



Manipulations of glucose/lipid metabolism and gut microbiota of resistant starch encapsulated *Ganoderma lucidum* spores in T2DM rats

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Abstract Our team previously demonstrated that *Ganoderma lucidum* spores (GLS) and resistant starch (RS) had hypoglycemic effects separately on type 2 diabetic mellitus (T2DM) rats. This work was to explore the effects of administering encapsulated GLS within RS (referred to as EGLS) in the T2DM rats, which were induced by streptozotocin (STZ). The EGLS was orally administered to rats for 28 days. The parameters of glycometabolism and lipometabolism were evaluated, and fecal microbiota

composition was investigated. The results showed that EGLS significantly enhanced glycometabolism and lipometabolism parameters in T2DM rats, which might be associate with the enhancement of the glucose and lipid metabolism, insulin secretion, and glycogen synthesis and reduced lipogenesis. Furthermore, the intervention of EGLS also reduced the *Proteobacteria* community and improved dysfunctional gut microbiota. This study indicated EGLS may be a potential candidate for dietary intervention to modulate diabetes.

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Introduction

Diabetes mellitus (DM), one of representative chronic metabolic diseases, showed fasting and postprandial hyperglycemia in the body. In accordance with the World Health Organization (2014), disease of diabetes is an ever-increasing global problem with a presence of 9% among adults, and type 2 diabetes mellitus (T2DM) represents 90% of global diabetes. With increasing incidence especially in Asia and Europe, current treatment strategies for T2DM to reduce hyperglycemia and insulin resistance include the use of synthetic based recombinant insulin and pharmacotherapy. However, these medicines may be associated with drug-resistance, hypoglycemia, weight gain dropsy and other side effects. Alternative or synergistic therapies may be beneficial. Traditional Chinese medicines have been widely used for the treatment of diabetes with a long history. Traditional remedies have been assessed in rat models of diabetes remedies, which are relatively cheaper and more effective with almost no side effects compared to

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synthetic agents (Pan et al., 2013). Such medicinal plants could also serve as a good source for drug design and herbal medicines.

Ganoderma lucidum (GL), commonly known as Lingzhi, was the most studied medicinal mushroom species all over the world. It has been used to cure various kind diseases in China, Korea, Japan and many other countries for many years. Intervention of GL exerted hypoglycemic and hypolipidemic effects in streptozotocin-induced diabetic rats (Bach et al., 2018). *Ganoderma lucidum* spores (GLS) are the mature germ cells of *Ganoderma lucidum* and have been associated with the control of blood glucose levels, decrease of plasma glucose concentrations and release of insulin in vivo (Pan et al., 2013). However, therapeutic-benefits mechanisms have not been fully elucidated. Complexing GLS with other bioactive ingredients is a novel approach to enhance the therapeutic benefit through synergistic action. Among them, resistant starch (RS) is a type of cereal fiber fraction. It cannot be digested in the small intestine but can be fermented by microflora in the colon (Jiang et al., 2019). The RS improved blood glucose parameters through direct and indirect mechanisms, such as delayed gastric emptying and improved gut microbiota. The RS seems to be the most beneficial source for maintaining normoglycemia (Marques et al., 2020). We have previously demonstrated that GLS and RS had hypoglycemic effects separately on diabetic rats (Wang et al., 2015). Encapsulation can increase the stability and also modify the release characteristics of essential contents. It was reported that starch based delivery of the bioactive compounds to the gut may prolong their release (Janaswamy, 2014). Thus, in this study, encapsulated GLS was prepared using RS as a coating material and the final product was termed as encapsulated *Ganoderma lucidum* spores (EGLS). The dietary change and nutritional intervention could modify the human microbiome (Wilson et al., 2020). In the last few decades, there were growing evidences that the gut microbiome played an important role in the development of insulin resistance, T2DM and obesity (Xu et al., 2017). In the upcoming future, one of the emerging strategies for the prevention and control of diabetes may modulate gut microbiota through precise dietary strategies (Conteh and Huang, 2020). To evaluate the efficacy of the combined application of GLS and RS on the regulation of glucose metabolisms and lipids metabolisms and gut microbiota in T2DM rats, we explored the influence of EGLS on blood glucose level, blood lipid compositions, the expression of gene relating with glucose and lipid metabolisms and gut microbiota in STZ induced T2DM rats.

Materials and methods

Materials

STZ was obtained from Sigma-Aldrich (St. Louis, MO, USA). The wall-broken spore powder of GL was obtained from Chongqing Biotechnology Institute, Chongqing, China. RS, from high amylose maize starch (Hi-maize™), was provided by the National Starch and Chemical Company, NSW, Australia.

Preparation of RS encapsulated GLS

10% of RS slurry was prepared using Reverse Osmosis (RO) water, followed by thermal treatment in an autoclave at 121 °C for 2 h. After cooling down to 50 °C, GLS powder was slowly added to the slurry with vigorous agitation. The ratio of the two substrates (RS/GLS) used in this study was 3/1 (w/w). After complete dispersion, the

