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Complete List of Authors:	Tsai, Yi-Chen; National Taiwan University, Department of Animal Science and Technology Wang, Han-Tsung; Chinese Culture University, Department of Animal Science Hsu, Jih-Tay; National Taiwan University, Department of Animal Science and Technology Li, Yi-Hung; National Taiwan University, Department of Animal Science and Technology Chen, Ching-Yi; National Taiwan University, Department of Animal Science and Technology

Running title: Yeast with bacteriocin enhances lipid and glucose utilization

Yeast with bacteriocin from rumen bacteria enhances glucose utilization, reduces ectopic fat accumulation, alters cecal microbiota in dietary-induced obese mice

Yi-Chen Tsai[1], Han-Tsung Wang[2], Jih-Tay Hsu[1], Yi-Hung Li[1], and Ching-Yi Chen[1*]

[1]Department of Animal Science and Technology, National Taiwan University; No. 50, Lane 155, Sec 3, Keelung Rd, Taipei, Taiwan

[2]Department of Animal Science, Chinese Culture University; No. 55, Hwa-Kang Rd, Taipei, Taiwan

*Corresponding author:
Ching-Yi Chen
Department of Animal Science and Technology
National Taiwan University
Present address: 50, Lane 155, Sec 3, Keelung Rd, Taipei, Taiwan, 112
Email: ronichen@ntu.edu.tw
Tel: +886-2-33665283
FAX: +886-2-27324070

ABSTRACT

BACKGROUND: This study investigated the effect of yeast with bacteriocin (YB) on the homeostasis of lipid and glucose in diet-induced obese (DIO) mice. Seven-wk-old C57BL/6 male mice were fed with a Western diet for 24 weeks to induce obesity. These DIO mice were randomly assigned to 2 groups: obese control (WS) and WYB [0.125 μg YB/g body weight (BW)]. YB was administered daily to the WYB mice in the last 4 weeks, while an equal volume of normal saline was administered to the WS mice.

RESULTS: YB caused a significant reduction in BW, and in plasma levels of total cholesterol and glucose. Less hepatic lipid accumulation and smaller adipocytes were observed in WYB mice. WYB mice had higher lipid catabolism in liver and adipose tissue. Compared with WS mice, WYB mice had higher glycolysis in the liver and muscles. YB suppressed hepatic GLUT5 expression, altered the composition of cecal microbiota, and also caused more efficient carbohydrate utilization for energy expenditure.

CONCLUSION**:** YB resulted in body weight loss, promoted lipid catabolism and carbohydrate utilization, it also modulated cecal microbiota, and therefore partially improved the health of obese mice.

Key Words: Diet-induced obesity, Yeast, Ectopic fat accumulation, Carbohydrate utilization, Cecal microbiota, Albusin B, Bacteriocin.

INTRODUCTION

Obesity is a major worldwide epidemic and has become a public health problem in recent years because of its strong association with symptoms of metabolic syndrome, including insulin resistance, dyslipidemia, and hypertension, all of which are risk factors for type 2 diabetes, hepatic steatosis, and cardiovascular disease [1, 2]. The increasing prevalence of obesity has primarily been caused by dramatic changes in lifestyle, including a lack of physical activity and the westernization of diet patterns [3]. The Western diet, which is high in fat and sugars, is the major dietary issue for obesity prevalence [4].

Bacteriocins, the naturally proteinaceous peptides secreted by some bacteria, have a highly specific killing spectrum in order to compete against closely related bacteria or bacteria of the same species as them [5]. According to the definition made by Klaenhammer (1993), bacteriocins are subdivided into 4 classes: (I) lantibiotics, (II) small heat stable peptides, (III) large heat labile proteins, and (IV) complex proteins whose activity requires the association of carbohydrate or lipid moieties [6]. These peptides have been widely applied in food safety and food additives in recent years [7]. In fact, some of the beneficial effects of some probiotics, such as lactic acid bacteria, are delivered through the production of bacteriocins that improve the health of the host [8, 9]. Recent studies further demonstrated a weight-lowering effect of bacteriocin against obesity. Clarke et al. found that administration with bacterion-producing probiotics *Lactobacillus salivarius* UCC118 caused a decrease in the body weight gain via altering gut microbiota, when compared with an isogenic non-bacteriocin producing control[8]. Not only bacteriocins, some probiotics also modulate the weight gain of obese subjects. Administrating *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 to dietary-induced obese (DIO) mice caused more body weight loss [10]. In addition, the administration of lactic acid bacteria to DIO animals lowered fatty acid synthesis in the liver, inhibited the enlargement of visceral adipocytes, and reduced the accumulation of ectopic fat,

thereby retarding the adipose tissue dysfunction of the DIO animals [10-12].

Albusin B, a 32-kDa and heat-labile bacteriocin secreted from the ruminal cellulolytic bacteria *Ruminococcus albus* 7, is considered to be a Class III bacteriocin [5]. In a previous study, we successfully mass-produced albusin B via a yeast expression system [13], and named this albusin B-expressing yeast product as YB. Oral administration of YB to broilers resulted in better growth performance, intestinal absorption, and antioxidant defense [13, 14]. YB also modulated the gut microbiota by increasing cecal *Lactobacillus* counts and decreasing pathogenic populations [15]. In addition, this yeast product has exhibited a hypolipidemic and anti-oxidative stress role in healthy mice [16]. Because obese subjects often have hyperlipidemia and suffer from the disadvantages resulting from hyperlipidemia, such as cardiovascular diseases [17, 18], many recent studies have focused on the discovery of anti-hyperlipidemic agents as potential therapies for metabolic-related diseases [11, 19, 20]. Probiotics and bacteriocins could be one of the potential agents. Owing to its hypolipidemic ability, the YB was orally administered to DIO mice in this study so that its potential effects on lipid metabolism and gut microbiota in DIO mice could be elucidated.

MATERIALS AND METHODS

Materials

All chemicals and reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) unless otherwise indicated.

