DOI: 10.26599/FSHW.2024.9250097

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Polygonatum Sibiricum Saponin-Lactic acid bacteria combination attenuate hyperglycemia in T2DM mice by modulating amino acid metabolism

Jiayuan Luo[a], Mingjie Jia[a], Shuang Liang[a], Yangyang Chai[a,b,*], Yihong Bao[a,b,*], Shilong Jiang[c]

[a]*Colleage of life science, Northeast Forestry University, Harbin 150040, PR China*

[b]*Key Laboratory of Forest Food Resources Utilization of Heilongjiang Province, Harbin 150040, PR China*

[c]*Heilongjiang Feihe Dairy Industry, Harbin 150040, PR China*

ABSTRACT: Some researches have shown that the combination of plant extracts and probiotics may be a better way to treat type 2 diabetes mellitus (T2DM) than a single intervention. However, there are still relatively few relevant reports in this aspect. Therefore, this study aims to investigate whether the treatment of *polygonatum sibiricum* saponin (PSS) and lactic acid bacteria (LAB) combination can better manage T2DM. And the anti-diabetes mechanism of the combination was studied from the perspectives of glucose metabolism, microbiome and metabolome. The results showed that PSS+LAB could better improve FBG level, insulin sensitivity, lipid metabolism disorder, and liver function. Protein analysis showed that PSS+LAB treatment significantly increased the expression of p-PI3K/PI3K, p-AKT/AKT, GLUT2, IRS2, and GSK-3β in the liver of T2DM mice, while inhibiting the expression of FOXO1. This combination positively regulated the composition and abundance of the gut microbiota. Metabolomic analysis showed that the combination treatment exhibited more changes in gut microbiota metabolites compared to PSS treatment alone. The alteration of gut microbiota by LAB+PSS led to significant changes in alanine, aspartate and glucose metabolism pathways. This study may provide a theoretical basis for the combined application of plant extracts and probiotics for the management of T2DM.

Keywords: Probiotics; *Polygonatum Sibiricum* Saponin; T2DM; gut microbiota; metabolites

1. Introduction

In recent years, people's living standards have gradually improved, and more and more people suffer from diabetes due to the intake of high salt and sugar and the reduction of exercise [1]. According to the prediction of International Diabetes Federation (IDF), by 2030, the number of diabetics will increase to 643 million. More than 90% of these patients belong to type 2 diabetes (T2DM) [2, 3]. Insulin resistance is the main characteristic of T2DM. When insulin resistance occurs, peripheral tissues such as liver is not sensitive to the insulin, thereby reducing the ability of glucose metabolism and glycogen synthesis[4]. Although traditional hypoglycemic drugs can effectively reduce blood glucose, they have dependence and side effects. Therefore, more and more attention has been paid to the research of natural plant active compounds that can improve diabetes without side effects.

***Corresponding author**
chaiyang@824; baoyihong@163.com

Received 4 July 2023
Received in revised from 13 August 2023
Accepted 4 September 2023

Modern researches have proved that gut microbiota may be closely related to the pathogenesis of T2DM. When the gut microbiota is dysbiosis, its intestinal barrier function is disrupted and intestinal mucosal permeability increases[5, 6]. Endotoxins can then enter the blood and trigger an inflammatory response in the body. Inflammatory factors cause damage to pancreatic islet cells and contribute to the development of T2DM. The composition and structure of gut microbiota in diabetics and healthy people were statistically different. Studies have shown that the *Firmicutes* and *Proteobacteria* abundance in the intestine of diabetics increases, while the abundance of *Bacteroides* decreases[7]. After transferring fecal microbiota from healthy donors to T2DM patients through fecal microbiota transplantation experiment, the donor microbiota was effectively colonized in patients within four weeks. The fasting blood glucose was decreased significantly, insulin sensitivity was increased, and insulin resistance of patients was greatly improved[8]. This indicated that it might be an effective strategy to treat T2DM by regulating gut microbiota.

The Food and Agriculture Organization (FAO)/World Health Organization (WHO) in 2002 had defined probiotics a “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [9]. Taking probiotics can regulate gut microbiota, enhance intestinal barrier and maintain the integrity of intestinal epithelial cells. Probiotics have beneficial effects on metabolic diseases and immune diseases [10, 11]. Cabello Olmo et al. found that fermented food containing *Lactobacillus* is beneficial to help diabetes rats with glucose metabolism and maintain health [12]. Yan et al.'s research suggested that *Lactobacillus acidophilus* can regulate glucose metabolism in T2DM mice by regulating glycogen synthase kinase 3 beta [13]. Lee's study showed that *Lactobacillus plantarum* could protect islet cells and restore gut microbiota in mice with T2DM [14]. Through the meta-analysis of randomized controlled trials, Wang et al. found that probiotics can help regulate blood lipid abnormalities in T2DM patients, and found that compound probiotics may be more effective than single probiotics [15]. In addition, Zhao et al. found that the extracellular polysaccharides of *Lactobacillus plantarum* can promote the secretion of intestinal hormone peptides and glucagon-like peptide-1 [16]. Although the anti-diabetic effect of probiotics has been demonstrated, the anti-diabetic effect and mechanism of probiotics varies from strain to strain due to the strain specificity of their function. Therefore, it is necessary to explore in depth the effects and potential mechanisms of different probiotic strains or combinations with hypoglycemic functions. Then relevant therapeutic drugs or functional foods will be developed according to the different characteristics.

Polygonatum sibiricum is a plant of the family *Liliaceae*, widely distributed in the north temperate zone with a long history of consumption based on its edible and medicinal characteristics [17, 18]. *Polygonatum sibiricum* has been listed by the National Health Commission of the People's Republic of China as "the list of items that are both food and medicine". At present, *Polygonatum sibiricum* has many commercial food products, including solid drinks, wine, crisps, etc. *Polygonatum sibiricum* has a variety of bioactive compounds, including polysaccharides, saponins and flavonoids [19, 20]. Among them, saponin is an important bioactive component. *Polygonatum sibiricum* saponin (PSS) has been proven to have antioxidant, hypolipidemic and anti-tumour properties [21]. Our previous studies had demonstrated the hypoglycemic

properties of PSS [22]. At present, the application of natural plant compounds in combination with probiotics to intervene in T2DM has attracted the attention of researchers.

Therefore, this study aimed to explore the hypoglycemic ability of lactic acid bacteria (LAB) combined with PSS. Then, the potential mechanism of LAB combined with PSS to improve the hyperglycemia in T2DM mice from the perspective of gut microbiota and metabolome was explored. The ability of LAB + PSS combination to improve glucose metabolism was investigated. Pathological analysis was conducted on the liver, pancreas, and colon. We also explored the function of intestinal barrier. Finally, the gut microbiota and metabolome of mouse feces were analyzed to reveal the potential mechanism.

2. Materials and methods

2.1. Preparation of saponins

As our previous research, ultrasonic assisted extraction method was used to extract saponins from *polygonatum sibiricum*. And AB-8 macroporous resin was used to purify the saponins. 70% and 80% ethanol fraction were collected [23]. After the fractions were concentrated and dried, PSS is obtained. 70% and 80% ethanol fractions were collected and combined, and then concentrated and freeze dried to obtain PSS powder. It was stored at -20 ℃ until use. The saponin compositions of PSS were detected by LC-MS, and the specific composition was shown in the Supplementary Table 1.

2.2. Bacteria and culture conditions

Lactobacillus casei BYglAB01 and *Lactobacillus bulgaricus* BYbjAD01 were obtained from the Key Laboratory of Forest Food Resources Utilization of Heilongjiang Province in China (Harbin, China). Before the experiment, the strains were cultured in MRS broth at 37 ℃ for 18 h. In animal experiments, the bacteria were centrifuged and resuspended in normal saline for subsequent experiments.

2.3. HFD/STZ-induced T2DM mice model and experimental design

4-week-old male ICR mice were obtained from Qingdao Daren Fortune Animal Technology Co. Ltd. (Animal license number: SCXK (Lu) 2014-0007). All animal experiments were approved by Northeast Forestry University Ethical Committee for Research on Laboratory Animals (Harbin, China). The animals were fed under the following conditions: a 12h light/dark cycle, (23 ± 2)℃, and 50%±10% humidity. Subsequently, mice were randomly divided into a normal control group and a diabetes group. Normal mice were fed with basic diet (50% cornmeal, 15% white flour, 20% soybean cake, 10% bran, 0.3% multivitamin and 0.2% salt), while diabetes mice were fed with high-sugar and high-fat diet (15% yolk, 10% lard, 15% sucrose, 2% cholesterol, 0.2% sodium cholate and 57.8% basic diet). 4 weeks later, the mice in the diabetes group were fasted for 12 h and then injected 1% streptozotocin (STZ) solution (50 mg/kg BW). After 72h, the fasting blood glucose (FBG) of mice was detected. Mice with a level of FBG > 11.1 mmol/L were regarded as T2DM mice [24].

Diabetic mice were randomly divided into five groups (n=10): (i) Diabetes control (DC) group with normal saline; (ii) Metformin control (MC) group with metformin with a dose of 500 mg/kg; (iii) PSS

treatment group with PSS with a dose of 1000 mg/kg; (iv) Lactic acid bacteria treatment group (LAB) with *L. casei* BYglAB01, *L. bulgaricus* BYbjAD01 (2:1); (v) PSS and compound LAB treatment group (the LAB concentration was 1×10^{10} CFU per mL, 0.1 mL per day). The compound probiotics are *L. casei* BYglAB01 and *L. bulgaricus* BYbjAD01 in a ratio of 2:1. All mice were intervened for 4 weeks, and their body weight and FBG were tested weekly.

2.4. Oral glucose tolerance test (OGTT)

The OGTT was measured as described above after treatment [25]. Mice were given glucose (2.5 g/kg BW) by gavage after fasting overnight. Then, the blood glucose was determined at 0, 30, 60, 120 h. The result of OGTT was presented by the area under the curve (AUC): $AUC = (0.5\times G_{0\ h}+G_{0.5\ h}+G_{1\ h}+0.5\times G_{2\ h}) \times 30$.

