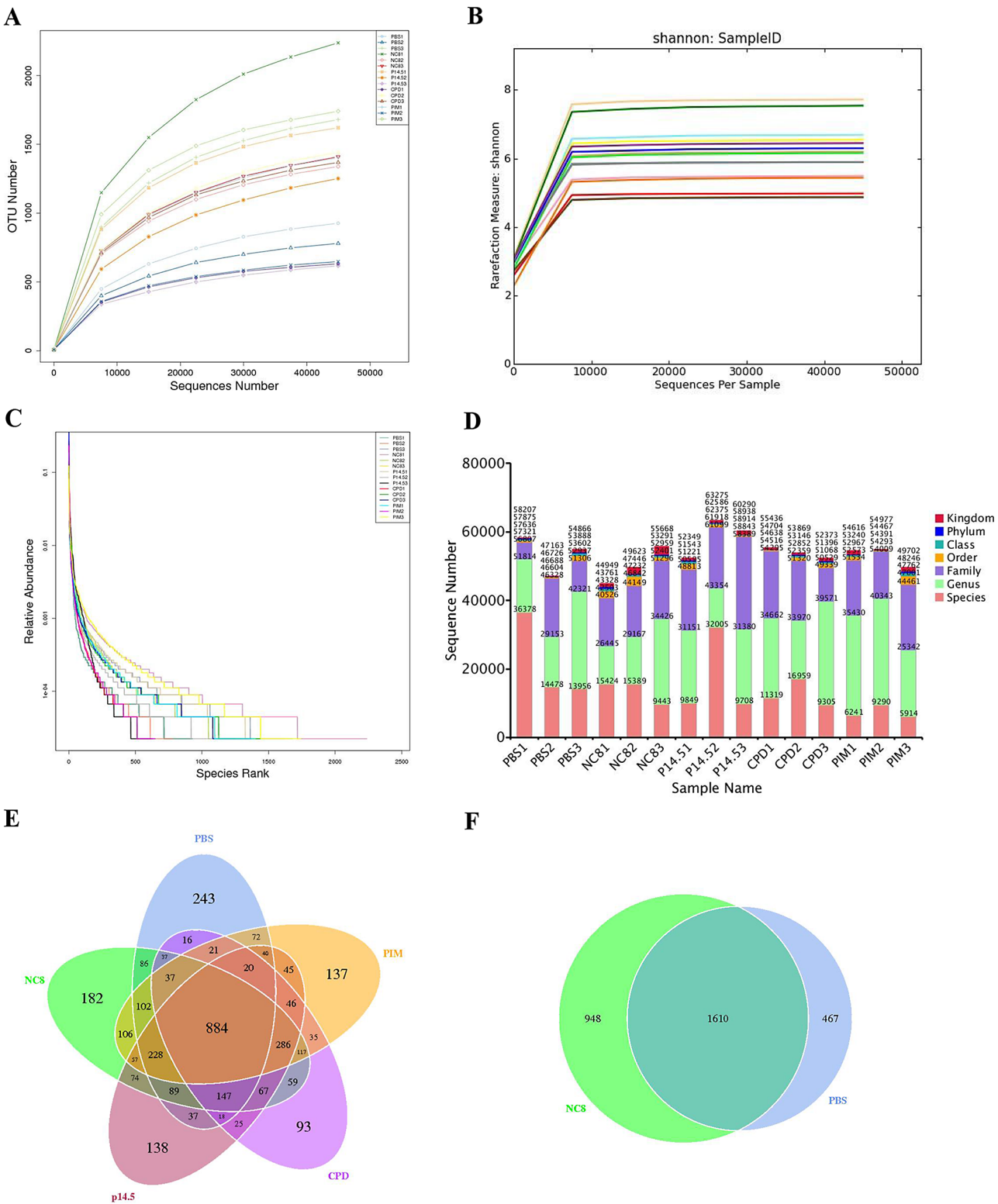


Fig. 1 Statistical analysis of intestinal microbial OTU by recombinant lactic acid bacteria. **(A)** Statistics of the number of sample OTUs. **(B)** Shannon-Wiener analysis of sequencing data. **(C)** Species accumulation curves. **(D)** Quantitative analysis of species annotation of samples at each level. **(E)** OTU analysis at 97% similarity in different groups. **(F)** OTU analysis at 97% similarity in NC8 and PBS groups. Abbreviations: NC8, samples from NC8-pLP-S group; P14.5, samples from NC8-pLP-S-p14.5 group; CPD, samples from NC8-pLP-S-CTA1-p14.5-D-D group. PIM, samples from NC8-pLP-S-p14.5-IL-33-Mus group

