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### Effect of dietary supplementation of *Bacillus subtilis* B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice<sup>\*#</sup>

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Abstract: While a high-fat diet (HFD) is assumed to be related to fat-mediated oxidative stress decreasing antioxidant enzyme activity, probiotics are believed to have positive effects on the regulation of HFD-induced obesity as well as lipid metabolism, energy homeostasis, and anti-oxidation. Because Bacillus subtilis B10 has beneficial effects on the abnormal lipid metabolism and the oxidative stress in HFD-induced obese mice, ICR mice were randomly assigned into an HFD group and the HFD was supplemented with 0.1% (w/w) Bacillus subtilis B10 (HFD+B10 group). Thereafter, 30-d treatments were run, and then hepatic lipid level and antioxidant status were measured. The expression of genes related to lipid metabolism and oxidative stress in the liver was determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). We found that HFD-induced obese mice treated with B10 showed a decrease in weight gain, serum glucose activity as well as hepatic triglyceride (TG), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) activities. In addition, the gene expressions of antioxidant genes, glutathione reductase (GR), xanthine oxidase (XO), heat-shock protein 90 (Hsp90), and lipid synthesis gene 3 -hydroxysteroid- 24 reductase (DHCR24) in the HFD+B10 group were down-regulated, suggesting alleviation of oxidative stress, while the lipolysis gene 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), energy metabolism gene peroxisome proliferator-activated receptor (PPARa) and the gene encoding tumor-suppressor protein p53 were up-regulated. The regulatory and positive effect of dietary supplementation of probiotic B10 suggests that it has a beneficial effect on the homeostasis of the lipid metabolism and on alleviating oxidative stress in HFD-induced obese mice.

Key words:Bacillus subtilis, High-fat diet, Oxidative stress, Lipid metabolismdoi:10.1631/jzus.B1400342Document code: ACLC number: \$816.3

### 1 Introduction

Obesity has been recognized as a major underlying factor in the pathogenesis of several diseases, including metabolic syndrome, insulin resistance, type 2 diabetes, hypertension, dyslipidemia, atherosclerosis, and several forms of cancer. It has become a serious public health problem both in industrialized countries and developing countries (Matsuda and Shimomura, 2013). As well as congenital genetics, obesity is also caused by acquired environmental

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factors (Tsai et al., 2014), especially high fat intake (Rizvi et al., 2003). Many reports have demonstrated that obesity is linked to an obvious oxidative stress (Furukawa et al., 2004; Matsuzawa-Nagata et al., 2008). Charradi et al. (2013) found that obesity is strongly related to systemic oxidative stress and was in particular concerned with the relationship between altered glutathione homeostasis and organ lipotoxicity associated with diet-induced obesity. Moreover, antioxidant supplementation showed beneficial effects in preventing dyslipidemia disease caused by a high-fat diet (HFD) (Gorinstein et al., 2006). Oxidative stress is an imbalance between oxidants and antioxidants (Sies, 1997). It can result in DNA hydroxylation, protein denaturation, lipid per-oxidation, apoptosis, tissue damage (Valko et al., 2006), and a disruption in the redox signaling pathway (Kamata and Hirata, 1999).

Several strategies have been used to prevent obesity, including diet control, exercise, behavior therapy and medication. Probiotics, which are defined as "live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host" (Araya et al., 2002), could modulate the intestinal flora and have some anti-oxidative and lipid-lowering abilities (Endo et al., 2013; Everard et al., 2014). Bacillus subtilis is an intestinal microorganism that may grow in the gut and consume oxygen to maintain an anaerobic environment for the prevention or therapy of gastrointestinal disorders (Hu et al., 2014). In addition, Bacillus subtilis is preferred due to its higher resistance to harsh environments and capacity for long-term storage at ambient temperature (Hong et al., 2005). Previously, we have reported that Bacillus subtilis B10 possessed positive effects on the anti-oxidative defense of cells in vitro (Li et al., 2013) and those findings lead us to further investigate the regulatory effect of Bacillus subtilis B10 on the antioxidative and lipid-lowering capacities of HFDinduced obese mice.