2.5. Measurement of biochemical parameters

Blood samples were centrifuged to obtain serum (3000g, 10 min). The fasting serum insulin was measured with an Elisa kit (Andy gene Technology Co., Ltd., Beijing, China). QUICKI was calculated as previously described [26]. High-density lipoprotein cholesterol (HDL-C), triacylglycerols (TG), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), alanine aminotransferase (ALT), and aspartate aminotransfer (AST), glucagon-like peptide-1 (GLP-1), peptide YY (PYY), glycosylated hemoglobin A1c (HbA1c), C peptide, lipopolysaccharide (LPS) and D-Lactic acid (D-LA) in serum were measured with commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Histopathological analysis

The livers, pancreas and colon were washed with normal saline. The tissue was fixed in 4% paraformaldehyde solution and then embedded in paraffin wax. Part of the liver tissue, pancreas tissue and colon tissue were stained with hematoxylin-eosin (H&E). Another part of the liver tissue was stained with periodic acid-schiff (PAS). Images were captured with a light microscope (Moticam 3000).

2.7. Western blotting analysis

Total proteins from liver tissue were extracted by RIPA lysis buffer (containing protease inhibitor, PMSF). Proteins (60 μg) were separated by 15% SDS-PAGE and transferred onto PVDF membranes. The membranes were incubated with 5% skimmed milk for 2h and incubated with primary antibodies (β-actin, PI3K, AKT, IRS2, GSK-3β, FOXO1, GLUT2) overnight at 4℃. The membranes were incubated with secondary antibodies for 1 h at room temperature after washing with TBST for three times [27]. Finally, protein bands were observed through enhanced chemiluminescence.

2.8 mRNA expression analysis by RT-qPCR

Total RNA was extracted from the colon using TRIzol and reverse transcribed into cDNA using PrimeScript RT premix [28]. Real time quantitative polymerase chain reaction was performed with a real-time fluorescence quantitative gene amplification instrument (qTOWER3G, Analytick Jena AG, Germany) using SYBR premix Ex Taq. The primers for the target gene were synthesized by synthesized by Shanghai Shenggong Technology Co., Ltd. (Shanghai, China). GADPH: forward sequence (F):

ACGGCAAATTCAACGGCACAG, reverse sequence (R): AGACTCCACGACATACTCAGCAC. Claudin-1: F: GGGGACAACATCGTGACCG, R: AGGAGTCGAAGACTTTGCACT. Occludin: F: CTGGATCTATGTACGGCTCACA, R: TCCACGTAGAGACCAGTACCT. ZO-1: F: GCCGCTAAGAGCACAGCAA, R: TCCCCACTCTGAAAATGAGGA. TLR2: F: TTTGCTCCTGCGAACTCC, R: GCCACGCCCACATCATTC. MUC2: F: GCCCACCTCACAAGCAGTAT, R: GTCATAGCCAGGGGCAAACT. MUC4: F: TCCTCACCAGCACATTGACC, R: TTGAGACATGCTGGGTGTCC.

2.9. Analysis of gut microbiota

Colon content was obtained from all mice in the colon after the end of the experiment. The genomic DNA of the sample was extracted using the E.Z.N.A.® Stool DNA Kit (D4015, Omega, Inc., USA). The V3-V4 region of the 16S rDNA gene was amplified with specific primers (343F 5'- TACGGRAGGCAGCAG -3' and 798R 5'- AGGGTATCTAATCCT-3'), and then the purity and concentration of DNA were detected by agarose gel electrophoresis. The quality of the amplicons was visualized by gel electrophoresis and purified using AMPure XP beads (Agencourt) for further PCR amplification. After a second purification using AMPure XP beads, the final amplicons were quantified using the Qubit dsDNA Detection Kit (12642ES76, Yeasen Biotechnology Co., Ltd., China). Equal amounts of purified amplicons were combined and used for subsequent sequencing.

The original data is in FASTQ format. Cutadapt software cuts the primer sequence from the raw data sequence. Then, DADA2 is used to conduct quality control analysis for the qualified double-ended raw data in the previous step according to the default parameters of QIIME 2 to obtain the representative sequence and ASV abundance table. QIIME2 calculated alpha diversity and beta diversity, and the indices they included reflected the complexity and diversity of the microbiota. The representative read of each ASV was selected using the QIIME2 package [29]. All representative reads were annotated and blasted against Silva database Version 138 using q2-feature-classifier.

2.10 Metabolomics Profiling of Fecal Samples

The metabolomics analysis of fecal samples was performed using gas chromatography-mass spectrometry (GC-MS) (7890A-5975C, Agilent, USA). Place 60 mg of fecal sample into a 1.5 mL centrifuge tube and add 600 μL methanol aqueous solution (v: v=4:1) (L-2-chlorophenylalanine, 0.3 mg/mL, soluble in methanol). Use a homogenizer (60Hz, 2 min) to homogenize the sample. Then, the sample was ultrasonically extracted for 10 min and left to stand at -20 ℃ for 30 min. Afterwards, centrifuge the sample at 13000 r/min and 4 ℃ for 15 min. Then, 400 μL supernatant was added into a glass vial and was evaporated using a centrifugal concentration dryer. 80 μL of methoxylamine hydrochloride pyridine solution (15 mg/mL) was subjected to vortex shaking for 2 min and then subjected to oximation reaction in a shaking incubator at 37 ℃ for 60 min. The sample was removed, 50 μL of BSTFA derivatization reagent was added, 20 μL of hexane was vortex shaken for 2 min, and the reaction was carried out at 70℃ for 60 min. After removing the sample, it was placed at room temperature for 30 min for GC-MS metabolomics analysis.

The raw GC/MS data obtained is imported into the software MS-DIAL, which performs peak detection, peak identification, characterisation, peak alignment, wave filtering and interpolation of missing values. Metabolite characterisation was based on the LUG database. In each sample, all peak signal intensities were segmented and normalised according to a filtered internal standard with an RSD greater than 0.3. After data normalisation, redundancy removal and peak merging were performed to obtain a data matrix. The matrix was imported into R for principal component analysis (PCA) to observe the overall distribution between samples and the stability of the overall analysis process. Orthogonal partial least squares discriminant analysis (OPLS-DA) and partial least squares discriminant (PLS-DA) were used to differentiate metabolites that differed between groups. Variable projection importance (VIP) values obtained from the OPLS-DA model were used to rank the overall contribution of each variable to group discrimination. Differential metabolites with VIP values greater than 1.0 and p-values less than 0.05 were selected.

2.11. Statistical Analysis

All experiments were conducted in parallel at least three times. Data are presented as mean ± standard deviation (SD) and analyzed using SPSS 22.0 software (Chicago, USA). For statistical comparisons between all groups, one-way analysis of variance (ANOVA) and Tukey's test for statistical comparisons were used, with $P < 0.05$ considered significant. Correlations between gut microbiota (genus level) and other serum parameters were visualized with Spearman's correlation.

3. Results

3.1. Effects on serum parameters of T2DM mice

As shown in Fig.1A, after successful modeling, the blood glucose of the DC group increased from 15.35 mmol/L to 25.80 mmol/L. The blood glucose in the NC group was stable and maintained at a low level. The blood glucose of other groups of mice decreased gradually. In the 4-week, the blood glucose of the MC group, PSS group, LAB group, and PSS+LAB group decreased by 33.65%, 20.57%, 17.90%, and 26.10%, respectively, compared with that of the 0-week. Meanwhile, the MC group had the fastest drop in blood glucose after the mice were given glucose, followed by the PSS group, LAB group, and PSS+LAB group. However, there was no significant difference in OGTT results among these three groups (Fig.1B). The changes in HbA1c also reflected similar results (Fig.1C).

As shown in Fig.1D, the insulin level of the DC group is significantly higher than that of other groups. The insulin level in the PSS+LAB group was the lowest in T2DM mice, 48.19% lower than in the DC group. After treatment, the level of C-peptide was also significantly decreased. After taking PSS+LAB, the level of C-peptide was approached that of normal group and positive control group taking metformin (Fig.1E). The quantitative insulin sensitivity check index (QUICKI) was calculated according to the FBG and insulin values. The QUICKI value of the DC group was 0.83 times that of the NC group. This indicated that T2DM mice had low insulin sensitivity and they have apparent insulin resistance. After the treatment, the QUICKI value of mice was significantly higher than that of the DC group, indicating that the insulin resistance of T2DM mice

was relieved. The above results showed that metformin, PSS, and LAB could positively regulate blood glucose and insulin secretion in T2DM mice. Especially the combination of PSS and LAB was used, this combination has the best intervention effect on insulin resistance in mice. The levels of gastrointestinal hormones GLP-1 and PYY were also affected, but there was no significant difference in PYY levels among the four treatment groups (Fig.1G&H).

In addition, the blood lipid levels of mice were also affected. Compared with the NC group, the levels of TC, TG, and LDL-C in the serum of the DC group increased significantly; The level of HDL-C decreased significantly ($P < 0.05$). TC, TG, and LDL-C levels were significantly decreased, and HDL-C levels were significantly increased in other groups ($P < 0.05$). The results showed that although metformin and PSS have regulatory effects on dyslipidemia in mice, LAB and the combination of LAB and PSS have more significant positive effects on lipid metabolism.

