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Polyunsaturated fatty acids from microalgae *Spirulina platensis* modulates lipid metabolism disorders and gut microbiota in high-fat diet rats

Tian-Tian Li[a,1], Ai-Jun Tong[a,1], Yuan-Yuan Liu[a], Zi-Rui Huang[a], Xu-Zhi Wan[a], Yu-Yang Pan[a], Rui-Bo Jia[a], Bin Liu[a,b,*], Xin-Hua Chen[b], Chao Zhao[a,b,c,d,**]

[a] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China
[b] Key Laboratory of Marine Biotechnology of Fujian Province, Institute of Oceanology, Fujian Agriculture and Forestry University, Fuzhou, 350002, PR China
[c] Fujian Province Key Laboratory for the Development of Bioactive Material from Marine Algae, Quanzhou Normal University, Quanzhou, 362000, China
[d] Institute of Chinese Medical Sciences, State Key Laboratory of Quality Control in Chinese Medicine, University of Macau, Taipa, Macau, China

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ABSTRACT

Effects of *Spirulina platensis* 55% ethanol extract (SPL55) on lipid metabolism in high-fat diet-induced hyperlipidaemic rats were investigated. Ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry indicated that SPL55 was enriched with polyunsaturated fatty acids. Meanwhile, serum and liver lipid levels, including total triglyceride, total cholesterol, and low-density-lipoprotein cholesterol, were significantly decreased in hyperlipidaemic rats of SPL55. Analysis of tissue sections showed that SPL55 treatment could markedly inhibit hepatic lipid accumulation and steatosis. Moreover, SPL55 regulated the mRNA and protein expression levels of SREBP-1c, HMG-CoA, PEPCK, ACC, and AMPK genes involved in lipid metabolism. Furthermore, SPL55 led to decrease the abundances of *Turicibacter*, *Clostridium_XlVa*, and *Romboutsia*, which were positive correlation with lipid metabolism indicators, and has also enriched *Alloprevotella*, *Prevotella*, *Porphyromonadaceae*, and *Barnesiella*. These results provided evidence that SPL55 might be developed as a functional food to ameliorate lipid metabolic disorders and hyperlipidaemia.

1. Introduction

Lipid metabolism disorder (LMD), one of the pathogenesis of obesity, hyperlipidemia, hyperglycemia, hypertension, fatty liver, arteriosclerosis, and type 2 diabetes, which induces abnormally high levels of the blood and liver index including triglyceride (TG), total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-c), and low levels of high-density-lipoprotein cholesterol (HDL-c) (Fenet al., 2017). International Diabetes Federation has reported that approximately 25% of global adults have LMDs (Cornier et al., 2008). The incidence of LMD in Asian is increasing year by year, which draw a widely public attention (Nestel et al., 2007; Sugimoto et al., 2014) . Although allopathic hypolipidemic drugs had made great progress and were widely used in the market, they are unfortunately inevitably accompanied by side effects and contraindications (Waness et al., 2008). Especially, the number of drugs were reported to have certain toxic side effects on multiple organs of the body (Sarin et al., 2016). Recent research had indicated that the dietary factors act a key part in lipid lowering (Lim et al., 2017). Therefore, searching for new and natural cholesterol-lowering food active ingredients is highly urgent and would be widely marketed.

Nowadays more and more functional foods have derived from algae (Cardoso et al., 2015; Zhao et al., 2015, 2018b). *Spirulina platensis*, a microalga, belongs to cyanobacteria and is well known for its special active ingredients (Deng and Chow, 2010). The high abundance of antioxidants such as polysaccharide, active polypeptide, phycocyanin, vitamin, tocopherols, and phenolic compounds, especially palmitic acid and polyunsaturated fatty acids (PUFAs) were detected from *S. platensis* (Herrero et al., 2010; Jaime et al., 2015; Ramadan and Ibrahim, 2008) . In addition, the therapeutic effect of *Spirulina* was shown that it can alleviate the toxicity of drugs. Therefore, it was selected as an interventional supplement in dietary intake for patients with inflammatory diseases, insulin resistance, type 2 diabetes and liver disease (Ku et al., 2013; Neyrinck et al., 2017). PUFAs are mostly focused on biological systems and coronary artery disease applications. Current nutritional research is increasingly trending to study on PUFAs, an essential

* Corresponding author. College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China.
** Corresponding author. College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China.
E-mail addresses: liubin618@hotmail.com (B. Liu), zhchao@live.cn (C. Zhao).
[1] Tian-Tian Li and Ai-Jun Tong contributed equally to this study.

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ingredient in the human diet (Ramadan and Ibrahim, 2008). However, the potential mechanisms of regulating LMDs by PUFAs from *S. platensis* remain unclear and rarely reported.

In recent years, more and more studies (Zhao et al., 2019) indicated that gut microbiota contributed to the regulation of the organism energy metabolism and serum lipid levels. Moreover, the abundances of specific bacteria were shown to be negatively related to BMI and other biochemical indices but positively related to HDL-c. Drugs can also alter the gut microbiota and regulate the state of the human body. Algae ethanol extracts have shown to ameliorate LMDs by altering the intestinal microbiota (Fu et al., 2015). In this study, high-fat rats model has been used to expound the hypolipidemic mechanisms of PUFAs from *S. platensis*, including the analysis of weight gain, serum and liver index, gut microbiota structure and gene expression levels related to lipid metabolism.