Alpha diversity analysis of gut microbes

To evaluate alpha diversity, we examined the Chao1 index (Fig. 2A), and observed species index (Fig. 2B), the Shannon–Wiener index (Fig. 2C), and Good's coverage (Fig. 2D). Compared with the PBS group, treatment with NC8 or recombinant *L. plantarum* significantly increased species richness, as indicated by the observed species and Chao1 index. According to Good's coverage analysis, the NC8 group had a much lower coverage than the other groups. Meanwhile, the Shannon–Wiener index data revealed that the NC8 and PIM groups had

significantly enhanced species diversity compared to the other groups. These findings suggest that recombinant *L. plantarum* can influence the diversity of gut bacteria.

Beta diversity analysis of gut microbes

The OTU information of the same categorization was consolidated and processed, and a distance matrix was produced using the phylogenetic relationship between OTUs based on the species annotation results of all samples and the abundance information of OTUs. The beta diversity of gut bacteria was analyzed using differences

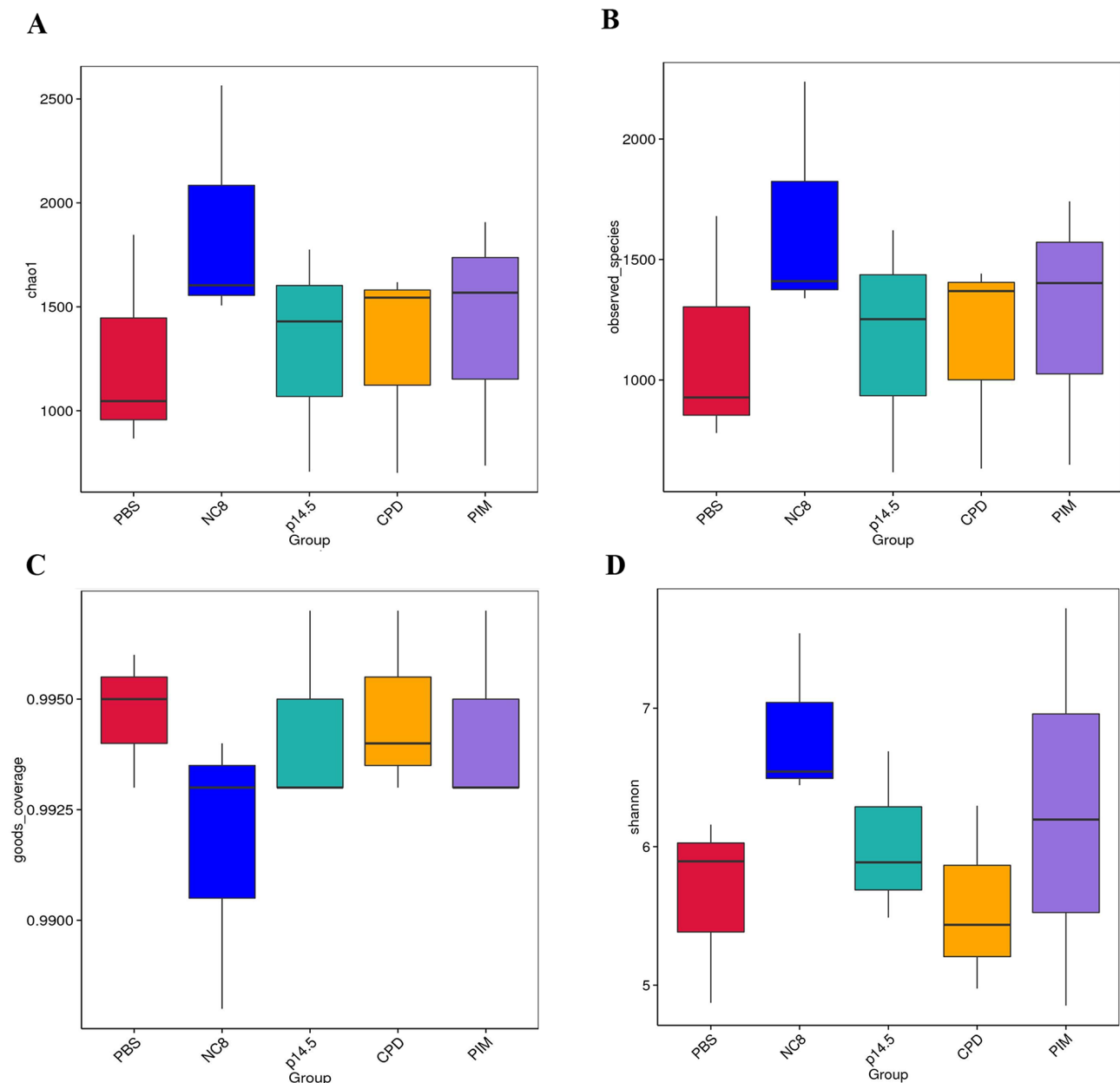


Fig. 2 Alpha diversity analysis of gut microbes. Chao1 (A) and the observed number of species (B) were used as richness estimators. Good's coverage analysis (C) and Shannon–Wiener index (D) was used as a diversity estimator. Abbreviations NC8, samples from NC8-pLP-S group; P14.5, samples from NC8-pLP-S-p14.5 group; CPD, samples from NC8-pLP-S-CTA1-p14.5-D-D group. PIM, samples from NC8-pLP-S-p14.5-IL-33-Mus group

in species diversity, nonmetric multidimensional scaling (NMDS), principal component analysis (PCA), and principal coordinates analysis (PCoA). The coefficient of difference between the samples in the PBS and PIM groups was higher, indicating increased microbial diversity (Fig. 3A). Similar results were observed for NMDS (Fig. 3B), PCA (Fig. 3C), and PCoA (Fig. 3D). Supplementation with recombinant *L. plantarum* significantly increased the intersample distance and structural variations in species composition compared with the PBS group.

Recombinant *L. Plantarum* alter the species distribution of gut microbes

Multiple sequence alignment was used to extract representative sequences from the top 100 genera, and the phylogenetic relationships of species at the genus level as well as the relative abundance of species at the phylum and genus levels were evaluated. According to the phylogenetic relationship analysis, the abundance of *Lactobacillus*, *Lachnospiraceae*-NK4A136, and *Helicobacter* was significantly increased in each group (Fig. 4A). The abundance of *Campylobacterota* was slightly lower in the recombinant *L. plantarum* group than in the PBS group, whereas *Proteobacteria* abundance was significantly higher in the NC8 and PIM groups (Fig. 4B).