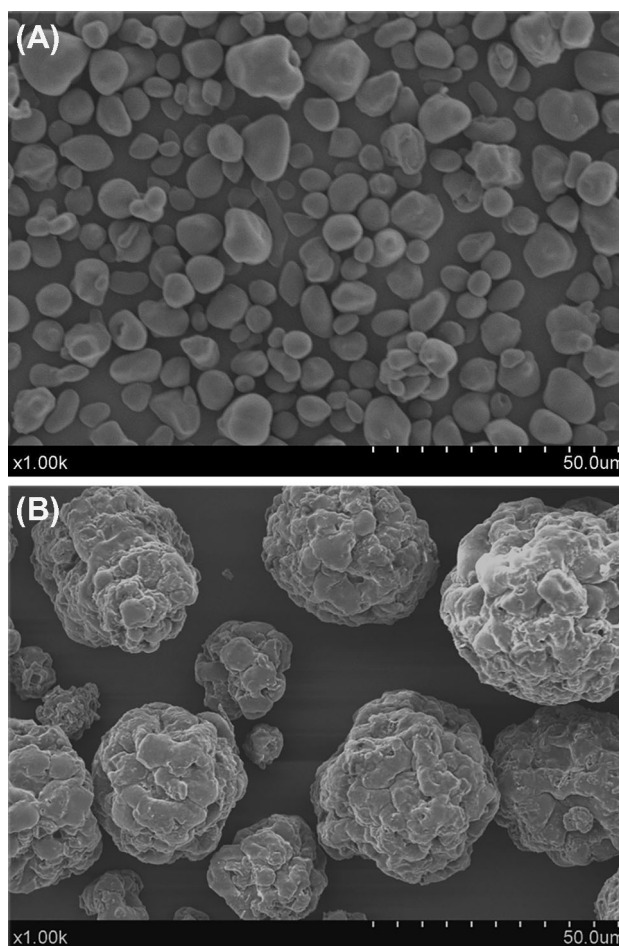


Fig. 1 Morphology of EGLS with scanning electron microscope. (A) Before encapsulation; (B) after encapsulation

mixture was then spray dried with 230 °C for air intake, 180 °C for air output. The final product (ECLS) was collected (Fig. 1) and used in the STZ-induced T2DM rats for intervention.

Animals and experimental design

Twenty-four healthy Sprague–Dawley rats (male, 190 ± 10 g weight) were provided by Chinese Military Medical Science Academy (Beijing, China). Rats were randomly divided into three different groups: NC: normal control, normal rats with basal diet; MC: model control, diabetic rats with basal diet and IG: Intervention group, diabetic rats with an intervention of ECLS (10.5 g/kg bw/day). The basal diet consisted of the AIN-76 diet (Si et al., 2017).

The SD rats were housed in plastic cages (4 rats/cage) in controlled conditions of light (12/12 h light/dark cycle), humidity ($55 \pm 5\%$) and temperature (at 25 °C) with free access to drinking water. After fed with the basal diet for one week, the rats were fasted for 12 h. STZ was dissolved in citrate buffer (0.1 mol/L, pH 4.0). The STZ (50 mg/kg b.w) was intravenous injected in rats except for the normal control group. Normal control rats were injected with the same volume of citrate buffer. After the injection, food was not provided to these animals until 2 h later. After 72 h STZ injection, blood was drawn from the rat tail vein, and fasting blood glucose (FBG) was detected with a glucometer (ACCU-CHEK, ROCHE, Basel, Switzerland). The FBG above 16.7 mmol/L in rats was considered as the diagnostic standard of diabetes and these diabetic rats were included in this experiment (Zhou et al., 2015). During the next four weeks, FBG and body mass was measured every week (Zhou et al., 2015). After 28 days, samples of feces, liver tissue and serum were collected and stored at -80 °C until further analysis. During the experiments, all rats survived. No casual or obvious sign of toxicity was observed (Si et al., 2017).

Biochemical analysis

Before sacrifice, rat blood samples were taken from the femoral artery. High-density lipoprotein-cholesterol (HDL-c) (Beijing North Kangtai clinical Reagent Co., China, F003-2), total cholesterol (TC) (Dong'ou diagnosis Products Co Ltd, F002-1), and triglyceride (TG) (Dong'ou diagnosis Products Co Ltd, Zhejiang, China, F001-1) concentrations were measured according to the instructions of their corresponding kits (Jiancheng Biological Engineering Institute, Nanjing, China), respectively.

Expression analysis of genes involved in glucose and lipid metabolism

After being immediately frozen in liquid nitrogen, rat liver was stored at -80 °C before RNA extraction. TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from liver tissues, and DNase I (Ambion, Austin, USA) was used to remove the contaminating genomic DNA. Total RNA was transcribed to cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) (Shang et al., 2017). Real-time quantitative PCR (RT-PCR) reactions were performed using a DyNAmo Flash SYBR Green Kit (Finnzymes, Espoo, Finland) on CFX96 RT-PCR Detection System (Bio-Rad, Rich-mond, CA, USA) (Shang et al., 2017). RT-PCR was performed under the following conditions: at 95 °C for 10 min for initial denaturation, 40 cycles at 95 °C for 30 s, 58 ~ 60 °C for 30 s, 72 °C for 30 s, and 72 °C for 10 min. To normalize target gene expression, the 18S rRNA gene was measured as an internal control. The relative transcript quantity was calculated according to the method of $2^{-\Delta\Delta CT}$ (Shang et al., 2017). Each reaction was carried out for three replicates. The primer sequences of the genes used for RT-PCR were shown in Table 1.

Determination of fecal microbial composition

After twenty-eight days of the feeding, the rat feces were collected within half an hour in fresh sterilized cages and frozen immediately in liquid nitrogen. Feces were stored at -80 °C until further experiments.

16S rDNA amplicon sequencing of gut microbiota and operational taxonomic unit (OTU) analysis were performed in Shanghai Majorbio Bio-Pharm Technology Co., Ltd. PCR primers flanking the V1–V3 hyper variable region of bacterial 16S rDNA were amplified. The barcoded fusion forward primer was 27F 5'-AGAGTTTGATCCTGGCT-CAG-3' and the reverse primer was 533R 5'-TTACCGCGGCTGCTGGCAC-3'. High quality sequence length was archived and uploaded to QIIME for further study. Sequences sharing $> 97\%$ V1–V3 gene sequence identity were defined as an OTU using UCLUST de novo clustering in Quantitative Insights into Microbial Ecology (QIIME). Shannon diversity indices and Chao 1 richness, describing the alpha diversity were determined by the OTUs. The Venn diagram showed the similarity and differences among the three groups. Unweighted clustering was performed using principal component analysis (PCA) to show beta diversity. Cluster analysis, venn diagram, heatmap representation and PCA were performed via R Project. Taxonomic classification (genus, family, order,