2. Materials and methods

2.1. Extraction of S. platensis 55% ethanol extracts

S. platensis powders were purchased from Fuqing King Dnarmsa *Spirulina* Co., Ltd. (Fuqing, China) and extracted with absolute ethanol and 55% (v/v) ethanol aqueous solution sequentially with 300 W based on a ratio of composition to liquid of 1:10 (w/v) at 45 °C for 30 min. The extracts (SPL55) were obtained by freeze-drying after concentration and used for the determination of ingredients and gavaged in rats.

2.2. UPLC-QTOF-MS/MS analysis of SPL55

Based on the former study (Huang et al., 2018), the mobile phases selected for this experiment were the solvent A (0.1% formic acid (v/v) in water) and solvent B (0.1% formic acid (v/v) in acetonitrile). The UPLC elution condition was optimized as follows: 99% of solvent A (0–0.25 min), 99-1% of solvent A (0.25–16.25 min), 1% of solvent A (16.25–17.00 min), 1–99% of solvent A (17.00–17.01 min), and 99% of solvent A (17.01–20.00 min) at a flow rate of 0.45 mL/min. The required sample was 1.0 μL. The m/z range was scanned from 50 to 1200 Da using the LockSpray model. The detailed conditions were as follows: capillary voltage at 3000 V, cone voltage at 45 V, source offset of 80 V, collision energy of 10–40 eV, ion source temperature of 120 °C, nebulization gas flow of 800 L/h at 800 °C, cone gas flow of 50 L/h, and nebulizer gas flow of 6.5 Bar. The data were collected and acquired using Mass Lynx V 4.1 (Waters, Millford, MA, USA).

2.3. Animals and dietary treatments

Male wistar rats (170 ± 20 g) were purchased from the Experimental Animal Centre of Shandong Province (Jinan, China). The rats were fed in a suitable temperature room (27 ± 1 °C) at 60 ± 10% relative moisture with a standard diet. The ethics review committee of Fuzhou General Hospital of Nanjing Military Region put forward the ethical approval opinion (FZZY-2016–019). After a week of adaptation, the rats were sorted into four treatment groups according to random rules as follows (n = 8/group): normal fat diet (NFD), high-fat diet (HFD), HFD-fed rats treated with silymarin (Sym), and SPL55 (150 mg/kg·day) groups. NFD group was given a basal diet, while HFD, Sym, and SPL55 groups were given high-sucrose/high-fat diet. SPL55 groups were gavaged with 2 mL of SPL55 (150 mg/kg). Similarly, NFD and HFD groups were gavaged with 2 mL of 0.9% saline. Based on the theory of traditional Chinese medicine, the gavage dose of SPL55 used in this experiment was calculated by the amount of crude drug conversion (Guo et al., 2018).

2.4. Serum samples collection

Through the experiment period, the body weights of rats were measured at 4th and 8th week to determine the differences. After fasting and anesthesia at 4th and 8th week, blood was obtained from the heart and separated serum at 3500 rpm for 10 min at 4 °C and then stored at low temperature for further study (Foster et al., 2016).

2.5. Liver homogenate preparation and histopathological analysis

After rapid freezing, the appropriate amount of liver tissue was homogenized with a certain volume of saline, and then the liver portion was used for routine analysis of liver histology by conventional hematoxylin and eosin (H&E) staining (Cheng et al., 2017).

2.6. Biochemical assays

The levels of TC, TG, HDL-c, LDL-c, alanine transaminase (ALT), aspartate transaminase (AST), and free fatty acids (FFA) were determined by using the relevant assay kits according to the instructions (Nanjing Jiancheng Institute of Biotechnology, China).

2.7. Quantitative real-time polymerase chain reaction (RT-qPCR) analysis

Using an RNA extraction kit (Takara, Japan) to extract total RNA from the liver tissues, cDNA was synthesized using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). The specific primers for RT-qPCR of adenosine 5′-monophosphate-activated protein kinase-α (AMPK-α), sterol regulatory element-binding transcription factor-1c (SREBP-1c), 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA), phosphoenolpyruvate carboxykinase (PEPCK), and acetyl CoA carboxylase (ACC) genes were listed in Table S1. Relative quantifications of target genes transcripts were completed in 96-well reaction plates with SYBR® Premix Ex Taq™ II kit (Takara, Japan) by using the AB7300 Real-Time PCR system (Applied Biosystems, USA). The relative mRNA levels were normalized to the level of β-actin gene in each sample and expressed as values of relative expression compared to that of the NFD group. Relative levels of target mRNAs were determined using the 2-ΔΔCt method and normalization (Hernández-Rodas et al., 2017; Hu et al., 2018; Li et al., 2018; Zhao et al., 2018a).

2.8. Western blot analysis

The liver cells of the rats were accurately weighed and prepared. After lysis on ice for 40 min and centrifuging at 12000 rpm, protein issues lysates were subjected using SDS-PAGE. Proteins were transferred to PVDF membranes and handled with no protein blocking solution for 2 h. Then they were incubated with primary rabbit polyclonal antibodies, including anti-HMGCR, anti-SREBP-1, anti-AMPK-α (Sangon, Shanghai, China) and anti-GAPDH (Sangon, Shanghai, China) for 16 h at 4 °C. GAPDH was used as the loading control. After washing four times with TBST buffer, the membrane was treated with the appropriate IgG secondary antibody at 37 °C for 1.5 h. The target protein band was developed with ECL chromogenic kit to observe the results (Beyotime, Shanghai, China). The grey value of protein band was analyzed by Image J (National Institutes of Health, USA).