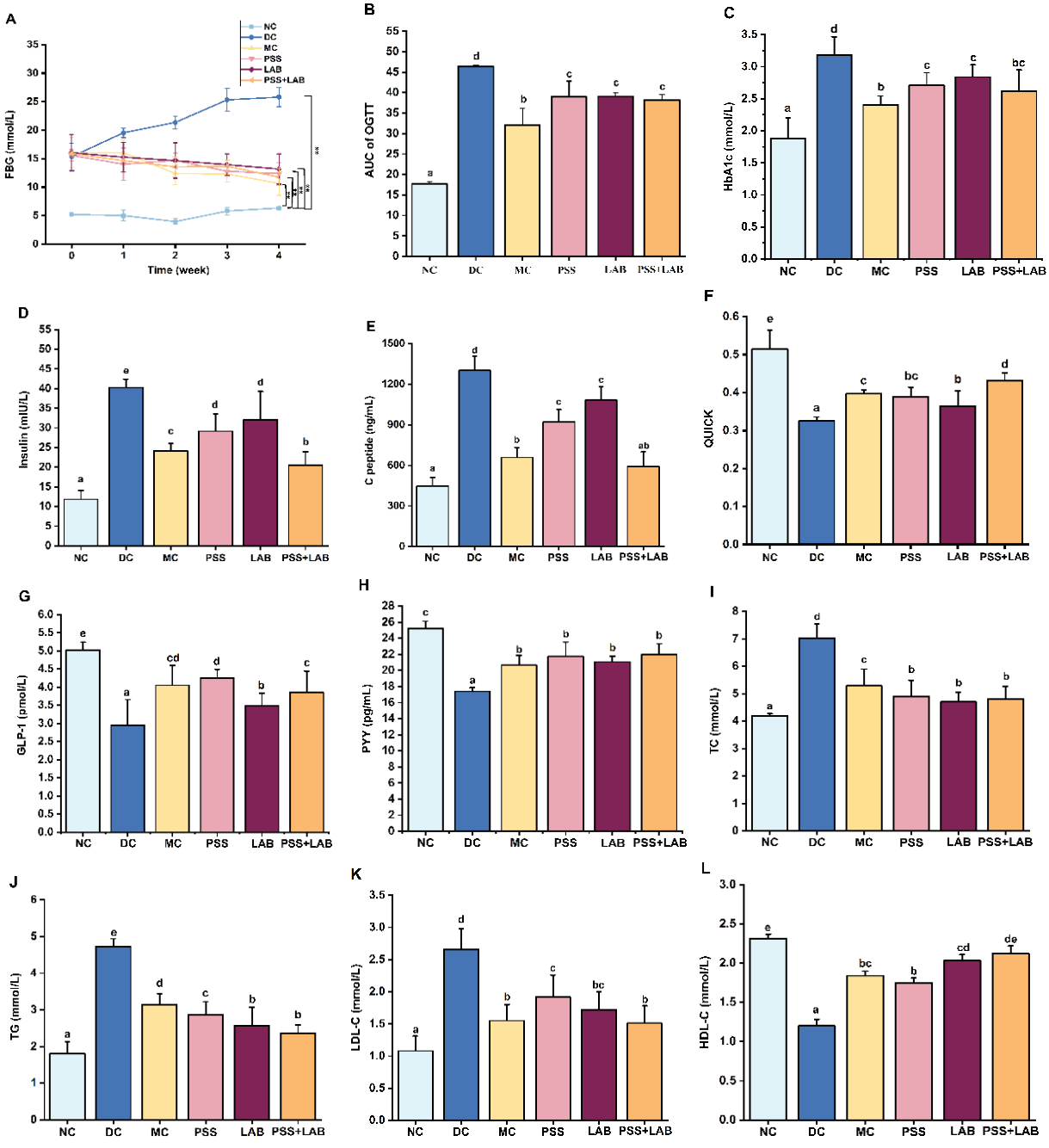


Fig.1. Effects on the hyperglycemia, glucose tolerance, and insulin resistance in mice (n=10). Fasting blood glucose (A), AUC of OGTT (B), HbA1c (C), insulin (D), C peptide (E), QUICKI (F), GLP-1 (G), PYY (H), TC (I), TG (J), LDL-C (K), and HDL-C (L). Different lowercase letters mean significant difference ($P < 0.05$).

3.2. Effects on liver and pancreas pathological changes

The liver and pancreas of mice were observed by H&E staining. In the NC group, the hepatocytes were regularly aligned, the cytoplasm and nucleus were clearly stained, and no inflammatory cell infiltration was observed. However, in the DC group, the liver cells were disorderly arranged, and many steatosis vacuoles were found. In addition, it was observed that inflammatory cells clustered together. After the supplement of metformin, PSS, LAB, and PSS+LAB, the liver abnormalities in the DC group were alleviated. Especially in the PSS group and PSS+LAB group, the steatosis in the liver tissue was obviously mitigated. Similar results were obtained in the determination of liver function indicators in mice. Compared with the NC group, serum AST and ALT activities in the DC group were significantly higher than those in the NC group. This indicated that the liver of T2DM mice was damaged. After 4 weeks of treatment with metformin, PSS, and LAB, AST and ALT activities were significantly reduced, indicating that the hepatic function of mice was significantly improved. In particular, the serum AST activity of the MC group and the serum ALP activity of the PSS+LAB group were close to those of the NC group.

The distribution of glycogen in the liver was observed by PAS staining. The glycogen in the liver of the NC group was purplish-red and evenly stained, indicating that there was a large amount of glycogen stored in the liver. The glycogen accumulation in the DC group was reduced, indicating that glycogen synthesis might be inhibited. After the intervention, the glycogen amount of T2DM mice was observed to increase, indicating that the glucose conversion rate to glycogen might increase. The pathological changes in the pancreas were also evaluated. The pancreas tissue in T2DM mice had cytoplasmic porosity and cavitation, accompanied by hyperemia and a decrease in islet cells. The treatment significantly alleviated the injury of the pancreas and partially restored the islet morphology.

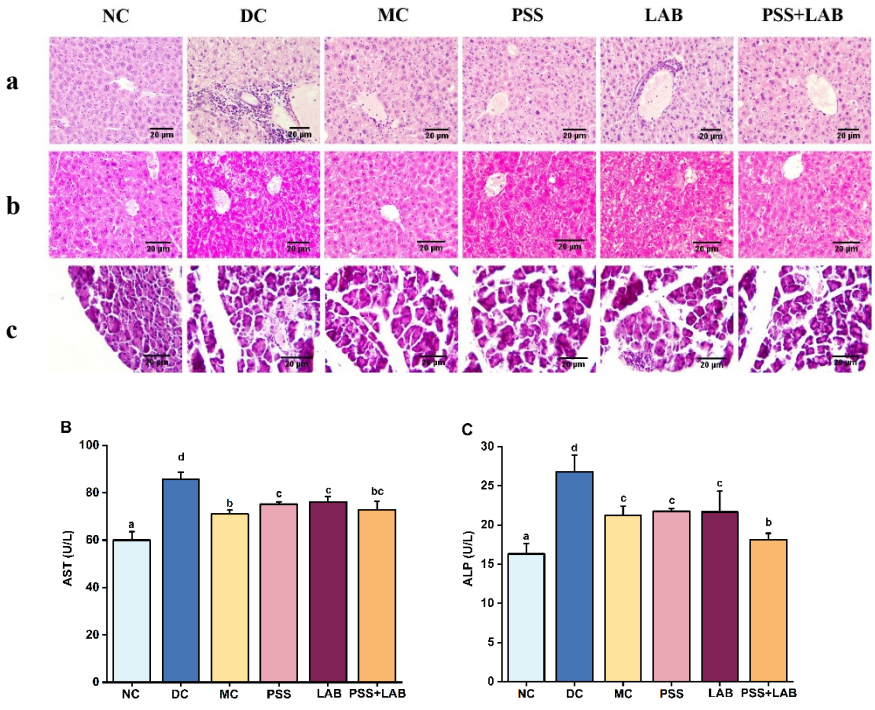


Fig.2. Pathological changes in liver and pancreas and of mice (n=10). Histological observation (A): a, H&E staining of liver; b, PAS staining of liver; c, H&E staining of pancreas (C). Level of AST in the liver (B), Level of ALP in the liver (C). Different lowercase letters mean significant difference ($P < 0.05$).

3.3. Effect on IRS2/PI3K/AKT signaling pathways in liver

The expression levels of related proteins in PI3K/AKT signaling pathway were detected by western blot analysis. Compared with the NC group, PI3K and AKT expression in the DC group were significantly inhibited by 40.64% and 31.46%, respectively (Fig.6, $P < 0.05$). Meanwhile, the expression of GLUT2, IRS2 was also significantly decreased (55.65%, 77.12%) ($P < 0.05$). Metformin, PSS, LAB, and PSS+LAB could effectively reverse these changes. PSS has a more significant effect on the expression of FOXO1 ($P < 0.05$). However, the expression of IRS2 and GSK-3β in the LAB and PSS+LAB groups was significantly higher than that in the PSS group, especially the expression of IRS2 in the PSS+LAB group was 9.33 times, 3.02 times, and 1.57 times of the DC group, MC group, and PSS group, respectively. The ratio of p-PI3K/PI3K and p-AKT/AKT also significantly increased after PSS+LAB treatment.

