Ameliorating effects of Sporidiobolus pararoseus extract on dyslipidemia in mice with high-fat diet-induced obesity

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Original Article

Ameliorating effects of *Sporidiobolus pararoseus* extract on dyslipidemia in mice with high-fat diet-induced obesity

Running title: Hyperlipaemia-moderating effect of *S. p.* extract

Chao Du¹,²*, Danyu Ying³, Yahui Guo³, Yuliang Cheng³, Mei Han¹, Weiguo Zhang³, He Qian¹,²*

¹School of Food Engineering, Ludong University, 186 Middle Hongqi Road Yantai, Shandong Province, 264025, P. R. China
²School of Food Science and Technology, Jiangnan University; ³School of Biotechnology, Jiangnan University

¹⁸⁰⁰ Lihu Avenue Wuxi, Jiangsu Province, 214122, P. R. China

<table>
<thead>
<tr>
<th>Authors’ name</th>
<th>Email addresses</th>
<th>Affiliations</th>
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<tbody>
<tr>
<td>Chao Du</td>
<td><a href="mailto:516826782@qq.com">516826782@qq.com</a></td>
<td>School of Food Engineering, Ludong University &amp; School of Food Science and Technology, Jiangnan University</td>
<td>Ludong University, 186 Hongqi Road Yantai, Shandong Province, 264025, P. R. China</td>
<td>+86-535-669 5491</td>
</tr>
<tr>
<td>Danyu Ying</td>
<td><a href="mailto:593924769@qq.com">593924769@qq.com</a></td>
<td>School of Food Science and Technology, Jiangnan University</td>
<td>Jiangnan University</td>
<td>+86-510-8532 8713</td>
</tr>
<tr>
<td>Yahui Guo</td>
<td><a href="mailto:601609769@qq.com">601609769@qq.com</a></td>
<td>School of Food Science and Technology, Jiangnan University</td>
<td>Jiangnan University</td>
<td>+86-510-8532 8713</td>
</tr>
<tr>
<td>Yuliang Cheng</td>
<td><a href="mailto:wxfoodcyl@126.com">wxfoodcyl@126.com</a></td>
<td>School of Biotechnology, Jiangnan University</td>
<td>Jiangnan University</td>
<td>+86-510-8532 8713</td>
</tr>
<tr>
<td>Mei Han</td>
<td><a href="mailto:hanmei.918521@163.com">hanmei.918521@163.com</a></td>
<td>School of Biotechnology, Jiangnan University</td>
<td>Jiangnan University</td>
<td>+86-510-8532 8713</td>
</tr>
<tr>
<td>Weiguo Zhang</td>
<td><a href="mailto:zhangwg168@126.com">zhangwg168@126.com</a></td>
<td>School of Food Science and Technology, Jiangnan University</td>
<td>Jiangnan University</td>
<td>+86-510-8532 8713</td>
</tr>
<tr>
<td>He Qian</td>
<td><a href="mailto:amtf168168@126.com">amtf168168@126.com</a></td>
<td>School of Food Science and Technology, Jiangnan University</td>
<td>Synergetic Innovation Center Of Food Safety and Nutrition, Jiangnan University</td>
<td>+86-510-8532 8713</td>
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*Corresponding author; E-mail: 516826782@qq.com; ruindu@163.com
Tel: +86-510-8532 8713; Fax: +86-510-8532 9081;