2.9. The changes of gut microflora by SPL55

The contents of intestine were frozen with liquid nitrogen and then maintained at −80 °C for further study (Meslin et al., 1999). The V_3–V_4 hypervariable regions of 16S rRNA gene from gut microbiota were amplified using universal primers (Li et al., 2016). The sequencing library was sequenced using a 2 × 300 format configuration by IonS5TMXL platform. The interactions of different operational taxonomic units (OTUs) and the composition of dominant species were performed by MiSeq control software. The initial classification analysis was conducted on Illumina's Base Space cloud computing platform.

2.10. Statistical analysis

The data for each group gained from experiments were reported as the mean ± SD and analyzed by SPSS program (SPSS, Chicago, IL, USA). The differences among groups were examined with one-way ANOVA followed by Student's t-test. A significant difference between data is indicated when the *p-value* is less than 0.05. Spearman's rank correlation method was used to analyze main interaction relationships between gut microbiota and lipid parameters (Henschel et al., 2015).

3. Results

3.1. Characterization of potent major compounds

The phytochemical analysis of SPL95 resulted in the isolation of ten major polyunsaturated fatty acids (Fig. S1). Further peaks were observed at different retention times from 0.79 to 12.48 min but attempts were made to identify these components explicitly based on Q-TOF/MS (Fig. S2). MS analysis confirmed the proposed presence of palmitoleic or linoleic acids (Table S2). Partial fragment ions on m/z were consistent with previously reported data. The specific structure of each peak was determined by comparing those MS spectral data reported in the literature (Feng et al., 2018; Herrero et al., 2007).

3.2. Effects of SPL55 on serum and liver lipid metabolism

After 8 weeks, the body mass indices of all groups were changed with time. Body weight and its gain were shown in Fig. 1A & B. At the middle experiment (4th week), there was a remarkable difference between the SPL55 and NFD groups ($p < 0.05$). Finally (8th week), the body weight of the rats with high-fat intake was higher than that in NFD, Sym, and SPL55 groups ($p < 0.01$). After 8 weeks, all experimental rats gained weight. However, the rats fed with SPL55 and Sym did not gain weight as rapidly as HFD. SPL55 improved overweight after 8-weeks of treatment than that in rats treated with HFD diet. The plasma lipid profile results were summarized in Fig. 1C. Compared with the HFD group, the serum TG and TC levels of rats in SPL55 group were decreased significantly by 42.3% and 25.1% with SPL55 supplement, respectively ($p < 0.01$). A similar trend was found for HDL cholesterol levels, which were increased by 19.4% ($p < 0.05$), and 62.5% ($p < 0.01$) in the SPL55 group at 4th or 8th week. Specially, the differences of the LDL-c levels in SPL55 and Sym groups were shown a very strong regulation ($p < 0.01$).

The liver lipid distributions were indicated in Table 1, which showed that the rat model of non-alcoholic fatty liver has been successfully established. The therapeutic effect of the SPL55 supplement was determined by a decrease in lipid parameters such as TC, TG, LDL-c, and FFA levels while an increase in HDL-c level ($p < 0.01$). ALT as a marker of liver injury was markedly decreased in SPL55 group, which was in line with the decreased AST level in liver ($p < 0.01$). Interestingly, the HFD group showed higher levels of ALT and AST, which indicated that the rat model was in a state of liver dysfunction. However, SPL55 significantly reduced these two parameters ($p < 0.01$). The results showed that SPL55 could significantly ameliorate serum and liver lipid profiles.

3.3. SPL55 attenuated HFD induced hepatic steatosis

Histopathological analysis of the rats' H&E staining of liver sections was shown in Fig. 1D. The intact hepatic lobular structure, the hepatic sinus without pathological defects, and the nucleus of normal morphology showed that the liver tissue of the normal fed group was in a state of healthy (Fig. 1D). Conversely, the gap junctional channels in hepatocytes in the HFD group became wider. Cell wall loosened or death, necrosis, and autolysis were also found (Fig. 1D). In SPL55 and Sym groups, the hepatic sinus was stacked neatly and the damage of