Table 1 Primer sequences for gene amplification

Gene		Primer sequence (5'-3')	Product size (bp)	Accession No
18S rRNA	Forward:	AAACGGCTACCACATCCAAG	159	NR_046237
	Reverse:	TTGCCCTCCAATGGATCCT		
ACC	Forward:	CAACCACTACGGCATGACTCA	155	NM_022193
	Reverse:	CGCAGAAGCAGCCCATTACTT		
FAS	Forward:	TGCTCCCAGCTGCAG	107	NM_017332
	Reverse:	GCCCGGTAGCTCTGGGTGTA		
Acox1	Forward:	CAAGGAGAGTGCTACGGGTTA	137	NM_017340
	Reverse:	TTCAGGTAGCCGTTATCCAT		
SREBP-1	Forward:	GCAAGGCCATCGACTACATC	161	XM_213329
	Reverse:	TTTCATGCCCTCCATAGACAC		
Insig 1	Forward:	TTGTCGGCTTATTGTATCCCT	147	NM_022392
	Reverse:	GCACATTATTGGCGAAATCT		
Insig 2	Forward:	GGCGGAAGGAGAGACGGAGTC	128	NM_178091
	Reverse:	AAGCCAGGAACACGCCAATGA		
Acly	Forward:	GCAGACCAGAAGGGCGTGAC	135	NM_016987
	Reverse:	CACACTGCCTGGGCGATACAG		
Fads1	Forward:	GTTTGTGTGGGTGACGCAGAT	117	NM_053445
	Reverse:	TTGAAGGCTGACTGGTGAACG		
Gpam	Forward:	CCT GTG GGC ATC TCG TAT GAT	122	NM_017274
	Reverse:	TTC CGC AGC ATT CTG ATA AC		
Dgat1	Forward:	CAGATGGGGCTGCTGCTACAT	175	NM_053437
	Reverse:	GGCGGCACCACAGATTGACAT		
GS2	Forward:	GACTGAGCAGGGCTTTTCC	90	NM_013089
	Reverse:	GAGGAGGGCCTGGGATACTT		
GYG1	Forward:	TCGCCAGCCCACAGGTT	90	NM_031043.2
	Reverse:	CACCACTGTCCAAGACATCTACCA		
PEPCK	Forward:	GAAAGTTGAATGTGTGGGTGAT	79	NM_198780
	Reverse:	TTCTGGGTTGATGGCCCTTA		
G6PCI	Forward:	GTATGGATTCCGGTGCTT	132	NM_013098
	Reverse:	AATGCCTGACAAGACTCCA		

class, and phylum) was performed using Mothur via the silva database.

Statistical analysis

Statistical analysis was performed by using SPSS 11.0 statistical software. One-way analysis of variance (chi-square test) was used for biochemical results. Pooled t-tests were used for bacterial abundance. $P < 0.05$ was considered significant.

Results and discussion

Effects of EGLS on body mass in diabetic rats

As a useful experimental animal model for studying the hypoglycemic activity, the STZ-induced diabetes in rats is characterized by a severe weight loss and some associated diabetic complications. The initial body mass among the three groups were similar. During four weeks experimental period, the body mass of diabetic rats (MC group) was significantly lower than that of NC group ($P < 0.05$). Unusual weight loss is the characteristic of diabetes and may be related to the fact that carbohydrates are not used as an energy source (Clark et al., 2007). Although there was no statistically significant difference in the two diabetic

groups from groups between MC and IG ($P > 0.05$), the body mass recovery was most evident for IG group.

Effects of EGLS on blood glucose levels

Hyperglycemia was observed after the injection of STZ. Glucose level was usually above 30 mmol/L within 72 h of STZ injection, which was the symptom of insulin-dependent diabetes. In the following four weeks, the blood glucose level of MC group was consistently higher than that of NC group ($P < 0.05$) (Table 2). During the four weeks, the blood glucose level of NC and MC rats did not change significantly ($P > 0.05$). The blood glucose level in the IG-treated animals had decreased (21.9% reduction) comparing with its initial level from the third week, and it also statistically significantly lower than the blood glucose level of MC group ($P < 0.05$). This was consistent with the findings of our previous work, in which the administration of either RS or GLS contributed to an anti-hyperglycemic effect (REF). Nevertheless, the intervention time needed to be extended for further research (Wang et al., 2015; Zhou et al., 2015).

Effect of EGLS on the expression of glucose metabolism genes

Results presented in Fig. 2A demonstrated the effects of EGLS on the expression of glucose-metabolism-related genes in diabetic rats. Hepatic glycogen synthesis and gluconeogenesis are the two main metabolic pathways associated with maintaining FBG levels. The storage of glucose as glycogen is controlled partly by the glycogen synthesis related enzymes-glycogenin 1 (*GYGI*) and glycogen synthase 2 (*GS*). In this study, *GS* expression in the diabetic control rats showed prominently to be reduced comparing with non-diabetic rats, while a slight reduction was also observed in *GYGI* expression (Fig. 2A). Glycogenin is a core protein in glycogen particles and *GYGI* is involved in the initial steps of glycogen synthesis (Laforêt et al., 2017). Hence, in this T2DM model, the suppressed of glycogen synthesis in the liver may be related with the sequential steps of glycogen synthesis, especially the decreased expression the *GS*. The decreased activity or

expression of *GS* in diabetes was previously reported (Mukundwa et al., 2015; Oyenihni et al., 2019). This study found that, after intervening with EGLS, the glycogen-synthesis-related genes, such as *GS2* and *GYGI* mRNA levels, were all significantly up-regulated compared to those of diabetic rats and normal rats ($P < 0.05$), indicating a progressive stimulation of glycogen synthesis during EGLS administration. In T2DM, the dysregulation of glycogen and glucose metabolism is crucial mechanisms. It was proposed the activation of *GS* activity was the therapeutic mechanisms of drugs for T2DM (Kaidanovich-Beilin and Eldar-Finkelman, 2006). Our result also suggested that the hypoglycemic activity of EGLS may be related with the inhibition of glycogen synthesis by reducing *GS2* and *GYGI* expression.