The research was conducted in the School of Food Science and Technology of Jiangnan University.
Abstract:
The study aims to elucidate the intervention effect of Sporidiobolus pararoseus (S. p.) extract on the lipid metabolism in Kunming mice with high-fat diet-induced obesity with Max EPA as a control. Ten mice were randomly selected from 60 mice as control group and the remaining 50 mice were fed with a high-fat diet to establish a dyslipidemia model. After 4 weeks, these 50 mice were randomly divided into 5 groups: high-fat model group, Max EPA group, and the 3 groups of different doses of S. p. extract (LD, MD and HD). After eight weeks, the mice were sacrificed and related parameters were measured. Compared with the high-fat group, S. p. extract (HD) group showed the significantly decreased body weight and serum TC, TG and LDL-c levels as well as the increased HDL-c level. RT-PCR results showed that the mRNA expression of SREBP-1c, FAS and ACC in S. p. extract supplementation group was lower than those in high-fat diet group whereas the expression of CPT-1 in S. p. extract supplementation group was higher than that of the high-fat diet group. Our results implied that the intake of S. p. extract might benefit the patients with dyslipidemia. Therefore, S. p. extract may be developed as a dietary supplement for ameliorating lipid metabolism of obese people.

Keywords:
Sporidiobolus pararoseus extract; Lipid metabolism; Dyslipidemia; High-fat diet-induced obesity; Unsaturated Fatty Acids

Abbreviations:
TC: Total Cholesterol
TG: Triglyceride
HDL-c: High-Density Lipoprotein cholesterol
LDL-c: Low-Density Lipoprotein cholesterol
S. p.: Sporidiobolus pararoseus
S. p. extract-LD: low dose of S. p. extract
AI: Atherosclerosis Index
H&E: Hematoxylin And Eosin
SD: Standard Deviation
HFD: High-Fat Diet
FFAs: Free Fatty Acids
SREBP-1c: Sterol Regulatory Element-Binding Protein 1c
ACC: Acetyl-Coa Carboxylase
CPT-1: Carnitine Palmitoyltransferase 1
1. Introduction

Dyslipidemia is characterized by elevated total cholesterol (TC) and triglyceride (TG) and decreased
high-density lipoprotein cholesterol (HDL-c) levels and usually co-exists with obesity. (Kim et al. 2016;
Shih et al. 2013) Recently, dyslipidemia has become an increasingly serious social problem, and the
treatment cost of obesity-related illness represents a significant expenditure in national healthcare
budgets in both developed and developing countries. (Jall et al. 2017; Jing et al. 2013; Lee et al. 2013a)
The over-consumption of industrialized foods containing rich fat is directly implicated in the above
situation. The dyslipidemia individuals have the particular risk of Type 2 diabetes, hyperlipidemia,
cardiovascular problems and certain cancers. (Bae et al. 2017; Choi et al. 2011; Vinuesa et al. 2016)
Given the low efficacy and side effects of drugs, more attention had been paid to dietary prevention and
2015)

*Sporidiobolus pararoseus* (*S. p.*) belongs to the class *Urediniomycetes* and can biosynthesize
intracellular oil in abundance (about 50% of dry weight). (Han et al. 2016a; Han et al. 2012; Han et al.
2016b) *S. p.* extract contains large amounts of unsaturated fatty acids and other nutrient substances,
such as carotenoids, squalene and ergosterol. (Du et al. 2017a, b) Unsaturated fatty acids have a
favorable effect on the regulation of blood lipids and can also effectively prevent cardiovascular
diseases. (Siscovick et al. 2017) Ergosterol is a plant sterol and has been used as a novel functional
ingredient for it can lower plasma cholesterol level by inhibiting the absorption of cholesterol.
(Gil-Ramírez et al. 2014) Carotenoids are well known as natural antioxidants, which have inhibition
effects on the proliferation of various tumor cells. (Du et al. 2015, 2016) Accordingly, *S. p.* extract may
have the good regulation effect on blood lipids, but its regulation effect has not been reported.
Recently, emerging evidences have shown that sterol regulatory element-binding proteins, such as SREBP-1c, are the target of a variety of treatments for metabolic diseases. SREBP-1c plays an important role in the regulation of cholesterol and fatty acid biosynthesis. Moreover, it also targets the genes involved in fatty acid metabolism, such as FAS, ACC and CPT-1. (Li et al. 2016) To our knowledge, the role of \textit{S. p.} extract in SREBP-1c signaling regulation has not been reported.