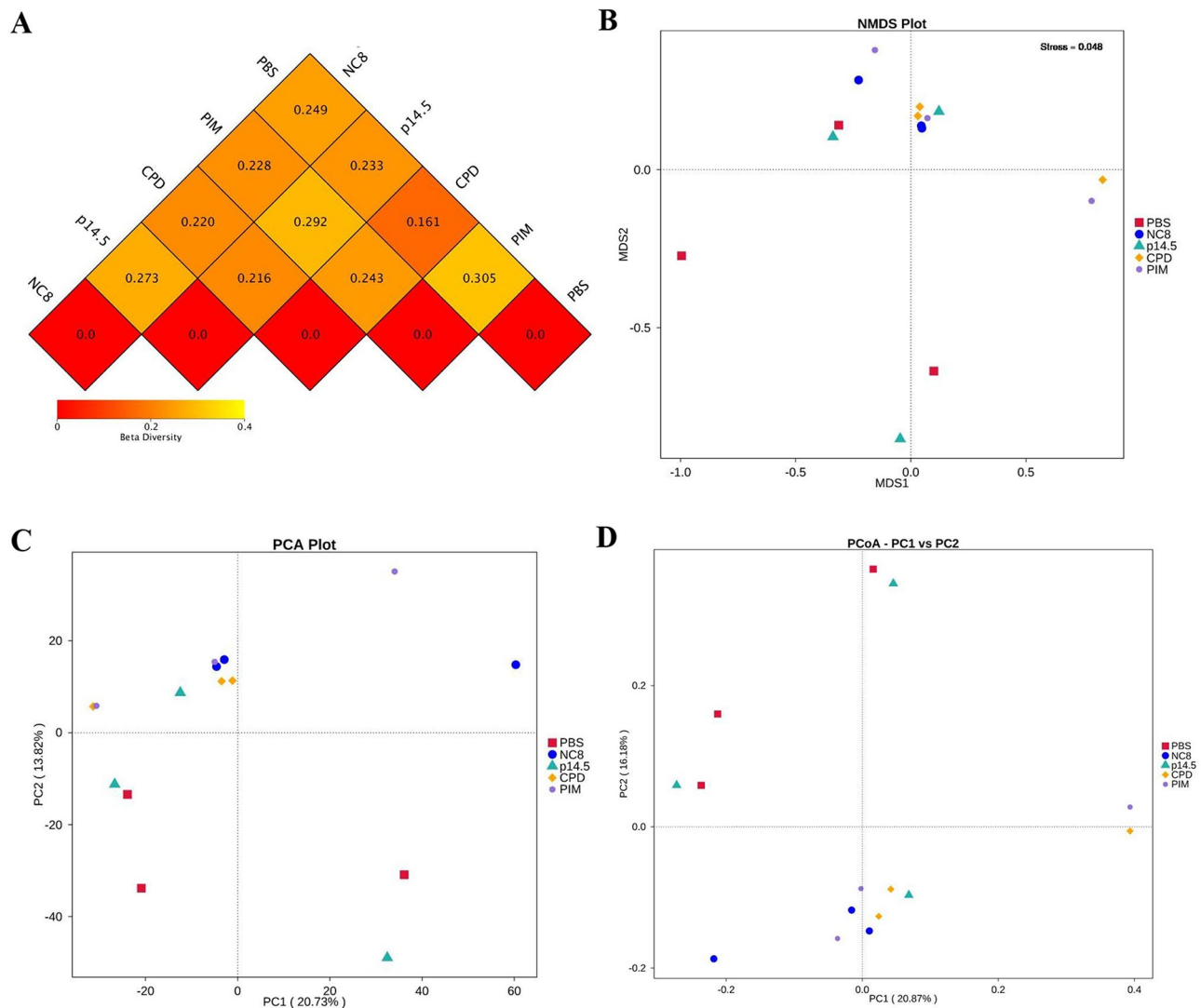


Fig. 3 Beta diversity analysis of gut microbes. **(A)** Differences in species diversity between samples. **(B)** NMDS analysis of samples based on Bray-Curtis distance. **(C)** PCA analysis of samples based on Euclidean distance. **(D)** PCoA analysis of samples based on Weighted Unifrac and Unweighted Unifrac distance. *Abbreviations* NC8, samples from NC8-pLP-S group; P14.5, samples from NC8-pLP-S-p14.5 group; CPD, samples from NC8-pLP-S-CTA1-p14.5-D-D group. PIM, samples from NC8-pLP-S-p14.5-IL-33-Mus group

