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Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats

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ABSTRACT

The contributions to hypoglycemic function and gut microbiota regulation by water and ethanol extracts of the microalgae *Chlorella pyrenoidosa* and *Spirulina platensis* were determined. An ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analysis indicated that most of the compounds in the 55% ethanol extracts of *C. pyrenoidosa* (CP55) and *S. platensis* (SP55) were polyunsaturated fatty acids. After an 8-week high-fat high-sucrose diet with *C. pyrenoidosa* and *S. platensis* supplementation, glucose tolerance was improved, and the composition of the gut microbiota was altered. The diversity of the gut bacterial community was evaluated using 16S rRNA gene pyrosequencing. *C. pyrenoidosa* supplementation increased the abundance of *Ruminococcus*, *Parasutterella*, and *Erysipelotrichacea* and decreased the abundance of *Lactobacillus*, *Turicibacter*, and *Blautia*; *S. platensis* supplementation increased the abundance of *Oscillibacter*, *Parasutterella*, and *Alloprevotella* and decreased the abundance of *Turicibacter*. Moreover, *Erysipelotrichacea* and *Ruminococcus* were uniquely increased in *C. pyrenoidosa* treatment groups. Thus, CP55 and SP55 may be developed as effective natural food materials for preventing diabetes, and *Ruminococcus* may play a vital role in the treatment of diabetes.

1. Introduction

Diabetes, which is currently the most prevalent metabolic disease, is characterized by high blood sugar levels. This disease can result in a variety of serious complications affecting the retina, nervous system, legs, kidneys, and cardiovascular system (Wild et al., 2004). With global economic growth and the increasing human lifespan, diabetes has become a worldwide epidemic. The International Diabetes Federation predicted that over 642 million adults will suffer from diabetes by 2040 (Chan et al., 2017). There is currently no cure for diabetes, but a variety of strategies can be used to control the condition, including diet modification and drug therapy (Dall et al., 2014; Verspohl, 2012). Metformin and sulfonylurea drugs have been widely used in clinical diabetes treatment as effective hypoglycemic drugs to regulate the blood glucose levels (Chaudhury et al., 2017). However, most of these anti-diabetic drugs have the potential to cause low blood glucose and often can cause high-risk side effects (Tzoulaki et al., 2009). Therefore, developing novel therapeutic drugs for diabetes has become an urgent problem at the moment (Zhao et al., 2018b).

There are over 200,000 species in the ocean, which together account for 80% of the total number of species on Earth. Marine biological resources contain a wide variety of biologically active substances with novel structures and unique functions. These resources can support corresponding drug research and development (Zhao et al., 2018a). Microalgae, as a major producer in aquatic ecosystems, have provided various biologically active natural substances, including anti-diabetic, anti-oxidant, antibacterial, and antiviral drugs (Wang et al., 2018; Zhao et al., 2015). Natural substances derived from microalgae possess tremendous pharmacological properties and have the potential to cure and prevent diabetes. *Chlorella pyrenoidosa* and *Spirulina platensis*, two major

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microalgae species from the green algal classes Chlorophyceae, are known for their unique biochemical profiles and alleged potent biological activity. *C. pyrenoidosa* contains β-carotene, chlorophylls, polysaccharides, and polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic and docosahexaenoic acids (Wan et al., 2018). Further, *S. platensis* has considerable anti-oxidant components, including β-carotene, tocopherols, phycocyanin, and phenolic compounds, as well as microelements and PUFAs, especially γ-linolenic acid (Li et al., 2018). They have also been reported to possess a variety of pharmacological effects, such as anti-tumor (Wang et al., 2010), anti-oxidant (Hu et al., 2007; Hwang et al., 2011), anti-inflammatory (Abdel-Daim et al., 2015; Guzmán et al., 2003), and anti-diabetic effects (Nasirian et al., 2018; Shibata et al., 2003). Their hypoglycemic activities have also been extensively reported. *C. pyrenoidosa* and *S. platensis* could be used as natural hypoglycemic food ingredients without side effects.

The intestinal flora regulates host physiological functions through its metabolites, such as short-chain fatty acids, endotoxins, sterols, xenobiotics, and total bile acids. The intestinal flora thus behaves like an endocrine organ, and the imbalance of gut microbiota can cause a range of metabolic syndromes (Sommer and Bäckhed, 2013). Previous research has suggested that the composition of gut microorganisms is related to the prevalence of diabetes and that an imbalance in Bacteroidetes and Firmicutes could cause various metabolic syndromes (Guo et al., 2010; Naseer et al., 2014). Thus, diabetes can be prevented and treated by improving blood glucose levels and maintaining the composition of intestinal microbes. In the present study, the hypoglycemic effects of *C. pyrenoidosa* and *S. platensis* water and ethanol extracts for high-fat, high-sucrose-fed rats were compared. Moreover, the gut microbiota essential in mediating the effects of *C. pyrenoidosa* and *S. platensis* on the diabetic metabolism were also thoroughly investigated.