The preparation of albusin B-expressing yeast product

Procedures for the preparation of albusin B-expressing yeast product were similar to those described previously [13, 21]. The *alb*B gene (GenBank accession number AF469209) of albusin B from *R. albus* 7 was amplified by polymerase chain reaction (PCR). Using *Eco*RI,

the PCR-generated fragment was cut from the pYEX-S1 vector and cloned into the filled-in *Eco*RI site to yield plasmid pYEX-alb. To mass production, *alb*B was transformed into *Saccharomyces cerevisiae* DBY 747 (ATCC 204 659) to form *S. cerevisiae* DBY 747 albB. The mass production (300 L) medium for *S. cerevisiae* DBY 747 albB was YEPD (1 % yeast extract, 1% peptone, 20% D-glucose). The yeast was fermented at pH 5 and 30 °C, the airflow rate was 0.5 vvm and the agitation rate was 120 rpm. After 48 h of fermentation, the yeast products were recovered by centrifugation and spray dried for future use. The concentration of albusin B was measured by the following procedure. The dried yeast with albusin B was dissolved in cold 50 mM Tris-buffer (pH 8.0) (1:5, w/v) and the yeast cell was disrupted by ultrasonicator. After centrifugation at 13,500 x g for 20 min, the supernatant was collected and separated by ÄKTA FPLC (GE Healthcare, Pittsburgh, PA, USA) with Hi-Trap DEAE column (GE Healthcare, Pittsburgh, PA, USA) in Tris-buffer system (pH 8.0). The fraction with albusin B activity (that had been identified by SDS-PAGE and Western blotting method) was collected and assayed the protein concentration by micro-BCA assay kit (Thermo Scientific, Waltham, MA, USA). The concentration of albusin B prepared was 1 mg of bacteriocin protein per gram of product yeast dry mass. The term "YB" was used to represent the product of yeast with albusin B. The initial count of live yeast with YPD agar (242720, Difco, Detroit, Michigan) in YB product was $3.2\pm0.2 \times 10^8$ CFU/g.

Animal treatment

All animal procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan University. Forty 5-wk-old C57BL/6 male mice were obtained from the Laboratory Animal Center, National Taiwan University College of Medicine. After a 2-wk acclimation period, mice were fed with a Western diet (W) containing 45.7% fat and 20.0% sucrose (4.60 Kcal/g)(TestDiet® 58V8, Richmond, IN, USA) *ad libitum* for 24 weeks. These mice (body weight: 46.0 ± 1.8 g) were randomly assigned into two groups: obese

control (WS) and WYB (0.125 μg YB/ g body weight). YB was administered daily by gavage to WYB mice in the last 4 weeks. An equal volume of normal saline was administered to the WS mice in the last 4 weeks. Each treatment group of 20 mice (5 mice/ cage) was housed in a room at 25°C ± 1°C under a 12-hour light/ 12-hour dark cycle. Body weight (BW) and food intake measurements were recorded weekly. At the end of the experiment, blood samples were collected. Samples from the liver, epididymal adipose tissue, and muscle of femurs were collected and stored at -80°C until analyzed.

Blood biochemical parameters assay

Blood samples were obtained from the facial vein of mice in a starved state at the end of experiment. Ethylenediaminetetraacetic acid disodium salt dihydrate was used as an anticoagulant. Plasma samples were collected by centrifugation at 2,500 × g, 4°C for 10 minutes and stored at -80°C for use. Plasma triglyceride, total cholesterol, and high density lipoprotein levels were determined through enzymatic assay kits (Fortress Diagnostics, Antrim, Northern Ireland, UK) using the colorimetric method according to the manufacturer's instructions.

Analysis of histological image

Samples of liver and epididymal adipose tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of 6 μm thickness were stained with hematoxylin and eosin. The photomicrographs of slices were quantified by the ImageJ software (NIH Image, Bethesda, MD, USA).

mRNA extraction and real-time polymerase chain reaction (PCR)

Total RNA was extracted from the samples of liver, epididymal adipose tissue, and muscle of femurs using the TRI reagent (Applied Biosystem, Grand Island, NY, USA)[16]. Two

μg of total RNA was reverse transcripted with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem, Grand Island, NY, USA) into cDNA. Then each cDNA was amplified by using SYBR Fast Master Mix (KAPA Biosystem, Woburn, MA, USA) and the StepOnePlus^{TM} Real-time PCR System (Applied Biosystem, Grand Island, NY, USA). The PCR cycling conditions were an initial denaturing step of 95°C for 6 min, followed by 40 cycles of 95°C for 10 s, individual annealing temperature for 30 s, 72°C for 30 s, and a final extension step at 74°C for 10 min. All primer sequences used and annealing temperatures are listed in Table 1. The relative expression levels were calculated according to the formula $2^{-\Delta CT}$ and normalized using the expression of the β-actin housekeeping gene in the same sample [22].

Respiratory quotient (RQ) assay

Mice were fasted overnight, and then placed in a metabolic chamber individually for the measurement of 24-hour oxygen (O_2) consumption and carbon dioxide (CO_2) production by the PhysioScan Metabolic System (AccuScan, Columbus, OH, USA). Food was not provided, and water was accessed freely. Room air was pumped into the chambers at a rate of 0.5 L/min. RQ was calculated as the ratio of VCO_2 to VO_2.

Cecal microbiota assay

Fresh cecal contents were weighed and mixed with 9-fold volume of sterile anaerobic diluents immediately [23]. After the diluted samples were homogenized, each cecal microbial sample was serial diluted before inoculated onto Petri dishes of sterile agar. The plate media used were Reinforced Clostridial agar (CM0151, Oxoid, Hampshire, England, UK) for *Clostridium*, *Eubacterium* Selective (ES) agar for *Eubacterium* [24], *Lactobacillus* selective media (7234A, Accumedia, Lansing, MI, USA) for *Lactobacillus*, Lombard-Dowell (LD) - esculin agar for *Bacteriodes* [25], *Bifidobacterium* iodoacetate medium 25 for *Bifidobacterium*

[26], Wilkins - Chalgren (WC) agar (7233A, Accumedia, Lansing, MI, USA) for total anaerobes. All anaerobic culture procedure were carried out in an anaerobic chamber (Coy Laboratory Products Inc., USA) under 97% CO_2/3% H_2 atmosphere at 39°C. Total aerobes were determined by 3M™ Petrifilm™ Aerobic Count Plates (6400, 3M, St. Paul, MN, USA). The colony-forming units (CFU) of plate were measured at least 1 mm in diameter by colony counter (aCOLyte SuperCount, Symbiosis, Frederick, MD, USA).