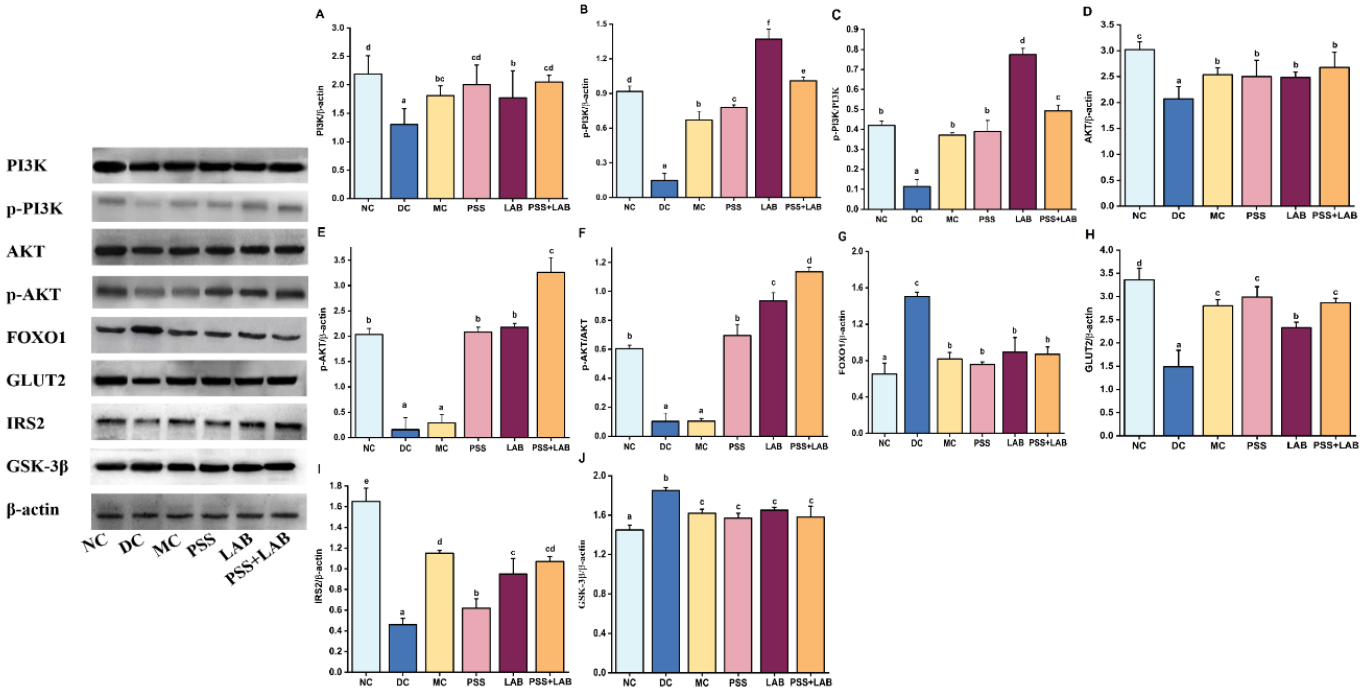


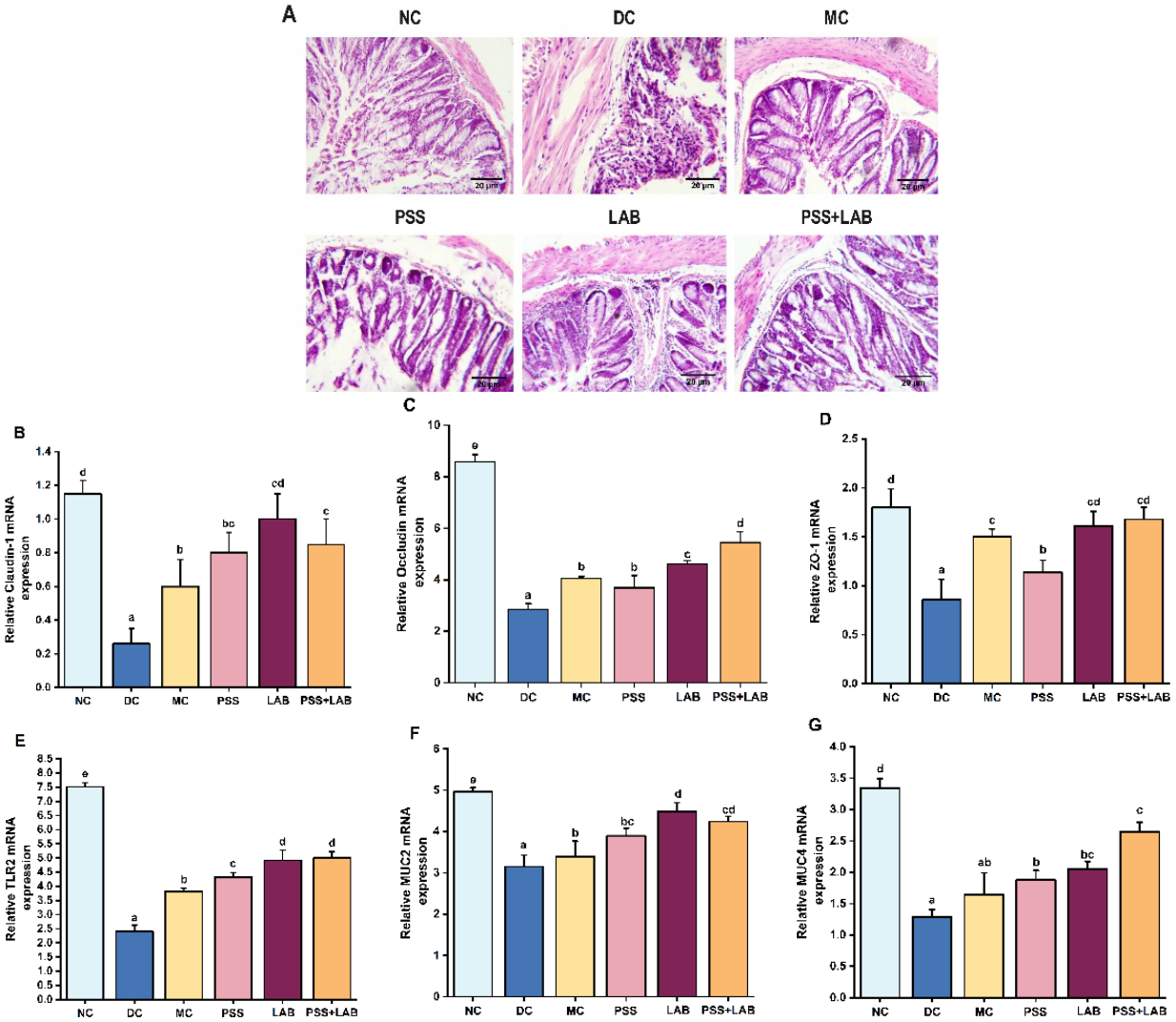
Fig.3. Effects on the expression of key proteins in the IRS2/PI3K/AKT signaling pathways in the liver of mice (n=10). PI3K (A), p-PI3K (B), p-PI3K/PI3K (C), AKT (D), p-AKT (E), AKT/p-AKT (F), FOXO1 (G), GLUT2 (H), IRS2 (I), GSK-3β (J). Different lowercase letters mean significant difference ($P < 0.05$).

3.4. Effect on gut barrier function

The H&E staining results of the intestine were shown in Fig.4A. Compared with the normal group, the DC group showed significant damage to the mucosal and muscular layers. The intestinal villi and crypts were significantly damaged, and the arrangement of glands is disordered. It indicating that T2DM can cause damage to the colon tissue in mice and damage to the integrity of the intestine. After administration of LAB to mice, the intestinal condition was significantly improved. In LAB group and PSS+LAB group, the mice colon crypt was complete, the glands were arranged neatly, and the goblet cell were complete, which was similar to the

morphology of the normal group colon. The intestinal tissue status of mice treated with PSS only also improved, but the intervention effect was weaker than that of the LAB group and PSS+LAB group.

Subsequently, the effects of tight junction proteins (TJPs), TLR2, and mucin mRNA expression in mice were detected. Compared with the DC group, the mRNA expression of TJPs, TLR2, and mucin were significantly increased in all treatment groups. In the LAB group, the mRNA expression levels of Claudin-1 and MUC2 were higher than those in other treatment groups, while in the PSS+LAB group, the expression levels of Occludin, ZO-1, and MUC4 were higher than other groups. In addition, a significant increase in the levels of D-LA and LPS in the blood of DC group mice was detected, indicating impaired intestinal barrier function in the mice. After treatment, the levels of D-LA and LPS significantly decreased, especially in the LAB and PSS+LAB groups, which were close to the NC group. All of the above indicated that PSS and LAB could repair the intestinal barrier function in T2DM mice.



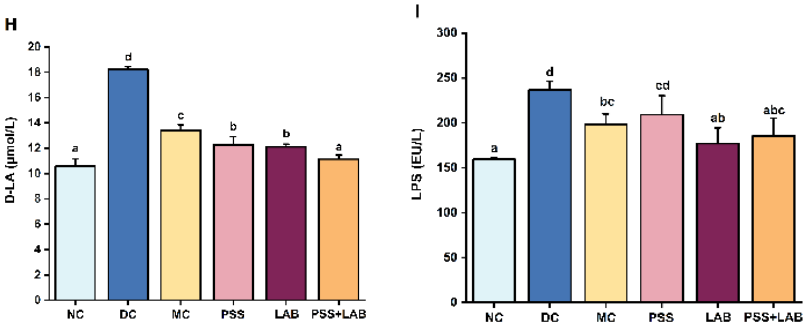


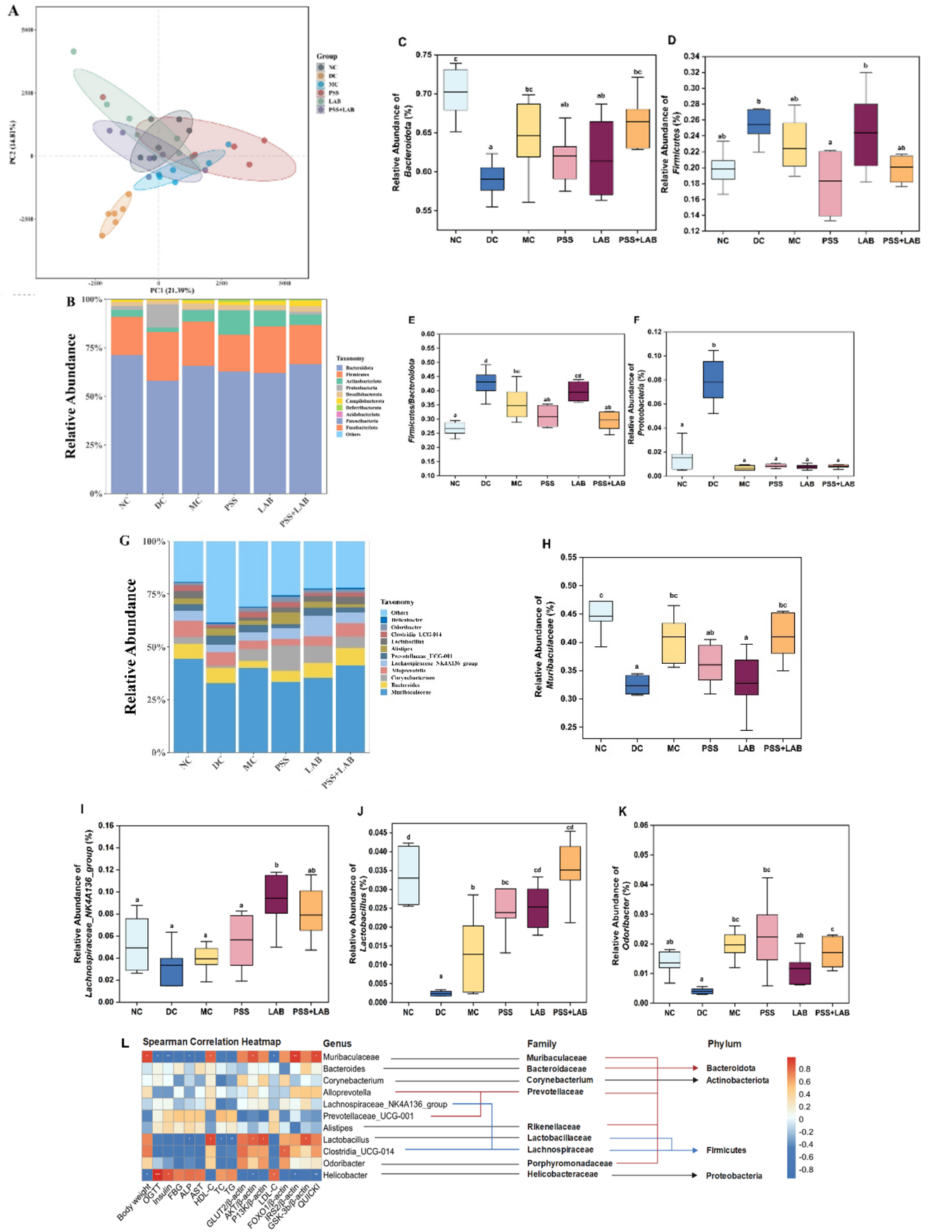
Fig.4 Effects on the *gut barrier function* of mice (n=10). H&E staining of colon (A), relative Claudin-1 mRNA expression (B), relative Occludin mRNA expression (C), relative ZO-1 mRNA expression (D), relative TLR2 mRNA expression (E), relative MUC2 mRNA expression (F) , relative MUC4 mRNA expression (G), D-LA level in serum (H), LPS level in serum (I). Different lowercase letters mean significant difference ($P < 0.05$).