2. Materials and methods

2.1. Preparation of C. pyrenoidosa and S. platensis extracts

C. pyrenoidosa and *S. platensis* powders were purchased directly from King Dnarmsa *Spirulina* Co. Ltd (Fuqing, China). *C. pyrenoidosa* ethanol extract (CP55) was extracted using 55% absolute alcohol at a ratio of 1:10 (w/v) at 50 °C for 1 h. *C. pyrenoidosa* water extract (CPWE) was extracted using distilled water at a ratio of 1:10 (w/v) at 80 °C for 1 h. After that, CP55 and CPWE were centrifuged, filtered, concentrated by rotary evaporator, and freeze-dried for further study. *S. platensis* 55% ethanol extract (SP55) and water extract (SPWE) were prepared using the same methods. Structural characterization of the major compounds in the *C. pyrenoidosa* and *S. platensis* extracts were determined by UPLC-Q-TOF-MS/MS in our previous studies (Li et al., 2018; Wan et al., 2018). The typical major polyunsaturated fatty acid (PUFA) constituents of the extracts were classified.

2.2. Animals

Forty-eight male rats (180 ± 10 g) were purchased from WuShi Experimental Animal Center (Fuzhou, China). The animals were housed in a standard environment (light-dark cycle consisting of 12 h each), 60% humidity, and 27 °C with a normal-chow diet. All experimental protocols followed the guidelines for laboratory animal welfare ethics and daily animal care. The Ethics Review Committee of College of Food Science, Fujian Agriculture and Forestry University provided ethical approval for this study (No. FS-2017-002). After one week of adaptive feeding, the rats were randomly divided into six groups and fed standard or high-fat high-sucrose chow. The six groups included two untreated control groups that were fed either the normal fat diet (NFD) or the high-fat high-sucrose diet (HFHS) and four HFHS-fed groups that were treated with 150 mg/kg·day of either CP55, CPWE, SP55, or SPWE (the CP55, CPWE, SP55, and SPWE groups, respectively). The standard chow diet provided for the NFD group contained 13.5% energy from fat (Lab Diet 5001; Lab Diet, USA), while the HFHS chow provided to the other groups contained 67% normal diet, 20% sucrose, 10% lard, and 3% cholesterol. The CP55, CPWE, SP55, and SPWE groups were gavaged with 2 mL of either water or ethanol extract every day for eight weeks. The NFD and HFHS groups were gavaged with 2 mL 0.9% saline.

2.3. Sample collection

The fasting blood glucose (FBG) of the rats was determined at the 0-week and after 8-weeks. After eight weeks of treatment with *C. pyrenoidosa* and *S. platensis* water and ethanol extracts, all rats were fasted overnight (10 h) with free drinking water prior to the oral glucose tolerance test (OGTT). Rats were gavaged with CP55, CPWE, SP55 or SPWE and given 2 g/kg oral glucose. Then, blood samples were collected and measured with a blood glucose meter at 0, 0.5, and 2 h after the SP55, SPWE, CP55, or CPWE administration to monitor any possible changes in blood glucose concentration (BGC) after receiving the *C. pyrenoidosa* and *S. platensis* extract treatments. Cecal samples were collected directly in sterile 5 mL cryotube and immediately transferred to liquid nitrogen for 30 s; the samples were then stored at −80 °C for further study. The values of the area under the curve (AUC) were calculated as follows: $AUC = 0.5\ (BGC\ 0\ h + BGC\ 0.5\ h) \times 0.5 + 0.5\ (BGC\ 2\ h + BGC\ 0.5\ h) \times 1.5$.

2.4. DNA extraction and preparation and 16S rRNA sequencing from cecal samples

The gut microbiota DNA from cecal samples of rats was extracted using the QIAamp-DNA Stool Mini Kit (Qiagen, Hilden, Germany). The diluted genomic DNA was used as a template, and specific primers with barcode regions based on the selected sequencing region were used to perform PCR to amplify the V3-V4 hypervariable region of the 16S rRNA gene. The primers were designed as follows: F: 5′-CCTACGGRRBGCASCAGKVRVGAAT-3′ and R: 5′-GGACTACNVGGGTWTCTAATCC-3′. Next, 16S rRNA sequencing was performed using the Ion S5 System (Thermo Fisher Scientific). The library was constructed using the Ion Plus Fragment Library Kit (48 rxns, Thermo Fisher) and subjected to Qubit quantification and library testing.