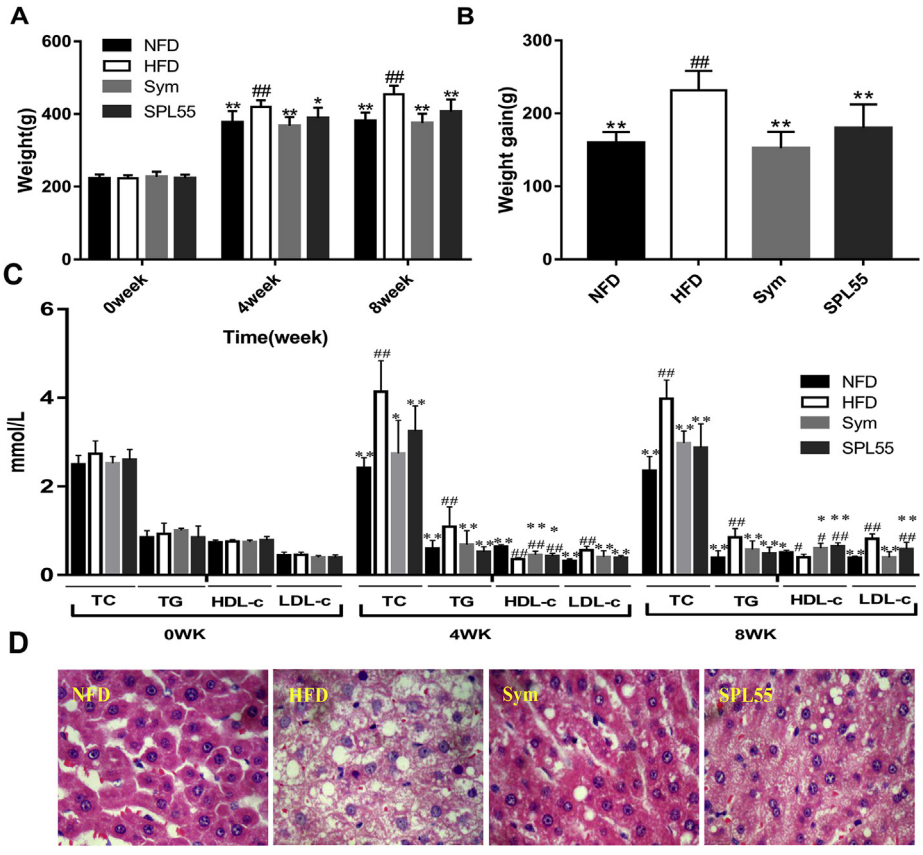

Fig. 1. Biochemical assays and liver parameters analysis during 8-week. (**A** and **B**) Changes in the body weight of rats. Data are expressed as mean ± SD (n = 8). $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, compared with NFD group; $^{*}p < 0.05$ and $^{**}p < 0.01$, compared with HFD group; **(C)** Serum lipid levels of rats; **(D)** Histopathological analysis of rat at 400 × magnification. **Note:** NFD, normal fat diet group; HFD, high-fat diet group; Sym, rats treated with silymarin group, SPL55, HFD-fed rats treated with SPL55 group; TG, triglyceride; TC, total cholesterol; HDL-c, high-density-lipoprotein cholesterol; LDL-c, low-density-lipoprotein cholesterol.

Table 1
Effects of SPL55 on TC, TG, HDL-c, LDL-c, ALT, AST, and FFA levels in the liver of rats.

Group	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	ALT (U/gprot)	AST (U/gprot)	FFA (μmol/gprot)
NFD	1.51 ± 0.23**	0.69 ± 0.05**	0.96 ± 0.10*	0.40 ± 0.03*	23.12 ± 1.47**	24.39 ± 4.77**	280.26 ± 60.56**
HFD	3.38 ± 0.38$^{\#\#}$	1.64 ± 0.06$^{\#\#}$	0.39 ± 0.23$^{\#}$	0.68 ± 0.32$^{\#}$	84.08 ± 5.70$^{\#\#}$	66.54 ± 6.83$^{\#\#}$	799.38 ± 94.70$^{\#\#}$
Sym	2.05 ± 0.08**	0.86 ± 0.18**	0.80 ± 0.31*	0.48 ± 0.18*	73.76 ± 15.25$^{\#\#}$	28.33 ± 11.83**	160.57 ± 19.60**
SPL55	1.92 ± 0.61**	0.70 ± 0.09**	0.94 ± 0.09**	0.47 ± 0.18**	31.31 ± 9.33**	23.74 ± 7.41**	215.09 ± 72.41**

Note: TG, triglyceride; TC, total cholesterol; HDL-c, high-density-lipoprotein cholesterol; LDL-c, low-density-lipoprotein cholesterol. ALT, alanine transaminase; AST, aspartate transaminase; FFA, free fatty acids. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the NFD group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the HFD group.

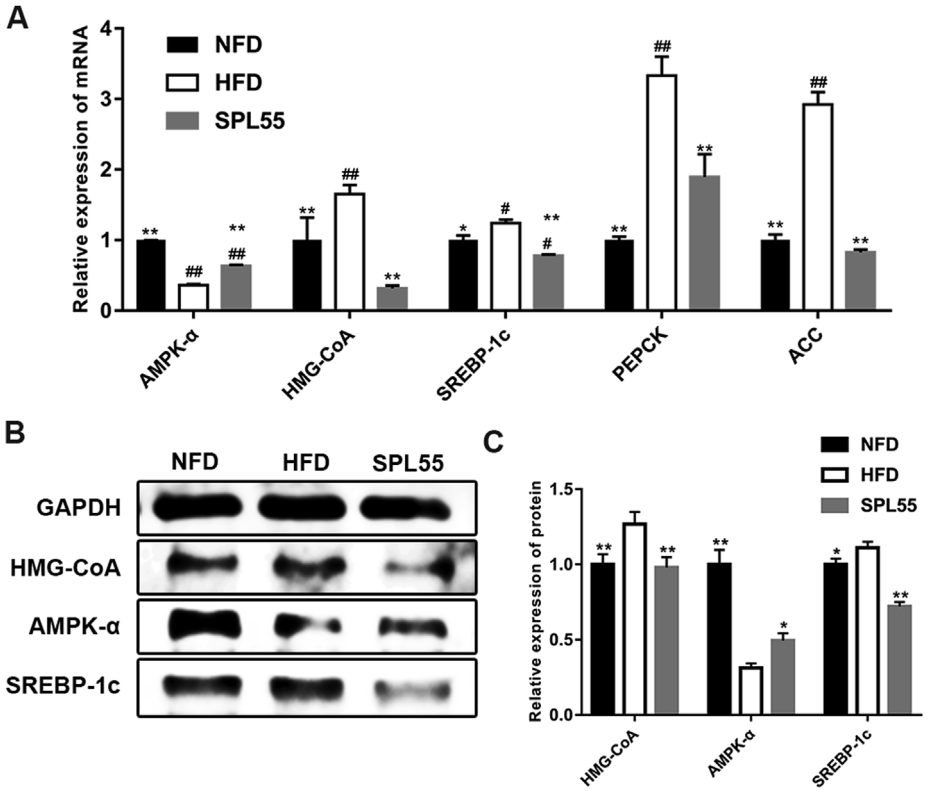

Fig. 2. The mRNA and protein expressions levels involved in lipid metabolism as determined using real-time PCR (**A**) and western blotting (**B**, **C**). The differences were assessed by ANOVA and denoted as follows: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the NFD group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the HFD group.