3.5. Effect on gut microbiota diversity and composition

As shown in Fig.5A, the principal component analysis (PCA) showed that the distribution of samples within groups was concentrated, and the microbial composition of the DC group was significantly different from the other five groups. This showed that PSS, *L. casei* BYglAB01 and *L. bulgaricus* BYbjAD01 could effectively regulate the gut microbiome structure in T2DM mice. Compared with the NC group, the microbiome of T2DM mice was changed significantly; the abundance of *Bacteroidota* was decreased significantly, the abundance of *Firmicutes* and *Proteobacteria* was increased significantly, and the ratio of *Firmicutes*/*Bacteroidota* was also increased significantly ($P < 0.05$). However, these changes were reversed after the treatment. At the genus level, we also found changes in specific microbiota in different groups. In the DC group, *Prevotellaceae_UCG-001* and *Alistipes* were significantly enriched. After the treatment of PSS+LAB, the abundance of *Lactobacillus* and *Odoribacter* increased by 6.93 times and 7.18 times, respectively, compared with the DC group. After the treatment of *L. casei* BYglAB01 + *L. bulgaricus* BYbjAD01, the abundance of *Lachnospiracea_ NK4A136* was significantly higher than that of the DC group ($P < 0.05$), while there was no significant difference in other groups, although there was also an increase. In addition, PSS could significantly increase the abundance of *Corynebacterium* in the gut microbiota of T2DM mice.

In order to study the interaction between gut microbiota and physiological and biochemical indicators, spearman correlation analysis was carried out on different genus microorganisms and serum indicators. As shown in Fig.5L, *Muribaculaceae*, *Lactobacillus, Odoribacter are positively* correlated with the HDL-C levels and QUICKI but negatively correlated with OGTT, Insulin, FBG, TC and TG. *Helicobacter was* was significantly positively correlated with OGTT and insulin level($P < 0.05$).

Spearman correlation analysis was conducted on 7 key indicators of intestinal barrier function and the level of gut microbiota genera to study their relationship (Fig.5M). The results showed that *Muribaculaceae* and *Lactobacillus* was positively correlated with intestine tight junction protein, and *Lactobacillus* was significantly positively correlated with the mRNA expression of toll like receptor and mycoprotein ($P < 0.05$). *Helicobacter* and *Prevotellaceae_UCG-001* are negatively correlated with LDS and L-DA levels. In addition, *Helicobacter* was negatively correlated with the expression of Occludin mRNA expression ($P < 0.05$).


A
Group
NC
DC
MC
PSS
LAB
PSS+LAB
PC1 (21.39%)
PC2 (14.81%)
B
Relative Abundance
Taxonomy
C
Relative Abundance of Bacteroidota (%)
D
Relative Abundance of Firmicutes (%)
E
Firmicutes/Bacteroidota
F
Relative Abundance of Proteobacteria (%)
G
Relative Abundance
Taxonomy
H
Relative Abundance of Muribaculaceae (%)
I
Relative Abundance of Lachnospiraceae_NK4A136_group (%)
J
Relative Abundance of Lactobacillus (%)
K
Relative Abundance of Odoribacter (%)
L
Spearman Correlation Heatmap
Genus
Family
Phylum
Muribaculaceae
Bacteroides
Corynebacterium
Alloprevotella
Lachnospiraceae_NK4A136_group
Prevotellaceae_UCG-001
Alistipes
Lactobacillus
Clostridia_UCG-014
Odoribacter
Helicobacter
Prevotellaceae
Rikenellaceae
Lactobacillaceae
Lachnospiraceae
Porphyromonadaceae
Helicobacteraceae
Bacteroidota
Actinobacteriota
Firmicutes
Proteobacteria
Body weight
OGTT
Insulin
FBG
ALP
AST
HDL-C
TG
GLUT2/β-actin
AKT/β-actin
PI3K/β-actin
LDL-C
FOXO1/β-actin
IRS/β-actin
GSK-3b/β-actin
QUICKI

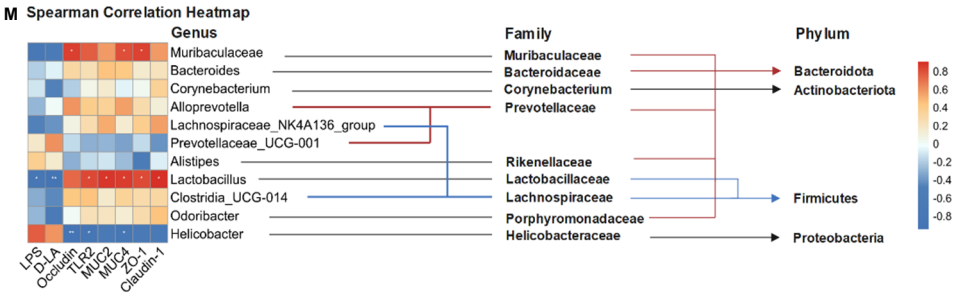


Fig.5. Effects on the gut microbiota in diabetic mice (n=6). Primary component analysis (PCA) based on weighted UniFrac (A), relative abundance at the phylum level (B), relative abundance of *Bacteroidota* (C), relative abundance of *Firmicutes* (D), *Firmicutes*/*Bacteroidota* (E), relative abundance of *Proteobacteria* (F), relative abundance at the genus level (G), relative abundance of *Muribaculaceae* (H), relative abundance of *Lachnospiraceae_NK4A136_group* (I), relative abundance of *Lactobacillus* (J), relative abundance of *Odoribacter* (K), Heatmap of Spearman's correlations analysis between the gut microbiota at genus level and T2DM related indicators in T2DM mice (L), Heatmap of Spearman's correlations analysis between the gut microbiota at genus level and gut barrier function related indicators in T2DM mice (N) . Different lowercase letters mean significant difference ($P < 0.05$).

3.5. Effect on the intestinal metabolites of gut microbiota in T2DM mice

By using non-targeted metabolomics analysis based on GC-MS, a total of 342 metabolites were detected. PCA analysis showed significant separation between the DC group and other groups (Fig.6A). There were significant changes in intestinal metabolites in normal mice and diabetes mice. After PSS+LAB treatment, a significant callback effect was observed, close to the NC group. Especially, LAB alone treatment showed significant changes compared to the DC group, but also separated from the NC group.

Quantitative and qualitative analysis of detected differential metabolites. All groups and DC groups had upregulated and downregulated differential metabolites. In terms of quantity, the LAB and DC groups had the highest number of differential metabolites, followed by the PSS+LAB group, indicating that LAB and PSS had a significant impact on intestinal metabolites. Compared with DC group, 16 metabolites showed significant changes after PSS treatment alone, 35 fewer metabolites than PSS+LAB group. Among them, mannose 6-phosphate, 2-aminoheptanedioic acid, quinic acid, d-xylitol, 2-monoolein, and propane-1,3-diol were significantly enriched in the DC group, while gallic acid, glucosamine acid, glucose-6-phosphate, and alpha-aminoadipic acid were significantly enriched in the PSS+LAB group. Alpha-aminoadipic acid was the key metabolite with the greatest contribution.

Fig.6D showed the enriched metabolic pathways. The metabolic pathways related to amino acid metabolism and carbohydrate metabolism are the most enriched. Among them, alanine, aspartate and glutamate metabolism, and lysine degradation are the two pathways with the most significant changes. The correlation between gut microbiota and its metabolites was analyzed. The results indicated that *Muribaculaceae*, *Lactobacillus*, *Clostridia*_UCG-014 were associated with more metabolites compared to other generas. *Lactobacillus* has a significant positive correlation with glucose-6-phosphate. *Corynebacterium* was negatively correlated with 2-aminoheptanedioic acid and mannose 6-phosphate. *Prevotellaceae*_UCG-001 was negatively correlated with gallic acid and glucosaminic acid.

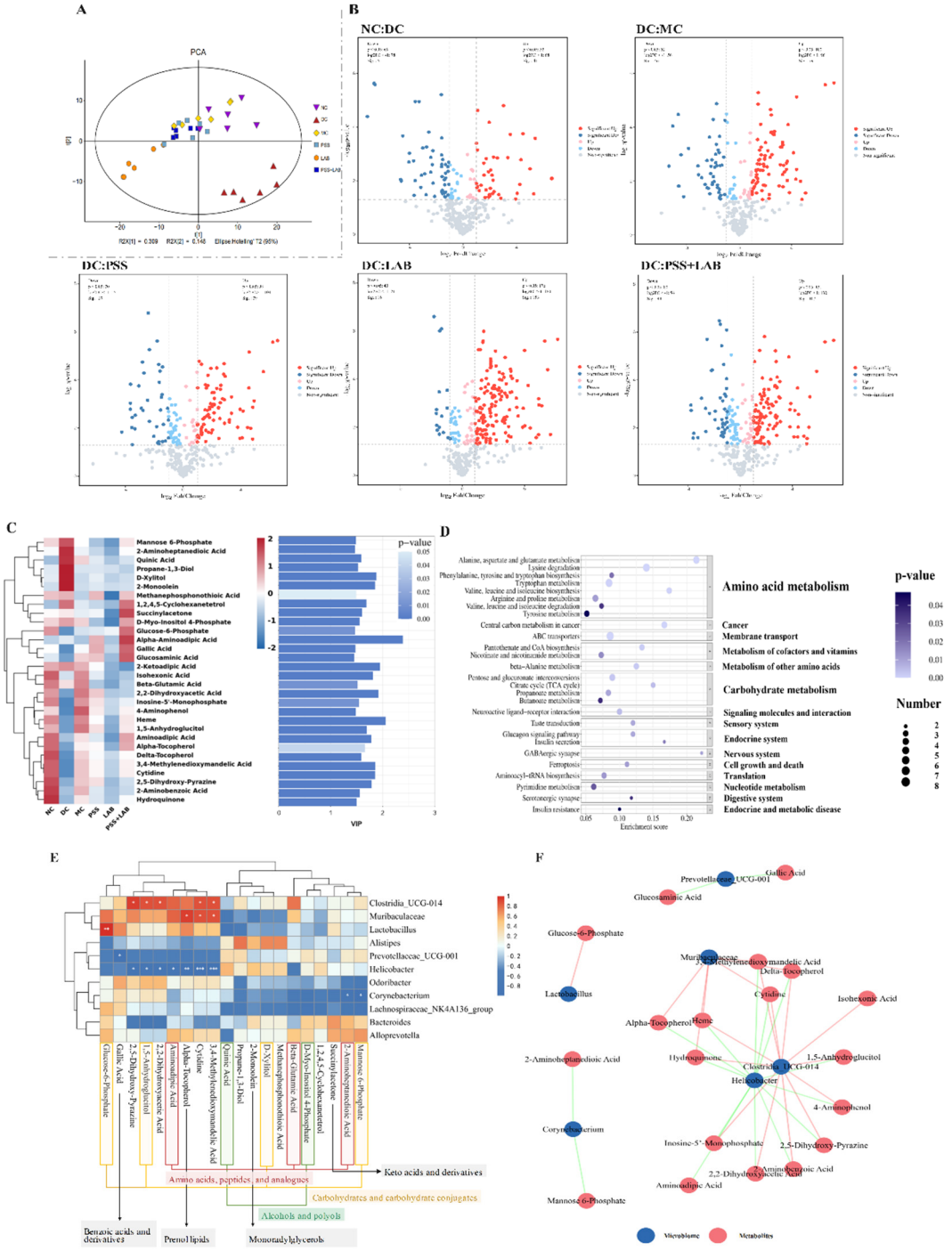


Fig.6. Effect on the intestinal metabolites of gut microbiota in T2DM mice (n=6). Primary component analysis (A), the number of differential metabolites between groups (B), cluster heatmap and bar graph of metabolites (C), KEGG enrichment pathway analysis (D), Heatmap of Spearman's correlations analysis between the gut microbiota and metabolites (E), correlation network (F).