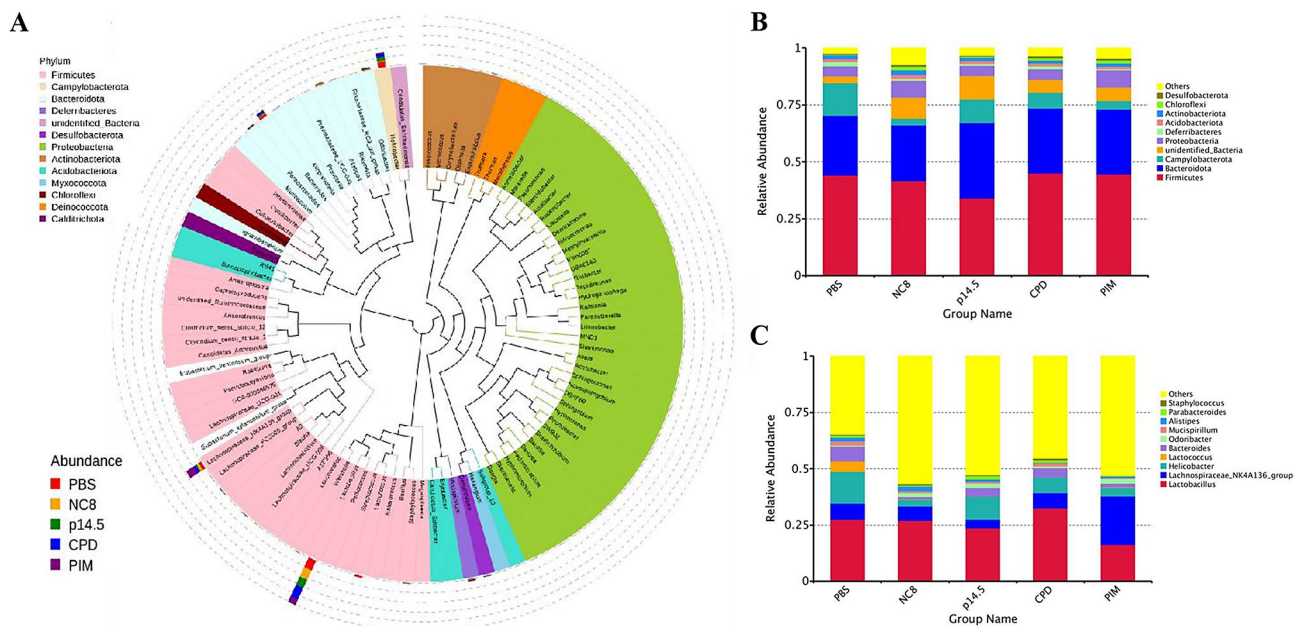


Fig. 4 Species distribution of gut microbes. **(A)** Obtain and analyze the phylogenetic relationship of species at the genus level by multiple sequence alignment (top100). **(B)** Relative abundance of species based on phylum level between groups. **(C)** Relative abundance of species based on genus level between groups. Abbreviations NC8, samples from NC8-pLP-S group; P14.5, samples from NC8-pLP-S-p14.5 group; CPD, samples from NC8-pLP-S-CTA1-p14.5-D-D group. PIM, samples from NC8-pLP-S-p14.5-IL-33-Mus group

The abundance of *Helicobacter* was much lower in the recombinant *L. plantarum* group than in the PBS group, whereas *Lachnospiraceae*-NK4A136 group abundance was significantly higher in the PIM group (Fig. 4C).

Functional analysis of gut microbes

The functional information of the prokaryotic genome in the KEGG database, annotated using the UProC and PAUDA approaches, correlates with the SILVA database to achieve functional annotation of the SILVA database. Subsequently, the function of gut microbes was explored using functional clustering analysis (Fig. 5A) and the relative abundance of gene functions at Level 1 (Fig. 5B), and Level 2 (Fig. 5C) annotation levels. According to the results of the functional clustering analysis, the gut microbial gene functions in the NC8 and P14.5 groups were significantly enriched in infectious diseases and cancer pathways, respectively. The immune system, metabolism, and energy metabolism pathways were significantly enriched in the gut microbial gene functions in the PIM group. There were no significant differences in the gene functions of all groups at Level 1 and 2 annotations. These findings suggest that recombinant *L. plantarum* have the potential to activate gut microorganisms.

Recombinant *L. Plantarum* PIM-induced gut microbes enhance immune function in mice

According to the results of gene function enrichment, the gut microbiota, which is regulated by the recombinant

L. plantarum PIM, regulates the immune system. Gut microbes interact with the immune system in various ways. To investigate the immunological pathways mediated by the gut microbes, an FMT model was created (Fig. 6A). The results showed that gut microbiota mediated by recombinant *L. plantarum* PIM increased the number of CD4⁺ T cells (Fig. 6B and C) in the MLN and IgA⁺ B cells (Fig. 6F and G) in Peyer's patches but not in CD8⁺ T cells (Fig. 6D and E). The expression of IgG (Fig. 6H) and IgG1 (Fig. 6I) in the serum, and sIgA (Fig. 6K) in feces increased during the same period. The expression levels of IgG2a (Fig. 6J) did not change significantly. In conclusion, the immune function of gut microbes mediated by recombinant *L. plantarum*, PIM, was verified.