Statistical analysis

All experimental data were analyzed by SAS® 9.2 (SAS Institute, Cary, NC, USA) using GLM procedures. The Duncan's multiple range test was used to assess the differences of variance among groups. Results were expressed as mean ± standard error (SE). *P* values < 0.05 were considered significant.

RESULTS

Effect of YB on physiological parameters

In the preliminary experiment, the DIO mice were administrated individually with various dosage of YB (0, 0.0625, 0.125, 0.25, and 0.625 μg YB/ g BW) for 4 weeks, and found that YB caused various degrees in BW loss (44.5±2.5, 41.3±2.3, 37.5±1.9, 39.8±1.8 45.1±3.9 g, respectively). The results showed that 0.125μg YB/ g BW caused the greatest lowering effect on BW; accordingly, this YB dosage was applied in the present study. The BW was not significantly different between treatments in the initial of experiment (46.0 ± 1.8 g). After oral YB administration for 4 weeks, WYB mice exhibited a significant decrease in BW when compared with WS mice (**Fig. 1A**). Mice orally administrated YB had a significant decrease in plasma levels of total cholesterol, LDL, and glucose compared to WS mice (**Fig. 1B**). The YB supplementation had no effect on the feed intake of DIO mice during the

experiment period (data not shown).

Effect of YB on lipid metabolism

WYB mice had less hepatic lipid accumulation (**Fig. 2A**). To explore the mechanism underlying the action of YB, the genes associated with lipid metabolism in the liver were determined. Administration of YB decreased hepatic gene expression of FABP1 (**Fig. 2B**). Compared with WS group mice, WYB mice had a higher transcript abundance of acyl-CoA oxidase (ACO) and HMG-CoA reductase (HMGCR) in the liver. These results demonstrate that YB decreased hepatic lipid accumulation by inhibiting fatty acid uptake and increasing fatty acid oxidation.

In terms of the morphology of epididymal adipose tissue, mice administered YB had smaller adipocytes than WS mice (**Fig. 3A**). Compared with WS mice, WYB mice had higher percentages of adipocytes smaller than 1000 μm^2 in size and lower percentages of those larger in size (3000-10000 μm^2). Gene expressions associated with lipolysis and lipogenesis were further analyzed to elucidate the action of YB. The YB treatments suppressed mRNA levels of lipogenic gene diglyceride acyltransferase 1 (DGAT1) in epididymal adipose tissue as compared to WS mice (**Fig. 3B**). WYB mice had lower hormone sensitive lipase (HSL) expression but higher adipose triglyceride lipase (ATGL) expression in adipose tissue than WS mice. In brief, oral administration of 0.125 μg YB/ g BW reduced lipogenesis and increased lipolysis in the epididymal adipose tissue, and therefore caused smaller adipocytes in the DIO mice.

Effect of YB on carbohydrate metabolism

The effects of YB on the homeostasis of carbohydrate metabolism in the obese mice were further studied. Administration of YB did not regulate the glucose transport protein 2 (GLUT2) expression in the liver, whereas it decreased hepatic GLUT5 expression (transporter

for fructose uptake) as compared to WS group (**Fig. 4A**). WYB mice exhibited higher mRNA expression of glucose kinase (GK), but exhibited no change of mRNA expression related to hepatic gluconeogenesis (glucose-6-phosphatase, G6Pase). Gene expressions of glucose uptake (GLUT4) and glycolysis (hexokinase and pyruvate kinase) in skeletal muscle were determined as well (**Fig. 4B**). The results showed that WYB mice exhibited no significant difference in GLUT4 expression but had higher expression of hexokinase (HK) and pyruvate kinase (PK) in skeletal muscle than did the WS group.

To investigate the effect of YB on systemic energy utilization in DIO mice, the respiratory quotient (RQ) values of the mice prior to administration of YB and at the end of the experiment were determined. There were no significant differences in RQ for WS mice during the experiment period (data not shown). After administration of YB for 4 weeks, the RQ value for a 24-h period was significantly shifted from 0.73 to 0.8, indicating that YB caused more carbohydrate used as an energy source in obese mice (**Fig. 5**). To summarize, YB increased systemic glucose utilization by increasing hepatic and muscular glycolysis.

Effect of YB on cecal microbiota

The results of cecal microbiota composition in mice were shown in Table 2. The bacterial counts of *Clostridium* and *Bifidobacterium* were significantly higher in WYB group than WS group. Mice administrated with YB had no significant difference in the count of *Eubacterium*, *Lactobacillus*, and *Bacteroides* as compared to WS mice. The ratio of Firmicutes to Bacterioidetes (F/B) is considered a biomarker for obesity. The result showed that YB treatments decreased ratio of F/B in the obese mice. These results gave more evidences that YB treatment regulated energy metabolism via modulating gut microbiota composition in the obese mice.

DISCUSSION

The present study demonstrated that YB caused decreases in plasma glucose, cholesterol, and body weight in DIO mice. In addition, YB diminished ectopic fat deposition, enhanced systemic glucose utilization, and modified the composition of cecal microbiota, all of which contributed to health improvement in the DIO mice.