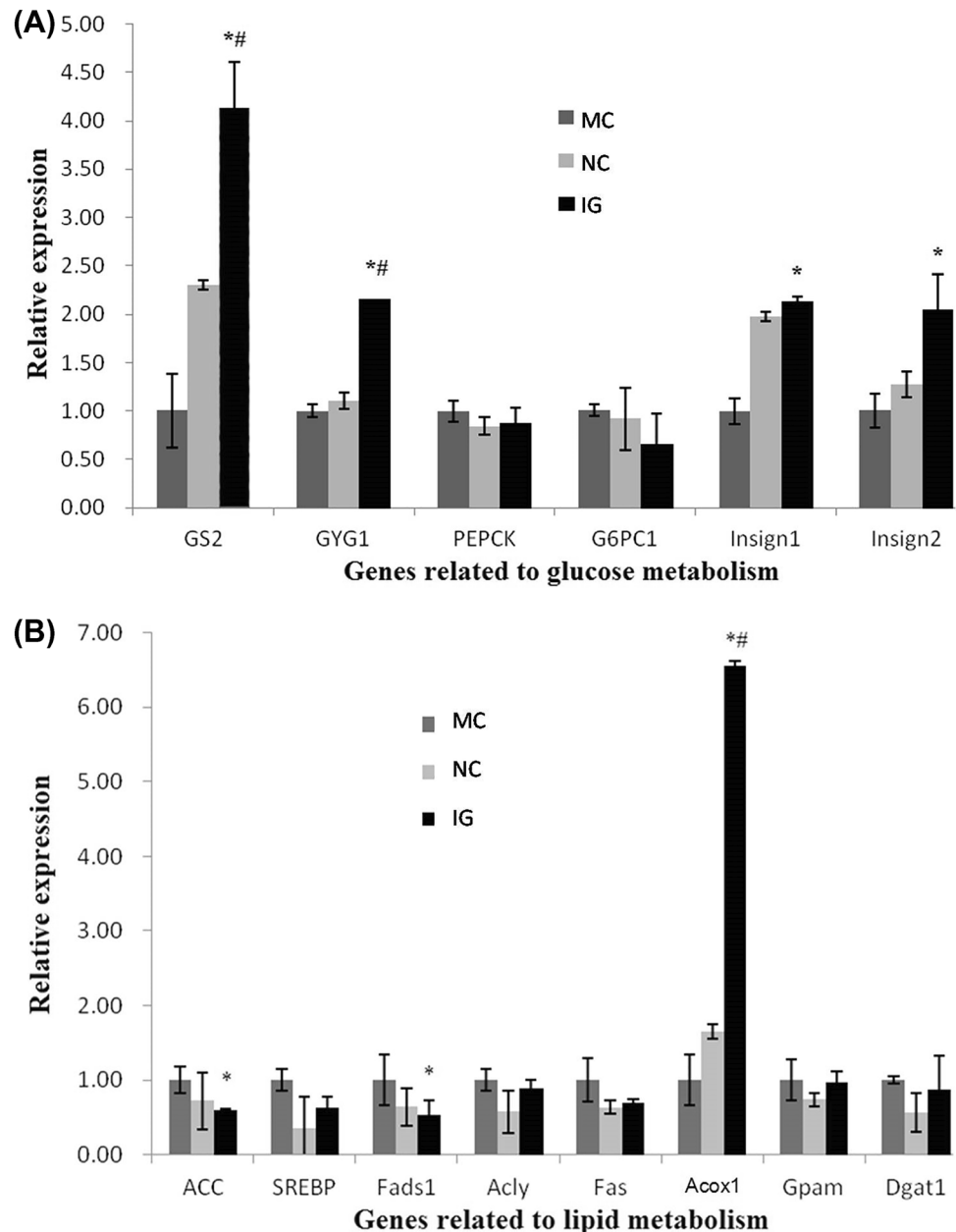
Isoform 1 of the catalytic subunit of glucose-6-phosphatase (*G6PC1*) and phosphoenolpyruvate carboxykinase (*PEPCK*) are the two key regulatory enzymes in gluconeogenesis. In this study, *G6PC1* mRNA levels were shown to be lower in the IG compared to the MC and NC groups, but there was no significant difference among the three groups. Similarly, previous researches had reported that the hepatic mRNA level of *G6PC* was also lower in GL or RS treated groups compared with a diabetic MC group (Sun et al., 2018; Xiao et al., 2012). However, no related report regarding the effect of GL and RS was found. *G6Pase* has catalytic effect on the ultimate reaction in hepatic glucose production, of which the inhibition is expected to reduce hepatic glucose production, irrespective of the contribution of gluconeogenesis or glycogenolysis (Zheng et al., 2012). Consequently, EGLS could also suppress the hepatic glucose output by reducing *G6PC* expression production in diabetic rats, which may be enhanced by a synergistic effect of RS and GLS. *PEPCK* catalyzes the rate-controlling step of gluconeogenesis, and is a key enzyme in maintaining blood glucose homeostasis (Hanson and Reshef, 1997). In this study, the *PEPCK* mRNA level was similar in the MC and NC groups (1.00 ± 0.11 and 0.84 ± 0.09). This result was in contrast to the previous reports that hepatic *PEPCK* activity of diabetic mice was prominently higher comparing with the normal mice (Gómez-Valadés et al., 2006; Pan et al., 2013). The reason may be related with the difference of

Table 2 Effect of EGLS administrations on blood glucose level in diabetic rats

Group	Blood glucose concentration (mmol/L)				
	72 h	1 W	2 W	3 W	4 W
NC	5.9 ± 0.6 ^a	5.8 ± 0.8 ^a	5.7 ± 0.3 ^a	5.8 ± 0.8 ^a	6.2 ± 0.5 ^a
MC	32.3 ± 1.0 ^b	30.0 ± 3.3 ^b	30.1 ± 1.6 ^b	31.0 ± 3.0 ^b	32.2 ± 1.7 ^b
IG	31.5 ± 2.2 ^b	30.1 ± 2.7 ^b	30.2 ± 4.1 ^b	27.2 ± 5.5 ^b	24.6 ± 2.8 ^c

Results were expressed as means ± SD ($n = 8$, one-way ANOVA). Different superscript lowercase letters on the table indicate significant difference ($P < 0.05$)

Fig. 2 Hepatic gene expression profiles for glucose metabolism and lipid metabolism 2A: Hepatic gene expression profiles for glucose metabolism. Hepatic gene expression profiles for glucose metabolism. 2B: Hepatic gene expression profiles for lipid metabolism. Hepatic gene expression profiles for lipid metabolism. Changes in gene expression were determined in triplicate by qPCR. Data are presented as mean \pm SD. * $P < 0.05$ vs. MC, # $P < 0.05$ vs. NC. NC: normal control; MC: model control; IG: interventional group utilizing EGLS



animal. The influence of *PEPCK* to the diabetic rats needs further research. In contrast, *PEPCK* mRNA level was slightly down-regulated in IG group comparing with the MC group, although the difference was not significant. This result was partly in accordance with other studies that the administration of GLS alone could significantly reduce the expression of *PEPCK* in diabetic mice (Pan et al., 2013; Xiao et al., 2012).

