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Evidence from comparative genomic analyses indicating that *Lactobacillus*-mediated irritable bowel syndrome alleviation is mediated by conjugated linoleic acid synthesis

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Irritable bowel syndrome (IBS) is a chronic intestinal disorder accompanied by low-grade inflammation, visceral hypersensitivity, and gut microbiota dysbiosis. Several studies have indicated that *Lactobacillus* supplementation can help to alleviate IBS symptoms and that these effects are strain-specific. Therefore, this study aimed to investigate the key physiological characteristics and functional genes contributing to the IBS-alleviating effects of *Lactobacillus*. An IBS model was established by subjecting C57BL/6 mice to *Citrobacter rodentium* ingestion and water avoidance stress. *Lactobacillus* strains with different physiological characteristics were administered to mice intragastrically for 4 weeks (5×10^9 CFU/0.2 mL per mouse per day). Indicators of colonic inflammation, visceral hypersensitivity, and gut microbiota were also evaluated. Finally, differences in functional genes between *Lactobacillus* strains were analyzed by a comparative genomic analysis, and the relationships between the physiological characteristics, functional genes, and IBS-alleviating effects of the strains were quantified using correlation analysis. Among the eight tested *Lactobacillus* strains, only *Lactobacillus plantarum* CCFM8610 significantly inhibited the expression of IL-1β, IL-6, PAR-2, and mast cell tryptase. *L. plantarum* CCFM8610 also significantly increased the intestinal barrier function, inhibited visceral hypersensitivity symptoms, and modulated the gut microbiota diversity and composition. The correlation analysis of factors associated with the IBS-alleviating effects of *Lactobacillus* revealed the ability to synthesize conjugated linoleic acid as the most strongly associated physiological characteristic and COG1028-related genes as the most strongly associated functional genes. In conclusion, these findings can facilitate the rapid screening of *Lactobacillus* strains with IBS-alleviating effects and lay a foundation for studies of the related mechanisms.

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1. Introduction

Irritable bowel syndrome (IBS) is a disorder characterized by recurrent abdominal pain associated with bowel movements and is accompanied by bloating and changes in bowel habits.[1] For affected patients, these urgent clinical symptoms are inconvenient in terms of work efficiency and quality of life and lead to a desire for a complete resolution of the condition.[2] Estimates suggest that IBS patients are willing to give up 10 years of life to cure their clinical symptoms.[3] IBS is currently a worldwide epidemic, with a prevalence of approximately 8.1% in North America and 9.6% in Asia.[4] In the United States, the medical costs associated with IBS have increased to US$1.66 billion per year.[5] In the UK, patients' mean annual costs associated with the diagnosis and treatment of IBS symptoms are £316.20.[6] Besides, the quality of life of IBS patients is greatly affected by their clinical symptoms. Frequent abdominal pain, bloating, and bad bowel habits make IBS patients avoid places without bathrooms and reluctant to travel.[7] Harsh food avoidance and severe activity impairment force IBS patients to be frequently absent from work and social activities.[8] Meanwhile, more than one-third of IBS patients who met

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the IBS Rome III criteria still have not been formally diagnosed with IBS.[9] Therefore, an ideal IBS treatment is needed to alleviate this massive economic pressure and quality of life burden.

Probiotics are often used as dietary ingredients because they are generally recognized as safe and exhibit characteristics beneficial to health. Several studies have focused on the effects and mechanisms by which probiotics alleviate intestinal diseases.[10–12] In IBS animal models, probiotics were proven to regulate the function of intestinal dendritic cells, protect the intestinal barrier, inhibit pro-inflammatory cytokine (*e.g.*, IL-1β and IL-6) expression, and reduce visceral hypersensitivity.[13–15] Many clinical trials have also indicated that supplementation with probiotics significantly improves the quality of life of IBS patients by alleviating bloating and abdominal pain symptoms and modulating gut microbiota disorders.[16–18] Other studies have shown that the ingestion of probiotics such as *Lactobacillus plantarum* 299v, *L. acidophilus* LA5, *Bifidobacterium animalis* BB12, and *L. helveticus* R0052 did not improve the clinical symptoms of IBS patients, could not restore the diversity of gut microbiota, and had no significant effect on the quality of life and negative emotions.[19–22]

The above evidence suggests that the ability of probiotics to alleviate IBS is strain-specific, and this specificity may be attributable to the following reasons. First, the physiological characteristics of probiotic strains may affect their health-promoting effects. For example, excellent gastrointestinal tolerance and intestinal cell adhesion could help the strain to survive in the intestine and exert its probiotic effects.[23] Better gastrointestinal tolerance would also enhance the ability of a probiotic strain to inhibit pro-inflammatory cytokines.[24] Second, the unique metabolites produced by specific strains may contribute to strain-specific differences in efficacy. Studies have shown that probiotic strains that produce ropy exopolysaccharide (EPS) can restore the composition of the host's intestinal mucosal barrier and gut microbiota, whereas strains that do not produce EPS exhibit a lesser capacity for these effects.[25] Probiotic strains with a strong ability to synthesize conjugated linoleic acid (CLA) may have an advantage over ordinary strains in terms of preventing pathological damage to the colon.[26] Third, previous studies indicated that the variable expression of functional genes was associated with strain-specific differences in health-promoting effects *in vivo*. In the host intestine, the expression of *EF-Tu*, *mapA*, and *mub* directly affects the gastrointestinal adhesion ability of *Lactobacillus*.[27] Moreover, in *L. plantarum*, the *cps* gene cluster was found to correlate with immune signal regulation in the host intestine.[28]

Therefore, this study was designed to verify whether the IBS-alleviating effects of bacterial probiotics are strain-specific. The study also explored the key physiological characteristics and functional genes that may affect the IBS-alleviating effects of probiotics.

2. Materials and methods

2.1. Bacterial strains and culture

The following *Lactobacillus* strains were used in this study: *L. casei* M2-03-F02-L4-1-5 (M25), *L. casei* CCFM30, *L. casei* V-CQYB6-170-M3 (VM3), *L. casei* M2-01-R02-S01 (M2S01), *L. casei* V-CQYoY1-157-M2 (VM2), *L. plantarum* N13, *L. plantarum* CCFM382, and *L. plantarum* CCFM8610. All strains were provided by the Culture Collections of Food Microbiology, Jiangnan University (Wuxi, China) and cultured in MRS broth for 20 h at 37 °C.

2.2. Animal experimental design

The experimental animals used in this study were adult male specific pathogen-free C57BL/6 mice (bodyweight 22–24 g). All of the mice were housed in an environment with temperature of 22–24 °C and humidity control of 40–70%, a 12 h light/dark cycle, and free access to standard commercial mouse food and distilled water. The experiments were approved by the Ethics Committee of Jiangsu Institute of Parasitic Diseases, China (JlPD-2019037), and were conducted under the guidelines set by the European Community (Directive 2010/63/EU).

This experiment was conducted as described by Ibeakanma *et al.*, with modifications.[29] One hundred mice were randomly divided into 10 groups (10 mice per group). The experimental duration was 30 days. On day 1 of the experiment, mice in the control group were administered 0.2 mL of sterile saline *via* gavage, whereas the remaining mice were administered *Citrobacter rodentium* DBS100 (1.2×10^{10} CFU/0.2 mL per mouse). From day 2 to day 8, all of the mice were injected subcutaneously with 0.5 mL of lactated Ringer's (LR) solution to prevent dehydration related to diarrhea.

From day 18 to day 30 of the experiment, all mice were subjected to water avoidance stress (WAS). WAS is considered to be a chronic psychological stress factor in this model. Repeated WAS can cause visceral hypersensitivity symptoms in mice, which manifested as the enhancement of visceral pain in the gut, that is, the decrease of visceral pain threshold.[15] It is a disorder of the central and peripheral nervous systems. The WAS device comprised a bucket with a diameter of 27 cm and a height of 33 cm. The bottom of the bucket held a drying platform with a diameter of 4 cm and a height of 9 cm. The bucket was filled with water to a level 1 cm below the platform. Each mouse was placed on the platform for 1 hour each day. If the mouse fell into the water, it was dried with a dry towel. Mice in the control group were placed on the platform for 1 hour in the absence of water.

In addition to the above treatment, from day 2 to day 29, the control and model groups were administered sterile saline (0.2 mL per mouse per day) by gavage. The other groups were administered a sterile saline suspension of the indicated *Lactobacillus* strain (5×10^9 CFU/0.2 mL per mouse per day) by gavage. The treatment provided to each group is shown in Table 1.

2.3. Visceral hypersensitivity assignment

In each group, the visceral hypersensitivity of colorectal distention (CRD) was measured using the abdominal withdrawal reflex (AWR) according to the method described by Chen *et al.*[30] The AWR experiment was performed after the mice fasted for 16 hours. During the test, the mice remained under

Table 1 Animal experimental protocol

Groups (no. of mice)	Experimental protocol
Control (n = 10)	Sterile saline + LR + WAS + sterile saline
Model (n = 10)	*Citrobacter rodentium* + LR + WAS + sterile saline
L. casei M25 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. casei* M25
L. casei CCFM30 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. casei* CCFM30
L. casei VM3 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. casei* VM3
L. casei M2S01 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. casei* M2S01
L. casei VM2 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. casei* VM2
L. plantarum N13 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. plantarum* N13
L. plantarum CCFM382 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. plantarum* CCFM382
L. plantarum CCFM8610 (n = 10)	*Citrobacter rodentium* + LR + WAS + *L. plantarum* CCFM8610

LR, lactated Ringer's solution; WAS, water avoidance stress.

isoflurane anesthesia. The air pressure was increased gradually to 0.1, 0.2, and 0.3 mL. In order to avoid the subjectivity of AWR experiment, the blind evaluation was carried out. Two researchers who did not know the grouping information scored the colorectal distention responses of the mice in each group.