YB reduces ectopic fat accumulation

Dietary-induced obesity is often accompanied by adipose tissue dysfunction, which is positively associated with the pathogenesis of metabolic-related diseases [18]. The classic function of adipose tissue is to store lipids; however, excessive lipid storage in the adipose tissue leads to adipocyte hypertrophy, hypoxia, and induced oxidative stress, endoplasmic reticulum stress, and inflammatory response within the adipose tissue, all of which impair the normal functions of the tissue, which is why these phenomena are termed adipose tissue dysfunctions [17, 27]. Ectopic fat, which is defined by excess adipose tissue in locations not classically associated with adipose tissue storage, is one of the consequences of adipose tissue dysfunction.

A lot of studies have indicated that consumption of probiotics inhibits hypertrophy and hyperplasia in adipocytes, which results in a decrease in the number of larger adipocytes and an increase in the number of smaller adipocytes in visceral adipose [11, 12]. In addition, administration of probiotics has been reported to decrease ectopic fat accumulation and therefore improve metabolic dysfunctions in DIO animals [11, 12]. In the present study, we found chronic Western diet-feeding induced ectopic fat accumulation in the liver and visceral adipose tissues. However, oral administration of YB inhibited fatty acid absorption and promoted fatty acid oxidation in the liver, in addition to suppressing lipogenesis and enhancing lipolysis in the adipose tissues, all of which accounted for the reduction of ectopic fat accumulation resulting from YB administration.

YB enhances systemic glucose homeostasis

The fate of fat in the cell is to form a lipid bilayer in the cell membrane, to be used as a fuel source, or to be stored as triglyceride when energy is oversupplied [28]. Long-term consumption of surplus fat causes the saturation of storage capacity in adipocytes; excess fat uptake further promotes *de novo* lipogenesis, lipid peroxidation, and massive generation of reactive oxygen species (ROS) [28, 29]. However, elevating the utilization of carbohydrates as an energy source in DIO animals has been reported to not only ease adipose tissue dysfunctions, but also to reduce circulating glucose and, therefore, decrease insulin resistance [29, 30]. In the present study, after oral administration of YB for 4 weeks, DIO mice used more carbohydrate as main energy source, together with increases of glycolysis in the liver and skeletal muscle. These changes caused by YB indicated the ability of YB to enhance the systemic glucose utilization of DIO mice.

Accumulating evidence has shown that high fructose consumption contributes to obesity and metabolic disorders [31]. Normally, fructose metabolism occurs in the splanchnic tissues. After fructose absorption, a large proportion of fructose is converted into glucose in the liver, which can be further converted into glycogen or triglyceride, and then shortly stored in the liver [31]. However, chronic excessive fructose supplementation increases the load on the liver, causing dyslipidemia, ectopic lipid deposition in the liver and muscle, insulin resistance, and impaired glucose homeostasis [31]. A 10% portion of the Western diet applied in this study consisted of fructose, and this long-term surplus fructose intake partially contributed to the impairment of glucose homeostasis in the DIO mice. However, YB administration inhibited the hepatic fructose uptake by suppressing GLUT5 expression, suggesting its novel role in regulating fructose metabolism of DIO mice. Taken together, YB improved the systemic glucose homeostasis of DIO mice by ameliorating glucose utilization and reducing hepatic fructose uptake.

Cholesterol-lowering effect

The possible mechanisms for plasma cholesterol reduction by probiotics could be: (1) decreased cholesterol absorption from the gut; (2) enhanced plasma cholesterol clearance; and (3) increased bile acid production and greater cholesterol excretion from the body in the form of bile acids [32]. The deconjugation of bile acids is responsible for the increase in fecal excretion of bile acid, which in turn has been reported to augment the demand of cholesterol for the *de novo* bile acid synthesis to replace the loss in feces [33]. In addition, some bacteria, such as *Bacteroides* spp., bifidobacteria, clostridia, and lactobacilli, suppress cholesterol absorption from the gut by deconjugating bile salts, and thereafter affect the cholesterol metabolism or directly assimilate cholesterol [33-35]. In this study, DIO mice administered YB displayed a higher hepatic expression of HMG CoA reductase, and a lower plasma LDL. In addition, the bacterial counting data demonstrated that YB administration increased the *Clostridium* and *Bifidobacterium* counts in the cecum of DIO mice (Table 2). Therefore, we postulated that YB caused a greater population of cecal *Clostridium* and *Bifidobacterium*, which deconjugated bile salts and increased the cholesterol excretion, and in turn increased the demand of cholesterol synthesis by stimulating HMG CoA reductase expression.

YB alters the composition of the cecal microbiota

This study showed a significant increase in the counts of *Clostridium* and *Bifidobacterium* in WYB group. The supplementation of YB product had effect on the composition of cecal microbiota. In our previous study in broiler, it indicated that YB supplementation at the same level resulted in about 3-fold increase in fecal yeast counts, compared to the yeast alone group (yeast without albusin B expression) (1.45 and 0.52 x 10^8 CFU/g, respectively)[36]. The improvement of anaerobic environment might result from the increase in total yeast counts after YB supplementation. In this study, the obligately anaerobic

bacterium *Bifidobacterium* count increased about 10^3 times in WYB group compared with WS group. On the other hand, higher tested bacteria in *Eubacterium, Lactobcillus* and *Bacteroides* and total anaerobic bacteria count number were observed in this study. These results might be related to that YB supplementation led to a more anaerobic environment in the cecum. Some studies reported that yeast supplementation modulated the gut microbiota by causing an increase in anaerobic bacteria and *Clostridium* counts [37, 38]. Moreover, higher yeast presence in the digestion tract had the ability to increase the oxygen scavenging and supply some growth factors [39]. Because the chemical composition and nutrition value of YB product are similar to the dry yeast powder, implying that the components of yeast might also play a role on YB-regulating effect against cecal microbiota. Accordingly, we postulated that the increasing number of total yeast in cecum induced a more strictly anaerobic environment and the YB lysate supplied some nutrients/ growth factors for other bacteria. Therefore, the YB supplementation may serve a role in the increase of *Clostridium* and *Bifidobacterium* counts in cecum.