Insulin-induced gene (*Insig*) play an important role in glucose homeostasis. In this study, T2DM caused a reduction in *Insig1* and *Insig2* expression in the diabetic control rats, and the decline of *Insig1* was more obvious. The blood glucose level reduction of diabetic rats may be

related with the relatively low expression of *Insig1* and *Insig2*, which suggested an inhibition of insulin production in the rats. However, there was no significant difference in comparison to normal rats. After taking the EGLS, the mRNA expressions of insulin induced genes (*Insig1* and *Insig2*) in IG were significantly up-regulated by a twofold increase comparing with the MC group ($P < 0.05$). Pancreas can produce insulin and promote the glucose uptake and synthesis of liver glycogen and muscle glycogen responding to insulin-stimulation. Some dietary fibers have the hypoglycemic properties, which are mainly induced by improving insulin secretion and protecting the pancreatic islet cells (Kabir et al., 2014). Enhanced activity and

secretion of insulin is beneficial to alleviate diabetic symptoms and pancreatic repair (Sun et al., 2018). This study showed that EGLS treatment directly affected glucose influx by significantly increasing the expression levels of *Insig1* and *Insig2* in T2DM rats. EGLS may interfere in T2DM with insufficient insulin secretion.

In conclusion, the gene expression of *GS2*, *GYG1*, *G6PC1*, *PEPCK*, *Insig1* and *Insig2* suggested that the hypoglycemic effects of EGLS may be related with the regulation of these key glucose metabolism enzymes involved in both promoting insulin production (*Insig1* and *Insig2*) and glycogen synthesis (*GS2* and *GYG1*) at the mRNA expression level.

Effects of EGLS on HDL-c, TC and TG concentrations

Following four weeks treatment, the HDL-c, TC and TG concentrations in the blood were detected and results were shown in Table 3. Among all three experimental groups, diabetic rats had the highest value of TC and TG and the lowest HDL-c value ($P < 0.05$). It is well known that dyslipidemia is associated with uncontrolled diabetes mellitus. The blood levels of TC and TG increased, while the HDL-C levels declined, contributing to secondary complications of diabetes (Elberry et al., 2015). In this research, diabetic rats exhibited a significant elevation of TG and TC and decline of HDL-C. The HDL-c value in the IG increased to 2.79 mmol/L, close to the standard level of 2.99 mmol/L ($P > 0.05$). Some earlier studies have also reported that RS and GL treatment can significantly increase the levels of HDL-c (Wang et al., 2015; Zheng et al., 2012). Our results further confirmed and the improvements in blood lipid profile could be associated with the reduced blood glucose levels seen in diabetic rats (Teng et al., 2012). The EGLS supplementation resulted in the elevation of HDL-C levels and reduction of TC and TG levels. These effects may be caused by lowering the

activity of lipolysis level or cholesterol biosynthesis enzymes (Gong et al., 2009).

Effects of EGLS on expression of lipid metabolism related genes

The effects of EGLS on the gene expression in diabetic rats were investigated (Fig. 2B). Comparing to MC, feeding with EGLS resulted in a decreased expression of lipogenesis associated genes, including *acetyl-CoA carboxylase glycogen (ACC)*, *fatty acid synthases (FAS)*, *ATP citrate lyase (Acl)* and *fatty acid desaturase (Fads1)*. In particular, *ACC* and *Fads1* gene expression was significantly decreased (0.59 ± 0.02 , 0.53 ± 0.20 fold). We propose that EGLS may efficiently inhibit the expression of *FAS* and subsequently reduce lipogenesis.

Fatty acid degradation occurs primarily via the β -oxidation cycle in most organisms, and *Acox1* is the rate-limiting enzyme of lipid degradation. In this study, the expression level of *Acox1* mRNA in EGLS-treated rats was significantly higher than the MC group (6.56 ± 0.06 fold). The up-regulation of *Acox1* expression in the liver may suggest that EGLS could reduce TG levels through promoting lipid oxidation via activation of β -oxidation pathway. Up-regulation of *Acox1* is associated with increased β -oxidation in diabetes animal models and obesity animal models. Chan et al. (2013) have shown that high fructose fed mice treated with a PPAR γ agonist had reduced insulin resistance and steatosis associated with increased *Acox1* (Chan et al., 2013). Up-regulation of *Acox1* and increased beta-oxidation were also seen in a protein kinase SKT25 KO mouse with an improvement in diet induced diabetes (Amrutkar et al., 2015).

For triglyceride-synthesis-related genes *Gpm* and *Dgat1*, rats in the IG showed decreases in their mRNA levels, although this difference in the expression was not statistically significant. In terms of gene expression related to cholesterol homeostasis, changes in *SREBP-1* mRNA levels were minor. However, *Insig1* and *Insig2* gene expressions improved greatly in EGLS-treated rats comparing with the diabetic model controls, and *Insig1* and *Insig2* had a great influence on the regulation of intracellular cholesterol and fat metabolism (Goldstein et al., 2006).

In summary, our biochemical and gene expression results suggest that EGLS may exert an anti-hyperlipidemic effect through enhancing lipid oxidation, suppressing triglyceride synthesis and regulating cholesterol homeostasis.