hepatocytes was significantly alleviated. Treatments with SPL55 and Sym also decreased fat droplets in liver tissue (Fig. 1D). SPL55 displayed protective effect and prevented the formation of fatty liver.

3.4. Effect of SPL55 on gene expressions in lipid metabolism

To identify the molecular mechanism underlying the observed changes in the accumulation of lipids, the mRNA and protein expression levels were evaluated *in vivo* with SPL55 treatment by RT-qPCR and western blot (Fig. 2). Compared with model group, the mRNA levels of liver SREBP-1c, ACC, PEPCK, and HMG-CoA genes were down-regulated significantly in SPL55 ($p < 0.01$), while AMPK-α was obviously up-regulated ($p < 0.05$) (Fig. 2A). The SREBP-1, HMG-CoA ($p < 0.01$), and AMPK-α ($p < 0.05$) had the same trends at the protein levels in lipid metabolism (Fig. 2B & C).

3.5. SPL55 modulated gut microbiota of high-fat-diet rats

To assess specific changes in the gut microbiota of experimental groups with treatments, the OTUs abundances were obtained by high throughput sequencing (Fig. 3). Firmicutes (62.02–81.51%), Bacteroidetes (9.76–27.01%), Proteobacteria (3.40–10.23%), Actinobacteria (0.63–3.68%), and Verrucomicrobia (0–0.27%) were the dominant bacterial taxa detected, whereas a small percentage (0.11–0.31%) was classified into unidentified bacteria at the phylum level (Fig. 3A). The relative abundance of the unclassified bacteria had only a small change throughout the experimental period. However, the relative abundance of Actinobacteria has changed significantly. The supplementation of a high fat diet revealed a significant change of Bacteroidetes (9.76%) and Firmicutes (81.51%). Firmicutes (62.02%) was significantly decreased and Bacteroidetes (27.01%) was significantly increased over time with SPL55 treatment. Meanwhile, the abundance of Verrucomicrobia could be significantly increased in the HFD group while showed no effect in the SPL55 group. Simultaneously, SPL55 had a certain improved effect on the variation after feeding with HFD (Fig. 3B), such as *Porphyromonadaceae*, *Prevotella*, *Ruminococcaceae*, *Bacteroides*, *Blautia*, and *Desulfovibrionaceae*. *Alloprevotella* and *Ruminococcus* were the most prominent increased changes detected after SPL55 administration at the genus level. *Allobaculum*, *Firmicutes*, *Clostridium_XlVa*, and *Lachnospiracea* were decreased in SPL55-fed rats. The proportion of *Blautia* after treatment with SPL55 was also increased compared with NFD.