Discussion

Trillions of bacteria colonize the gut, forming complex ecological communities that influence health and disease risks through their collective metabolic activities and host interactions [29, 30]. Variations in the microbiota can directly affect the physiological state of the body. For instance, when gnotobiotic mice were administered microbiota from obese and lean mice, the former developed obesity more rapidly [31]. Moreover, the composition and diversity of the gut microbes are associated with various diseases. The microbiota of IBD patients was significantly altered compared to that of the control group, with a loss of commensal bacteria, particularly members

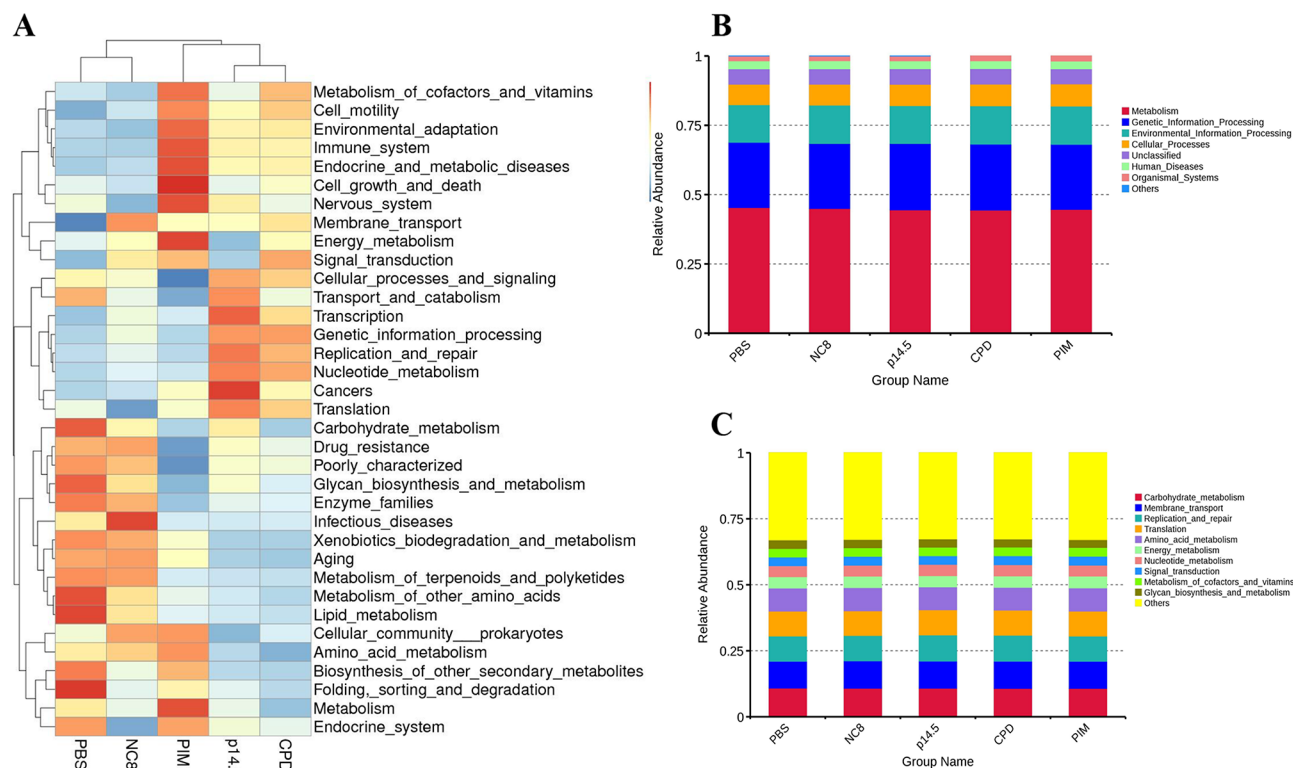


Fig. 5 Gene function prediction of gut microbes. (A) Relative abundance cluster analysis based on annotation-based KEGG database prokaryotes genome-wide functional information. Analyze the top 10 functional information of each grouping with the greatest abundance at the annotation level 1 (B) and level 2 (C), based on the database annotation results. *Abbreviations* NC8, samples from NC8-pLP-5 group; P14.5, samples from NC8-pLP-5-p14.5 group; CPD, samples from NC8-pLP-5-CTA1-p14.5-D-D group. P1M, samples from NC8-pLP-5-p14.5-IL-33-Mus group

of the phyla *bacillota* and *bacteroidota* [32]. Another study showed compositional differences in luminal and mucosal-associated microbiota between irritable bowel syndrome (IBS) patients and healthy controls, along with a significant reduction in microbial diversity in stool samples from IBS [33]. Antibiotics have also been linked to significant changes in gut microbiota diversity, including a substantial decrease in the proportion of butyrate-producing bacteria. This is the first direct evidence of a relationship between gut microbiota and antibiotic-related diarrhea [34]. In this study, supplementation with recombinant *L. plantarum* led to changes in gut microbial composition and diversity. This supports the notion that alterations in gut microbiota play a crucial role in regulating health and disease.