In our previous study, the result of Minimum Inhibitory Concentration (MIC) test indicated that MIC50 of partially purified albusin B to *Enterobacter* and *Salmonella* is about 0.5 and 0.75 mg/mL, respectively [40]. The result of antimicrobial activity assays (agar diffusion method) against *Lactobacillus acidophilus, Lactobacillus casei,* and *Bifidobacterium* sp. showed no inhibition effect of albusin B on tested lactic acid bacteria and *Bifidobacterium* sp.[36]. However, we found that albusin B supplementation stimulated the growth of these tested lactic acid bacteria. According to the MIC data, the antibiotic ability of albusin B in this feeding level might not play a dominant role in the composition change of microbiota. However, feeding YB in 75 to 125 ug/g BW/d (equal to 75-125 ng albusin B/g BW/d) showed a significant growth stimulation effect on cecal lactic acid bacteria in the laying hen[38]. According to our results of *in vitro* and *in vivo* digestion test, YB was decomposed in the digestive tract, and therefore released albusin B[36], indicating that the main effect of YB might

not accomplish through the live YB. Taken together, it is suggested that the signal or lactic acid bacteria growth enhancer property of albusin B might possess the important role in the composition change of microbiota.

Body weight-lowering effect

Several possible mechanisms for the BW-lowering effect have been proposed to elucidate the probiotic and prebiotic actions, including (1) inhibiting lipid absorption [41]; (2) increasing the thermogenic responses [42]; (3) reducing energy intake [43]; (4) decreasing lipogenesis [44]; and (5) increasing the lipolytic response [42]. We previously studied the effect of YB on healthy mice, and found that YB caused body weight loss by promoting lipid oxidation and suppressing lipid synthesis [16]. YB treatment lowered BW in DIO mice by the same mechanism through enhanced lipid oxidation and suppressed fatty acid absorption and lipogenesis.

A tight connection between obesity and gut microbiota composition has been reported. A decrease in the proportion of *Bacteriodetes* relative to total bacteria was found in the genetically obese mice [45]. DIO mice harbored an increase of *Firmicutes* and a decrease of *Bacteriodetes* in the cecal microbiota [46], whereas increasing the abundance of *Bacteroidetes* in DIO mice caused a lower BW [47]. In addition, DIO mice administrated with *Bifidobacterium* in the gut exhibited significant reduction of BW, fat mass, and hepatic lipid deposition [48-50]. These studies support the evidences that alteration of gut microbiota composition modulates metabolic regulation of obese animals, therefore results in BW-changing effect. The present study showed a positive effect of YB treatment on increasing the ratio of *Bacteriodetes* to *Firmucutes* and the abundance of *Bifidobacterium*, providing evidences that modulating the gut microbiota by YB may partially contribute to the weight loss of DIO mice.

Currently, only few drugs have been approved as anti-obesity agents, and the drug application needs more caution due to the side effects. To develop a more efficacious and safe

anti-obesity therapy, several phytochemicals and natural products have been evaluated. Orlistat, a well-known anti-obesity drug, caused 25% and 66% reduction in BW and serum cholesterol of DIO mice, while clerodane diterpene, isolated from *Polyalthia longifolia*, caused 25% and 73% reduction in BW and serum cholesterol [20]. Both them decreased hepatic lipid accumulation. Ferreira et al. (2011) reported that phloroacetophenone isolated from *Myrcia multiflora* caused 40%, 37%, and 46% reduction in BW, total cholesterol, and triglyceride serum level of DIO mice [51]. Additionally, the authors pointed that lovastatin, an anti-hyperlipidemia drug, decreased serum cholesterol and triglyceride without changing BW, demonstrating that not all the potential anti-obesity agents exhibited the similar efficacy as orlistate. Similar results were found in the study of Jonas et al. (2015)[52]. They demonstrated that 3,5-Diiodo-L-Thyronine reduced blood cholesterol and hepatic lipid accumulation without any effect on BW. The present study showed that YB caused more than 50% and 20% reduction in hepatic lipid accumulation and BW loss, implying its potential role as an anti-obesity agent.

One issue was brought out when considering YB as a potential anti-obesity agent. In the preliminary study, we found that 0.125μg YB/ g BW (low dosage) caused the greatest lowering effect on BW, while the high dose of YB (0.625μg YB/ g BW) did not exhibit similar effects on DIO mice as the low dose of YB did. One of the possible explanations for this might involve the mass-produced yeast system. Yeast is considered a growth promoter in farm animals, and the level of yeast supplementation affects animal performances. In this study, the YB feeding levels of the 0.125-0.625 μg YB/ g BW were equivalent to yeast supplementation levels of 1-5 g/ kg in a diet. Aluwong et al. (2013) indicated that 5 g/ kg yeast supplementation (*S. cerevisiae*) increased BW gain in broilers [53]. Shen et al. (2011) noted that supplementation of 5-7.5 g/ kg yeast in the diets of gestating sows resulted in higher back fat gain and BW weight gain [54]. A study of weanling piglets also indicated that yeast supplementation in diets (> 2 g/ kg) increased colon VFA concentrations, which

provided an extra energy source for metabolism and led to higher BW gain [55]. All these studies suggest that the actual levels of yeast supplementation in the diet may have contributed to the different results for body weight loss for the high dose and low dose of YB groups. That is, the high dose of YB increased the yeast supplementation and further augmented the action of yeast by increasing nutrient absorption, and therefore antagonized the anti-obesity ability of YB.

Taken together, the YB product could be considered as a probiotic and play a part in improving the ecosystem of digestive tract. The signal or growth enhancer property of albusin B might possess a role in the composition change of microbiota. Furthermore, the lysed YB could supply some growth factors to alter the cecal microbiota. On the other hand, the modification on lipid and glucose metabolism, and BW-lowering effect could be attributed to the lactic acid bacteria growth enhancing property of albusin B and the microbial modification effects of YB supplement

Conclusion

The present study demonstrated the beneficial effects of YB on health in DIO mice. Oral administration of 0.125 μg YB/ g BW to DIO mice decreased the body weight of DIO mice by reducing ectopic fat deposition, lowering blood cholesterol, increasing systemic glucose utilization, and elevating the *Bifidobacterium* population in cecal microbiota.