Table 3 Effect of EGLS administrations on blood TC, TG and HDL-c levels in diabetic rats (mmol/L)

Group	TG	TC	HDL-c
NC	0.285 ± 0.0^a	2.96 ± 1.2^a	2.99 ± 0.2^b
MC	2.915 ± 1.2^b	5.57 ± 1.9^a	1.32 ± 0.2^a
IG	0.644 ± 1.7^a	3.96 ± 0.3^a	2.79 ± 0.1^b

Results were expressed as means \pm SD (n = 8, one-way ANOVA). Different superscript lowercase letters on the table indicate significant difference ($P < 0.05$)

TG triglyceride, TC total cholesterol, HDL-C high density lipoprotein cholesterol

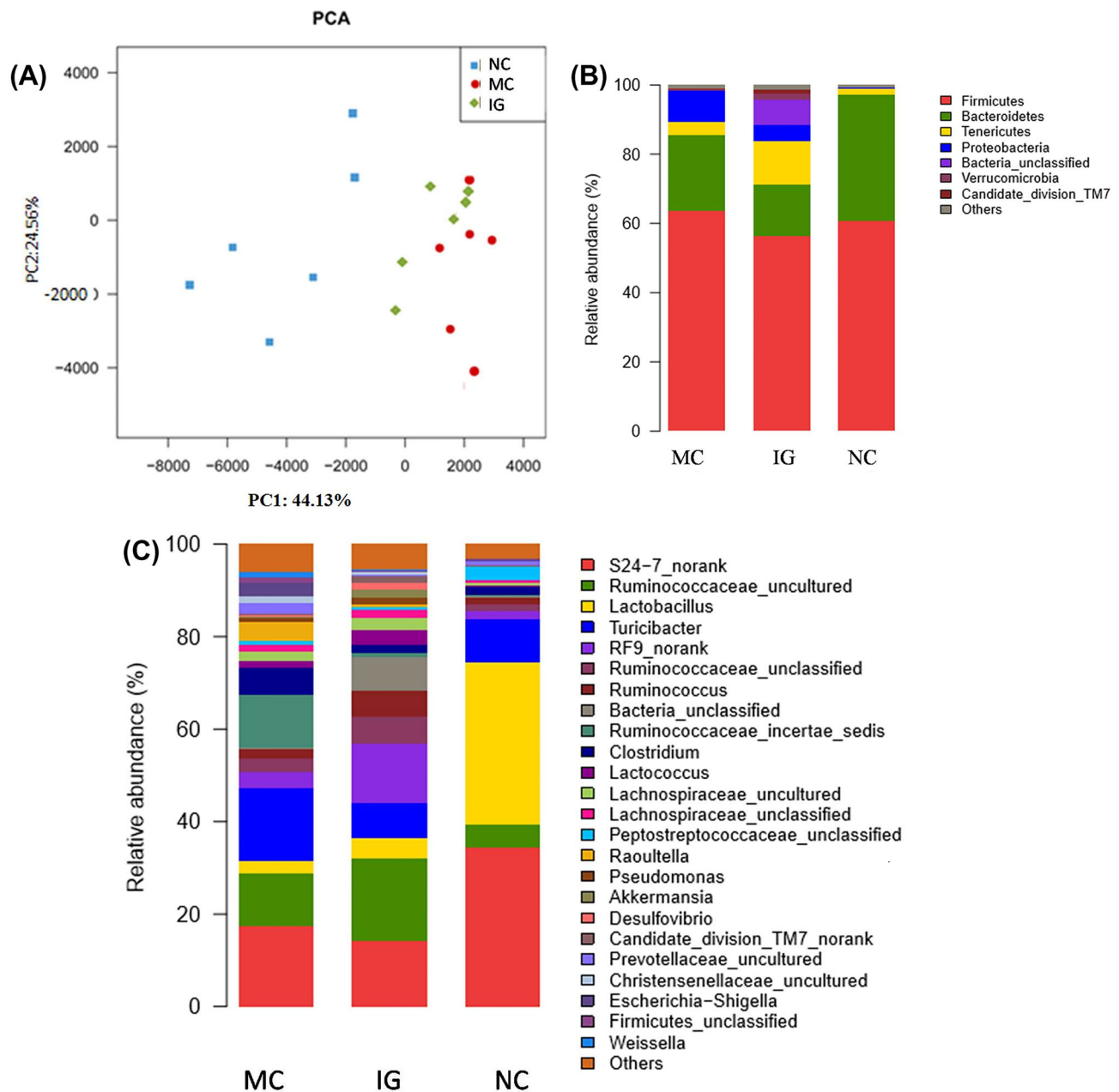


Fig. 3 Compositions analysis of the microbial communities. 3A: Principal component analysis (PCA) of samples based on the compositions of the microbial communities. 3B: Relative abundance of sequences belonging to different bacterial phyla. 3C: Relative

abundance of sequences belonging to different bacterial genus. NC: normal control; MC: model control; IG: interventional group utilizing EGLS

Effects of EGLS on gut microbiota composition

In total, 297,913 high-quality sequences were obtained through MiSeq sequencing analysis (Zhou et al., 2016). 6418 OTUs were identified per sample. All sequences were assigned to 14 different phyla and 142 different genus. The data was available through the NCBI/EBI/DBJ Short Read Archive (accession number ERA043547; <http://www.ncbi.nlm.nih.gov/ena/data/view/ERP000842>) (Shi et al., 2012).

The abundance of intestinal community was measured with the Chao index. The diversity of intestinal community was measured with the Shannon index. PCA showed a visual cluster of intestinal flora composition in different group (Fig. 3A).

The PCA analysis of gut community indicated that there was an obvious difference between normal rats and diabetic rats. Detailed analysis showed that both community abundance and diversity of diabetic model control group

were the highest, which was in accordance with the previous works. The quantity, composition and diversity of the intestinal flora changed significantly in diabetic rats (Larsen et al., 2010; Wirth et al., 2014). Following the administrations of EGLS, both community abundance and diversity decreased comparing with model control group, indicating that the EGLS intervention can effectively perturb the microflora diversity and abundance in diabetic rats, and this might contribute to an improvement of intestinal microflora.