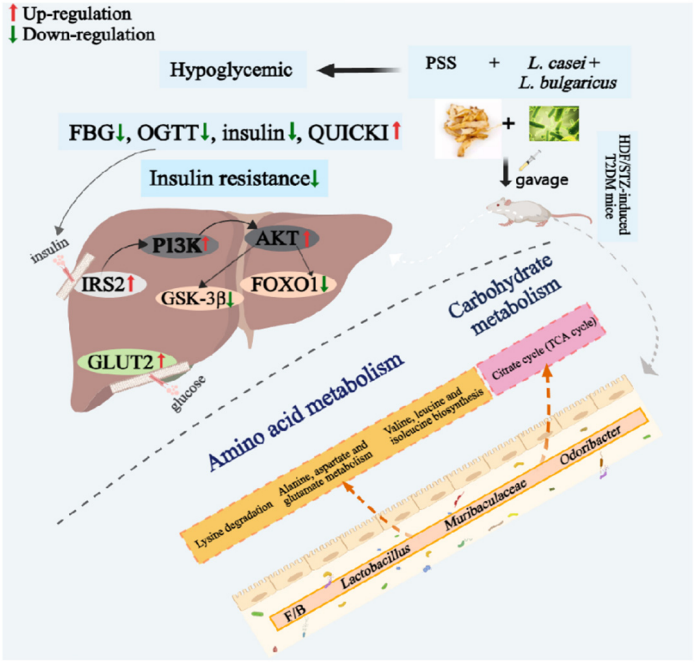


Fig.7 Schematic of PSS+LAB resistance to the mechanism of T2DM

4. Discussion

T2DM is a metabolic disease usually characterized by hyperglycemia caused by insulin insensitivity and insulin resistance. Drugs that can control blood glucose have been developed clinically, such as metformin and glimepiride. However, such drugs will have negative effects on bodies, such as weight surge, gastrointestinal discomfort, and dependence [30, 31]. Therefore, it is necessary to develop functional foods that are safe, non-toxic, and have no side effects. PSS and probiotics have hypoglycemic activity, and both can be used as safe and healthy food raw materials or ingredients. Therefore, this study investigated the hypoglycemic effect and mechanism of PSS combined with LAB.

Insulin resistance and hyperglycemia are typical characteristics of T2DM. In our study, compared with normal mice, blood glucose and serum insulin levels in diabetic mice significantly increased, and QUICKI values significantly decreased. It indicated that the mice have serious insulin resistance, islet β cell dysfunction, and insulin sensitivity reduction. Pathological observation of pancreatic islet tissue also confirmed this point. After the intervention of PSS+LAB, the blood glucose of mice decreased significantly while the glucose tolerance increased significantly, indicating that insulin resistance was relieved. The quantitative insulin sensitivity check index (QUICKI) is an essential index for evaluating insulin resistance [27]. The insulin level of the PSS+LAB group is lower than that of the PSS and LAB group, with a higher QUICKI value, indicating that the hypoglycemic performance has been improved after combined use. As a metabolic disease, T2DM is often accompanied by abnormalities in lipid metabolism in addition to disorders of glucose metabolism [32, 33]. TC、TG、LDL-C, and HDL-C are marker metabolites for blood lipid[34]. PSS+LAB can reduce the TC, TG, and LDL-C content and increase the HDL-C level in the serum. The combination of PSS and composite strains has a more significant effect on regulating lipid metabolism, especially on the level of TG and HDL-C. Meanwhile, the histological analysis of the liver also confirmed that the steatosis significantly decreased after the intervention. The above results showed that the lipid

metabolism of mice has also been positively affected. In addition to biochemical indicators, pathological observations also show that the combined use of PSS+LAB also restored damage to the liver and pancreas (Fig. 7).

IRS2/PI3K/AKT/FOXO1 and IRS2/PI3K/AKT/GSK-3β are vital insulin signaling pathways in T2DM and play a central role in regulating blood glucose [35]. Generally, insulin binding with insulin receptors will activate insulin receptor substrate (IRS) and then activate downstream signal pathway PI3K/AKT. Glycogen synthase kinase-3 beta (GSK-3β) and Forkhead box protein O1 (FOXO1) are essential substrates of AKT. Activated AKT can promote glycogen synthesis and reduce blood glucose by inhibiting the activity of GSK-3β [36]. Similarly, AKT can inactivate FOXO1 by phosphorylating it, thereby blocking downstream effector molecules and inhibiting gluconeogenesis [37]. Cui et al. found that insulin-like growth factor-1 (IGF-1) treatment enhanced the viability of STZ-treated islet cells [38]. IGF-1 treatment reversed the inhibition of STZ on the expression of IRS1, p-PI3K, p-Akt, and p-FOXO1, and the insulin secretion of islet cells increased significantly. It is suggested that IGF-1 can inhibit the islet β cell apoptosis and improve the dysfunction by activating IRS1/PI3K/AKT/FOXO1 pathway [39]. Our study confirmed that after the administration of PSS and compound strains, the expression of PI3K and AKT in the liver of diabetic mice increased significantly, and the expression of FOXO1 and GSK-3β decreased significantly. PSS has a more substantial pro-expression effect on PI3K than LAB, while LAB has a stronger pro-expression effect on IRS2 and an inhibitory effect on GSK-3β. However, combining the two shows a better regulation effect than the single-use. Glucose Transporter 2 (GLUT2) is the main transport protein for transferring glucose between the liver and blood [40]. When glucose concentration is too high, GLUT2 can promote glucose absorption, thereby reducing blood glucose [41]. We found that the expression of GLUT2 protein in the PSS group was significantly higher than that in the DC group and other treatment groups, and the expression of GLUT2 protein in the DC group was the lowest. Therefore, these findings suggest that PSS+LAB reduces insulin resistance and increases hepatic glycogen storage, which may depend on the activation of IRS2/PI3K/AKT/FOXO1, IRS2/PI3K/AKT/GSK-3β pathways, and GLUT2 protein target.

The intestinal barrier function is crucial for preventing diseases and maintaining body homeostasis. Intestinal epithelial cells will secrete mucin and tight junction protein to maintain normal intestinal permeability. Decreased secretion of mucin and tight junction protein will increase intestinal permeability [42]. Endotoxin-lipopolysaccharide (LPS) is the cell wall component of Gram-negative bacteria. D-lactic acid (D-LA) is a metabolite mainly produced by the fermentation of gut bacteria in the human body. The elevated levels of LPS and D-LA in the blood indicate impaired intestinal barrier function, and a large amount of LPS entering the blood can cause systemic inflammation and induce insulin resistance [43]. We observed that the combined treatment of LAB and PSS increased the expression of intestinal tight junction protein (Claudin-1, Occludin, ZO-1) and mucin (MUC2, MUC4) in T2DM mice, and decreased the expression of Toll like receptors (TLR2) that mediate immune and inflammatory responses, all of which indicated the recovery of intestinal barrier function. The gut microbiota is an important part of the intestinal microbiota barrier. The gut

microbiota provides energy for the intestinal epithelial cells through the digestion and absorption of nutrients, thus affecting the intestinal permeability. Therefore, next we evaluated the changes of gut microbiota.

The gut microbiota is closely related to the host's metabolism, and its abnormal state is inseparable from metabolic diseases. The imbalance of gut microbiota is another obvious feature of T2DM diabetes [44]. Type 2 diabetes patients showed a significant increase in the *Firmicutes*/*Bacteroidota* (F/B) ratio, and the F/B ratio was negatively correlated with glucose homeostasis [45]. Consistent with the above studies, we found that the DC group has a high abundance of *Firmicutes*, while the *Bacteroidota* has a low abundance. In the PSS+LAB group, the F/B ratio decreased significantly, accompanied by increased glucose tolerance. Shin et al. reported that harmful metabolism would destroy glucose homeostasis and lead to the massive reproduction of *Proteobacteria* [46]. In our study, *Proteobacteria* was enriched in the DC group, but this situation was reversed after the PSS and composite probiotics intervention. Smith et al. found that the population size of *Muribaculaceae* increased in mice treated with α-glucosidase inhibitors [47]. And the final products of *Muribaculaceae* fermentation include propionic acid which has the function of lipid-lowering and anti-inflammatory. Therefore, this may be one of the mechanisms of the anti-diabetes effect of α-glucosidase inhibitors. In our study, we also found that after the treatment of PSS and composite strains, the abundance of *Muribaculaceae* increased significantly, close to normal mice. Kameyamade et al. reported that *Lachnospiracea* might reduce the risk of T2DM by reducing the ketone body level [48]. *Lactobacillus* is a typical probiotic that can effectively regulate glycolipid metabolism. Previous studies have shown that *Lactobacillus* may improve hyperglycemia in diabetes patients through the glucagon signaling pathway [49]. In addition, the study of Park et al. speculated that the enrichment of *Lactobacillus* in the gut microbiota is the potential mechanism of the ability of EGCG to maintain glucose homeostasis [50]. Similarly, our study also showed similar results. The abundance of *Lactobacillus* in the PSS, LAB, and PSS+LAB groups were significantly higher than that in the DC group. In addition, our study also found that compared with the single effect, the combination of PSS and compound strains enriched a large amount of *Odoribactor* in the intestine of mice and was significantly higher than that of the DC and NC group. *Odoribactor* is believed to have the effect of anti-colorectal cancer [51]. Huber-Ruano et al. speculated that *Odoribacter* is a promising probiotic through the fecal bacteria transplantation experiment, which can improve the glucose tolerance of mice and the inflammatory level of obese mice [52].