2.5. Statistical analysis and bioinformatics

The experimental data for each group were displayed as mean ± SEM (n = 8). The statistical significance of all comparisons of rat-related experiments was determined using one-way analysis of variance (ANOVA) with Tukey's test correction. Statistically significant data produce a significant difference as determined by having a *p*-value of less than 0.05. In the process of bioinformatics analysis, clean reads of all samples were clustered using the Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/), and the sequencing was clustered into OTUs by default with 97% identity. Simultaneously, the sequences were screened using the sequences with the highest frequency in OTUs as representative. The changes in microbial flora structure were visualized using two-dimensional partial least squares discriminant analysis (PLS-DA) plots with forced grouping. The top species of the intestinal flora in the HFHS-fed rats were predicted by the variable importance in projection (VIP) method. The significant difference of the microbiota among the rats after NFD, HSHF, SP55, SPWE, CP55, and CPWE supplementation were revealed by an extended error bar plot with the using the STAMP (structural time series analyser, modeller and predictor) ($p < 0.05$). Spearman's rank test was used to analyze the correlation between the intestinal microbiota and serum glucose levels with the RStudio software. The visualized network of blood glucose indicators and cecal microbiota correlation was generated using Cytoscape 3.6.1.

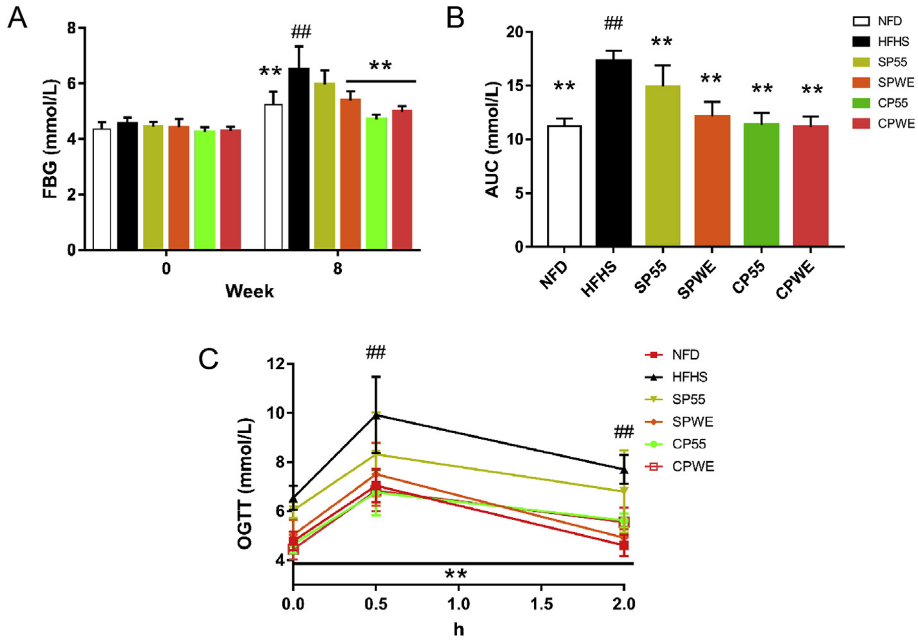

Fig. 1. Effects of SP55, SPWE, CP55, and CPWE on FBG (A), AUC (B), and OGTT (C) of HFHS-fed rats during the experimental period. NFD (White): normal fat diet; HFHS (black): high-fat high-sucrose diet; SP55 (yellow): 150 mg/(kg·day) *S. platensis* 55% ethanol extract; SPWE: 150 mg/(kg·day) *S. platensis* water extract; CP55 (green): 150 mg/(kg·day) *C. pyrenoidosa* 55% ethanol extract; CPWE (red): 150 mg/(kg·day) *C. pyrenoidosa* water extract. All *p* values were analyzed by one-way ANOVA with Tukey's correction and were shown as follows: $^{*}p < 0.05$ versus (VS) the HFHS group, $^{\#}p < 0.05$ VS the NFD group, $^{**}p < 0.01$ VS the HFHS group, and $^{\#\#}p < 0.01$ VS the NFD group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Effects of *C. pyrenoidosa* and *S. platensis* extracts on FBG levels of HFHS-fed rats

FBG, the most commonly used test for detecting diabetes, reflects islet β-cell function and basal insulin secretion. All rats in the study had similar FBG levels at the beginning of the experiment. After the 8-weeks treatment, the HFHS-fed rats exhibited higher FBG levels than the chow-fed rats. Moreover, the SPWE, CP55, and CPWE treatments distinctly reduced FBG levels compared to the untreated HFHS group ($p < 0.01$). However, SP55 supplementation did not have an obvious effect. In addition, the FBG levels in the *C. pyrenoidosa* groups were lower than those in the *S. platensis* groups, which indicated that the microalga *C. pyrenoidosa* might possess better hypoglycemic function than *S. platensis* (Fig. 1A).