Firmicutes and *Bacteroidetes* were dominant bacterial phyla in the three groups (Zhou et al., 2016). The abundance of *Proteobacteria* (the most identified as pathogenic bacteria) in diabetic rats was the highest (Fig. 3B) (Larsen et al., 2010; Wirth et al., 2014). The increased levels of *Proteobacteria* may suggest an inflammatory reaction in the gut of MC group, which may be involved in the onset of T2DM. The enrichment of *Proteobacteria* in the gut might be applied to predict T2DM (Karlsson et al., 2013). However, the ratio of *Proteobacteria* in EGLS treated rats was significantly reduced when compared with MC group (4.78% vs. 9.03%). Both RS and GL have shown previously to promote anti-inflammatory activity and the inflammation in the host can disrupt the intestinal microflora (Claudia et al., 2007; Haenen et al., 2013; Yan et al., 2014). It could be postulated that the reduction in blood glucose and improvements in cholesterol metabolism in the EGLS treated diabetic rats may be related with changes in faecal microbial community which promoted anti-inflammatory response.

The abundance of *Lactobacillus* was predominant in the normal rats (35.01%) at the genus level, and the data showed a sharp drop to 2.69% in MC group, which was also lower in the IG group (4.49%) (Fig. 3C). *Lactobacillus* is the most extensively studied probiotic strain, and has been shown to exert an anti-hyperglycemic effect in several rodent models (Park et al., 2015; Zhou et al., 2016). Park et al. (2015) also systematically demonstrated beneficial effects of *Lactobacillus* in the treatment of diabetes. The intervention of EGLS may increase the proportion of *Lactobacillus*, which may then contribute to an anti-hyperglycemic effect. The enrichment of *Lactobacillus* may be due to the GL component feeding (Xu et al., 2017).

Conclusion

This study suggests that the administration of EGLS may exert a synergistic influence on modulating hypoglycemic and hypolipidemic activities in the liver of diabetic rats by promoting gene expression which is associated with of glycogen synthesis (*GS2* and *GYG1*), glucose homeostasis and cholesterol homeostasis (*Insig1*, *Insig2*), lipid

oxidation (*Acox1*), and lipogenesis suppression (*ACC*, *Fads1*). The gut microbiota of diabetic rats was significantly different from that of normal rats. The gut microbiota in T2DM rats contributed to inflammation. EGLS treatment had a positive effect on the inflammation by reducing the abundance of *Proteobacteria*. The synergism of the RS and GLS could exert a positive effect on human metabolism. Thus, EGLS is a potential candidate for the prevention and therapy of T2DM.

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Declarations

Conflict of interest All authors declare no conflict of interest.

Ethics statement All national guidelines of laboratory animals were followed. The animals were approved by the Animal Care and Use Committee.

References

- Amrutkar M, Cansby E, Chursa U, Nuñez-Durán E, Chancón B, Ståhlman M, Fridén V, Mannerås-Holm L, Wickman A, Smith U. Genetic disruption of protein kinase STK25 ameliorates metabolic defects in a diet-induced type 2 diabetes model. *Diabetes* 64: 2791-2804 (2015)
- Bach EM, Martins, Cristina AM, Nascimento, Paloma AM, Wadt, Yamashita NS. Hypoglycemic and Hypolipidemic Effects of *Ganoderma lucidum* in Streptozotocin-Induced Diabetic Rats. *Medicines* (2018)
- Chan S, Sun R, Zeng X, Choong Z, Wang H, Watt M, Ye J. Activation of PPAR α ameliorates hepatic insulin resistance and steatosis in high fructose-fed mice despite increased ER stress. *Diabetes* 62: 1-11 (2013)
- Clark NG, Fox KM, Grandy S. Symptoms of diabetes and their association with the risk and presence of diabetes: findings from the Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD). *Diabetes Care* 30: 2868-2873 (2007)
- Claudia, Lupp, and, Marilyn, L., Robertson, and, Mark, E., Wickham. Host-Mediated Inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host and Microbe* (2007)
- Conteh A, Huang R. Targeting the gut microbiota by Asian and Western dietary constituents: a new avenue for diabetes. *Toxicology research* 9: 569-577 (2020)
- Elberry AA, Harraz FM, Ghareib SA, Gabr SA, Nagy AA, Abdelsattar E. Methanolic extract of *Marrubium vulgare* ameliorates hyperglycemia and dyslipidemia in streptozotocin-induced diabetic rats. *International Journal of Diabetes Mellitus* 3: 37-44 (2015)
- Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell* 124: 35-46 (2006)
- Gómez-Valadés AG, Vidal-Alabró A, Molas M, Boada J, Bermúdez J, Bartrons R, Perales JC. Overcoming Diabetes-Induced