The study aims to investigate the effect of \textit{S. p.} extract supplement on ameliorating dyslipidemia and regulating lipid metabolism in the high-fat diet-induced obese mice model. The effects of \textit{S. p.} extract supplement on dyslipidemia-related parameters, such as serum levels of TC, TG, HDL-c, and LDL-c, were evaluated. The histological changes and relative molecular mechanisms were also investigated.

2. Materials and Methods

2.1 Regents

\textit{Sporidiobolus pararoseus} yeast cells were cultivated and enriched according to a previously published method. (Han et al. 2012) Cell walls of \textit{S. p.} were broken by high-pressure homogenization and then the lipid was extracted with ethyl acetate. The general composition of \textit{S. p.} extract was determined by High Performance Liquid Chromatography (Supplementary material). Other chemicals and reagents with the commercially available highest degree of purity were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Mice and experimental design

Eight-week-old Kunming male mice obtained from SLAC Laboratory Animal (Shanghai, China) were used in all the experiments. The mice were housed in standard cages under the temperature (23 ± 2 °C) and humidity (60 ± 5 %) with a 12-h light/dark cycle and fed with commercial standard chow (SLAC
Laboratory Animal, Shanghai, China) and water ad libitum. After 1 week of acclimatization, 10 mice were randomly selected from 60 mice as the control group and the remaining 50 mice were fed with a high-fat diet (78.8% basic forage, 1% cholesterol, 20% lard and 0.2% cholic acid sodium) to establish an obesity model. After 4 weeks, the 50 mice were randomly divided into 5 experimental groups (n = 10) and fed with different diets (Table 1).

During the experimental process, S. p. extract, Max EPA or normal saline was administered intragastrically by gavage every day. The control group mice were fed with basic forage and intragastric administration was performed with normal saline once a day. Food consumption and weight gain were respectively measured daily and weekly. At the end of the experimental period, the mice were anesthetized with aether withholding food for 12 h, and blood was drawn from eyeball to determine the plasma biomarkers. The liver and epididymal white adipose tissue were removed after blood collection, rinsed with a physiological saline solution, weighed, and processed for histological (H&E) staining and further analysis. (Du et al. 2016; Jing et al. 2013)

All experimental procedures involving animals in this research were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). The approval of this experiment was obtained from the Institutional Animal Ethics Committee of Jiangnan University (Wuxi, China). All animal protocols were reviewed and approved by the Animal Care and Use Committee of Jiangnan University (JN No. 20160301-20160531[13]).

2.3 Serum chemistry analysis

Mice were fasted for 12 h before gathering blood samples for the serum chemistry analysis. The serum was collected and serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol
(HDL-c) and low-density lipoprotein cholesterol (LDL-c) were measured using a Roche Cobas C501 Automatic Analyzer (Roche, Ltd., Germany). Levels of atheroclerosis index (AI) were calculated according to the following formula: 

\[ AI = \frac{TC - HDL-c}{HDL-c}. \] 

(Lim et al. 2015)

### 2.4 Histological analysis of liver and white adipose tissue

Liver and epididymal white adipose tissue were removed from the mice and fixed in 10% buffered formalin. Tissues were processed and paraffin blocks were made according to conventional tissue processing steps. For morphological analysis, paraffin blocks were cut into 5-µm thick sections and mounted on the slides. The slides were subsequently stained with hematoxylin and eosin (H&E) for evaluation under a standard light microscope. (Li et al. 2016)

### 2.5 RNA extraction and quantitative real-time PCR

Total RNA was extracted using the TRIzol reagent (Shanghai Generay Biotech Co., Ltd., China). The concentration and purity of the RNA samples were determined spectroscopically. RNA was reversely transcribed into cDNA with Revert Aid TM M-Mu LV Reverse Transcriptase (Thermo Scientific, Rockford, USA) according to manufacturer’s instructions. For each target mRNA, 1 µL of cDNA was mixed with SYBR Green PCR Premix (TaKaRa, Dalian, China) and 0.8 µL of each specific forward/reverse primer in a final volume of 10 µL. The sequences of the primer sets are shown in Table 2 and synthesized by Generay Biotechnology (Shanghai, China). The PCR amplification was monitored in real time with the ABI 7900 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). Gene expression was quantified by the method described before. (Du et al. 2017a; Vinuesa et al. 2016)

### 2.6 Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Experimental data were analyzed by
one-way analysis of variance (ANOVA) using SPSS version 21.0. $P$-value < 0.05 was considered to be statistically significant.