2.4. Collection of feces, blood, and tissue samples

At the end of the experiment, fresh feces were collected and stored at −80 °C. The mice were then sacrificed under isoflurane. Serum was collected from centrifuged blood samples and stored at −80 °C. A portion of the colon was fixed with paraformaldehyde, and the remainder was stored at −80 °C.

2.5. Biochemical analyses of the colon and serum

After tissue collection, 0.1 g of colon tissue was weighed accurately, homogenized in ice-cold sterile saline, and subjected to centrifugation (3000*g*, 4 °C, 5 min). The supernatant was collected, and the levels of IL-1β, IL-6, IL-10, and TNF-α in the colon were determined using the corresponding ELISA kits (R&D Systems China Co., Ltd, Shanghai, China). The occludin level in the colon and the corticosterone level in the serum were measured using ELISA assay kits (SenBeiJia Biological Technology Ltd, Nanjing, China). The protease-activated receptor 2 (PAR-2) and mast cell tryptase levels in the colon were determined by using ELISA assay kits (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China).

2.6. Western blotting analysis

The western blotting analysis was conducted as per our previous research.[31] Antibodies specific for occludin (ab167161, 1 : 50 000, Abcam Ltd, Cambridge, MA, USA), PAR-2 (ab180953, 1 : 1000, Abcam Ltd), and β-actin (1 : 1000, Santa Cruz Biotechnology Co., Dallas, TX, USA) were used for immunoblotting.

2.7. Fecal DNA extraction, sequencing, and analysis

Fecal bacterial DNA was isolated using the FastDNA® Spin kit (MP Biomedicals Ltd, Santa Ana, CA, USA). The sequencing of the gut microbiota was conducted as described by Zhao *et al.*[32] A linear discriminant analysis effect size (LEfSe) analysis was used to analyze differences in the gut microbiota composition between the groups.

2.8. Histological evaluation

The colon samples were dehydrated in ethanol, sectioned (5 μm), embedded in paraffin, and stained with hematoxylin and eosin. A microscope (BA410E microscope, Motic China Group Ltd, Xiamen, China) was used to analyze the histopathological damage.

2.9. Comparative genomic analysis

The comparative genomic analysis was conducted as described by Cen *et al.*[33] The genomes were subjected to BLAST analysis against annotated proteins in the clusters of orthologous groups (COGs) protein database (http://www.cog.org) to identify the differences in functional genes between the *Lactobacillus* strains.

2.10. Correlation analysis

The correlation analysis was performed with reference to our previously published research.[31] Pearson correlation coefficients were used to quantify the correlation between the physiological characteristics, functional genes, and IBS-alleviating effects of *Lactobacillus* strains.

2.11. Statistical analysis

All of the data are expressed as means ± standard deviations (SD). A one-way analysis of variance (ANOVA) was used to analyze the results, and Tukey's multiple comparison test was used subsequently to determine the statistical significance. A P-value ≤0.05 was considered to indicate statistical significance.

3. Results

3.1. Effect of *Lactobacillus* strains on colon histopathological damage

Compared with mice in the control group, those in the model group exhibited mild inflammatory injuries in the colon, with characteristic mild inflammatory cell infiltration (Fig. 1). These pathological features were also observed in most of the

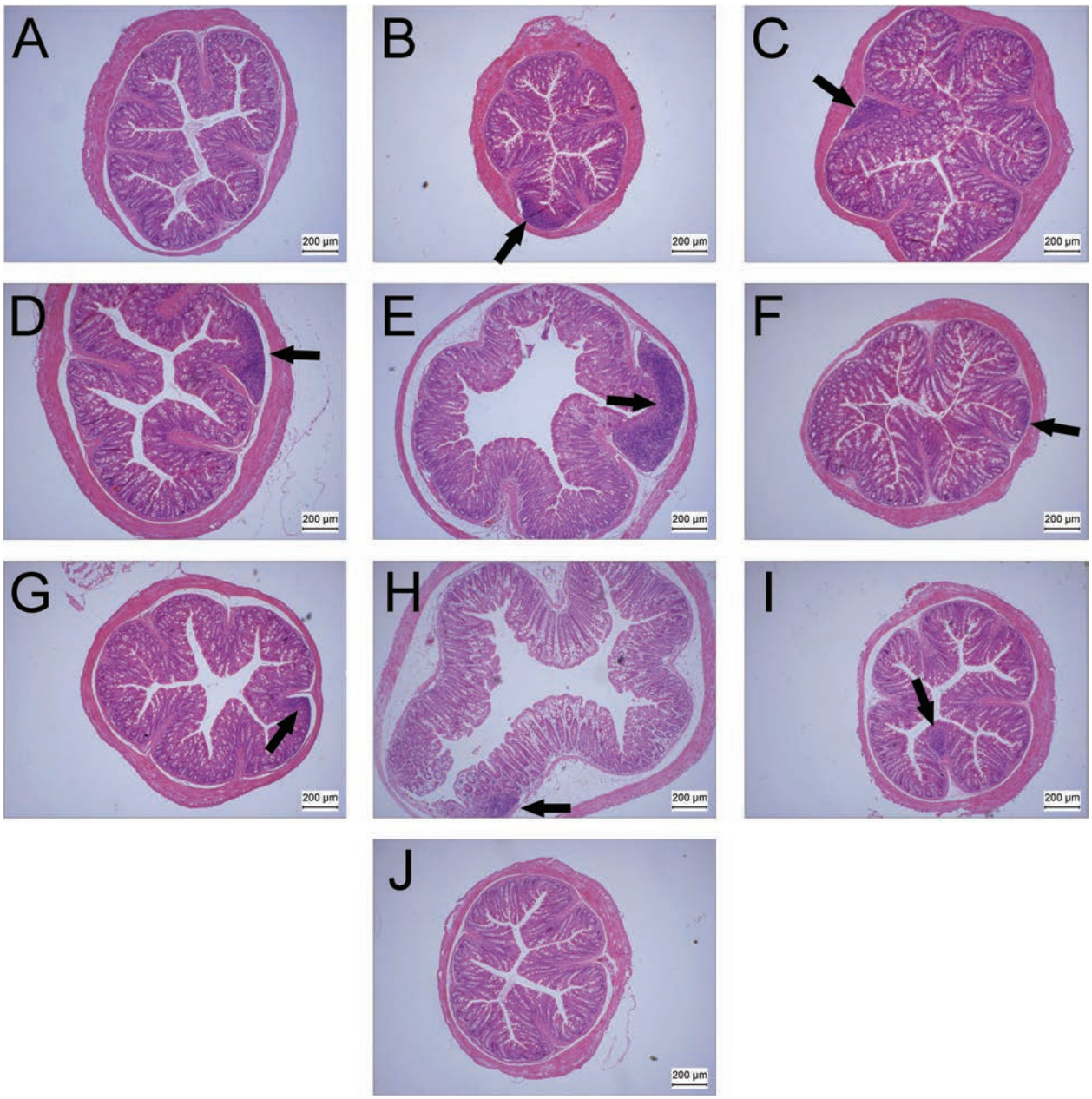

Fig. 1 Histopathological analysis of colon tissues (magnification = 40×; scale bar = 200 μm). (A) Control; (B) model; (C) *L. casei* M25; (D) *L. casei* CCFM30; (E) *L. casei* VM3; (F) *L. casei* M2S01; (G) *L. casei* VM2; (H) *L. plantarum* N13; (I) *L. plantarum* CCFM382; and (J) *L. plantarum* CCFM8610. Arrows indicate inflammatory cell infiltration.

Lactobacillus treatment groups. However, no apparent histopathological damage was observed in mice that were treated with *L. plantarum* CCFM8610, suggesting that this strain could potentially alleviate intestinal inflammation.

3.2. Effects of *Lactobacillus* strains on pro-inflammatory and anti-inflammatory cytokine expression

The ingestion of *Citrobacter rodentium* and exposure to chronic WAS induced immune disturbances in mice, which were manifested by significant increases in the production of IL-1β, IL-6, TNF-α, and IL-10 (Fig. 2A–D). The effects of the *Lactobacillus* strains on cytokine production varied significantly. *L. plantarum* CCFM8610 treatment led to significant decreases in the production of the pro-inflammatory IL-6 and TNF-α, which were restored to normal levels (Fig. 2B and C). This finding further indicated that *L. plantarum* CCFM8610 supplementation could inhibit intestinal inflammation. Of the tested strains, *L. casei* M2S01 most strongly promoted the production of the anti-inflammatory cytokine IL-10 (Fig. 2D). However, there was no significant difference between the model group and the *L. casei* M2S01 group.