This study implicated a therapeutical potential of YB to human for weight loss of control. However, one concern about the level of YB supplementation should be brought out, that is, high level of YB supplementation (0.625μg YB/ g BW) did not reduce the body weight of DIO mice. Accordingly, it should be cautious when using YB as a potential anti-obesity agent.

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Conflict of interest: None

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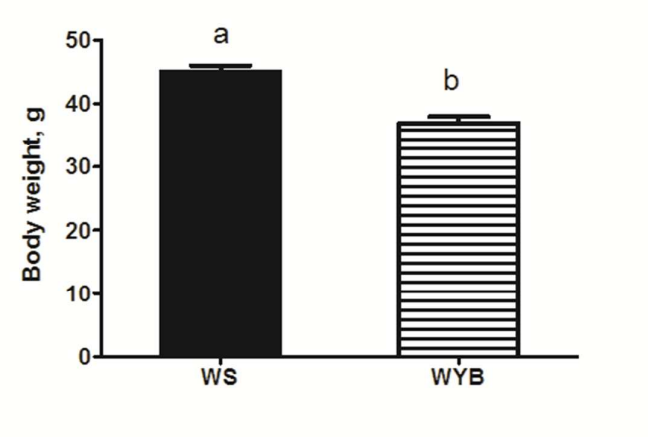
A



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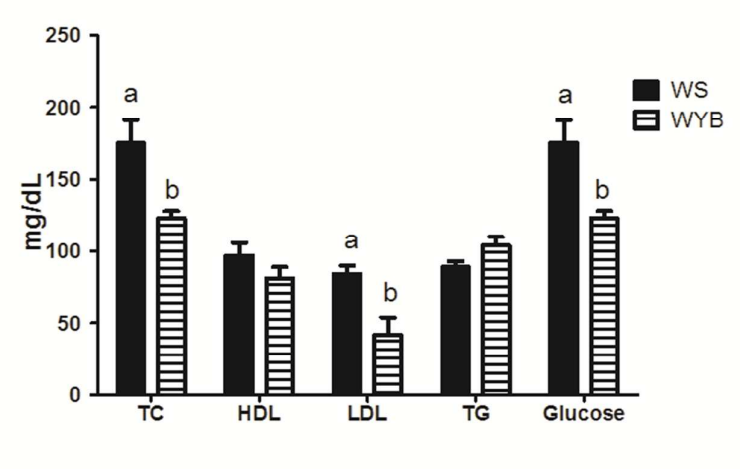
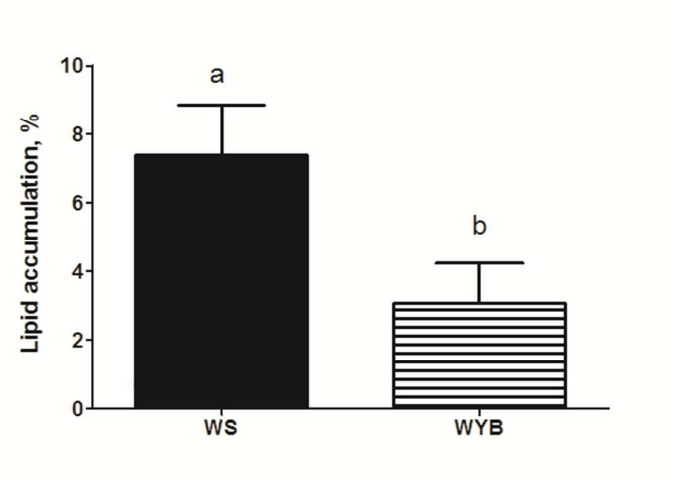


Fig 1. Body weight (A) and plasma parameters (B) of dietary-induced obese mice. Data are expressed as mean ± SE (n=20). [ab] Different letters represent significant differences (P<0.05). TC: total cholesterol. HDL: high density lipoprotein. LDL: low density lipoprotein. WS: Western diet + saline. WYB: Western diet + 0.125 μg YB/ g BW.

A



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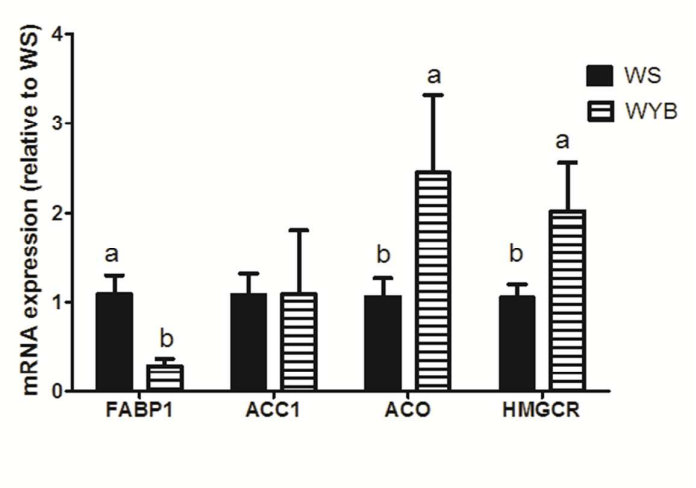
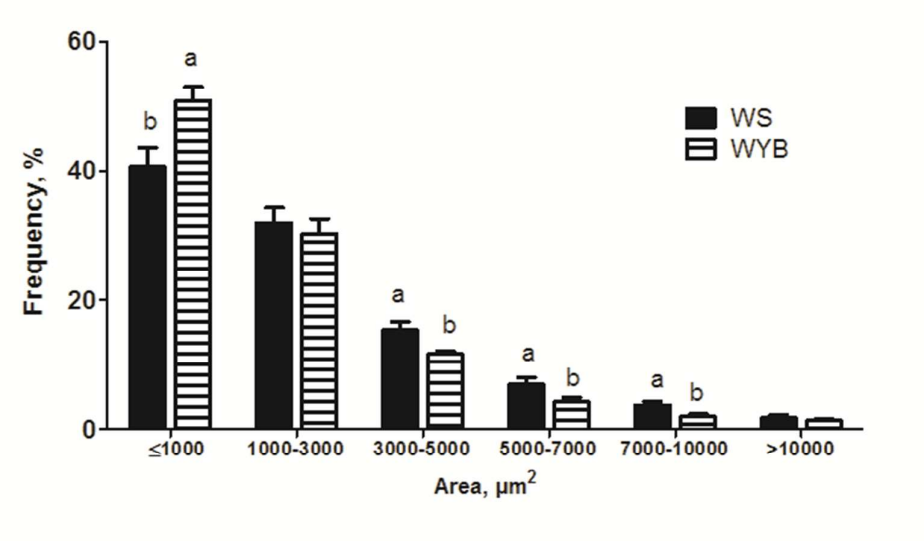