During the long evolutionary process, gut microbes and the host immune system interact and balance with each other [35]. External stimuli, such as bacteria or viruses, can modify and alter the function of the gut microbiota. The gut microbiota provides colonization resistance to pathogens through its metabolites, limiting pathogen expansion and spread [36]. Moreover, there is a bidirectional relationship between respiratory viral infection and the gut microbiota. Although respiratory viral infections can alter the gut microbiota, they also influence

adaptive immune responses to respiratory pathogens [37]. Gut dendritic cells transfer microbial antigens after colonization, causing microbiota-specific T lymphocytes to proliferate. These specific T cells act as effectors in the gut via the circulatory system, thereby protecting the body from relevant pathogens [38]. Our findings are consistent with those of previous studies. Supplementation with recombinant *L. plantarum* has immunomodulatory effects on the gut innate microbiota. CD_4^+ T cells were activated by recombinant *L. plantarum* P1M-mediated microbiota, likely because IL-33 controls DC cell activation, which then increases T cell activation. Furthermore, IgG class switching was regulated by the P1M-mediated recombinant *L. plantarum* microbiota, and enhanced expression of IgG and IgA in B cells could represent a role played by TH_2 cells.

The gut microbiota plays a significant role in regulating the mucosal immune system. IgA is produced in both T-cell-dependent and -independent manners, typically stimulated by dietary antigens and gut bacteria. Secretory IgA (sIgA) maintains microbial homeostasis in the intestinal mucosa and functions within the densely populated, moving, and highly dynamic ecology of the gut bacteria [39, 40]. Symptoms of sIgA deficiency include dysbiosis in the gut flora, increased abundance of related