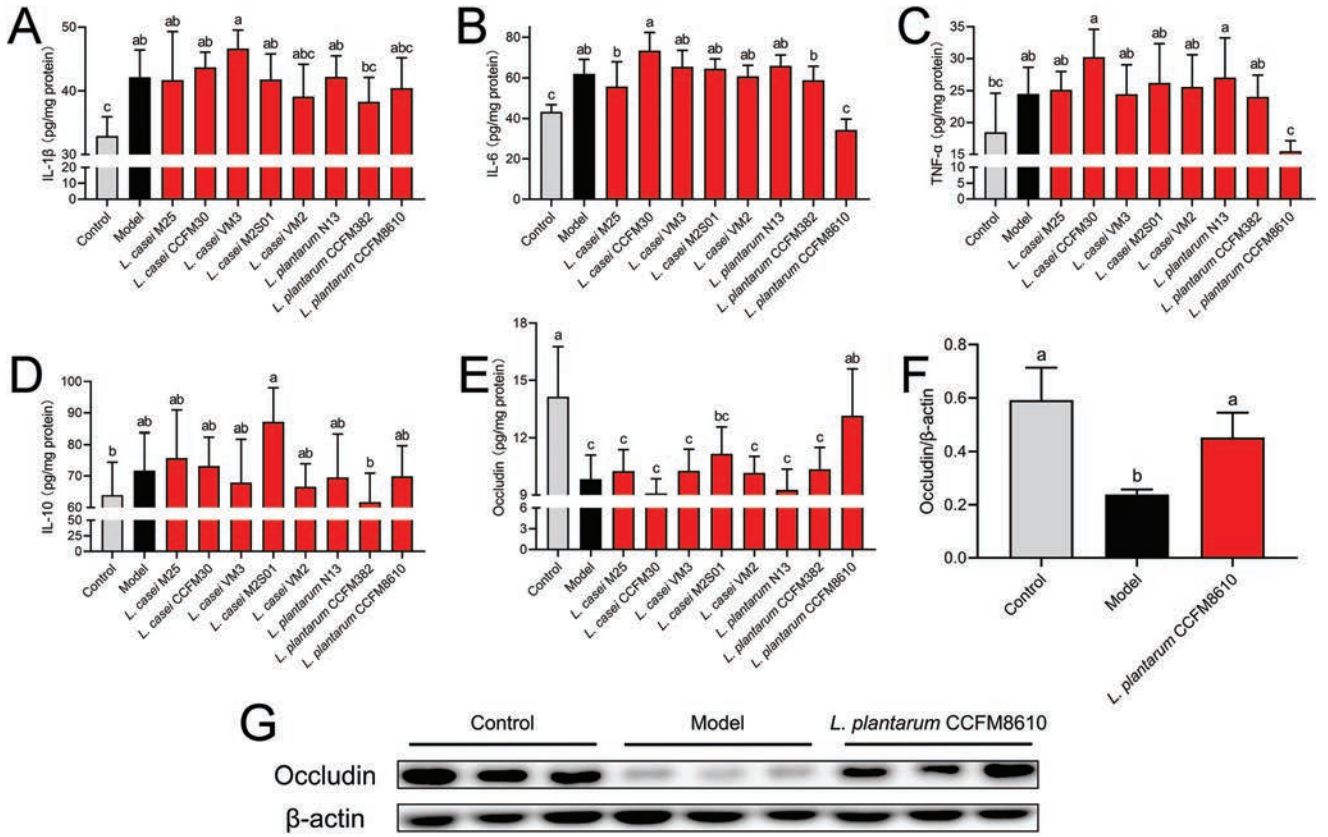

Fig. 2 Analysis of cytokines and occludin protein in colon tissue. ELISA analyses of (A) IL-1β; (B) IL-6; (C) TNF-α; (D) IL-10; and (E) occludin; (F) the relative gray value of occludin and β-actin; (G) western blotting analysis of occludin. Letters a–c indicate statistically significant differences ($p < 0.05$).

3.3. Effects of *Lactobacillus* strains on the intestinal barrier in the colon

An analysis of the colonic levels of occludin protein revealed significant changes in the model group ($p < 0.05$, Fig. 2E–G). After supplementation with *L. plantarum* CCFM8610, the occludin level was restored significantly. Other *Lactobacillus* strains did not significantly restore the occludin level.

3.4. Effects of *Lactobacillus* strains on visceral hypersensitivity

As shown in Table 1, treatment with *Citrobacter rodentium* and chronic WAS led to significant increases in the AWR score in the model group ($p < 0.05$, Fig. 3A–C). However, most *Lactobacillus* strains did not alleviate the symptoms of visceral hypersensitivity in mice. Only *L. plantarum* CCFM8610 was associated with significant reductions in the AWR scores at gas pressures of 0.2 and 0.3 mL ($p < 0.05$), indicating that this strain effectively alleviated visceral hypersensitivity.

3.5. Effects of *Lactobacillus* strains on the levels of mast cell tryptase and PAR-2 in the colon and corticosterone in the serum

As shown in Fig. 4, the highest levels of mast cell tryptase (Fig. 4A) and PAR-2 in the colon and corticosterone in the serum (Fig. 4B–E) were detected in the model group ($p < 0.05$ for all). However, the levels of all three indicators changed after the ingestion of *Lactobacillus* strains. Some *Lactobacillus* strains led to increases in the PAR-2 and corticosterone levels, although these changes were not statistically significant. Of the tested strains, *L. plantarum* CCFM8610 significantly decreased the levels of mast cell tryptase, PAR-2, and corticosterone ($p < 0.05$ for all, Fig. 4A–E). *L. casei* M2S01 also restored the corticosterone level in the serum ($p < 0.05$, Fig. 4D).

3.6. Effects of *Lactobacillus* strains on the gut microbiota diversity and composition

Compared with the control group, the model group exhibited a non-significant decrease in gut microbiota diversity (Fig. 5A and B). The oral administration of different *Lactobacillus* strains induced changes in the gut microbiota diversity to varying degrees. *L. plantarum* CCFM8610 induced the strongest beneficial effect. According to the observed OTU index and the Shannon index, the highest gut microbiota diversity was observed in the *L. plantarum* CCFM8610 group, and this level of diversity was significantly higher than that in the model group ($p < 0.05$, Fig. 5A and B). *L. plantarum* CCFM382 also led to a significant increase in the observed OTU index.

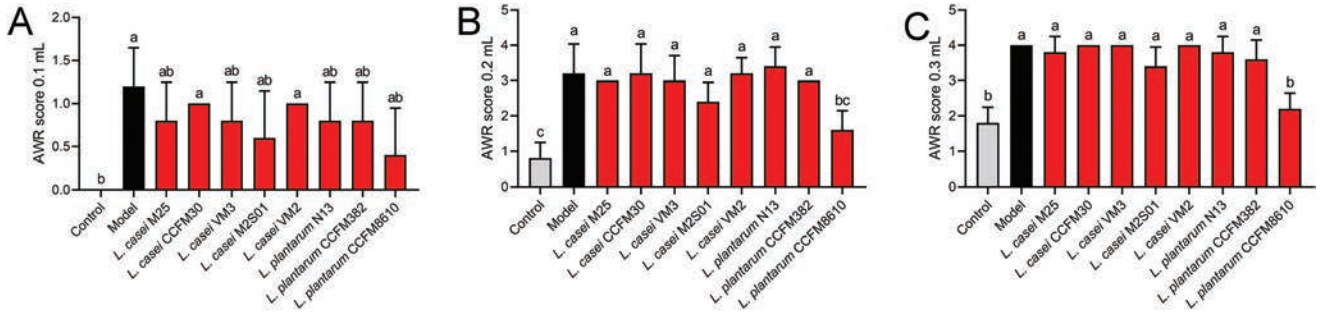

Fig. 3 Comparison of the abdominal withdrawal reflex (AWR) scores of different groups. Air pressures of (A) 0.1 mL; (B) 0.2 mL; and (C) 0.3 mL. Letters a–c indicate statistically significant differences ($p < 0.05$).