Fig 2. Lipid accumulation (A) and lipid metabolism-related gene expression (B) in the livers of dietary-induced obese mice. Data are expressed as mean ± SE (n=20). [ab] Different letters represent significant differences (P<0.05). WS: Western diet + saline. WYB: Western diet + 0.125 μg YB/ g BW. FABP1: fatty acid binding protein 1. ACC1: acetyl-CoA carboxylase 1. ACO: acyl-CoA oxidase. HMGCR: HMG-CoA reductase.

1

A



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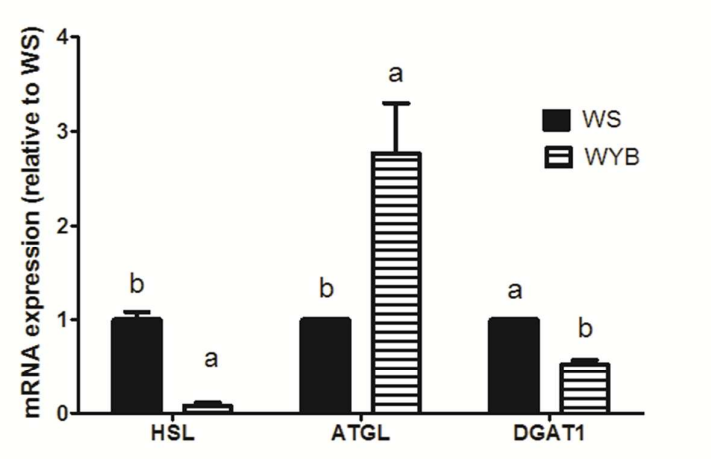
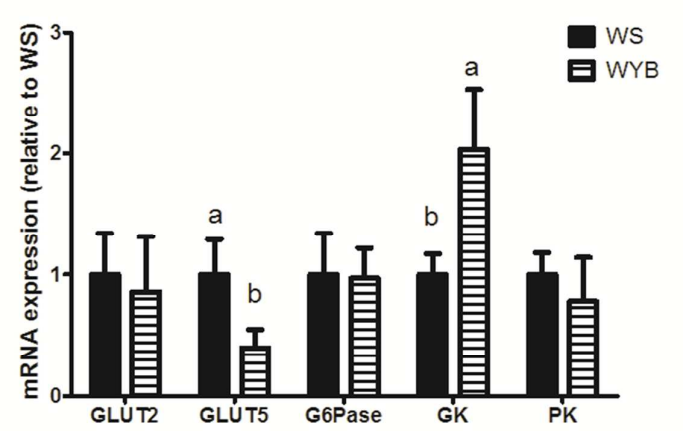


2 **Fig 3.** Frequency of adipocyte distribution (A) and lipid metabolism-related gene expression
3 (B) in the epididymal adipose tissue of dietary-induced obese mice. Data are expressed as
4 mean ± SE (n=20). [ab] Different letters represent significant differences (P<0.05). HSL:
5 hormone sensitive lipase. ATGL: adipose triglyceride lipase. DGAT1: diglyceride
6 acyltransferase 1. WS: Western diet + saline. WYB: Western diet + 0.125 μg YB/ g BW.

1

A



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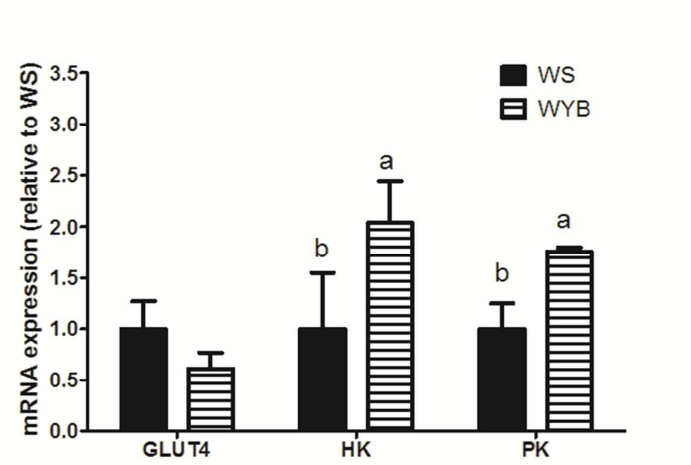

2

3 **Fig. 4** Carbohydrate metabolism-related gene expression in the liver (A) and muscle (B) of
4 dietary-induced obese mice. Data are expressed as mean ± SE (n=20). [ab] Different letters
5 represent significant differences (P<0.05). GLUT2: glucose transporter 2. GLUT5: glucose
6 transporter 5. G6Pase: glucose-6-phosphatase. GK: glucose kinase. PK: pyruvate kinase.
7 GLUT4: glucose transporter 4. HK: hexokinase. WS: Western diet + saline. WYB: Western
8 diet + 0.125 μg YB/ g BW.