- Hyperglycemia through Inhibition of Hepatic Phosphoenolpyruvate Carboxykinase (GTP) with RNAi. *Molecular Therapy* 13: 401-410 (2006)
- Gong F, Li F, Zhang L, Li J, Zhang Z, Wang G. Hypoglycemic effects of crude polysaccharide from purslane. *International journal of molecular sciences* 10: 880-888 (2009)
- Haenen D, Zhang J, Souza dS, C., Bosch G, Van dM, I. M., Van Arkel J, van den Borne JJG, Perez Gutierrez O, Smidt H, Kemp B. A Diet High in Resistant Starch Modulates Microbiota Composition, SCFA Concentrations, and Gene Expression in Pig Intestine. *Journal of Nutrition* 143: 274-283 (2013)
- Hanson RW, Reshef L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annual review of biochemistry* 66: 581-611 (1997)
- Janaswamy S. Encapsulation altered starch digestion: Toward developing starch-based delivery systems. *Carbohydrate polymers* 101: 600-605 (2014)
- Jiang F, Du C, Jiang W, Wang L, Du SK. The preparation, formation, fermentability, and applications of resistant starch. *International Journal of Biological Macromolecules* 150 (2019)
- Kabir AU, Samad MB, D'Costa NM, Akhter F, Ahmed A, Hannan JMA. Anti-hyperglycemic activity of *Centella asiatica* is partly mediated by carbohydrase inhibition and glucose-fiber binding. *BMC complementary and alternative medicine* 14: 31 (2014)
- Kaidanovich-Beilin O, Eldar-Finkelman H. Long-term treatment with novel glycogen synthase kinase-3 inhibitor improves glucose homeostasis in *ob/ob* mice: molecular characterization in liver and muscle. *Journal of Pharmacology and Experimental Therapeutics* 316: 17-24 (2006)
- Karlsson F, Tremaroli V, Nielsen J, Bäckhed F. Assessing the Human Gut Microbiota in Metabolic Diseases. *Diabetes* 62: 3341-3349 (2013)
- Laforêt P, Malfatti E, Vissing J. Update on new muscle glycogenesis. *Current opinion in neurology* 30: 449-456 (2017)
- Larsen N, Vogensen FK, Den Berg FV, Nielsen DS, Andreasen AS, Pedersen BK, Alsoud WA, Sorensen SJ, Hansen LH, Jakobsen M. Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. *PLOS ONE* 5 (2010)
- Marques AM, Linhares BS, Novaes RD, Freitas MB, Goncalves RV. Effects of the amount and type of carbohydrates used in type 2 diabetes diets in animal models: A systematic review. *PLoS ONE* 15: e0233364 (2020)
- Mukundwa A, Mukaratirwa S, Masola B. Effects of oleanolic acid on the insulin signaling pathway in skeletal muscle of streptozotocin-induced diabetic male Sprague-Dawley rats. *Journal of Diabetes* (2015)
- Oyenihi AB, Langa SOP, Mukaratirwa S, Masola B. Effects of *Centella asiatica* on skeletal muscle structure and key enzymes of glucose and glycogen metabolism in type 2 diabetic rats. *Biomedicine & Pharmacotherapy* 112: 108715 (2019)
- Pan D, Zhang D, Wu J, Chen C, Xu Z, Yang H, Zhou P. Antidiabetic, antihyperlipidemic and antioxidant activities of a novel proteoglycan from *Ganoderma lucidum* fruiting bodies on *db/db* mice and the possible mechanism. *PloS ONE* 8: e68332 (2013)
- Park K-Y, Kim B, Hyun C-K. *Lactobacillus rhamnosus* GG improves glucose tolerance through alleviating ER stress and suppressing macrophage activation in *db/db* mice. *Journal of clinical biochemistry and nutrition* 56: 240-246 (2015)
- Shang W, Si X, Zhou Z, Wang J, Strappe P, Blanchard C. Studies on the unique properties of resistant starch and chito-oligosaccharide complexes for reducing high-fat diet-induced obesity and dyslipidemia in rats. *Journal of Functional Foods* 38: 20-27 (2017)
- Shi Z, Wang L, Zhang H. Low Diversity Bacterial Community and the Trapping Activity of Metabolites from Cultivable Bacteria Species in the Female Reproductive System of the Oriental Fruit Fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae). *International Journal of Molecular Sciences* 13: 6266-6278 (2012)
- Si X, Zhou Z, Strappe P, Blanchard C. A comparison of RS4-type resistant starch to RS2-type resistant starch in suppressing oxidative stress in high-fat-diet-induced obese rats. *Food & Function* 8: 232-240 (2017)
- Sun H, Ma X, Zhang S, Zhao D, Liu X. Resistant starch produces antidiabetic effects by enhancing glucose metabolism and ameliorating pancreatic dysfunction in type 2 diabetic rats. *International journal of biological macromolecules* 110: 276-284 (2018)
- Teng BS, Wang CD, Zhang D, Wu JS, Pan D, Pan LF, Yang HJ, Zhou P. Hypoglycemic effect and mechanism of a proteoglycan from *Ganoderma lucidum* on streptozotocin-induced type 2 diabetic rats. *European Review for Medical and Pharmacological Sciences* 16: 166 (2012)
- Wang F, Zhou Z, Ren X, Wang Y, Yang R, Luo J, Strappe P. Effect of *Ganoderma lucidum* spores intervention on glucose and lipid metabolism gene expression profiles in type 2 diabetic rats. *Lipids in health and disease* 14: 49 (2015)
- Wilson AS, Koller KR, Ramaboli MC, Nesengani LT, O'Keefe SJD. Diet and the human gut microbiome: an international review. *Digestive Diseases and Sciences* 65 (2020)
- Wirth R, Bódi N, Maróti G, Bagyánszki M, Talapka P, Fekete É, Bagi Z, Kovács KL. Regionally distinct alterations in the composition of the gut microbiota in rats with streptozotocin-induced diabetes. *Plos One* 9 (2014)
- Xiao C, Wu Q-P, Cai W, Tan J-B, Yang X-B, Zhang J-M. Hypoglycemic effects of *Ganoderma lucidum* polysaccharides in type 2 diabetic mice. *Archives of pharmacal research* 35: 1793-1801 (2012)
- Xu S, Dou Y, Ye B, Wu Q, Wang Y, Hu M, Ma F, Rong X, Guo J. *Ganoderma lucidum* polysaccharides improve insulin sensitivity by regulating inflammatory cytokines and gut microbiota composition in mice. *Journal of functional foods* 38: 545-552 (2017)
- Yan G, Shuiling Z, Zhenming L, Hongyu X, Jin-Song S. Anti-inflammatory activity of mycelial extracts from medicinal mushrooms. *International Journal of Medicinal Mushrooms* (2014)
- Zheng J, Yang B, Yu Y, Chen Q, Huang T, Li D. *Ganoderma lucidum* polysaccharides exert anti-hyperglycemic effect on streptozotocin-induced diabetic rats through affecting β -cells. *Combinatorial chemistry & high throughput screening* 15: 542-550 (2012)
- Zhou Z, Wang F, Ren X, Wang Y, Blanchard C. Resistant starch manipulated hyperglycemia/hyperlipidemia and related genes expression in diabetic rats. *International Journal of Biological Macromolecules* 75: 316-321 (2015)
- Zhou Z, Ren X, Jiang Y, Zhang Q, Zhang M, Strappe P, Blanchard C. Responses of fecal bacterial communities to resistant starch intervention in diabetic rats. *Starch - Stärke* 68: 1008-1015 (2016)

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