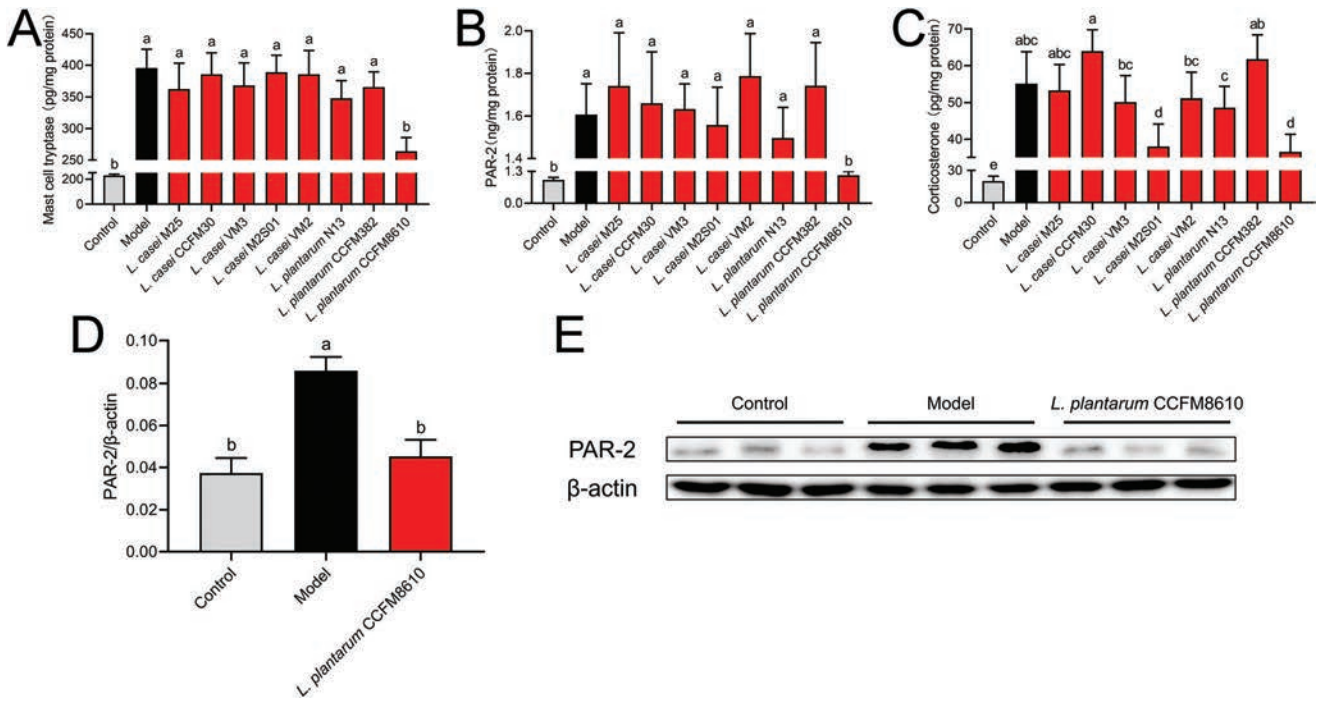

Fig. 4 Levels of mast cell tryptase and PAR-2 in the colon and corticosterone in the serum. ELISA analyses of (A) mast cell tryptase; (B) PAR-2; and (C) corticosterone; (D) the relative gray value of PAR-2 and β-actin; (E) western blotting analysis of PAR-2. Letters a–e indicate statistically significant differences ($p < 0.05$).

The LEfSe analysis revealed significant differences in the gut microbiota compositions of different groups (Fig. 5C). Exposure to *Citrobacter rodentium* and WAS induced changes in the gut microbiota in the model group, including a rapid decrease in the relative abundances of *Alistipes*, *Lachnoclostridium*, and other genera (Fig. 5D). However, supplementation with *L. plantarum* CCFM8610 led to significant modulation of the relative abundances of most genera (Fig. 5E).

3.7. Comparative analysis of functional genes of *Lactobacillus* strains

Homologous gene analysis of eight *Lactobacillus* strains identified 1321 core genes (Fig. 6A). Further comparison and analysis of the functional genes detected in *Lactobacillus* strains revealed that the *L. plantarum* CCFM8610 genome contained a significantly higher number of genes related to 10 primary functions, including amino acid transport and metabolism, when compared with the other tested strains (Fig. 6B). The results of gene annotation *via* the COG database showed that *L. plantarum* CCFM8610 differed significantly from the other strains in 14 COGs (Fig. 6C). This finding indicates that *L. plantarum* CCFM8610 might exhibit different physiological characteristics and functions relative to other strains.

3.8. Relationship between the IBS-alleviating effects, physiological characteristics, and genome

Pearson correlation coefficients were used to characterize the correlation between two indicators in this study. An analysis of

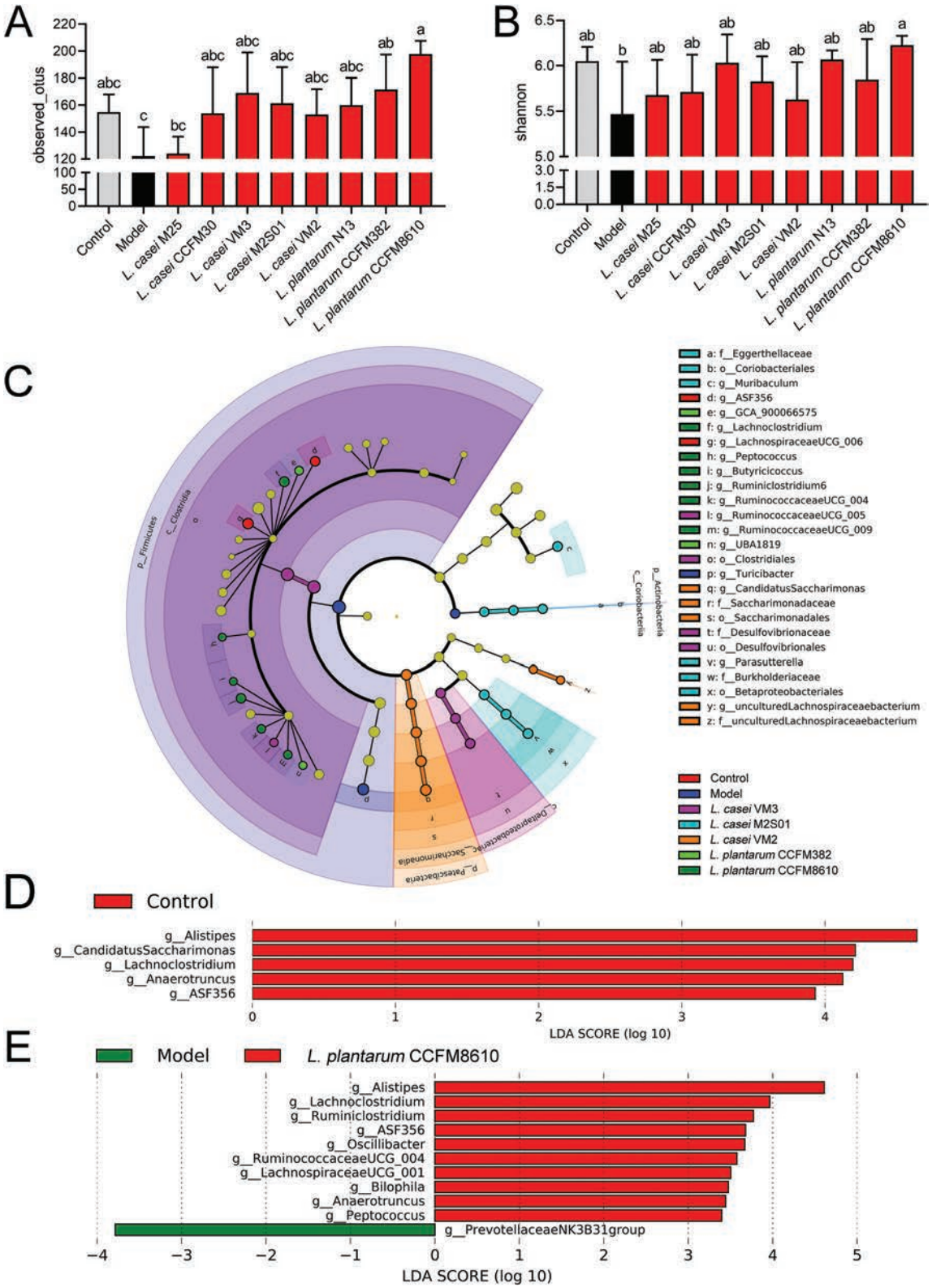

Fig. 5 Comparison of the gut microbiota diversity and composition between treatment groups. (A) Observed OTU index. (B) Shannon index. (C) Taxonomic cladogram. LDA scores at the genus level (D) in the control and model groups and (E) the model and *L. plantarum* CCFM8610 groups. Letters a–c indicate statistically significant differences ($p < 0.05$). LDA, linear discriminant analysis.