Changes in gut microbiota can lead to changes in metabolites. The results of metabonomics showed that the combination of LAB and PSS had a more significant effect on the metabolites of gut microbiota than that of PSS alone. Alpha-aminoadipic acid is a metabolite with significant difference between DC group and LAB+PSS group, which affects the lysine metabolic pathway. Previous studies have shown that gallic acid can prevent and treat hepatic and renal oxidative stress injury in diabetic rats, thereby alleviating the related T2DM complications [53]. Glucose-6-phosphate is an important intermediate product in glycolysis. Glycolysis is a common stage that all organisms must go through for glucose degradation. Gallic acid and glucose-6-phosphate were significantly up-regulated in LAB+PSS group. Further analysis of metabolic

pathways revealed that both amino acid metabolism and carbohydrate metabolism were affected. Among them, alanine, aspartate and glutamate metabolism pathways and lysine degradation pathways were significantly affected. Amino acid homeostasis was proved to be closely related to T2DM. Amino acids can regulate insulin secretion of islet β cells [54]. Glutamate, alanine and leucine may affect glucose metabolism by entering the tricarboxylic acid cycle [55]. And Spearman correlation analysis showed that *Lactobacillus* and *Muribaculaceae* were positively correlated with aminoadipic acid (amino acid metabolism pathway). The above results indicate that LAB and PSS can regulate type 2 diabetes by interfering with carbohydrate metabolism and amino acid metabolism.

5. Conclusion

In conclusion, the combination of PSS and probiotics can improve fasting blood glucose, lipid metabolism, and liver function damage in T2DM mice. The hypoglycemic mechanism of the complex may be related to the regulation of IRS2/PI3K/AKT/FOXO1 and IRS2/PI3K/AKT/GSK-3 β signal pathway and the regulation of gut microbiota in T2DM mice. In addition, the gut microbiome was significantly correlated with amino acid metabolism and carbohydrate metabolism. Our research shows that the combination of plant extracts and probiotics may be a more effective way to manage T2DM. The complex of PSS and lactobacillus can be developed as a functional dietary product.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by National Natural Science Foundation of China (grant number 32000252), Natural Science Foundation of Heilongjiang Province of China (grant number YQ2020C014), Natural Science Foundation of Heilongjiang Province of China (grant number ZD2019C002), National Key Research and Development Program of China (grant number 2017YFC1601901).

Reference

[1] Z. Chang, J. Xu, Y. Qin, Q. Zheng, L. Zhao, Y. Wang, Y. Zhang, Safety and efficacy of anti-hyperglycemic agents in patients with type 2 diabetes mellitus (T2DM): Protocol for an overview of systematic reviews based on network meta-analysis, PLoS One, 18 (2023) e0282143.

[2] E. Lecumberri, L. Nattero-Chavez, J. Quinones Silva, S. Alonso Diaz, E. Fernandez-Duran, B. Dorado Avendano, H.F. Escobar-Morreale, M. Luque-Ramirez, Impact of excluding hyperglycemia from international diabetes federation metabolic syndrome diagnostic criteria on prevalence of the syndrome and its association with microvascular complications, in adult patients with type 1 diabetes, Endocrine, 76 (2022) 601-611.

[3] K. Crawford, Review of 2017 Diabetes Standards of Care, Nurs Clin North Am, 52 (2017) 621-663.

[4] Q. Martin-Saladich, R. Simo, S. Aguade-Bruix, O. Simo-Servat, C. Aparicio-Gomez, C. Hernandez, C. Ramirez-Serra, M.N. Pizzi, A. Roque, M.A. Gonzalez Ballester, J.R. Herance, Insights into Insulin Resistance and Calcification in the Myocardium in Type 2 Diabetes: A Coronary Artery Analysis, Int J Mol Sci, 24 (2023).

[5] A.L. Cunningham, J.W. Stephens, D.A. Harris, Gut microbiota influence in type 2 diabetes mellitus (T2DM), Gut Pathog, 13 (2021) 50.

[6] M.S. Thomas, M. Calle, M.L. Fernandez, Healthy plant-based diets improve dyslipidemias, insulin resistance, and inflammation in metabolic syndrome. A narrative review, Adv Nutr, 14 (2023) 44-54.

[7] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat Rev Microbiol, 19 (2021) 55-71.

[8] Z. Wu, B. Zhang, F. Chen, R. Xia, D. Zhu, B. Chen, A. Lin, C. Zheng, D. Hou, X. Li, S. Zhang, Y. Chen, K. Hou, Fecal microbiota transplantation reverses insulin resistance in type 2 diabetes: A randomized, controlled, prospective study, Front Cell Infect Microbiol, 12 (2022) 1089991.

[9] S. Salminen, M.C. Collado, A. Endo, C. Hill, S. Lebeer, E.M.M. Quigley, M.E. Sanders, R. Shamir, J.R. Swann, H. Szajewska, G. Vinderola, Author Correction: The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, Nat Rev Gastroenterol Hepatol, 19 (2022) 551.

[10] M. Kechagia, D. Basoulis, S. Konstantopoulou, D. Dimitriadi, K. Gyftopoulou, N. Skarmoutsou, E.M. Fakiri, Health benefits of probiotics: a review, ISRN Nutr, 2013 (2013) 481651.

[11] C. Hill, Probiotics and pharmabiotics: alternative medicine or an evidence-based alternative?, Bioeng Bugs, 1 (2010) 79-84.

[12] M. Cabello-Olmo, M. Oneca, P. Torre, N. Sainz, M.J. Moreno-Aliaga, E. Guruceaga, J. Vicente Diaz, I.J. Encio, M. Barajas, M. Arana, A Fermented Food Product Containing Lactic Acid Bacteria Protects ZDF Rats from the Development of Type 2 Diabetes, Nutrients, 11 (2019) 2530.

[13] F. Yan, N. Li, J. Shi, H. Li, Y. Yue, W. Jiao, N. Wang, Y. Song, G. Huo, B. Li, Lactobacillus acidophilus alleviates type 2 diabetes by regulating hepatic glucose, lipid metabolism and gut microbiota in mice, Food & Function, 10 (2019) 5804-5815.

[14] Y.S. Lee, D. Lee, G.S. Park, S.H. Ko, J. Park, Y.K. Lee, J. Kang, Lactobacillus plantarum HAC01 ameliorates type 2 diabetes in high-fat diet and streptozotocin-induced diabetic mice in association with modulating the gut microbiota, Food Funct, 12 (2021) 6363-6373.

[15] C. Wang, C. Zhang, S. Li, L. Yu, F. Tian, J. Zhao, H. Zhang, W. Chen, Q. Zhai, Effects of Probiotic Supplementation on Dyslipidemia in Type 2 Diabetes Mellitus: A Meta-Analysis of Randomized Controlled Trials, Foods, 9 (2020) 1540.

[16] J. Zhao, L. Wang, S. Cheng, Y. Zhang, M. Yang, R. Fang, H. Li, C. Man, Y. Jiang, A Potential Synbiotic Strategy for the Prevention of Type 2 Diabetes: Lactobacillus paracasei JY062 and Exopolysaccharide Isolated from Lactobacillus plantarum JY039, Nutrients, 14 (2022) 377.

[17] Z. Chen, J. Luo, M. Jia, Y. Chai, Y. Bao, Polygonatum sibiricum saponin Exerts Beneficial Hypoglycemic Effects in Type 2 Diabetes Mice by Improving Hepatic Insulin Resistance and Glycogen Synthesis-Related Proteins, Nutrients, 14 (2022) 3250.

[18] C. Xiaowei, W. Wei, G. Hong, C. Hui, Z. Xiaofei, W. Haonan, W. Yumeng, Z. Xuelan, H. Chunchao, Review of Polygonatum sibiricum: A new natural cosmetic ingredient, Pharmazie, 74 (2019) 513-519.

[19] F. Shen, Z. Song, P. Xie, L. Li, B. Wang, D. Peng, G. Zhu, Polygonatum sibiricum polysaccharide prevents depression-like behaviors by reducing oxidative stress, inflammation, and cellular and synaptic damage, J Ethnopharmacol, 275 (2021) 114164.

[20] K. Yelithao, U. Surayot, W. Park, S. Lee, D.H. Lee, S. You, Effect of sulfation and partial hydrolysis of polysaccharides from Polygonatum sibiricum on immune-enhancement, Int J Biol Macromol, 122 (2019) 10-18.

[21] H.Y. Zhang, W.C. Hu, G.X. Ma, N.L. Zhu, X.B. Sun, H.F. Wu, X.D. Xu, A new steroidal saponin from Polygonatum sibiricum, J Asian Nat Prod Res, 20 (2018) 586-592.

[22] J. Luo, Y. Chai, M. Zhao, Q. Guo, Y. Bao, Hypoglycemic effects and modulation of gut microbiota of diabetic mice by saponin from Polygonatum sibiricum, Food Funct, 11 (2020) 4327-4338.

[23] J. Luo, L. Jiang, B. Gao, Y. Chai, Y. Bao, Comprehensive in silico analysis of the probiotics, and preparation of compound probiotics-Polygonatum sibiricum saponin with hypoglycemic properties, Food Chem, 404 (2023) 134569.

[24] S. Wu, J. Zuo, Y. Cheng, Y. Zhang, Z. Zhang, M. Wu, Y. Yang, H. Tong, Ethanol extract of Sargarsum fusiforme alleviates HFD/STZ-induced hyperglycemia in association with modulation of gut microbiota and intestinal metabolites in type 2 diabetic mice, Food Res Int, 147 (2021) 110550.

[25] F. Zhou, M.Z. Zhu, J.Y. Tang, J. Ou-Yang, B.H. Shang, C.W. Liu, J. Wang, Q. Liu, J.A. Huang, Z.H. Liu, Six types of tea extracts attenuated high-fat diet-induced metabolic syndrome via modulating gut microbiota in rats, Food Res Int, 161 (2022) 111788.

[26] J.W. Fleming, L.W. Fleming, C.S. Davis, Fixed-dose combinations in type 2 diabetes - role of the canagliflozin metformin combination, Diabetes Metab Syndr Obes, 8 (2015) 287-294.

[27] J. Hrebicek, V. Janout, J. Malincikova, D. Horakova, L. Cizek, Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention, The Journal of clinical endocrinology and metabolism, 87 (2002) 144-147.