### 2 Materials and methods

### 2.1 Bacterial strain

Bacillus subtilis B10 powder (10<sup>8</sup> colony-forming unit (CFU)/g) was prepared by Microbiology and

Genetic Engineering Laboratory, Institute of Feed Sciences, Zhejiang University, China. B10 strain was cultured on Luria-Bertani media, kept at 37 °C for 24 h and shaken at 180 r/min. Pure bacterial cells were collected after centrifugation at 5000g for 10 min at 4 °C and cooling. Thereafter, these cells were washed twice with sterile 0.85% (8.5 g/L) sodium chloride solution. Ultimately, the culture purity and identification were constantly checked by the spreading plate method (Nikoskelainen **et al.**, 2003).

### 2.2 Animals and diets

Thirty male ICR mice (7-8 weeks old, n=15 per group) were obtained from the Institutional Animal Care and Use Committee of Zhejiang University, China. All mice were housed in standard plastic cages (three mice per cage) and maintained under a 12-h light-dark cycle at constant temperature and humidity  $((23\pm1)$  °C and  $(55\pm5)$ %, respectively). One group of mice was fed with an HFD (92.8% normal diet, 2% cholesterol, 5% lard, and 0.2% cholate) and the other was kept on an HFD supplemented with 0.1% (w/w) B10 powder for 30 d. The normal diet was provided by Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and its nutrient components level is listed in a supplemental material Table S1. During the preparation of the B10 powder, starch was used to dilute B10 and the same amount of starch was also added to the HFD group to compensate for the difference in nutrient composition of the diets. All the mice were fed ad libitum and food intake was recorded. The experiment was approved by and performed in accordance with the guidelines of the local ethics committee.

### 2.3 Sample collection

After 30 d, mice (n=6) from each group (HFD and B10) fasted for 12 h, and then were anesthetized with diethyl ether. Mice were killed by cervical dislocation and the blood samples were taken from the inferior vena cava. After incubation at 4 °C for 30 min and centrifugation at 2000g for 20 min, the serum was obtained (Centrifuge 5804R, Eppendorf, Germany). Liver samples were dissected out and placed in liquid nitrogen. Samples were finally frozen and kept at -80 °C for no more than 2 months until further analysis.

### 2.4 Biochemical assays of serum

The contents of glucose, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) in serum were determined by spectrophotometry according to the kit manufacturer's instructions. The methods of those kits are presented in the supplemental material Data S1. The materials of glucose measurement were purchased from Rongsheng Bio-pharmaceutical Co., Ltd., Shanghai, China. Kits for serum TG and TC were purchased from Saike Bio-technology Co., Ltd., Ningbo, China. Kits for serum BUN, GOT, and GPT were bought from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. Materials for serum LDL-C and HDL-C were purchased from Beihuakangtai Clinical Reagent Co., Beijing, China.

### 2.5 Biochemical assays of the liver

Liver samples were homogenized with ice-cold physiologic saline (1:10, v/v) and centrifuged at 2000g for 10 min (Centrifuge 5804R, Eppendorf, Germany). Supernatant was collected for determination of the total anti-oxidant capability (T-AOC), anti-superoxide anion activity (ASOA), concentrations of TC, TG, LDL-C, HDL-C, 8-hydroxy-2'-deoxyguanosine (8-OHdG), glutathione (GSH), and thioredoxin reductase (TrxR), and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and xanthine oxidase (XO). Kits for hepatic TC and TG were obtained from Saike Biological Technology Co., Ltd., Ningbo, China. Kits for hepatic LDL-C and HDL-C were purchased from Beihuakangtai Clinical Reagent Co., Beijing, China. Kits for SOD, CAT, GSH-Px, XO, T-AOC, ASOA, and GSH were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. All the above parameters were determined by spectrophotometry according to the manufacturers' instructions. Enzyme-linked immunosorbent assay (ELISA) kits for 8-OHdG and TrxR were purchased from Bioleaf Biological Co., Ltd., Shanghai, China.