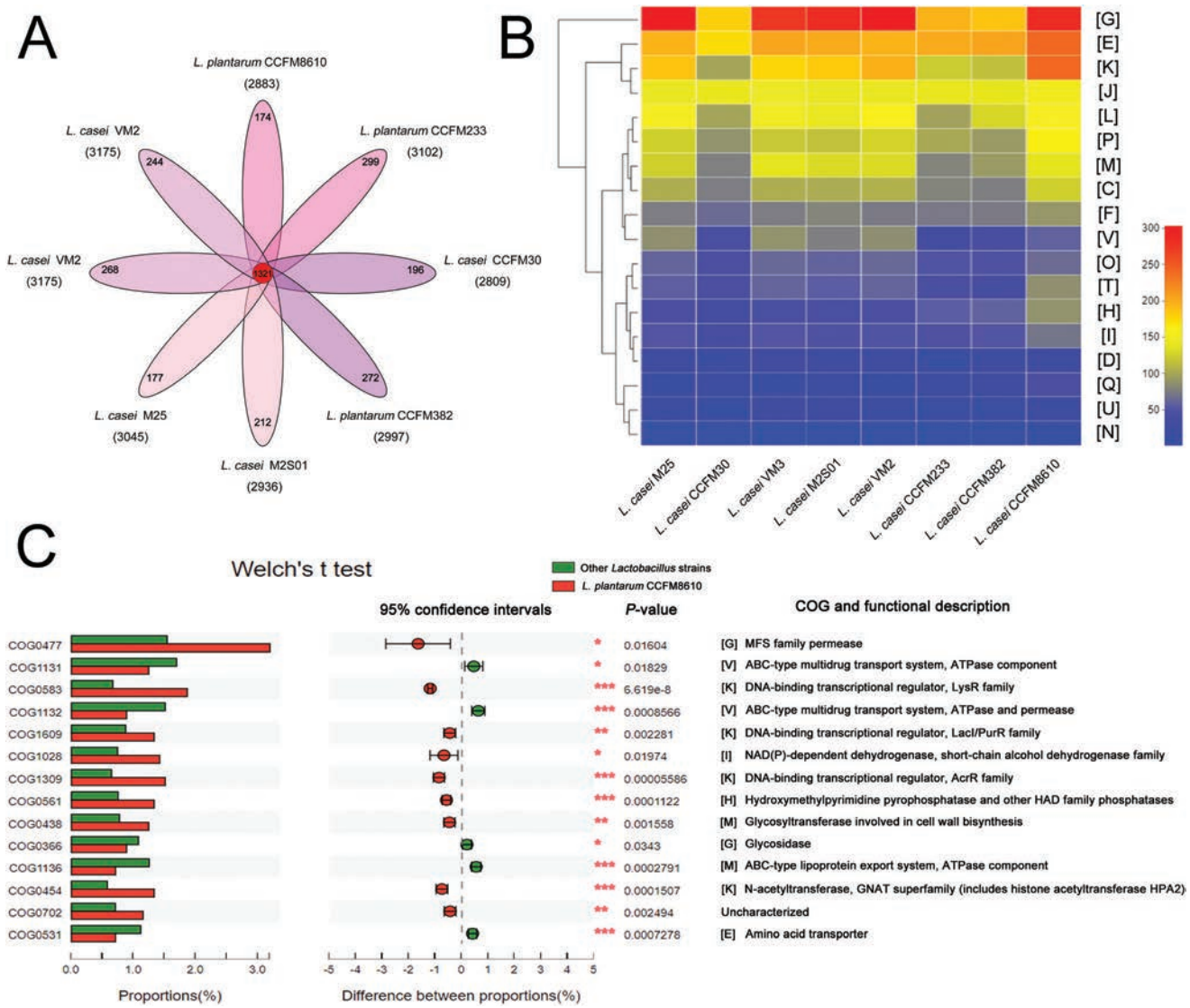

Fig. 6 Comparative analysis of the genomes of *Lactobacillus* strains. (A) Venn diagram. (B) Comparison of COG functional categories between *Lactobacillus* genomes. (C) Comparison of strain-specific COGs in *L. plantarum* CCFM8610 *vs.* other *Lactobacillus* strains. COG, cluster of orthologous groups. [C] Energy production and conversion, [D] cell cycle control, cell division and chromosome partitioning, [E] amino acid transport and metabolism, [F] nucleotide transport and metabolism, [G] carbohydrate transport and metabolism, [H] coenzyme transport and metabolism, [I] lipid transport and metabolism, [J] translation, ribosomal structure and biogenesis, [K] transcription, [L] replication, recombination and repair, [M] cell wall/membrane/envelope biogenesis, [N] cell motility, [O] post-translational modification, protein turnover and chaperones, [P] inorganic ion transport and metabolism, [Q] secondary metabolite biosynthesis, transport and catabolism, [T] signal transduction mechanisms, [U] intracellular trafficking, secretion, and vesicular transport, and [V] defense mechanisms.

correlation related to the *in vitro* physiological characteristics of the *Lactobacillus* strains revealed that the ability to synthesize CLA was most strongly correlated with the IBS-alleviating effects (Fig. 7A). In total, the ability to synthesize CLA was shown to correlate with nine indicators of the IBS-alleviating effects. For example, the levels of IL-6 and PAR-2 were negatively correlated with the ability to synthesize CLA (Pearson correlation coefficients $\leq$0.7 for all). Further analysis revealed a negative correlation between the production of EPS and the levels of IL-1β and IL-10. The gut microbiota diversity was also shown to be correlated negatively with the *Lactobacillus* generation time.

The analysis of correlation between *Lactobacillus* functional genes and IBS-alleviating effects revealed strong and consistent correlation (Fig. 7B). For example, all *Lactobacillus* genomes exhibited a negative correlation with IL-6 and a positive correlation with occludin. Lipid transport and metabolism, amino acid transport and metabolism, and nucleotide transport and metabolism were identified as the most relevant *Lactobacillus* genomic indicators associated

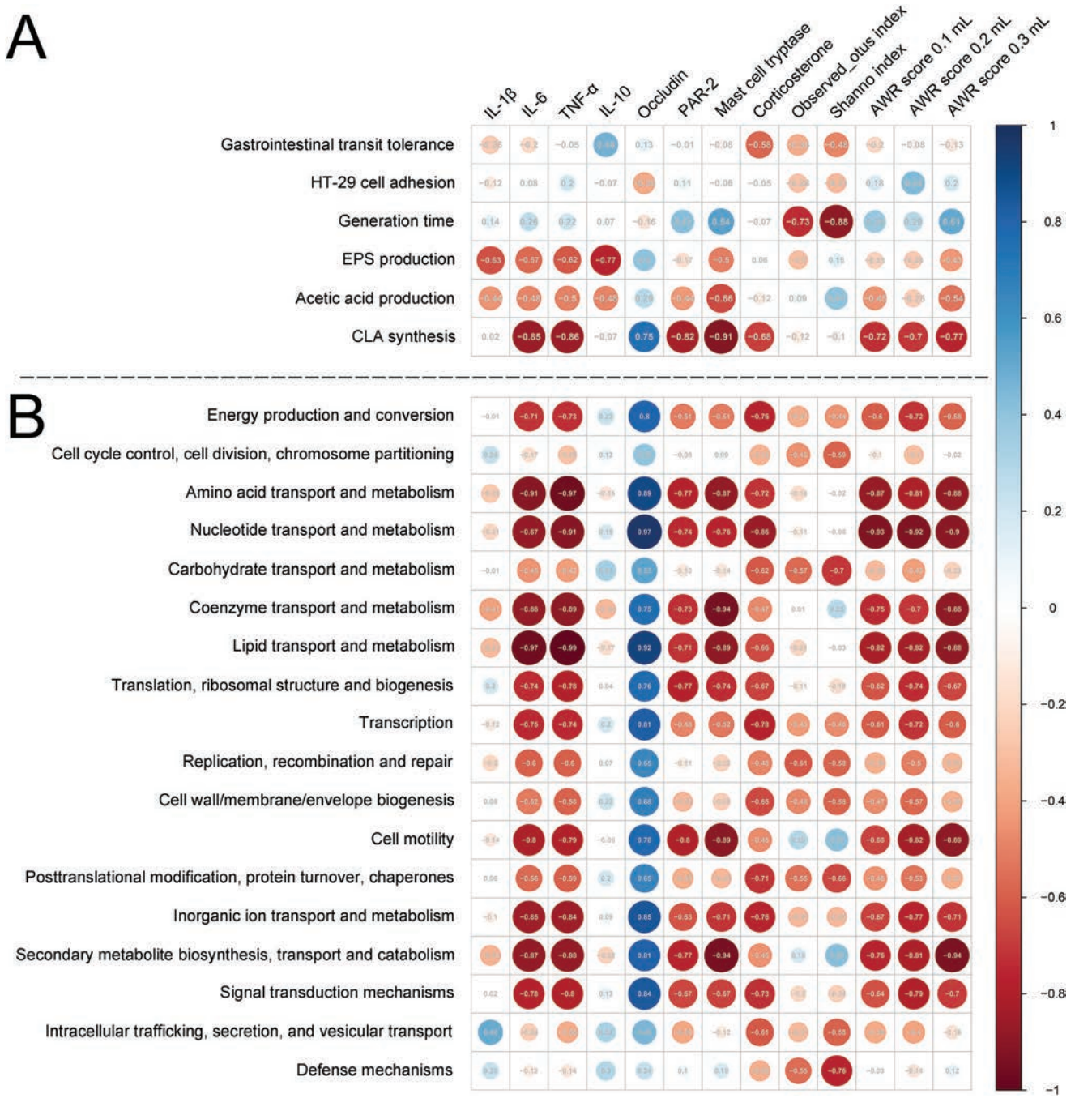

Fig. 7 Analysis of correlation between the physiological characteristics, functional genes, and IBS-alleviating effects of *Lactobacillus* strains. (A) Correlation between physiological characteristics and IBS-alleviating effects. (B) Correlation between functional genes and IBS-alleviating effects.

with the indicators of intestinal low-grade inflammation (*e.g.*, pro-inflammatory cytokines and occludin; absolute Pearson correlation coefficients >0.9 for all) and visceral hypersensitivity (*e.g.*, PAR-2 expression, mast cell tryptase, serum corticosterone, and AWR score; Pearson correlation coefficients ≤0.8 for all). Generally, the correlation between *Lactobacillus* genomic indicators and gut microbiota diversity indicators was weak.

4. Discussion

This study aimed to identify *Lactobacillus* strains with IBS-alleviating effects and analyze their key physiological characteristics and functional genes with respect to these effects. We selected eight *Lactobacillus* strains with different *in vitro* physiological characteristics[31,34] and performed a comparative genomic analysis. We also explored the IBS-alleviating effects

of these strains in animal models and evaluated the correlation between the *in vitro* physiological characteristics, functional genes, and IBS-alleviating effects of the strains.