3.2. Effects on OGTT in HFHS-fed rats

OGTT is an important indicator for investigating the severity of diabetes. The results revealed that the glucose tolerance of the HFHS control group was affected by a certain degree of damage after eight weeks of HFHS feeding. At 0 h, blood glucose was higher in the HFHS group than in the other groups (Fig. 1C). At 120 min, all treated groups exhibited significantly decreased serum glucose levels compared to the HFHS group. Further, the blood glucose levels of the *C. pyrenoidosa* groups were close to that of the NFD group. There was a significant difference in serum glucose levels between NFD and HFHS-fed rats. The microalgae treatment groups significantly reduced the AUC of glucose concentration compared to the HFHS group ($p < 0.01$) (Fig. 1B). These results suggest that oral *C. pyrenoidosa* and *S. platensis* supplementation ameliorates the impaired glucose tolerance of HFHS-fed rats.

3.3. Effects on the structure of gut microbiota in HFHS-fed rats

Changes in the component distributions of intestinal microbiota were analyzed using PLS-DA (Fig. 2A), which was used to construct a specific model for each class separately. The intestinal microbiota in the NFD, HFHS, SP55, SPWE, CP55, and CPWE groups were highly distinct. The HFHS group mainly accumulated in the positive first principal component (PC1) and displayed an obvious structural change compared to the NFD group. Meanwhile, the SP55, SPWE, CP55, and CPWE groups exhibited some recovery in variation even with HFHS feeding. The status of the microalgae *C. pyrenoidosa* and *S. platensis* treatment groups presented a similar trend as that of the normal group. The VIP scores predicted the significance of each microbiota in the projection with the PLS model and were often used for variable selection. The VIP scores of the intestinal microbes showed that the species *Oscillibacter* and *Erysipelotrichaceae* were the most likely microbes to lead to the population segregation of gut microbiota in HFHS-fed rats (Fig. 2B).

3.4. *C. pyrenoidosa* and *S. platensis* modulated gut microbiota of HFHS-fed rats

At the end of the 8-week treatment, specific changes in the cecal microbiota were compared by determining the abundance of bacterial populations among all groups. The components of gut microflora at the phylum level were detected. HFHS-fed rats had high levels of *Bacteroidetes*, *Actinobacteria*, and *Firmicutes*. However, after the 8-week treatment, supplementation with the microalgae *C. pyrenoidosa* and *S. platensis* increased the *Bacteroidetes* and reduced the *Actinobacteria* populations (Fig. 3A). Moreover, microalgae administration prevented the increase in the *Firmicutes/Bacteroidetes* ratio (Fig. 3B), which is a characteristic sign of micro-ecological disorder in intestinal microbes. Significantly, a higher abundance of *Verrucomicrobia* was observed in the CP55 and CPWE treatment groups compared to the other groups (Fig. 3A).

Bacteria with significantly different abundances are showed in an extended error bar plot (Fig. 3C). The characteristic microbes of the NFD group, such as *Turicibacter* and *Blautia*, were significantly lower than in the HFHS group, while the abundances of *Oscillibacter*, *Ruminococcus*, and *Ruminococcaceae* were higher. The composition of the intestinal microbiota underwent significant changes in HFHS-fed rats after treatment with *C. pyrenoidosa* and *S. platensis*. The SP55 treatment markedly reduced the relative abundances of *Turicibacter*, which were enriched in the HFHS group. It also significantly increased the abundances of *Oscillibacter*, *Parasutterella*, and *Alloprevotella*. Compared with HFHS group, the abundance of *Alloprevotella* increased, while *Lactobacillus* decreased in the SPWE group. In addition, after CPWE treatment, the abundances of *Ruminococcus*, *Akkermansia*, *Parasutterella*, *Erysipelotrichacea*, and *Oscillibacter* increased, while *Lactobacillus*, *Ruminococcaceae*, *Turicibacter*, and *Blautia* decreased. Interestingly,