## 2.6 RNA extraction and quantitative real-time polymerase chain reaction (PCR)

Liver tissues of approximately 100 mg were used for RNA extraction. RNA was extracted using the RNAiso plus method (TaKaRa, Dalian, China) according to the instructions of the manufacturer. Quantitative and qualitative analyses of isolated RNA were assessed from the ratio of absorbance at 260 and 280 nm by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and agarose gel electrophoresis (Sangon Biotech, Shanghai, China). Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using M-MLV reverse transcriptase (TaKaRa, Dalian, China). Transcriptional changes were then identified by quantitative PCR (qPCR), which was performed using the Premix Ex Taq<sup>TM</sup> with SYBR Green (TaKaRa, Dalian, China) as per the manufacturer's instructions, and the ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). The thermocycle protocol lasted for 30 s

Table 1 Primers used for real-time PCR

Gene	Primer sequence
GAPDH	F: 5'-GTTGTCTCCTGCGACTTCA-3'
	R: 5'-GCCCCTCCTGTTATTATGG-3'
$\beta$ -actin	F: 5'-CCTGTACGCCAACACAGTGC-3'
	R: 5'-ATACTCCTGCTTGCTGATCC-3'
Hsp90	F: 5'-CAAGAGGTTGATAGAGCGTTT-3'
	R: 5'-TTGTTCTCGGGACTTTGGA-3'
CATI	F: 5'-GGCATCCTAACCTCCAACCA-3'
	R: 5'-TGCTCATACACCATCCCACA-3'
PPARα	F: 5'-CTTTACATCAGTGTTTCCGTCAG-3'
	R: 5'-TCAAATTGCCACCGTTCTT-3'
PPARβ	F: 5'-GGTGACCCTCCTCAAGTATGG-3'
	R: 5'-GGTGTCCTGGATGGCTTCTA-3'
PPARγ	F: 5'-AGAACCTGCATCTCCACCTTA-3'
	R: 5'-CCACAGACTCGGCACTCAAT-3'
DHCR24	F: 5'-TACAAGAAGACCCATAAGAACATC-3'
	R: 5'-CCACAGACTCGGCACTCAAT-3'
HMGCS2	F: 5'-GGACCGTGCTCCCTTCTTAG-3'
	R: 5'-GAGCTCTTCGTGGGTTCTGT-3'
TRX2	F: 5'-GGAAACCAGAAGCCGAACA-3'
	R: 5'-GACAAGGAATAGAAGGGACAGAT-3'
GR	F: 5'-GTAGGAAGCCCACCACGAC-3'
	βR T459(F:5):per X2 1(oxiso(C9-C)m52.8(e)G5-C

X2 1

at 95 °C, followed by 40 cycles of 5 s denaturation at 95 °C, 34 s annealing/extension at 60 °C, and then a final melting curve analysis to monitor purity of the PCR product. Primer sequences for the mouse genes were designed and selected by Primer 5.0 and Oligo 7.0 softwares and the sequences are presented in Table 1. The  $2^{-\Delta\Delta C_q}$  method was used to estimate mRNA abundance.  $\Delta C_q$  is  $C_{q, \text{ target}}$ – $C_{q, \text{ reference}}$  and  $\Delta\Delta C_q$  is  $\Delta C_{q, \text{ treatment}}$ – $\Delta C_{q, \text{ control}}$ . Relative gene expression levels were normalized to those of the eukaryotic reference genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and  $\beta$ -actin.

### 2.7 Statistical analysis

Data are expressed as mean±standard deviation (SD) and were analyzed using the one-way analysis of variance (ANOVA) procedure of SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows by the Tukey test and the variable used is the administration of B10. Differences were considered statistically significant at P<0.05.

### 3 Results

## 3.1 Effects of dietary supplementation of *Bacillus subtilis* B10 on body weight and food intake of mice

While the final body weight and average daily weight gain for HFD+B10 group significantly (P<0.05) decreased compared with the HFD group,

no significant differences in daily food intake were observed between the two groups (Table 2).