As noted above, IBS is an intestinal disorder characterized by abdominal pain and distension and altered defecation habits. Several studies have indicated that IBS pathogenesis results from a combination of physiological and psychological factors.[4] In this study, we used a model in which the combination of *Citrobacter rodentium* and WAS exposure effectively simulated the physiological and psychological pathogenesis of IBS. Notably, the establishment of this model led to mild symptoms of inflammatory cell infiltration in the colon (Fig. 1), the increased expression of pro-inflammatory cytokines, and damage to the intestinal barrier function (Fig. 2). These results are consistent with the clinical symptoms of low-grade IBS-related inflammation and indicate that low-grade colonic inflammation was induced in the mice.[35,36]

In this study, WAS led to significant increases in the AWR scores of mice (Fig. 3). When we measured several characteristic indicators of visceral hypersensitivity, we observed a significant increase in the concentrations of PAR-2 in the colon and corticosterone in the blood. We also observed a significant increase in the release of mast cell tryptase (Fig. 4). These results suggest that chronic WAS exposure activated mast cells and the hypothalamic-pituitary-adrenal axis, which activated PAR-2 and induced continuous intestinal nerve excitation, eventually leading to visceral hypersensitivity. In addition to these symptoms of low-grade inflammation and visceral hypersensitivity, we also observed significant changes in the gut microbiota diversity and composition in the mice. This result was consistent with the findings from clinical trials of IBS, indicating that the mice exhibited the clinical IBS-related symptom of gut microbiota dysbiosis.[37]

We next investigated the abilities of *Lactobacillus* strains to alleviate the symptoms of low-grade inflammation, visceral hypersensitivity, and gut microbiota dysbiosis in mice, and observed significant differences between the strains. Particularly, most strains did not alleviate the symptoms of IBS. This observation was consistent with the findings from numerous IBS-related studies, which indicated that not all *Lactobacillus* strains can alleviate the clinical symptoms of IBS.[38,39] In fact, the ingestion of *Lactobacillus* strains such as *L. casei* CCFM30 and *L. casei* VM3 was shown to exacerbate colonic inflammation in mice. Compared with the model group, the mice treated with these two *Lactobacillus* strains exhibited a broader scope of colonic inflammatory cell infiltration (Fig. 1). Both strains also significantly increased the production of pro-inflammatory cytokines in the colon (Fig. 2). Similar results have been reported in previous studies of intestinal inflammation and may be attributable to negative interactions between *Lactobacillus* strains and intestinal immunity.[40,41]

In contrast, some strains, including *L. casei* M2S01, *L. plantarum* CCFM382, and *L. plantarum* CCFM8610, did alleviate the symptoms of IBS. However, we did not observe significant improvements associated with *L. casei* M2S01 and *L. plantarum* CCFM382 when we evaluated the histological characteristics and visceral hypersensitivity, suggesting that the regulatory effects of these two strains are not a key mechanism in the alleviation of IBS. In other words, *L. casei* M2S01 and *L. plantarum* CCFM382 do not possess the key physiological characteristics or functional genes required to alleviate IBS.

In our analysis, *L. plantarum* CCFM8610 exhibited the strongest IBS-alleviating effects. Supplementation with *L. plantarum* CCFM8610 significantly alleviated the characteristic IBS symptoms of low-grade inflammation and visceral hypersensitivity and modulated the gut microbiota dysbiosis. Previous studies have identified multiple mechanisms by which probiotics alleviate IBS. For example, *Escherichia coli* Nissle 1917 produces a secondary metabolite, analgesic peptide, that inhibits calcium flux and thereby alleviates the visceral pain associated with IBS.[42] Butyric acid-producing probiotic members of the genus *Roseburia* can enhance the intestinal barrier function and inhibit visceral hypersensitivity by increasing the butyric acid concentration in the intestine.[43] *Clostridium butyricum* can regulate the numbers and functions of lamina propria dendritic cells and thus alter the intestinal immune response to alleviate intestinal inflammation in IBS.[13] Given these findings and the experimental results from the above-mentioned ineffective strains, we speculated that *L. plantarum* CCFM8610 may possess a key physiological characteristic or functional gene that contributes to its IBS-alleviating mechanisms and effects.

To investigate this key physiological characteristic or functional gene of *L. plantarum* CCFM8610, we performed a comparative genomic analysis and identified significant differences between this and other strains in terms of functional genes (Fig. 6). Further analysis of the correlation between the *in vitro* physiological characteristics and IBS-alleviating effects of *Lactobacillus* identified the ability to synthesize CLA as the most strongly associated factor. We further analyzed the correlation between functional genes and IBS-alleviating effects and identified genes related to COG1028 as the most strongly associated functional genes. COG1028 is associated with lipid transport and metabolism and is annotated as a member of the short-chain alcohol dehydrogenase family according to the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov). Short-chain alcohol dehydrogenase is a multi-component linoleic acid isomerase and is considered necessary for the ability of *L. plantarum* to transform CLA.[44] Therefore, we believe that *L. plantarum* CCFM8610 benefits from an abundance of genes related to COG1028, which can promote the expression of short-chain alcohol dehydrogenases and confer an excellent ability to synthesize CLA.

CLA is a long-chain fatty acid with multiple health-promoting functions. Studies have demonstrated that CLA can achieve the dual physiological effects of inflammation regulation and immune activation in the gastrointestinal tract.[45,46] Mechanistically, these effects appear to rely on the CLA-mediated activation of peroxisome proliferator-activated receptors, which inhibit the NF-κB signaling pathway.[47] Moreover, animal experiment results demonstrated that CLA could also

exert anti-inflammatory effects by inhibiting the expression of pro-inflammatory cytokines, such as IL-6 and COX-2.[48] *L. plantarum* CCFM8610 was previously shown to possess an excellent CLA synthesis ability.[31] In this study, we proved that *L. plantarum* CCFM8610 had a significant advantage over the other tested strains because of the presence of COG1028-related genes. Our result verifies the excellent CLA synthesis ability of *L. plantarum* CCFM8610 at the genetic level. Similarly, the advantages of *L. plantarum* CCFM8610 with respect to genes related to nucleotide transport and metabolism and amino acid transport and metabolism can be considered advantageous in terms of gene transcription and translation. These genomic characteristics may further promote the synthesis of enzymes required for CLA transformation.

Based on our results, we speculate that *L. plantarum* CCFM8610 may alleviate the clinical symptoms of IBS through the high-yield production of CLA. Previous studies have indicated that CLA can improve intestinal inflammation by inhibiting the NF-κB signaling pathway activation and pro-inflammatory cytokine expression.[47,48] We believe that this mechanism also explains the ability of *L. plantarum* CCFM8610 to alleviate the low-grade inflammation associated with IBS. Our conclusion is supported by multiple reports describing the ability of high-yield CLA probiotic strains to alleviate intestinal inflammation.[49,50]

Healthy gut microbiota can improve the intestinal health of the host and also has specific health benefits on emotional regulation and immune regulation.[51] Other reports have also proven the regulatory effects of CLA on the gut microbiota.[52–54] Dietary supplementation with CLA can significantly increase the relative abundances of Firmicutes, *Bifidobacterium*, and *Odoribacter* and reduce the relative abundance of *Bacteroides*. In other words, the ability of *L. plantarum* CCFM8610 to regulate the gut microbiota may be mediated by CLA.[53,54] However, we have not identified any published studies on the ability of CLA to alleviate visceral hypersensitivity, and therefore, this relationship remains uncertain. Nevertheless, CLA has been reported to have significant inhibitory effects on anxiety and depression. In rats, the maternal ingestion of CLA could reduce anxiety by inhibiting lipid peroxidation in the brains of offspring.[55] The oral administration of CLA-rich goat milk products to rat dams was shown to prevent anxiety in their offspring.[56] In depression-related studies, dietary supplementation with CLA was shown to inhibit compensatory overactivation of the Nrf2 pathway and thus alleviate depression in mice.[57] This evidence suggests that CLA can alleviate negative emotions. This functional ability may explain the alleviation of visceral hypersensitivity symptoms. The brain and gut can communicate bidirectionally through the brain–gut axis,[58] such that stimulation in the gut can cause anxiety, depression, and other negative emotions in the brain. Conversely, negative emotions can exacerbate intestinal visceral hypersensitivity symptoms through the vagus nerve.[59] Therefore, we believe that physiologically, the strong ability of *L. plantarum* CCFM8610 to synthesize CLA may contribute to emotional regulation in mice and alleviate intestinal symptoms of visceral hypersensitivity. Moreover, the low-grade inflammation associated with IBS is thought to induce peripheral sensitization, which accelerates the development of long-term visceral hypersensitivity.[60] Therefore, the alleviating effects of CLA on low-grade intestinal inflammation may also be a potential mechanism for the alleviation of visceral hypersensitivity.

We have carried out a clinical trial of *L. plantarum* CCFM8610 in alleviating IBS with diarrhea (IBS-D) in the Tinghu People's Hospital of Yancheng, Jiangsu, China. The results showed that dietary supplementation of *L. plantarum* CCFM8610 could alleviate the clinical symptoms of IBS-D patients, improve the quality of life, and modulate gut microbiota dysbiosis. It is similar to this animal experiment's results, which indicates that *L. plantarum* CCFM8610 may have the same mechanism in the animal model and clinical model. In future experiments, CLA could be administered to mice in the form of dietary supplements to verify the ability of CLA to modulate low-grade inflammation, visceral hypersensitivity, and gut microbiota dysbiosis related to IBS. Moreover, another clinical trial would further be conducted to verify the underlying mechanisms of CLA in IBS.

However, this study did identify the physiological characteristics and functional genes in *Lactobacillus* that were most relevant to the IBS-alleviating effects. Future studies should apply molecular biological methods, such as polymerase chain reactions to identify relevant functional genes and GC-MS to detect CLA synthesis. These approaches may enable the rapid screening of *Lactobacillus* strains with IBS-alleviating effects.