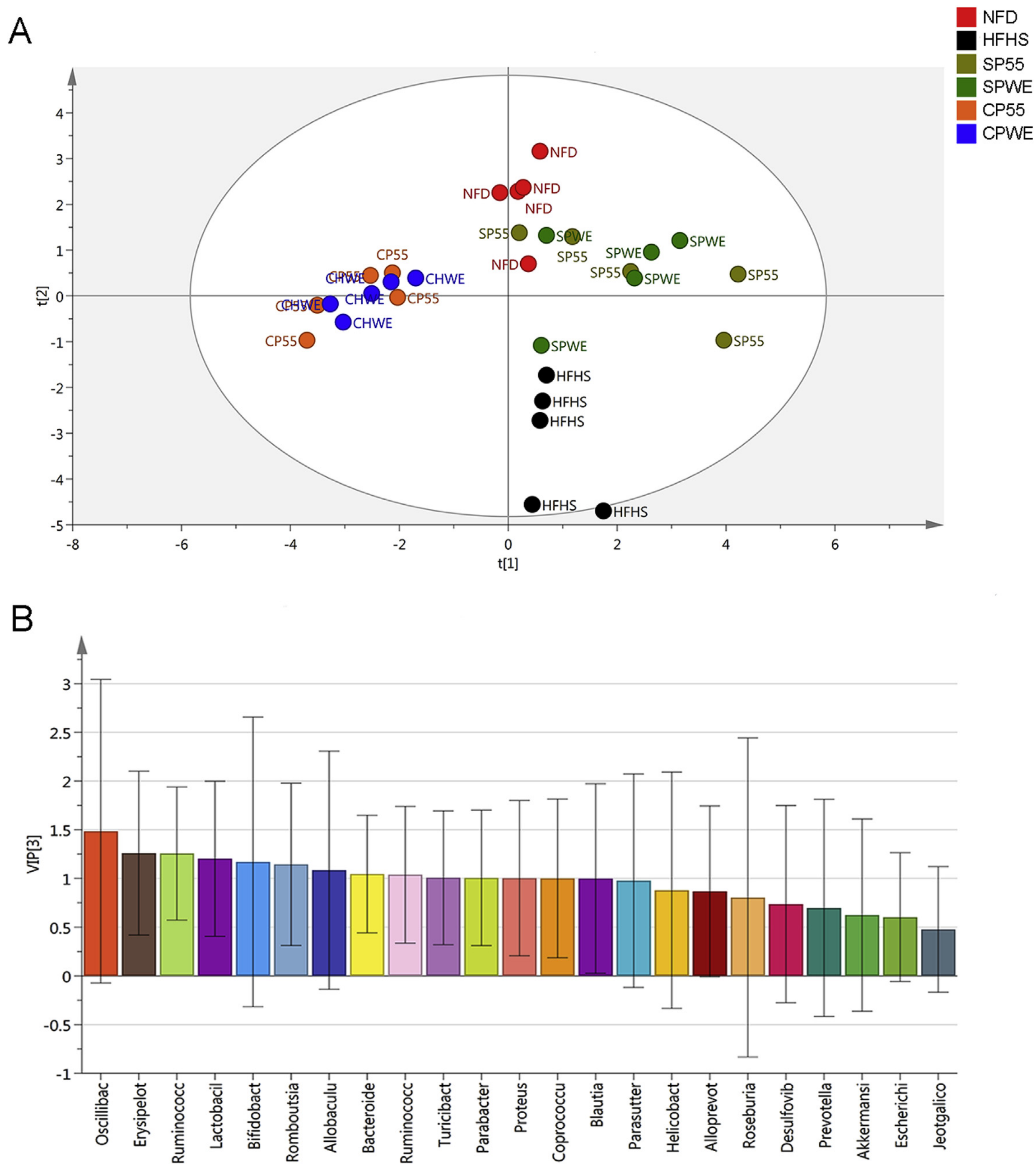

Fig. 2. *C. pyrenoidosa* and *S. platensis* improved diabetic gut microbiota disorder in HFHS-fed rats. Rats were randomly selected from each group for analyzing the changes. The partial least squares discriminant analysis (A) and variable importance in projection (VIP) (B) analyses are shown.

Erysipelotrichacea and *Ruminococcus* were unique for the *C. pyrenoidosa* and NFD rather than the *S. platensis* and HFHS groups. The results revealed that treatments with *C. pyrenoidosa* and *S. platensis* microalgae had notable effects on restoring the ecological balance of the intestinal flora. Additionally, *Erysipelotrichacea* and *Ruminococcus* might play a vital role in the treatment of diabetes.

3.5. Correlation between blood glucose levels and gut microbiota

The correlation between gut microbiota composition and biochemical parameters induced by treatment with *C. pyrenoidosa* and *S. platensis* was analyzed by nonparametric Spearman's test (Fig. 4). The network demonstrated that the microbes, including *Erysipelotrichacea*, *Blautia*, and *Turicibacter*, showed positive relationships with FBG levels, and *Oscillibacter* has displayed a negative correlation with the FBG and AUC levels after SP55 and SPEWE treatments. In the CP55 groups, *Ruminococcus* and *Parasutterella* were negatively correlated with FBG, and *Oscillibacter* was negatively correlated with AUC. Moreover, *Blautia* and *Turicibacter* were positively correlated with AUC, and *Lactobacillus* was positively correlated with FBG. After CPWE supplementation, *Blautia* showed a positive relationship with FBG and AUC, and *Lactobacillus* was positively related to FBG. Moreover, *Alloprevotella* and *Parasutterella* were negatively correlated with FBG, and *Oscillibacter* was negatively related to AUC. Interestingly, *Ruminococcus* was negatively correlated with FBG and AUC. These results indicate that the microbiota, especially *Ruminococcus* and *Oscillibacter*, play the important roles in the beneficial effects of the *C. pyrenoidosa* and *S. platensis* treatments.