# **3.2** Effects of dietary supplementation of *Bacillus subtilis* **B10** on lipid profile in the serum and liver of mice

Hepatic TC and TG of HFD-fed mice were effectively (P<0.05) decreased in B10-treated mice. In addition, the TC, LDL-C, and HDL-C levels in serum of the B10 treatment group were significantly (P<0.05) increased (Table 3). Besides, B10 supplementation also exhibited a significant decrease in the serum glucose concentration and GOT and GPT activities compared with the HFD group, while serum BUN was unaffected between the two groups (Table 4).

# **3.3** Effects of dietary supplementation of *Bacillus subtilis* **B10** on mice hepatic mRNA expression of genes associated with lipid metabolism of mice

The administration of B10 down-regulated the transcript levels of  $3\beta$ -hydroxysteroid- $\Delta 24$  reductase (DHCR24) significantly (P<0.05) and up-regulated that of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) remarkably (P<0.05). No significant differences were noted in the gene expression levels of carnitine acyltransferase I (CAT I), peroxisome proliferator-activated receptor  $\beta$  (PPAR $\beta$ ), and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) between the two groups (Fig. 1).

Table 2 Effects of dietary supplementation of Bacillus subtilis B10 on body weight and food intake of mice

Group	Initial body weight (g)	Final body weight (g)	Average daily gain (g)	Average food intake (g)
HFD	35.47±0.58	41.16±0.52 <sup>a</sup>	0.19±0.03 <sup>a</sup>	4.72±0.30
HFD+B10	35.13±1.20	$38.51 \pm 1.10^{b}$	$0.11 \pm 0.03^{b}$	5.02±0.30

Data are expressed as mean $\pm$ SD (n=15 for initial body weight, final body weight, and average daily gain; n=5 for average food intake). Different letters indicate a statistically significant difference between groups (P<0.05)

Table 3	Effects of dietary	supplementation of	of Bacillus	subtilis !	B10 on	lipid p	rofiles	in the	liver and	l serum	of mice

Group			Liver			
	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TG (mg/g)	TC (mg/g)
HFD	0.96±0.09	$3.14 \pm 0.12^{b}$	$0.32 \pm 0.08^{b}$	$1.63 \pm 0.11^{b}$	$20.35 \pm 1.38^{a}$	$6.72 \pm 0.63^{a}$
HFD+B10	$0.94 \pm 0.08$	$4.06 \pm 0.17^{a}$	$0.55 \pm 0.11^{a}$	$2.41{\pm}0.58^{a}$	$13.60 \pm 0.60^{b}$	$4.66 \pm 0.17^{b}$
HFD+B10	$0.94 \pm 0.08$	4.06±0.17 <sup>a</sup>	0.55±0.11 <sup>a</sup>	2.41±0.58 <sup>a</sup>	13.60±0.60 <sup>b</sup>	4.66±

Data are expressed as mean±SD (n=6). Different letters indicate a statistically significant difference between groups (P<0.05)

Group	Glucose (mmol/L)	BUN (mmol/L)	GOT (IU/L)	GPT (IU/L)
HFD	$8.62{\pm}1.00^{a}$	5.30±0.39	$4.43 \pm 0.28^{a}$	5.68±0.30 <sup>a</sup>
HFD+B10	$5.18 \pm 0.54^{b}$	5.28±0.22	$3.30\pm0.10^{b}$	$3.12 \pm 0.38^{b}$

Data are expressed as mean±SD (n=6). Different letters indicate a statistically significant difference between groups (P<0.05)



Table 5 Effects of dietary supplementation of Bacillus subtilis B10 on HFD-induced oxidative stress in the liver of mice

Group	T-AOC	TrxR	SOD	CAT	GSH-Px	GSH	ASOA	XO	8-OHdG
	(U/mg prot)	(mg/L)	(U/mg prot)	(U/mg prot)	(U/mg prot)	(mg/g prot)	(U/g prot)	(U/g prot)	(pg/ml)
HFD	$0.84{\pm}0.04$	$0.96 \pm 0.04$	$55.32 \pm 4.00$	$0.83 \pm 0.06$	597.15±31.74 <sup>c</sup>	$0.81 \pm 0.12$	$82.18{\pm}4.53$	$16.00{\pm}1.26$	$14.42{\pm}1.32^{a}$
HFD+B10	$1.04{\pm}0.17$	1.31±0.13	50.53±4.37	1.15±0.13	$1441.22{\pm}105.98^{a}$	$0.90 \pm 0.08$	$75.82 \pm 4.07$	$14.45 \pm 0.76$	$10.28{\pm}1.02^{b}$