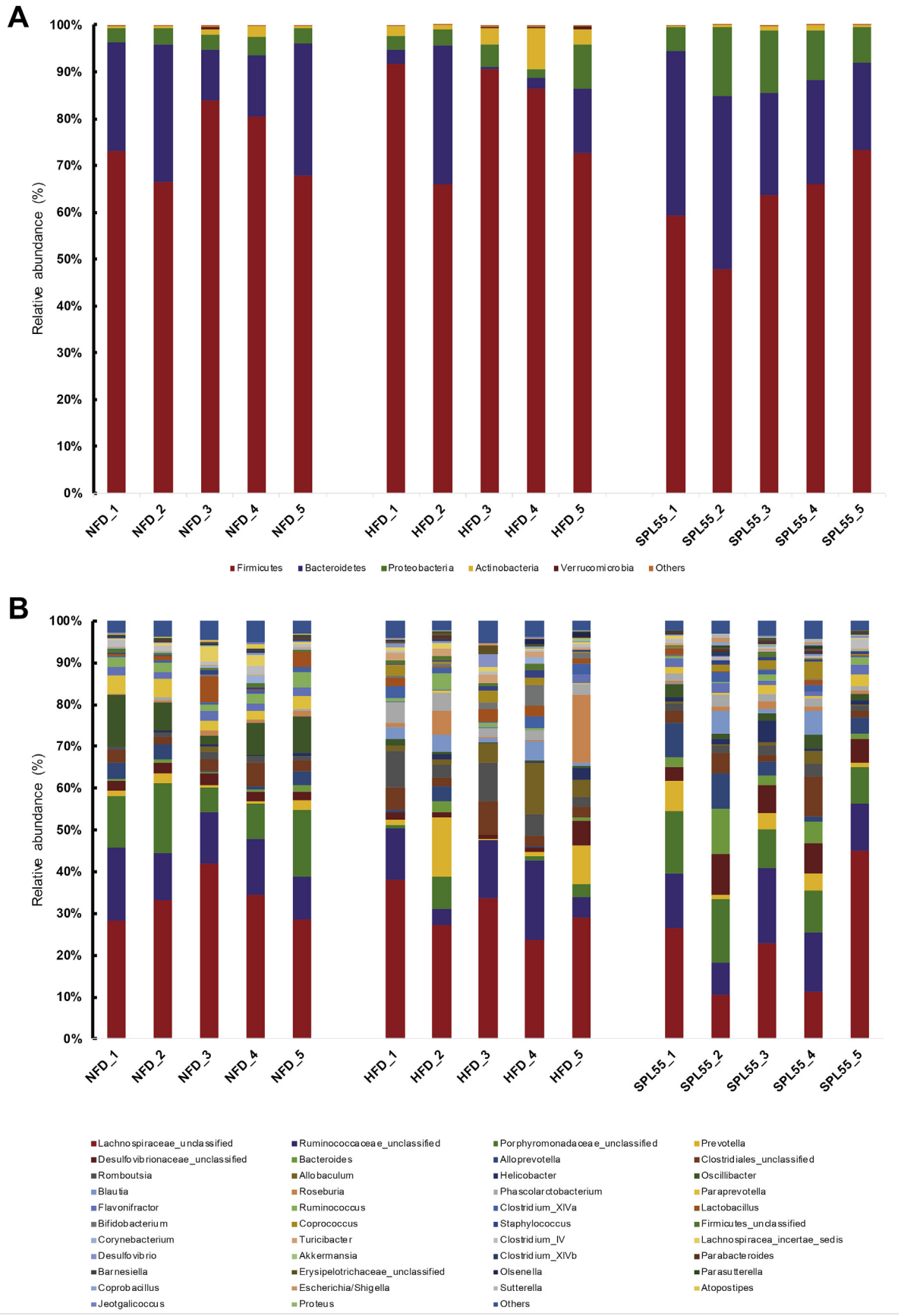

Fig. 3. Changes in the bacterial composition of rat intestinal contents at different genera. Five rats were randomly selected for analysis of gut microbiota. **A**, Composition of gut microbiota at phylum level; **B**, Composition of gut microbiota at genus level.