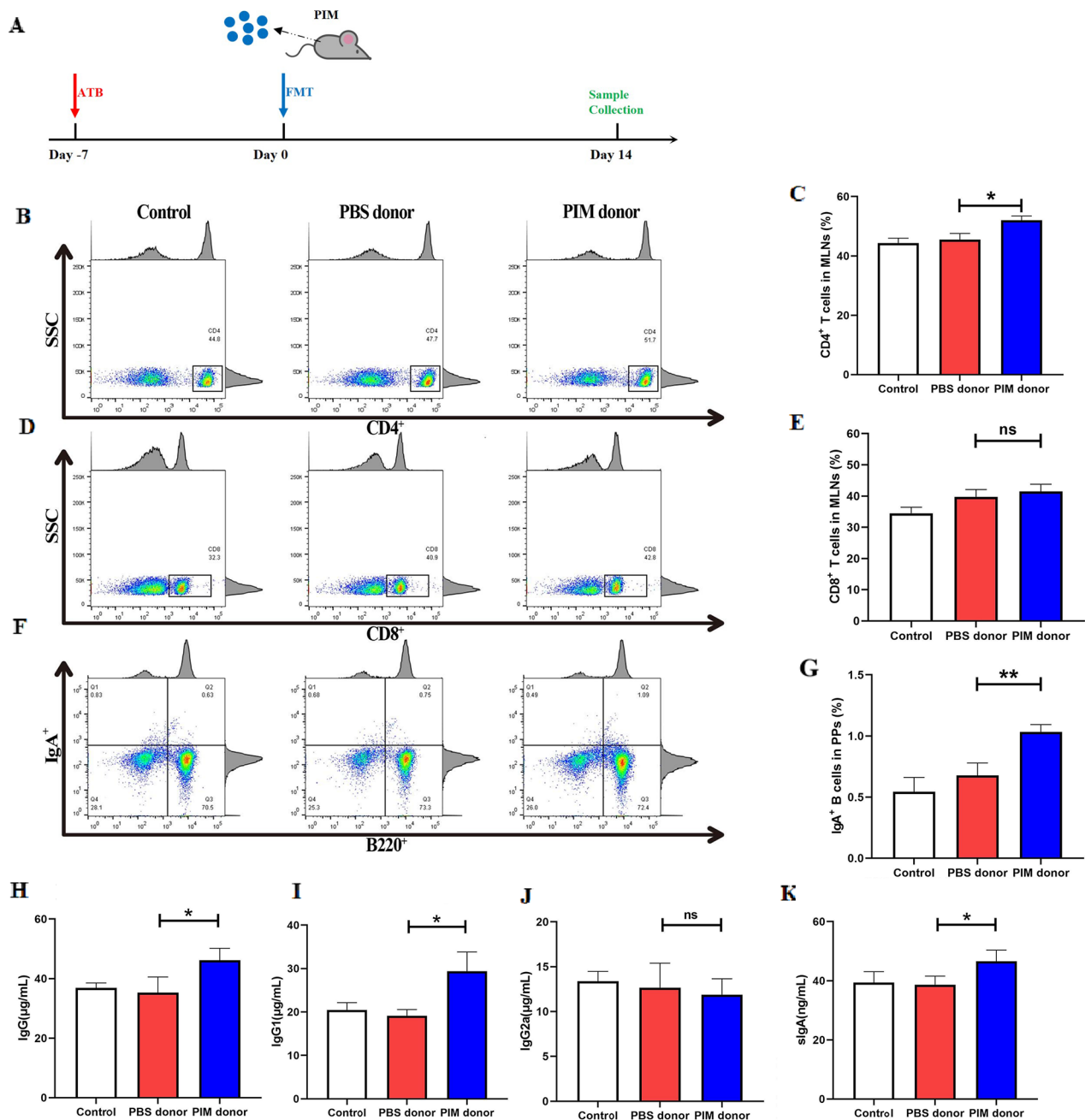


Fig. 6 Recombinant lactic acid bacteria PIM induces the immune function of gut microbes. Establishment of a mouse FMT model (**A**). At 14 days after FMT, the numbers of CD4⁺ (**B**, **C**) and CD8⁺ (**D**, **E**) T cells in MLN and IgA⁺ B cells (**F**, **G**) in Peyer's patches were detected by flow cytometry. The contents of IgG (**H**), IgG1 (**I**) and IgG2a (**J**) in serum and sIgA (**K**) in feces were detected by ELISA. Values are mean ± SEM (n = 3). *P < 0.05; **P < 0.01

pathogenic bacteria (e.g., *Proteus* and *Escherichia coli*), and insufficient quantity of specific IgA-targeted commensal bacteria [39, 40]. IgA-deficient mice showed comparable outcomes. *B. fragilis* requires an IgA response to inhabit a specific mucosal niche that mediates persistent gut colonization by eliminating external competitors [39, 40]. Our research also found a connection between the

gut microbiota and sIgA, suggesting that recombinant *L. plantarum* could influence the gut immune system.

Conclusion

In conclusion, we investigated the impact of recombinant *L. plantarum* on the composition, diversity, and function of the gut microbiota. We used FMT to evaluate the immunological function of the gut microbiota mediated

by recombinant *L. plantarum*. The intestinal microbiota, mediated by recombinant *L. plantarum*, increased the expression of serum IgG and IgG1 as well as the content of fecal sIgA. Simultaneously, CD4⁺ T cells in the MLN and IgA⁺ B cells in Peyer's patches were stimulated, revealing another function of recombinant *L. plantarum*.

We believe that the controls for non-recombinant strains (NCp-PLP-S, backbone vector only) will add important background to our research and help us gain a deeper understanding of the specific mechanisms of action of recombinant strains. Therefore, we are planning to include a control for non-recombinant strains in future experiments in order to more fully evaluate the comparative efficacy of the two strains.

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Not applicable.

Author contributions

CW and QH designed the study; JX and QH was involved in the acquisition of data, analysis, and figure preparation; FX and QH helped revise the manuscript; FT and JR supervised the study; JX drafted the original paper. All authors read and approved the final manuscript.

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Data availability

The raw data reported in this manuscript have been deposited in Jilin Agricultural University, Changchun, China and the NCBI Sequence Read Archive (SRA) database (BioProject: PRJNA839929).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Institutional Review Board Statement

The animal experiments met the requirements of the Animal Management and was approved by the Ethics Committee of Jilin Agricultural University.

Competing interests

The authors declare no competing interests.

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