Data are expressed as mean±SD (n=6). Different letters indicate a statistically significant difference between groups (P<0.05). prot: protein

# **3.4 Effects of dietary supplementation of** *Bacillus subtilis* **B10 on hepatic antioxidant biochemical values and DNA damage of mice**

As shown in Table 5, the administration of B10 lowered concentrations of hepatic 8-OHdG remarkably (P<0.05), increased T-AOC, GSH, and TrxR levels and the CAT activity slightly, and significantly elevated, by a factor of 1.4, the GSH-Px activity (P<0.05).

# **3.5** Effects of dietary supplementation of *Bacillus subtilis* **B10** on hepatic mRNA expression of genes associated with oxidative stress of mice

It was found that the transcript levels of GR, XO and Hsp90 genes in the liver were much less for the B10 group than for the HFD group (P<0.05). However, an increased hepatic gene expression of p53 was also observed in the B10 treatment group (P<0.05). There was no significant difference between the two groups in the transcript levels of SOD, glutamylcysteine synthetase (GCS), and thioredoxin reductase 2 (TRX2) (Fig. 2).

### 4 Discussion

A 30-d supplementation of B10 is able to decrease mice body weight, although there is no difference in food intake between the two groups (Table 2). The decrease in body weight induced by probiotics is in agreement with the results of other research (Park et al., 2013; Wang et al., 2015). Although we did not compare the digestive capacity and nutrient absorption of mice between the HFD and HFD+B10 groups, previous research showed that the digestive enzyme activity was unchanged between animals fed an HFD or HFD supplemented with probiotics (An et al., 2011). So we conjecture that the lower body weight gain caused by B10 was due to its modulation of lipid metabolism other than because of



Fig. 2 Effect of dietary supplementation of *Bacillus subtilis* B10 on the expression of mRNA associated with antioxidation in mice liver of the HFD (high-fat diet) and HFD+B10 (high-fat diet with 0.1% *Bacillus subtilis* B10) groups

All mRNA quantification data are normalized to the reference gene. Gene expression levels are expressed as values relative to that of the HFD group. Data are expressed as mean $\pm$ SD (n=6). Different letters indicate a statistically significant difference between groups (P<0.05)

different food intake or digestion. In recent years, numerous investigations have shown that probiotics can be considered as biotherapeutics in treating abnormal lipid metabolism (Park et al., 2013; Xin et al., 2014). In our study, hepatic TC, TG and serum HDL-C, glucose, activities of GOT and GPT are much less than those of HFD-fed mice (Tables 3 and 4). In fact, GPT is an enzyme related to the liver fat accumulation (Westerbacka et al., 2004). From the point of view of Eckel et al. (2005), once fatty liver disease occurs, widespread lipid will accumulate in muscles and other organs, which facilitates the metabolic syndrome. As GPT could be a sensitive marker of hepatic dysfunction associated with metabolic syndrome, even in the range below the current limit (Jeong et al., 2004), the low GPT activity in the B10 treatment group may imply that B10 could be involved in the alleviation of the susceptibility to metabolic syndrome in HFD-fed mice. Nevertheless, the significant increases of TC and LDL-C in the serum were in contrast to the findings of Panda et al. (2006),

in which serum TC and LDL were reduced by dietary supplementation of Lactobacillus sporogenes. However, the study of Novak et al. (2011) also found an enhanced serum LDL-C level. In their opinion, the lowered concentrations of cecal propionic and butyric acids in Bacillus-fed chickens might be the reason for higher levels of LDL compared with the control, because products of bacterial fermentation, specifically short chain fatty acids, may inhibit cholesterol synthesis in the plasma cholesterol in the liver (Rafter, 2002).