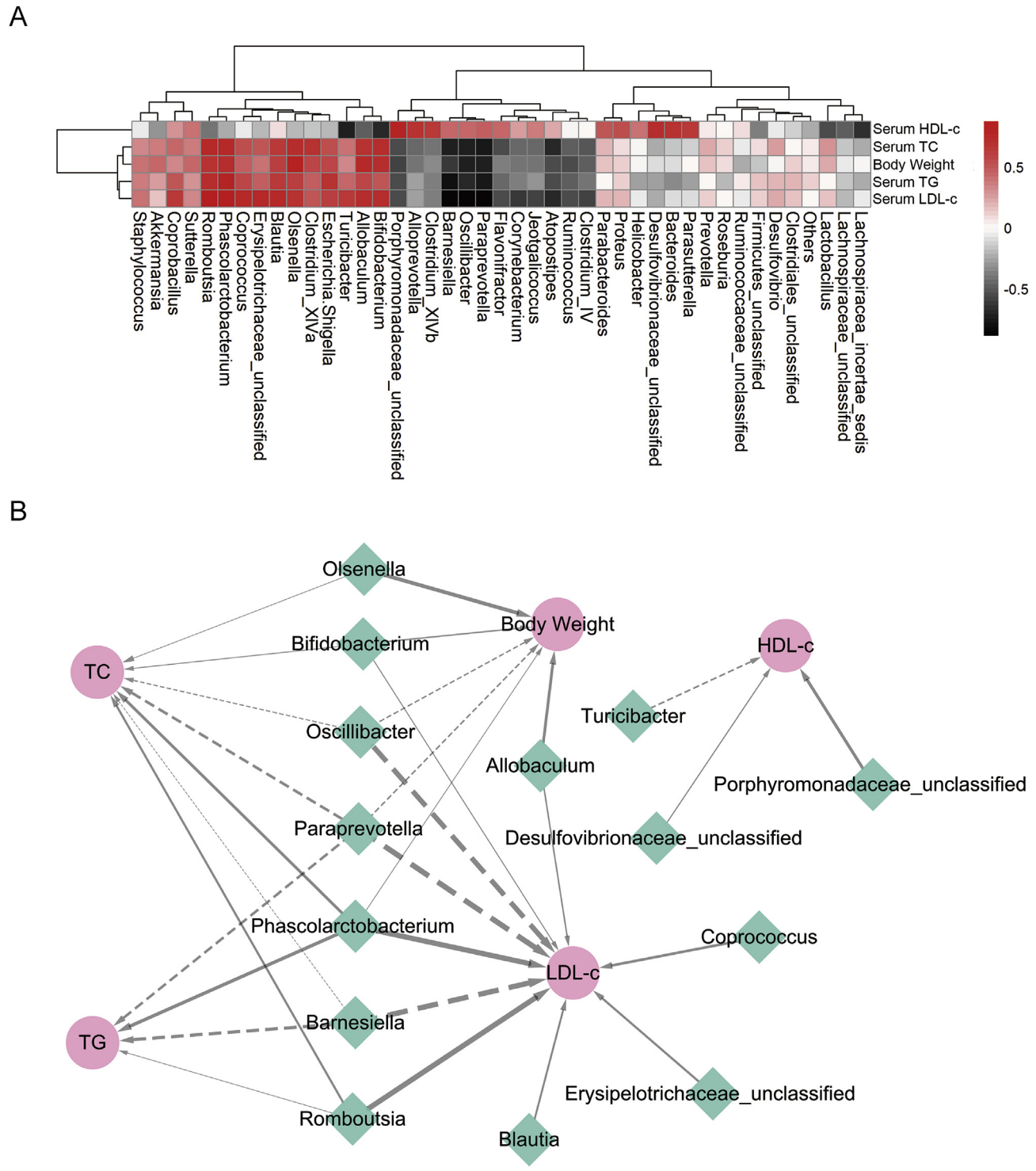

Fig. 4. Correlations between the gut microbiota and lipid metabolic parameters and visualization of the correlation network in NFD, HFD and SPL55 groups. **(A)** Statistical Spearman's correlations between the gut microbiota of significant differences and lipid metabolic parameters. **Note:** The intensity of the colour represents the degree of association between gut microbiota of significant differences and MetS-associated parameters; **(B)** Functional grouped network between microbiota and lipid parameters. **Note:** Biochemical indicators and the bacteria are shown in pink circles node and mint green diamonds node, respectively. The solid grey line and dotted grey line represent positive and negative correlation, respectively. In addition, the linewidth indicates the strength of the correlation. The significant edge thickness is scaled between the minimum and maximum scores shown using the Spearman's correlation test ($|r| > 0.7$, FDR adjusted $p < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.6. Correlations of biochemical data and key phylotypes of gut microbiota

Correlation analysis between the gut microbiota and biochemical indexes by Spearman's algorithm was performed (Fig. 4A). The characteristic microbes including *Turicibacter*, *Romboutsia*, *Phascolarctobacterium*, *Erysipelotrichaceae*, *Firmicutes*, and *Clostridium* XVIII had a large content in the HFD group. These microbes had a positive correlation with serum TG and TC, while had a negative correlation with HDL-C. As shown in Fig. 4B, the relative abundances of *Porphyromonadaceae* and *Desuifovibrionaceae* had a positive correlation

with serum HDL-c, but *Turicibacter* was negative with serum HDL-c. The bacteria of *Coprococcus*, *Erysipelotrichaceae*, *Blautia*, *Allobaculum*, *Bifidobacterium*, *Romboutsia*, and *Phascolarctobacterium* were strongly positively associated with serum LDL-C, while the *Barnesiella*, *Oscillibacter,* and *Paraprevotella* were showed a negative correlation to serum LDL-c. Interestingly, body weight was positively correlated with *Olsenella*, *Allobaculum*, *Bifidobacterium*, and *Phascolarctobacterium*, while negatively correlated with *Oscillibacter* and *Paraprevotella.* Moreover, serum TG was showed positive relationships with *Romboutsia* and *Phascolarctobacterium*, but negative with *Barnesiella* and *Paraprevotella*. The results revealed that *S. platensis* 55% ethanol extract might have an effect on restoring the ecological imbalance of gut microbiota and maintain its healthy state.

4. Discussion

Unhealthy diets, especially those with excessive fat intake, can lead to obesity. Fortunately, high-fat fed rats treated with SPL55 had significant weight loss. This situation is closely related to the enhancement of bioenergy metabolism after ingestion of *Spirulina*. The PUFAs from *Spirulina* contributed to the research progress of anti-obesity. An abnormally elevated blood lipid level can lead to life-threatening diseases. The bad habit of continuously eating high calorie diet is closely related to the prevalence of excessive metabolic syndrome (Buettner et al., 2006). As reported, consumption of HFD significantly increased levels of serum TG, TC, and LDL-c, simultaneously, reduced HDL-c levels, which was similar to the conclusions of previous studies (Miller et al., 2011). Interestingly, during the 8-week trial, SPL55 treatment stabilized serum HDL-c levels increased by high calorie intake and relatively effective extenuated blood lipids levels such as TC and TG. Disease researches indicated that LDL-c and TG as the key risky indicators of heart and cerebrovascular diseases play a key role in the innovative process of intervention and treatment of disease methods (Koyama et al., 2012). *Spirulina* was used interacts with other lipid-lowering substances had an auxiliary therapeutic function in the relief of LMDs. The changes of biochemical indicators may be associated with healthy morphological alterations in rats' liver. A high-fat diet can promote the accumulation of fat droplets in rats and cause fatty liver. Histopathological analysis revealed the differences of hepatic lipid accumulation and structures in liver tissue. With treatment of SPL55, the changes in the structure of the liver tissue were regulated and lipid droplets were significantly reduced. The data illustrated that SPL55 could improve the liver FFA, AST, and ALT in obesity rats, which indicated that it could ameliorate fatty liver in high-fat fed rats.