5. Conclusion

In summary, this study aimed to screen *Lactobacillus* strains with IBS-alleviating effects and investigate the associated key physiological characteristics and functional genes. We used an animal model of IBS to determine the abilities of *Lactobacillus* strains to alleviate IBS and analyzed the correlation between the *in vitro* physiological characteristics, functional genes, and IBS-alleviating effects of these strains. We identified *L. plantarum* CCFM8610 as a strain with significant abilities to alleviate the IBS symptoms of low-grade inflammation and visceral hypersensitivity and modulated the gut microbiota dysbiosis. Our correlation analysis identified the CLA synthesis ability and COG1028-related genes as the most relevant physiological characteristic and functional genes, respectively, in this context. The observed relationships between a high CLA synthesis ability, COG1028-related genes, and IBS-alleviating effects will contribute to the rapid screening of *Lactobacillus* species with IBS-alleviating effects and lay a foundation for future investigations of the underlying mechanisms.

Conflicts of interest

All of the authors declare that they have no conflicts of interest.

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References

1 A. C. Ford, B. E. Lacy and N. J. Talley, Irritable Bowel Syndrome, *N. Engl. J. Med.*, 2017, **376**, 2566–2578.
2 J. L. Buono, R. T. Carson and N. M. Flores, Health-related quality of life, work productivity, and indirect costs among patients with irritable bowel syndrome with diarrhea, *Health. Qual. Life. Outcomes*, 2017, **15**, 35.
3 C. Canavan, J. West and T. Card, Review article: the economic impact of the irritable bowel syndrome, *Aliment. Pharmacol. Ther.*, 2014, **40**, 1023–1034.
4 K. A. Gwee, U. C. Ghoshal and M. Chen, Irritable bowel syndrome in Asia: pathogenesis, natural history, epidemiology, and management, *J. Gastroenterol. Hepatol.*, 2018, **33**, 99–110.
5 H. M. Staudacher and K. Whelan, The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS, *Gut*, 2017, **66**, 1517–1527.
6 R. L. Akehurst, J. E. Brazier, N. Mathers, C. O'Keefe, E. Kaltenthaler, A. Morgan, M. Platts and S. J. Walters, Health-related quality of life and cost impact of irritable bowel syndrome in a UK primary care setting, *Pharmacoeconomics*, 2002, **20**, 455–462.
7 S. Ballou, C. McMahon, H.-N. Lee, J. Katon, A. Shin, V. Rangan, P. Singh, J. Nee, M. Camilleri and A. Lembo, Effects of irritable bowel syndrome on daily activities vary among subtypes based on results from the IBS in America Survey, *Clin. Gastroenterol. Hepatol.*, 2019, **17**, 2471–2478.
8 D. M. Brenner and G. S. Sayuk, Current US Food and Drug Administration-approved pharmacologic therapies for the treatment of irritable bowel syndrome with diarrhea, *Adv. Ther.*, 2020, **37**, 83–96.
9 G. S. Sayuk, R. Wolf and L. Chang, Comparison of symptoms, healthcare utilization, and treatment in diagnosed and undiagnosed individuals with diarrhea-predominant irritable bowel syndrome, *Am. J. Gastroenterol.*, 2017, **112**, 892–899.
10 M. E. Sanders, D. J. Merenstein, G. Reid, G. R. Gibson and R. A. Rastall, Probiotics and prebiotics in intestinal health and disease: from biology to the clinic, *Nat. Rev. Gastroenterol. Hepatol.*, 2019, **16**, 605–616.
11 F. De Filippis, E. Pasolli and D. Ercolini, The food-gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health, *FEMS Microbiol. Rev.*, 2020, **44**, 454–489.
12 M. S. do Carmo, C. itapary dos Santos, M. C. Araújo, J. A. Girón, E. S. Fernandes and V. Monteiro-Neto, Probiotics, mechanisms of action, and clinical perspectives for diarrhea management in children, *Food Funct.*, 2018, **9**, 5074–5095.
13 Q. Zhao, W.-R. Yang, X.-H. Wang, G.-Q. Li, L.-Q. Xu, X. Cui, Y. Liu and X.-L. Zuo, *Clostridium butyricum* alleviates intestinal low-grade inflammation in TNBS-induced irritable bowel syndrome in mice by regulating functional status of lamina propria dendritic cells, *World J. Gastroenterol.*, 2019, **25**, 5469–5482.
14 R. Ding, W. Goh, R. Wu, X. Yue, X. Luo, W. W. T. Khine, J. Wu and Y. Lee, Revisit gut microbiota and its impact on human health and disease, *J. Food Drug Anal.*, 2019, **27**, 623–631.
15 A. Ait-Belgnaoui, I. Payard, C. Rolland, C. Harkat, V. Braniste, V. Théodorou and T. A. Tompkins, *Bifidobacterium longum* and *Lactobacillus helveticus* synergistically suppress stress-related visceral hypersensitivity through hypothalamic-pituitary-adrenal axis modulation, *J. Neurogastroenterol.*, 2018, **24**, 138–146.
16 C. J. Martoni, S. Srivastava and G. J. Leyer, *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium lactis* UABla-12 improve abdominal pain severity and symptomology in irritable bowel syndrome: randomized controlled trial, *Nutrients*, 2020, **12**, 363.
17 C. Cremon, S. Guglielmetti, G. Gargari, V. Taverniti, A. M. Castellazzi, C. Valsecchi, C. Tagliacarne, W. Fiore, M. Bellini and L. Bertani, Effect of *Lactobacillus paracasei* CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: A pilot randomized clinical trial, *United Eur. Gastroenterol. J.*, 2018, **6**, 604–613.
18 S. P. Shin, Y. M. Choi, W. H. Kim, S. P. Hong, J.-M. Park, J. Kim, O. Kwon, E. H. Lee and K. B. Hahm, A double blind, placebo-controlled, randomized clinical trial that breast milk derived-*Lactobacillus gasseri* BNR17 mitigated diarrhea-dominant irritable bowel syndrome, *J. Clin. Biochem. Nutr.*, 2018, **62**, 179–186.
19 C. Stevenson, R. Blaauw, E. Fredericks, J. Visser and S. Roux, Randomized clinical trial: effect of *Lactobacillus plantarum*, 299v on symptoms of irritable bowel syndrome, *Nutrition*, 2014, **30**, 1151–1157.
20 K. Hod, R. Dekel, N. Aviv Cohen, A. Sperber, Y. Ron, M. Boaz, S. Berliner and N. Maharshak, The effect of a multispecies probiotic on microbiota composition in a clinical trial of patients with diarrhea–predominant irritable bowel syndrome, *Neurogastroenterol. Motil.*, 2018, **30**, e13456.
21 L. M. Begtrup, O. B. S. de Muckadell, J. Kjeldsen, R. d. Christensen and D. E. Jarbøl, Long-term treatment with probiotics in primary care patients with irritable bowel syndrome-a randomised, double-blind, placebo controlled trial, *Scand. J. Gastroenterol.*, 2013, **48**, 1127–1135.

22 A. R. Romijn, J. J. Rucklidge, R. G. Kuijer and C. Frampton, A double-blind, randomized, placebo-controlled trial of *Lactobacillus helveticus* and *Bifidobacterium longum* for the symptoms of depression, *Aust. N. Z. J. Psychiat.*, 2017, **51**, 810–821.

23 C. Dunne, L. O'Mahony, L. Murphy, G. Thornton, D. Morrissey, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald and C. Daly, *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings, *Am. J. Clin. Nutr.*, 2001, **73**, 386s–392s.

24 R. Li, Y. Zhang, D. B. Polk, P. M. Tomasula, F. Yan and L. Liu, Preserving viability of *Lactobacillus rhamnosus* GG *in vitro* and *in vivo* by a new encapsulation system, *J. Controlled Release*, 2016, **230**, 79–87.

25 S. Yan, B. Yang, J. Zhao, J. Zhao, C. Stanton, R. P. Ross, H. Zhang and W. Chen, A ropy exopolysaccharide producing strain *Bifidobacterium longum* subsp. longum YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation, *Food Funct.*, 2019, **10**, 1595–1608.

26 J. Wang, H. Chen, B. Yang, Z. Gu, H. Zhang, W. Chen and Y. Q. Chen, *Lactobacillus plantarum* ZS2058 produces CLA to ameliorate DSS-induced acute colitis in mice, *RSC Adv.*, 2016, **6**, 14457–14464.

27 V. Subramaniyan and K. Gurumurthy, Diversity of probiotic adhesion genes in the gastrointestinal tract of goats, *J. Cell. Biochem.*, 2019, **120**, 12422–12428.

28 I.-C. Lee, G. Caggianiello, I. I. van Swam, N. Taverne, M. Meijerink, P. A. Bron, G. Spano and M. Kleerebezem, Strain-specific features of extracellular polysaccharides and their impact on *Lactobacillus plantarum*-host interactions, *Appl. Environ. Microbiol.*, 2016, **82**, 3959–3970.

29 C. Ibeakanma, F. O. Cortes, M. M. Morales, T. Mcdonald, I. Spreadbury, N. Cenac, F. Cattaruzza, D. J. Hurlbut, S. Vanner and N. W. Bunnett, Brain-gut interactions increase peripheral nociceptive signaling in mice with post-infectious irritable bowel syndrome, *Gastroenterology*, 2011, **141**, 2098–2108.

30 Q. Chen, Y. Ren, J. Lu, M. Bartlett, L. Chen, Y. Zhang, X. Guo and C. Liu, A novel prebiotic blend product prevents irritable bowel syndrome in mice by improving gut microbiota and modulating immune response, *Nutrients*, 2017, **9**, 1341.