4. Discussion

Microalgae are considered preventive nutrients and are used for

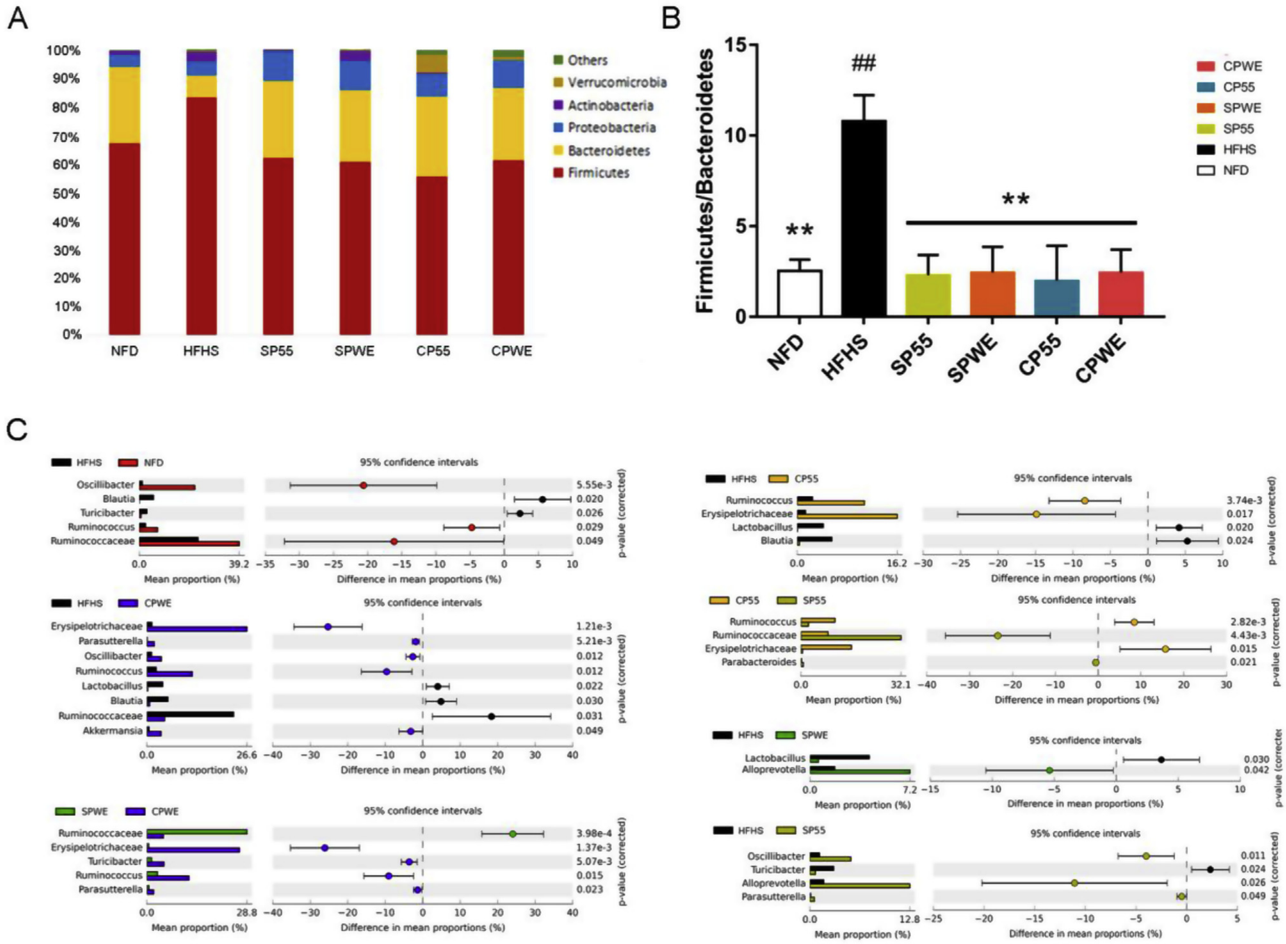

Fig. 3. Effects of *C. pyrenoidosa* and *S. platensis* treatment on changes in the intestinal microbiota of HFHS-fed rats at the phylum level (A). The ratio of Firmicutes to Bacteroidetes (B). The extended error bar plot shows the gut microbiota exhibiting significant differences among average proportions of bacterial taxa (C). HFHS (black) VS NFD (red), HFHS (black) VS CPWE (blue), HFHS (black) VS CP55 (orange), HFHS (black) VS SP55 (yellow), HFHS (black) VS SPWE (green), CP55 (orange) VS SP55 (yellow), and CPWE (blue) VS SPWE (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