3. Results

3.1 Effects of *S. p.* extract supplement on body weight and food intake

Average weekly weight gain for 8 weeks in the *S. p.* extract (LD, MD and HD) supplementation groups was significantly lower than that in the high-fat diet (HFD) group (1.28±0.15, 1.11±0.18, 1.00±0.10 vs 1.56±0.21 g/week). Among various *S. p.* extract groups of different doses, *S. p.* extract HD group particularly exhibited the more significant response of suppressing weight gain (Fig. 1a). Moreover, *S. p.* extract-LD supplement exhibited a similar effect on the weight gain as Max EPA in the end of the experiment. In addition, *S. p.* extract supplement did not affect food intake (Fig. 1b) in all high-fat diet groups. (Lee et al. 2013b)

3.2 Effects of *S. p.* extract supplement on serum lipid profiles

To determine the dyslipidemia-ameliorating effects of *S. p.* extract on HFD-fed mice, circulating lipid levels in serum, including TC, TG, HDL-c, and LDL-c, were measured (Fig. 2). HFD led to significantly higher TC and LDL-C levels ($P<0.05$) compared with the control group, whereas *S. p.* extract supplement resulted in a significant reduction in serum levels of TC and LDL-c compared with the HFD group. In contrast, serum TG or HDL-C levels showed no significant difference among the groups. Atheroclerosis index (AI) had been considered as an important indicator of vascular risks and significant predictors of atherosclerosis. Therefore, AI values of all groups were also determined. The AI values of the HFD group were significantly higher than that of the control group. Besides, the *S. p.* extract (LD, MD and HD) supplements effectively reduced AI values by 5.80%, 27.03% and 37.94%
3.3 Effects of *S. p.* extract supplement on hepatic and adipose tissue morphology

*S. p.* extract supplement reduced the hepatic lipid droplets and average epididymal adipocyte size compared with the HFD group. In mouse livers of the control group, normal lobular architecture and cellular structures were accompanied by a few fat droplets (Fig. 3a). In contrast, the livers of HFD mice exhibited severe steatosis, whereas *S. p.* extract supplementation groups exhibited a lower grade of steatosis and a different distribution of fat droplets. Moreover, the *S. p.* extract-HD supplementation groups had the much smaller average epididymal adipocyte size compared with the HFD group (Fig. 3b). (Norris et al. 2017)

3.4 Effects of *S. p.* extract supplement on the mRNA expressions of SREBP-1c, FAS, ACC, and CPT-1 in liver

To investigate the molecular mechanisms of *S. p.* extract supplement in the regulation of lipid metabolism in liver tissues, gene expressions of SREBP-1c, FAS, ACC, and CPT-1 were analyzed (Fig. 4). These genes play key roles in fatty acid synthesis and \( \beta \)-oxidation. (Li et al. 2016) As shown in Fig. 4, mRNA expressions of SREBP-1c, FAS, ACC and CPT-1 were up-regulated in the HFD group compared with the control group \( (P<0.05) \). In contrast, *S. p.* extract supplement exhibited significantly lower mRNA levels of SREBP-1c, FAS, and ACC than those in the HFD group \( (P<0.05) \) and the significantly higher expression levels of CPT-1 than that in HFD group. Interestingly, the regulating effects on the mRNA expression of certain genes of groups treated with *S. p.* extract-HD were comparable to that of Max EPA.