SPL55 may be activated the AMPK signaling pathway in lipid metabolism. The serine kinase AMPK is an emerging drug target for LMDs that is a critical player of glucose and lipid metabolism. And the lipid metabolism disorders can be regulated by its own downstream genes, such as SREBP-1c, PEPCK, ACC, and HMG-CoA (Grahame Hardie, 2014; Kim et al., 2017). SREBP-1c, one of the main forms of SREBPs, prefer to activate the genes of fatty acid synthesis-associated transcription (Cheng et al., 2017). HMG-CR, the rate-limiting enzyme of cholesterol biosynthesis through transforming HMG-CoA into mevalonic acid through the mevalonate pathway, is regulated through a negative feedback mechanism (Deboseboyd, 2008) and increases the decompose rate of plasma LDL. Moreover, high LDL-c concentrations can induce some metabolic syndrome, such as atherosclerosis symptoms (Tobert, 2003). PEPCK has been considered one key pathway for hepatic gluconeogenesis. Interestingly, the transcriptional expression of PEPCK usually up-regulated by hormones over fasting, while significantly down-regulated when intake of insulin or glucose (Yang et al., 2009). It may be as a potential therapeutic target to a cure for LMDs (Gómez-Valadés et al., 2008). p-AMPK phosphorylates ACC coupled with the intracellular malonyl-CoA content decreased (Brahma Naidu et al., 2016). ACC is a critical lipid metabolism-related enzyme. In the present work, the ACC expression level after oral administration of SPL55 was close to that of the NFD group, probably because of AMP activation, which can inhibit fat accumulation and reduce glucose and lipid levels by regulating ACC inactivated. It was showed that the ACC expression could be regulated in HFD-fed rats with SPL55 supplement. Moreover, the down-regulation of expression of HMG-CoA was one of the reasons for improving serum index in SPL55 group. Furthermore, the expression of gene SREBP-1c has significantly changed the level of TG, which led to the inhibition of cholesterol and fatty acid synthesis (Shao et al., 2013). It was found that the genes expression in the liver were significantly regulated, which facilitated the accumulation of TG and TC. Rats with treatment of SPL55 had lower expression levels of SREBP-1c, ACC, and HMG-CoA. The results showed that SPL55 could improve HFD-fed induced hyperlipidemic rats through AMPK-related signaling pathway, which provided a theoretical basis for the industrialization of *Spirulina* as a food supplement.

The microbiome metabolites can control adiposity and affect many physiologiesbased on their functions. These metabolites include the indigestible nutrients from food, synthesizing vitamins, and promoting intestinal homeostasis (Dolan and Chang, 2016). The intestinal flora can directly explain the change of blood lipid levels with its effects on the development of atherosclerosis. The gut microbiota of the experimental rats was clearly analyzed to elucidate the role of active ingredients in SPL55 for improving hyperlipidemia. SPL55 has enriched a series of gut microbiota including *Prevotella*, *Porphyromonadaceae*, *Barnesiella*, and *Parasutterella*. There is a potential co-occurrence or exclusion relationship between the intestinal flora and the lipid profile. *Prevotella* as a key microbia was negatively correlative with serum biochemical indexes. Their bidirectional interaction makes an importance role on the physiology of the whole organism. *Prevotella* species can digest and absorb more complex compounds and promote the biosynthesis of bile acids, wihich reflects their high abilities to resist LMD (Nakayama et al., 2015). A similar study revealed that food intake was associated with the increased abundance of *Prevotella* compared to both lean and obese controls. *Prevotella* can also regulate lipid levels and bile acid metabolism (Fu et al., 2015). The genus *Barnesiella* from the Porphyromonadaceae family enhances the survival of the host due to its special improvement in the microbial community composition (Ubeda et al., 2013). Besides, a favorable lipid profile was developed and related to the enriched contents of *Alloprevotella* and *Ruminococcus* genera by SPL55 supplement. Moreover, these bacteria have a negative correlation with some metabolic diseases such as the non-alcoholic fatty liver and LMDs. They can also indirectly produce short-chain fatty acids, which can stimulate gastrointestinal motility to protect the intestinal mucosal barrier and regulate energy metabolism and insulin resistance (Brown et al., 2003; Shang et al., 2017). The effect of the probiotic intervention in obese individuals showed that the high abundance of Bacteroides in the intestinal was related to reducing obesity (Dewulf et al., 2013). Howover, SPL55 supplementation significantly increased the intestinal microbial diversity, especially in *Firmicutes* and *Bacteroides*. In addition, the dynamic body mass index and blood lipid changes in high-fat rats were positively associated with the *Firmicutes*. The increased abundance of Porphyromonadaceae by SPL55 supplement may have a link with the obesity-related LMDs (Henao-Mejia et al., 2012). The abundances of *Turicibacter* and *Clostridium* XVIII were raised by a high-fat diet, while SPL55 reduced abundance of these bacteria to some extent. *Turicibacter* may have a bad impact on the healthy function of the gut and maintaining serum metabolic index. Moreover, the higher numbers of *Clostridium* XVIII in gut microbiata can cause a series of gastrointestinal diseases and physiological dysfunction and further induce high-fat-related inflammatory reactions (Ley et al., 2005). The potential use of SPL55 may be used to cure the LMDs and revealed that potent regulation of the intestinal microbiota was associated with its promotion during metabolic disease. The results have provided the evidence for anti-hyperlipidemia and reducing the probability of developing LMDs.

5. Conclusions

PUFAs from SPL55 had significant effective in regulating lipid metabolism in HFD-fed wistar rats. SPL55 has ameliorated hepatocyte abnormality and improved the expression levels of lipid genes by up-regulating AMPK-α and down-regulating SREBP-1c and HMG-CoA as potent natural hypolipidemic bioactive molecules. Moreover, SPL55 was further investigated by studying the change of gut microbiota and molecular mechanisms. Its regulation of gut microbiota was also carried out by enhancing the development of beneficial bacteria, such as *Prevotella*, *Barnesiella*, and *Paraprevotella*.

Author contributions

TL, AT and YL had done the research work and interpreted the results, ZH and XW critically analyzed the important data of microbiota. BL and CZ contributed in designing the experiment and research. YP, RJ and XC provided guidance for publication.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of interests

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.

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Transparency document

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Appendix A. Supplementary data

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