their hypoglycemic and therapeutic effects (Zhao et al., 2015). The present evidence shows that daily treatment of HFHS-fed rats with PUFA-rich water and ethanol extracts of *C. pyrenoidosa* and *S. platensis* was sufficient to reduce FBG levels and ameliorate glucose tolerance. Previous work has reported that *C. pyrenoidosa* and *S. platensis* supplementation produced a great amount of pharmacological effects, which included the prevention of diabetes, regulation of the balance of host energy metabolism, and production of a therapeutic effect for metabolic syndrome in animal models. However, it has remained unclear which of the two microalgae species is better for lowering blood glucose. Our results revealed that *C. pyrenoidosa* and *S. platensis* effectively reduced fasting blood glucose levels and significantly strengthened glucose tolerance in HFHS-fed rats. The fasting blood glucose test, a glucose oxidase method, can be used to specifically measure the true blood glucose concentration. Blood glucose levels are kept relatively stable through regulation by the nervous system and hormones. In turn, an imbalance in these adjustments would cause blood glucose disorders. Long-term abnormal blood glucose levels could impair insulin function and cause diabetes (Bortheiry et al., 1994). The oral glucose tolerance test is a glucose load experiment used to determine the function of islet β-cells and the body's ability to regulate blood glucose. Clinically, patients with abnormal blood glucose are often administered this test to diagnose abnormal glucose metabolism (Wang et al., 2016). The present results indicated that the hypoglycemic effect of *C. pyrenoidosa* treatment was more significant than that of *S. platensis* treatment and that the blood glucose levels of rats treated with *C. pyrenoidosa* groups were closer to those of normal chow-fed rats than *S. platensis*-treated rats.

Previous research has reported that *C. pyrenoidosa* and *S. platensis* could regulate the composition of gut microbiota. The gut microbiota played an important role in preventing the onset of insulin resistance and type 2 diabetes by affecting host energy metabolism, glucose regulation, and inflammation (Wen et al., 2008). The *Firmicutes* abundance improved, as did that of *Bacteroidetes*, the abundance of which was found to be reduced in rats fed the high energy diet. An imbalance in the ratio of *Bacteroidetes* to *Firmicutes* caused metabolic disorder diseases (Lambeth et al., 2015). Further, *Firmicutes* played a major role in absorbing calories from the diet and storing fat in gut cells (Kelder et al., 2014). The microbial population of the rat's cecal contents was mainly composed of bacteria. *Bacteroidetes* and *Firmicutes* accounted for more than 90% of bacteria present, which was similar to the human intestinal flora (Martínez et al., 2013). In the present investigation, the abundances of the *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* phyla changed significantly in the HFHS-fed rats. The results demonstrated that the ratio of *Firmicutes/Bacteroides* in the HFHS group was markedly increased compared to the NFD group and the groups treated with *C. pyrenoidosa*, and *S. platensis*. The abundances of *Oscillibacter* and *Parasutterella* increased and that of *Turicibacter* decreased after *C. pyrenoidosa* and *S. platensis* treatment. *O. valericigenes* belongs to the clostridial cluster IV, which is commonly found in the digestive tracts of