4. Discussion:
Dyslipidemia is a metabolic disorder, which is characterized by abnormally high levels of serum triglyceride (TG) and/or cholesterol (TC), increased low-density lipoprotein (LDL) cholesterol, and decreased high-density lipoprotein (HDL) cholesterol. (Jing et al. 2013; Norris et al. 2017)

*Sporidiobolus pararoseus* (*S. p.*) extract contains large amounts of unsaturated fatty acids as well as other nutrient substances, such as carotenoids, squalene and ergosterol. (Du et al. 2017a, b) Both unsaturated fatty acids and ergosterol had a favorable effect on the regulation of lipid metabolism, thus effectively preventing dyslipidemia and related diseases. (Gil-Ramírez et al. 2014; Siscovick et al. 2017)

In the present study, we investigated the effects of *S. p.* extract on obesity and dyslipidemia and analyzed the underlying mechanisms with high-fat diet-induced (HFD) obese mice. We found that *S. p.* extract significantly reduced body weight gain and plasma TC and LDL-c levels. Furthermore, *S. p.* extract regulated the mRNA expressions of lipid metabolism-related genes in the *S. p.* extract supplementation group compared with those in HFD-induced obese mice. These results suggest that *S. p.* extract possesses ameliorating effect on dyslipidemia in obese mice.

In our study, eight-week-old Kunming mice were fed with either basic forage or high-fat diet (HFD) for 12 weeks. There was no statistically significant difference in initial mouse weight among the experimental groups. The effects of the HFD on weight gain were observed after the model was successfully established. *S. p.* extract supplement significantly reduced final body weight compared with mice fed with HFD alone under the same food intake, indicating that *S. p.* extract had an attractive effect on the body weight control of HFD-induced obesity. (Choi et al. 2011)

Previous studies suggested that HFD-induced obesity changed lipid metabolism including metabolic parameters such as TC, TG, HDL-c and LDL-c. Increased levels of TC and LDL-c accompanied by low levels of HDL-c were major risk factors for atherosclerosis and cardiovascular diseases. (Kim et al. 2013)
Thus, the increased level of \((TC-HDL-c)/HDL-c\) was considered as major predictors of atherosclerosis. (Lim et al. 2015) In the study, \(S. p.\) extract supplement significantly improved the lipid profiles by lowering the levels of TC and LDL-c in MD and HD groups, morphological improvements in livers and epididymal white adipose tissues were also observed through histological analysis. These results indicated that \(S. p.\) extract had beneficial effects on ameliorating dyslipidemia through body weight control and lipid metabolism regulation.

To further understand the beneficial effects of \(S. p.\) extract on lipid metabolism in obese-related disorders, it is necessary to investigate the molecular mechanisms. The liver is a major fat metabolizing organ where saturated free fatty acids (FFAs) are synthesized, esterified, and packaged into low-density lipoprotein for export, thereby preventing the retention of fat. Excessive dietary fat intake can increase serum FFAs levels and enhance the delivery of FFAs to the liver. As a result, these excessive FFAs are commonly associated with metabolic syndromes, including obesity, dyslipidemia and atherosclerosis. (Lim et al. 2015; Park et al. 2015)