Genes of lipid synthesis (PPARy, DHCR24, PPAR $\beta$ ), lipolysis (HMGCS2, CAT I), and energy metabolism (PPAR $\alpha$ ) are involved in lipid metabolism. The obese mice induced by the HFD have association with abnormal lipid metabolism in the liver, including altered acetyl-CoA carboxylase 1 (ACC1), fatty acid synthetase (FAS), fasting-induced adipose factor (FIAF), and PPAR $\gamma$  gene expression (Kang et al., 2013; Xin et al., 2014). In our study, HFD also induced abnormal lipid metabolism compared with mice fed a normal diet (data not shown), including the up-regulation of lipid synthesis genes and downregulation of lipolysis. For the HFD+B10 group, the mRNA expression of hepatic PPAR $\alpha$  and HMGCS2 is increased while DHCR24 mRNA expression decreases (Fig. 1). Thus, it could be deduced that the positive effect of B10 on lipid reduction may be due to the up-regulation of lipolysis and the downregulation of lipid synthesis. This is consistent with the study of Kang et al. (2013) and Park et al. (2013), in which the transcript level of PPAR $\alpha$  was also increased and the expression of some lipid synthesis and lipolysis genes was regulated by probiotics.

The abnormal lipid metabolism induced by high fat intake could also affect pro- and anti-oxidative balances in many tissues (Marczuk-Krynicka et al., 2009). The altered lipoprotein profiles enhance the synthesis and accumulation of intramyocellular TG, resulting in an increased synthesis of toxic fatty aciddelivered metabolites, causing enhanced lipid peroxides, hepatic membranes damage, and oxidative stress (Newsholme and Krause, 2014). Gao et al. (2011) demonstrated that HFD-fed ICR mice supplemented with lactic acid bacteria could enhance the activities of SOD and GSH-Px. In the present study, the GSH-Px activity was also significantly up-regulated in HFD+B10 group (Table 5). Moreover, the increased enzyme activity was accompanied by the regulation of genes related to antioxidant enzymes and oxidative stress. XO is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and could generate reactive oxygen species, leading to oxidative stress (Ardan et al., 2004). On the other hand, the p53, a tumor-suppressor protein, exhibits antioxidant activities to eliminate oxidative stress and ensure cell survival in response to low levels of oxidative stresses through the p53-regulated gene GPX1, manganese superoxide dismutase (MNSOD) or other pathways (Sablina et al., 2005; An et al., 2008; Liu and Xu, 2011; Chen et al., 2012; Wu et al., 2014). As we expected, the transcript level of XO in mice treated with B10 being obviously down-regulated compared with HFD group, p53 expression is markedly up-regulated (Fig. 2). Moreover, as mentioned above, the activity of GPX was significantly increased (Table 5), which means, p53 may exert the antioxidant activity via the increment of GPX activity after B10 treatment. Our findings show that B10 has a positive regulatory effect on lipid profile, GSH-Px activity and p53, XO expression. All these alterations caused by B10 administration result in the attenuation of oxidative stress and DNA damage, which was verified by the decreased expression of antioxidantrelated genes (GR and Hsp90) and lowered 8-OHdG level, a major product of DNA oxidation (de Souza-Pinto et al., 2001). Based on our study, the antioxidative activity of B10 is reflected in the increased activity of GSH-Px, the regulation of antioxidantrelated genes, like XO and p53 as well as the lipid profile and may be the modulation of gut microbiota. Accumulating evidences showed a strong correlation between gut microbiota and metabolic syndrome (Araya et al., 2002; Everard et al., 2014) and therefore, further research is needed to clarify the effect of B10 on the modulation of gut microbiota.

### **5** Conclusions

In conclusion, **Bacillus subtilis** B10 could decrease body weight gain of diet-induced obese mice by improving lipid metabolism and oxidative stress status.

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### **Compliance with ethics guidelines**

Kai LEI, Ya-li LI, Yang WANG, Jing WEN, Hong-zhao WU, Dong-you YU, and Wei-fen LI declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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### List of electronic supplementary materials

Table S1Compositions of mice normal dietData S1Methods for biochemical parameter detection



**B10**