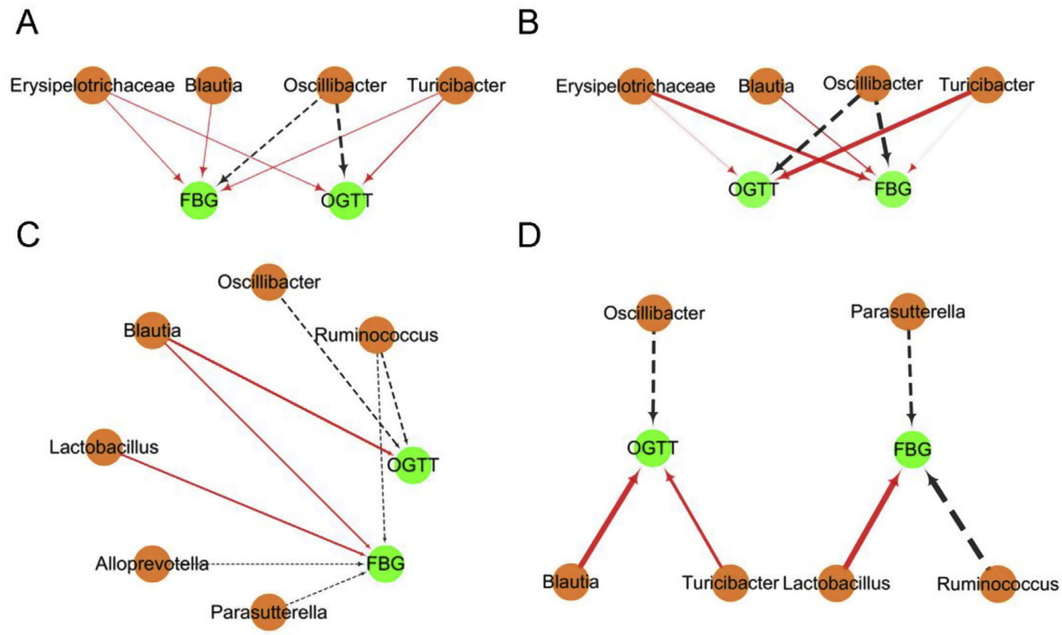

Fig. 4. A network revealing the correlations among blood glucose levels and the significant intestinal flora of each group. The solid red line corresponds to the positive correlation, and the dotted black line corresponds to negative correlation. The width of the line represents the intensity of correlation. A: SP55, NFD, and HFHS groups. B: SPWE, NFD, and HFHS groups. C: CP55, NFD, and HFHS groups. D: CPWE, NFD, and HFHS groups. ($|r| > 0.5$, FDR adjusted $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

animals and humans. *Oscillibacter* controlled the degradation of fiber by cellulases and produced butyric acid from glucose, ribose, and xylose (Lee et al., 2012). Moreover, butyric acid could be directly absorbed and used to repair intestinal mucosa and enteritis. *Parasutterella* species are Gram-negative coccobacilli (circular and rod-shaped) that are strictly (or obligate) anaerobic and are non-motile (Nagai et al., 2009). Noble et al. found that increased *Parasutterella* abundances could increase sugar and alcohol consumption (Noble et al., 2017), which was consistent with the current result. *Turicibacter* belongs to the phylum *Firmicutes* and has been tentatively placed in the class Erysipelotrichia (Auchtung et al., 2016). *Turicibacter* has been reported to be related to the trinitrobenzene sulfonic acid-induced colitis and diabetic rat model and might also affect intestinal health and cause various diseases, including diabetes, inflammation, and neuroinflammation (Jones-Hall et al., 2015). The abundances of *Erysipelotrichacea* and *Ruminococcus* were increased following the administration of *C. pyrenoidosa* extracts compared to *S. platensis* extracts. Further analysis revealed that the abundances of *Ruminococcus* were significantly increased in the NFD group than HFHS group. *Ruminococcus* is a genus of bacteria in the class Clostridia that is found in significant numbers in the intestines of humans and animals. *Ruminococcus* as a probiotic could bind and transport mucins as well as degrade the constituent sugars. Yan et al. revealed that the abundance of *Ruminococcus* was increased in type 2 diabetic rats after total saponin treatment (Blandino et al., 2016; Yan et al., 2017). The present results show that *C. pyrenoidosa* treatment had a more effective hypoglycemic effect than *S. platensis* supplementation, which may be connected to *Ruminococcus* and the potential of these bacteria to regulate blood glucose levels.

5. Conclusions

Most notably, to the authors' knowledge, this is the first study to compare the effectiveness of PUFA-rich water and ethanol extracts of the microalgae *C. pyrenoidosa* and *S. platensis* on the control of hypoglycemic function and regulation of the gut microbiota in high-fat high-sucrose-fed rats. The present results provide compelling evidence for long-term involvement these functions in HFHS-fed rats and suggest that *C. pyrenoidosa* treatment is more effective in counteracting hypoglycemia in rats than *S. platensis* treatment. Moreover, *C. pyrenoidosa* and *S. platensis* supplementation maintained the number of beneficial bacteria in the intestines, such as *Oscillibacter*, *Parasutterella*, and *Ruminococcus*, as well as reduced the abundances of *Blautia* and *Turicibacter*. Specifically, *Ruminococcus* might be the key bacteria related to the regulation of diabetes by comparison with the alternation of gut microbiota observed among the *C. pyrenoidosa*, *S. platensis*, and NFD treatment groups. The present work provides novel experimental evidence and a therapeutic window for the prevention and treatment of diabetes mellitus. Further studies are needed to investigate the hypoglycemic mechanism of *Ruminococcus* in the metabolic improvements induced by *C. pyrenoidosa* supplementation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.04.017.

Transparency document

Transparency document related to this article can be found online at doi:10.1016/j.fct.2019.04.017

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