9

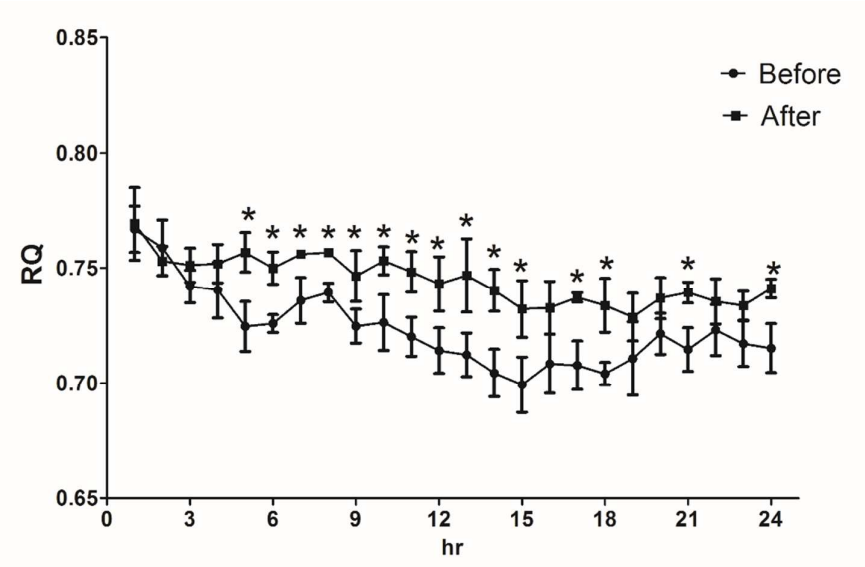

Fig 5. Respiratory quotient (RQ) values of mice. Before: day 0 of YB feeding; after: the end of the experiment. Values for each group are means ± SE (n=6). * indicates statistically significant differences (P<0.05). WYB: Western diet + 0.125 μg YB/ g BW.

Table 1 Primer sequences of genes for real-time PCR

Gene	Access number	Primer sequences	Products (bp)	Annealed Temperature, (℃)
ACC1 (Acetyl-CoA carboxylase 1)	NM_133360.2	Forward:5'-TAATGGGCTGCTTCTGTGACTC-3' Reverse: 5'-CTCAATATCGCCATCAGTCTT-3'	146	62
ACO (Acyl-CoA oxidase)	NM_015729.2	Forward: 5'-GACCCACAAGCCCTTGCCAGG-3' Reverse: 5'-CCATCAGGCTTCACCTGGGCGT-3'	150	62
ATGL (Adipose triglyceride lipase)	NM_001163689.1	Forward: 5'-TATCCGGTGGATGAAAGAGC-3' Reverse: 5'-CAGTTCCACCTGCTCAGACA-3'	112	62
β-actin	NM_007393.3	Forward: 5'-TGTTACCAACTGGGACGACA-3' Reverse: 5'-CTTTTCACGGTTGGCCTTAG-3'	130	62
DGAT (Diacylglycerol acyltransferase)	NM_010046.2	Forward: 5'-TGGGTGGCCAGGACAGGAGTAT-3' Reverse: 5'-CCAGTGGGACCTGAGCCATCATG-3'	121	62
FABP1 (Fatty acid binding protein 1)	NM_017399.4	Forward: 5'-TCGGTCTGCCGGAAGAGCTCA-3' Reverse: 5'-TGGACCCAGCGGTGATGGTGA-3'	105	62
GK (Glucose kinase)	NM_010292.4	Forward: 5'-TGTCGCAGGTGGAGAGCGACT-3' Reverse: 5'-TCACAGGCACGGCGCACAAT-3'	112	62
GLUT4 (Glucose transporter 4)	NM_009204.2	Forward: 5'-CCACCAGACCCGCCCTTTGC-3' Reverse: 5'-GGGGTTCCCCATCGTCAGAGC-3'	174	62
GLUT5 (Glucose transporter 5)	NM_019741.3	Forward: 5'-CAGCGCAGGCGTGAAAAGCG-3' Reverse: 5'-TGGTGTTCTGCAGCGCCAGT-3'	191	62
HK2 (Hexokinase 2)	NM_013820.3	Forward: 5'-GGGCATGAAGGGCGTGTCCC-3' Reverse: 5'-CCAGGTCAAACTCCTCTCGCCG-3'	182	62
HSL (Hormone sensitive lipase)	NM_010719.5	Forward: 5'-ATGGAGCCGGCCGTGGAATC-3' Reverse: 5'-AACGCTGAGGCTTTGATCTTGCC-3'	119	62
PKLR (Pyruvate kinase: liver/RBC)	NM_013631.2	Forward: 5'-ACATGCGATTGCCCGGGAGG-3' Reverse: 5'-GACCTCGGTTGGGTCACGGC-3'	196	62
PKm (Pyruvate kinase: muscle)	NM_011099.3	Forward: 5'-GCACCTGATTGCCCGAGAGGC-3' Reverse:5'-GGCAGCTTCTGTGGGGTCGC-3'	103	62

Table 2 Cecal microbiota (log CFU/ g cecum content) [1] of DIO mice in response to oral administration of YB[2]

Type	WS	WYB
Total aerobes	7.70 ± 0.78	8.46 ± 0.99
Total anaerobes	7.63 ± 1.19	9.12 ± 0.25
Firmicutes		
Clostridium	5.13 ± 1.24^{b}	8.47 ± 0.10^{a}
Eubacterium	7.57 ± 1.09	8.34 ± 0.32
Lactobacillus	7.96 ± 1.40	9.06 ± 0.25
Bacteroidetes		
Bacteroides	3.51 ± 1.75	5.44 ± 1.23
Actinobacteria		
Bifidobacterium	5.30 ± 1.31^{b}	8.41 ± 0.26^{a}
Bacteroidetes/Firmicutes	0.06 ± 0.06^{b}	0.24 ± 0.04^{a}

[1] CFU: colony-forming unit.

[2] Values for each group are means ± SE (n=5). Different letters represent significant difference in the same row (P<0.05). WS: Western diet + saline. WYB: Western diet + 0.125 μg YB/ g BW.