[28] N. Huang, Y. Wei, M. Liu, Z. Yang, K. Yuan, J. Chen, Z. Wu, F. Zheng, K. Lei, M. He, Dachaihu decoction ameliorates septic intestinal injury via modulating the gut microbiota and glutathione metabolism as revealed by multi-omics, J Ethnopharmacol, 312 (2023) 116505.

[29] E. Bolyen, J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, H. Alexander, E.J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J.E. Bisanz, K. Bittinger, A. Brejnrod, C.J. Brislawn, C.T. Brown, B.J. Callahan, A.M. Caraballo-Rodriguez, J. Chase, E.K. Cope, R. Da Silva, C. Diener, P.C. Dorrestein, G.M. Douglas, D.M. Durall, C. Duvallet, C.F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J.M. Gauglitz, S.M. Gibbons, D.L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G.A. Huttley, S. Janssen, A.K. Jarmusch, L. Jiang, B.D. Kaehler, K.B. Kang, C.R. Keefe, P. Keim, S.T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M.G.I. Langille, J. Lee, R. Ley, Y.X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B.D. Martin, D. McDonald, L.J. McIver, A.V. Melnik, J.L. Metcalf, S.C. Morgan, J.T. Morton, A.T. Naimey, J.A. Navas-Molina, L.F. Nothias, S.B. Orchanian, T. Pearson, S.L. Peoples, D. Petras, M.L. Preuss, E. Pruesse, L.B. Rasmussen, A. Rivers, M.S. Robeson, 2nd, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S.J. Song, J.R. Spear, A.D. Swafford, L.R. Thompson, P.J. Torres, P. Trinh, A. Tripathi, P.J. Turnbaugh, S. Ul-Hasan, J.J.J. van der Hooft, F. Vargas, Y. Vazquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K.C. Weber, C.H.D. Williamson, A.D. Willis, Z.Z. Xu, J.R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, J.G. Caporaso, Author Correction: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2, Nat Biotechnol, 37 (2019) 1091.

[30] C. Ning, Y. Jiao, J. Wang, W. Li, J. Zhou, Y.-C. Lee, D.-L. Ma, C.-H. Leung, R. Zhu, H.-M.D. Wang, Recent advances in the managements of type 2 diabetes mellitus and natural hypoglycemic substances, Food Science and Human Wellness, 11 (2022) 1121-1133.

[31] J. Wang, W. Yu, J. Xu, L. Feng, H. Yang, X. Liu, [Study on the prevalence of lipid metabolism disorders and quantitative analysis of apolipoproteins in T2DM patients], Wei Sheng Yan Jiu, 45 (2016) 587-592.

[32] Q. Zhang, R.L. Li, L.Y. Wang, T. Zhang, D. Qian, D.D. Tang, C.X. He, C.J. Wu, L. Ai, Hydroxy-alpha-sanshool isolated from Zanthoxylum bungeanum Maxim. has antidiabetic effects on high-fat-fed and streptozotocin-treated mice via increasing glycogen synthesis by regulation of PI3K/Akt/GSK-3beta/GS signaling, Front Pharmacol, 13 (2022) 1089558.

[33] X. Wang, T. Xian, X. Jia, L. Zhang, L. Liu, F. Man, X. Zhang, J. Zhang, Q. Pan, L. Guo, A cross-sectional study on the associations of insulin resistance with sex hormone, abnormal lipid metabolism in T2DM and IGT patients, Medicine (Baltimore), 96 (2017) e7378.

[34] L. Zeng, F. Zhong, Z. Chen, G. Li, Q. Zhu, Polygonatum sibiricum polysaccharides protect against obesity and non-alcoholic fatty liver disease in rats fed a high-fat diet, Food Science and Human Wellness, 11 (2022) 1045-1052.

[35] Y. Liu, K. Tanabe, D. Baronnier, S. Patel, J. Woodgett, C. Cras-Meneur, M.A. Permutt, Conditional ablation of Gsk-3beta in islet beta cells results in expanded mass and resistance to fat feeding-induced diabetes in mice, Diabetologia, 53 (2010) 2600-2610.

[36] C. Chen, S. Tan, T. Ren, H. Wang, X. Dai, H. Wang, Polyphenol from Rosaroxburghii Tratt Fruit Ameliorates the Symptoms of Diabetes by Activating the P13K/AKT Insulin Pathway in db/db Mice, Foods, 11 (2022) 636.

[37] W. Yang, W. Liao, X. Li, W. Ai, Q. Pan, Z. Shen, W. Jiang, S. Guo, Hepatic p38alpha MAPK controls gluconeogenesis via FOXO1 phosphorylation at S273 during glucagon signalling in mice, Diabetologia, 66 (2023) 1322-1339.

[38] F. Cui, X. He, IGF-1 ameliorates streptozotocin-induced pancreatic beta cell dysfunction and apoptosis via activating IRS1/PI3K/Akt/FOXO1 pathway, Inflamm Res, 71 (2022) 669-680.

[39] J.S. Gauer, S. Tumova, J.D. Lippiat, A. Kerimi, G. Williamson, Differential patterns of inhibition of the sugar transporters GLUT2, GLUT5 and GLUT7 by flavonoids, Biochem Pharmacol, 152 (2018) 11-20.

[40] G.D. Holman, Structure, function and regulation of mammalian glucose transporters of the SLC2 family, Pflugers Arch, 472 (2020) 1155-1175.

[41] Z. Zhou, D. Wang, X. Xu, J. Dai, G. Lao, S. Zhang, X. Xu, A. Dinnyes, Y. Xiong, Q. Sun, Myofibrillar protein-chlorogenic acid complexes ameliorate glucose metabolism via modulating gut microbiota in a type 2 diabetic rat model, Food Chem, 409 (2023) 135195.

[42] J. Huang, J. Huang, Y. Li, Y. Wang, F. Wang, X. Qiu, X. Liu, H. Li, Sodium Alginate Modulates Immunity, Intestinal Mucosal Barrier Function, and Gut Microbiota in Cyclophosphamide-Induced Immunosuppressed BALB/c Mice, J Agric Food Chem, 69 (2021) 7064-7073.

[43] Y. Zhang, Y. Peng, L. Zhao, G. Zhou, X. Li, Regulating the gut microbiota and SCFAs in the faeces of T2DM rats should be one of antidiabetic mechanisms of mogrosides in the fruits of Siraitia grosvenorii, J Ethnopharmacol, 274 (2021) 114033.

[44] M.A.I. Al-Obaide, R. Singh, P. Datta, K.A. Rewers-Felkins, M.V. Salguero, I. Al-Obaidi, K.R. Kottapalli, T.L. Vasylyeva, Gut Microbiota-Dependent Trimethylamine-N-oxide and Serum Biomarkers in Patients with T2DM and Advanced CKD, J Clin Med, 6 (2017) 3250.

[45] L. Liu, J. Zhang, Y. Cheng, M. Zhu, Z. Xiao, G. Ruan, Y. Wei, Gut microbiota: A new target for T2DM prevention and treatment, Front Endocrinol (Lausanne), 13 (2022) 958218.

[46] N.R. Shin, T.W. Whon, J.W. Bae, Proteobacteria: microbial signature of dysbiosis in gut microbiota, Trends Biotechnol, 33 (2015) 496-503.

[47] B.J. Smith, R.A. Miller, T.M. Schmidt, Muribaculaceae Genomes Assembled from Metagenomes Suggest Genetic Drivers of Differential Response to Acarbose Treatment in Mice, mSphere, 6 (2021) e0085121.

[48] K. Kameyama, K. Itoh, Intestinal colonization by a Lachnospiraceae bacterium contributes to the development of diabetes in obese mice, Microbes Environ, 29 (2014) 427-430.

[49] T. Wu, Y. Zhang, W. Li, Y. Zhao, H. Long, E.M. Muhindo, R. Liu, W. Sui, Q. Li, M. Zhang, Lactobacillus rhamnosus LRa05 Ameliorate Hyperglycemia through a Regulating Glucagon-Mediated Signaling Pathway and Gut Microbiota in Type 2 Diabetic Mice, J Agric Food Chem, 69 (2021) 8797-8806.

[50] J.M. Park, Y. Shin, S.H. Kim, M. Jin, J.J. Choi, Dietary Epigallocatechin-3-Gallate Alters the Gut Microbiota of Obese Diabetic db/db Mice: Lactobacillus Is a Putative Target, J Med Food, 23 (2020) 1033-1042.

[51] B.S. Oh, W.J. Choi, J.S. Kim, S.W. Ryu, S.Y. Yu, J.S. Lee, S.H. Park, S.W. Kang, J. Lee, W.Y. Jung, Y.M. Kim, J.H. Jeong, J.H. Lee, Cell-Free Supernatant of Odoribacter splanchnicus Isolated From Human Feces Exhibits Anti-colorectal Cancer Activity, Front Microbiol, 12 (2021) 736343.

[52] I. Huber-Ruano, E. Calvo, J. Mayneris-Perxachs, M.M. Rodriguez-Pena, V. Ceperuelo-Mallafre, L. Cedo, C. Nunez-Roa, J. Miro-Blanch, M. Arnoriaga-Rodriguez, A. Balvay, C. Maudet, P. Garcia-Roves, O. Yanes, S. Rabot, G.M. Grimaud, A. De Prisco, A. Amoruso, J.M. Fernandez-Real, J. Vendrell, S. Fernandez-Veledo, Orally administered Odoribacter laneus improves glucose control and inflammatory profile in obese mice by depleting circulating succinate, Microbiome, 10 (2022) 135.

[53] L.S. de Oliveira, G.R. Thome, T.F. Lopes, K.P. Reichert, J.S. de Oliveira, A. da Silva Pereira, J. Baldissareli, C. da Costa Krewer, V.M. Morsch, M.R. Chitolina Schetinger, R.M. Spanevello, Effects of gallic acid on delta - aminolevulinic dehydratase activity and in the biochemical, histological and oxidative stress parameters in the liver and kidney of diabetic rats, Biomed Pharmacother, 84 (2016) 1291-1299.

[54] H.M. Sickmann, H.S. Waagepetersen, A. Schousboe, A.J. Benie, S.D. Bouman, Obesity and type 2 diabetes in rats are associated with altered brain glycogen and amino-acid homeostasis, J Cereb Blood Flow Metab, 30 (2010) 1527-1537.

[55] P. Newsholme, K. Bender, A. Kiely, L. Brennan, Amino acid metabolism, insulin secretion and diabetes, Biochem Soc Trans, 35 (2007) 1180-1186.