Therefore, we investigated the gene expressions related to lipid metabolism in liver. Sterol regulatory element-binding protein 1c (SREBP-1c) plays a great role in fatty acid synthesis and is highly expressed in adipose, liver and brain tissues of mice and humans. SREBP-1c enhances the expression levels of other lipogenic genes, including FAS, an enzyme which can stimulate the de novo synthesis of fatty acids and catalyze the synthesis of saturated FFAs. Moreover, FAS also plays an important role in regulating body weight and dietary intake. During dyslipidemia, when FFA synthesis is stimulated by some enzymes such as FAS and acetyl-CoA carboxylase (ACC, a regulator of fatty acid oxidation), the processes of mitochondrial and peroxisomal \(\beta\)-oxidation are suppressed. Under normal circumstances, peroxisomal \(\beta\)-oxidation is a minor pathway of fatty acid oxidation compared with the mitochondrial
β-oxidation. However, when an animal is under a lipid-overloaded state and metabolic lipids are
dysregulated, peroxisomal β-oxidation is stimulated. Carnitine palmitoyltransferase 1 (CPT-1) is a
rate-limiting enzyme in mitochondrial fatty acid oxidation and the increased expression of CPT-1 is
associated with an up-regulation of fatty acid β-oxidation. (Jing et al. 2013; Lee et al. 2013b; Wakutsu
et al. 2012) In the study, the gene expressions of SREBP-1c, FAS and ACC were found to be reduced in
liver tissues from S. p. extract supplementation group compared with their mRNA expression in HFD
only mice, while the expression level of CPT-1 was increased. In S. p. extract-HD group, SREBP-1c,
FAS and ACC gene expressions were respectively significantly reduced by 20.31%, 18.73%, and
30.26%, whereas CPT-1 expression in S. p. extract-HD group was enhanced by 149.44% compared
with that in the HFD group. These results implied that S. p. extract supplement ameliorated
dyslipidemia and obesity through FFA synthesis inhibition and fatty acid oxidation acceleration.

In conclusion, the data in the study demonstrated that S. p. extract supplement reduced body weight
gain and prevented the development of dyslipidemia through improving blood lipid profiles and
lowering lipid accumulation in the liver of mice with an HFD. Furthermore, these effects appeared to
be mediated through the inhibition of the mRNA expressions of SREBP-1c, FAS and ACC as well as
the activation of CPT-1 expression. Thus, these results provide valuable insights into the possible
application of S. p. extract as a dietary supplement for the prevention of obesity-related metabolic
disorders, and further studies are ongoing.

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Conflict of interest:

The authors declare that they have no competing interests.
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Figure Legends:

Figure 1 Supplementary effects of *S. p.* extract on (a) body weight and (b) food intake in mice fed with a high-fat diet. Data are expressed as mean ± SD (n = 10). After the dyslipidemia model was established, male Kunming mice were fed with or without various doses of *S. p.* extract (0.8 g/kg bw, 1.6 g/kg bw and 3.2 g/kg bw) by oral gavage once daily for eight weeks.

Figure 2 *S. p.* extract improved serum lipid profile and dyslipidemia in mice with high-fat diet-induced obesity. (a–d) Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) concentrations. (e) Effects of *S. p.* extract on atherosclerosis index (AI) in obese mice. Data are expressed as mean ± SD (n = 10). The values with different letters are significantly different (*P*<0.05). *S. p.* extract–LD (low dose of *S. p.* extract, 0.8 g/kg bw), *S. p.* extract –MD (middle dose of *S. p.* extract, 1.6 g/kg bw); *S. p.* extract–HD (high dose of *S. p.* extract, 3.2 g/kg bw).

Figure 3 Hematoxylin and eosin staining of (a) hepatic tissue and (b) adipose tissue in mice from different treatment groups. Original magnification, ×200.

Figure 4 mRNA expression of SREBP-1c, FAS, ACC and CPT-1 in the liver of mice from different treatment groups. Data are expressed as mean ± SD (n = 6). The values with different letters are significantly different (*P*<0.05). β-actin was used as the control. *S. p.* extract–LD (low dose of *S. p.* extract, 0.8 g/kg bw), *S. p.* extract –MD (middle dose of *S. p.* extract, 1.6 g/kg bw); *S. p.* extract–HD (high dose of *S. p.* extract, 3.2 g/kg bw).
Figure 1

254x93mm (300 x 300 DPI)
Figure 2

247x123mm (300 x 300 DPI)
Figure 3

236x229mm (300 x 300 DPI)
Figure

280x191mm (300 x 300 DPI)
### Table 1 Intragastric administration substances and doses in each group

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### Table 2 The sequence of primers used for quantitative real-time PCR

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<td>ACC</td>
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