31 Y. Liu, Y. Sheng, Q. Pan, Y. Xue, L. Yu, F. Tian, J. Zhao, H. Zhang, Q. Zhai and W. Chen, Identification of the key physiological characteristics of *Lactobacillus plantarum* strains for ulcerative colitis alleviation, *Food Funct.*, 2020, **11**, 1279–1291.

32 J. Zhao, F. Tian, S. Yan, Q. Zhai, H. Zhang and W. Chen, *Lactobacillus plantarum* CCFM10 alleviating oxidative stress and restoring the gut microbiota in d-galactose-induced aging mice, *Food Funct.*, 2018, **9**, 917–924.

33 S. Cen, R. Yin, B. Mao, J. Zhao, H. Zhang, Q. Zhai and W. Chen, Comparative genomics shows niche-specific variations of *Lactobacillus plantarum* strains isolated from human, *Drosophila melanogaster*, vegetable and dairy sources, *Food Biosci.*, 2020, **35**, 100581.

34 Y. Liu, Y. Li, X. Yu, L. Yu, F. Tian, J. Zhao, H. Zhang, Q. Zhai and W. Chen, Physiological characteristics of *Lactobacillus casei* strains and their alleviation effects against inflammatory bowel disease, *J. Microbiol. Biotechnol.*, 2020, **30**, DOI: 10.4014/jmb.2003.03041.

35 R. Choghakhori, A. Abbasnezhad, A. Hasanvand and R. Amani, Inflammatory cytokines and oxidative stress biomarkers in irritable bowel syndrome: Association with digestive symptoms and quality of life, *Cytokine*, 2017, **93**, 34–43.

36 N. Bertiauxvandaele, S. B. Youmba, L. Belmonte, S. Lecleire, M. Antonietti, G. Gourcerol, A. M. Leroi, P. Dechelotte, J. Menard and P. Ducrotte, The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype, *Am. J. Gastroenterol.*, 2011, **106**, 2165–2173.

37 R. Duan, S. Zhu, B. Wang and L. Duan, Alterations of gut microbiota in patients with irritable bowel syndrome based on 16S rRNA-targeted sequencing: a systematic review, *Clin. Transl. Gastroenterol.*, 2019, **10**, e00012.

38 T. Ringelkulka, J. W. Mcrorie and Y. Ringel, Multi-Center, double-blind, randomized, placebo-controlled, parallel-group study to evaluate the benefit of the probiotic *Bifidobacte*rium infantis, 35624 in non-patients with symptoms of abdominal discomfort and bloating, *Am. J. Gastroenterol.*, 2017, **112**, 145–151.

39 S. Ludidi, D. Jonkers, C. J. M. Koning, J. W. Kruimel, L. Mulder, I. B. V. Der Vaart, J. M. Conchillo and A. A. M. Masclee, Randomized clinical trial on the effect of a multispecies probiotic on visceroperception in hypersensitive IBS patients, *Neurogastroenterol. Motil.*, 2014, **26**, 705–714.

40 Y. Cui, H. Wei, F. Lu, X. Liu, D. Liu, L. Gu and C. Ouyang, Different effects of three selected *Lactobacillus* strains in dextran sulfate sodium-induced colitis in BALB/c mice, *PLoS One*, 2016, **11**, e0148241.

41 B. Zheng, J. Van Bergenhenegouwen, H. J. G. Van De Kant, G. Folkerts, J. Garssen, A. P. Vos, M. E. Morgan and A. D. Kraneveld, Specific probiotic dietary supplementation leads to different effects during remission and relapse in murine chronic colitis, *Benefic. Microbes*, 2016, **7**, 205–213.

42 T. Perezberezo, J. Pujo, P. Martin, P. L. Faouder, J. Galano, A. Guy, C. Knauf, J. Tabet, S. Tronnet and F. Barreau, Identification of an analgesic lipopeptide produced by the probiotic *Escherichia coli* strain Nissle 1917, *Nat. Commun.*, 2017, **8**, 1314.

43 J. Zhang, L. Song, Y. Wang, C. Liu, L. Zhang, S. Zhu, S. Liu and L. Duan, Beneficial effect of butyrate-producing Lachnospiraceae on stress-induced visceral hypersensitivity in rats, *J. Gastroenterol. Hepatol.*, 2018, **34**, 1368–1376.

44 B. Yang, H. Qi, Z. Gu, H. Zhang, W. Chen, H. Chen and Y. Chen, Characterization of the triple–component linoleic acid isomerase in *Lactobacillus plantarum* ZS2058 by

genetic manipulation, *J. Appl. Microbiol.*, 2017, **123**, 1263–1273.

45 N. P. Evans, S. A. Misyak, E. M. Schmelz, A. J. Guri, R. Hontecillas and J. Bassaganya-Riera, Conjugated linoleic acid ameliorates inflammation-induced colorectal cancer in mice through activation of PPARγ, *J. Nutr.*, 2010, **140**, 515–521.

46 S. Borniquel, C. Jadert and J. O. Lundberg, Dietary conjugated linoleic acid activates PPARγ and the intestinal trefoil factor in SW480 cells and mice with dextran sulfate sodium-induced colitis, *J. Nutr.*, 2012, **142**, 2135–2140.

47 P. Delerive, P. Gervois, J.-C. Fruchart and B. Staels, Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators, *J. Biol. Chem.*, 2000, **275**, 36703–36707.

48 M. A. Belury, Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action, *Annu. Rev. Nutr.*, 2002, **22**, 505–531.

49 Y. Chen, Y. Jin, C. Stanton, R. P. Ross, J. Zhao, H. Zhan, B. Yang and W. Chen, Alleviation effects of *Bifidobacterium breve* on DSS-induced colitis depends on intestinal tract barrier maintenance and gut microbiota modulation, *Eur. J. Nutr.*, 2020, DOI: 10.1007/s00394-020-02252-x.

50 J. Bassaganya-Riera, M. Viladomiu, M. Pedragosa, C. De Simone, A. Carbo, R. Shaykhutdinov, C. Jobin, J. C. Arthur, B. A. Corl and H. Vogel, Probiotic bacteria produce conjugated linoleic acid locally in the gut that targets macrophage PPARγ to suppress colitis, *PLoS One*, 2012, **7**, e31238.

51 M. Cole and M. A. Augustin, Food Safety and Health, *Engineering*, 2020, **6**, 391–392.

52 L. J. den Hartigh, Conjugated linoleic acid effects on cancer, obesity, and atherosclerosis: a review of pre-clinical and human trials with current perspectives, *Nutrients*, 2019, **11**, 370.

53 T. M. Marques, R. Wall, O. O'Sullivan, G. F. Fitzgerald, F. Shanahan, E. M. Quigley, P. D. Cotter, J. F. Cryan, T. G. Dinan and R. P. Ross, Dietary trans-10, cis-12-conjugated linoleic acid alters fatty acid metabolism and microbiota composition in mice, *Br. J. Nutr.*, 2015, **113**, 728–738.

54 Y. Chen, B. Yang, R. P. Ross, Y. Jin, C. Stanton, J. Zhao, H. Zhang and W. Chen, Orally administered CLA ameliorates DSS-induced colitis in mice via intestinal barrier improvement, oxidative stress reduction, and inflammatory cytokine and gut microbiota modulation, *J. Agric. Food Chem.*, 2019, **67**, 13282–13298.

55 M. P. Queiroz, M. S. Lima, M. F. F. T. Melo, C. C. D. S. Bertozzo, D. F. Araújo, G. C. B. Guerra, R. C. R. E. Queiroga and J. K. B. Soares, Maternal suppplementation with conjugated linoleic acid reduce anxiety and lipid peroxidation in the offspring brain, *J. Affective Disord.*, 2019, **243**, 75–82.

56 J. K. B. Soares, A. P. R. Melo, M. C. Medeiros, R. C. R. E. Queiroga, M. A. D. Bomfim, E. C. A. Santiago and R. A. Guedes, Anxiety behavior is reduced, and physical growth is improved in the progeny of rat dams that consumed lipids from goat milk: an elevated plus maze analysis, *Neurosci. Lett.*, 2013, **552**, 25–29.

57 L. Cigliano, M. S. Spagnuolo, F. Boscaino, I. Ferrandino, A. Monaco, T. Capriello, E. Cocca, L. Iannotta, L. Treppiccione and D. Luongo, Dietary supplementation with fish oil or conjugated linoleic acid relieves depression markers in mice by modulation of the Nrf2 pathway, *Mol. Nutr. Food Res.*, 2019, **63**, 1900243.

58 J. F. Cryan, K. J. O'Riordan, C. S. Cowan, K. V. Sandhu, T. F. Bastiaanssen, M. Boehme, M. G. Codagnone, S. Cussotto, C. Fulling and A. V. Golubeva, The microbiota-gut-brain axis, *Physiol. Rev.*, 2019, **99**, 1877–2013.

59 G. Fond, A. Loundou, N. Hamdani, W. Boukouaci, A. Dargel, J. Oliveira, M. Roger, R. Tamouza, M. Leboyer and L. Boyer, Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis, *Eur. Arch. Psychiatry Clin. Neurosci.*, 2014, **264**, 651–660.

60 B. Adam, T. Liebregts, J. M. Gschossmann, C. Krippner, F. Scholl, M. Ruwe and G. Holtmann, Severity of mucosal inflammation as a predictor for alterations of visceral sensory function in a rat model, *Pain*, 2006, **123**, 179–186.

