Lactobacillus paracasei ssp. *paracasei* YBJ01 reduced D-galactose–induced oxidation in male Kuming mice

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ABSTRACT

We investigated in vitro and in vivo antioxidant activity of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (LPSP-YBJ01) isolated and identified from fermented yogurt. Strain LPSP-YBJ01 had stress tolerance against acidity, bile salt, and osmotic pressure. Five in vitro antioxidant assays were used to evaluate antioxidant activity of LPSP-YBJ01, which could scavenge free radicals (2,2-diphenyl-1-picrylhydrazyl and hydroxyl) and superoxide anion in vitro. In addition, strain LPSP-YBJ01 had stronger antilipid peroxidation activity and weak reducing power in vitro. We measured in vivo antioxidant activity of LPSP-YBJ01 in an oxidation mouse model induced by D-galactose injection. Strain LPSP-YBJ01 significantly increased serum superoxide dismutase (SOD), glutathione peroxidase, and total-antioxidant capability, and inhibited generation of malondialdehyde in a dose-dependent manner. In addition, strain LPSP-YBJ01 also increased the hepatic and splenic protein expressions of some antioxidant enzymes such as catalase, Cu/Zn-SOD, and Mn-SOD in mice treated with D-galactose. Thus, LPSP-YBJ01 had antioxidant activity in vitro and in vivo and may be a useful probiotic.

Key words: *Lactobacillus paracasei* ssp. *paracasei* YBJ01, in vitro antioxidant, D-galactose, oxidation mice

INTRODUCTION

As the human lifespan increases, research will be focused on issues facing the elderly, such as oxidative stress, which contributes to tumors, Alzheimer's disease, type 2 diabetes, obesity, cardiovascular disease,

and age-related macular degeneration (Bakala et al., 2012; Badgujar et al., 2016). Under normal physiological condition, free radicals and reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\bullet OH$), and peroxynitrite ($ONOO^-$) are generated from aerobic metabolism (Bonomini et al., 2015); however, an imbalance between endogenous antioxidants and accumulation of ROS causes oxidative stress. Chronic oxidative stress could induce degenerative disorders such as carcinogenesis, rheumatoid arthritis, atherosclerosis, neurodegenerative diseases, and Alzheimer's disease in humans (Chen et al., 2010; Callaway and Jiang, 2015; Castaneda et al., 2017). Normally, excess ROS are scavenged by endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase, and nonenzyme glutathione, coenzyme Q10, and uric acid and perturbations in these systems may cause ROS accumulation and oxidative stress (Chen et al., 2015).

Lactic acid bacteria (LAB) are probiotics (Das and Goyal, 2015). In food manufacturing, LAB are also used as starters for food fermentation. *Lactobacillus paracasei*, *Lactococcus lactis*, and *Lactobacillus plantarum* isolated from fermented products, such as Brazilian kefir and a Sikkim fermented beverage, reportedly had high total antioxidant activity (Leite et al., 2015; Das and Goyal, 2015). Current research suggests diets rich in antioxidants may promote health and prevent disease (He et al., 2017). Thus, we used LAB to study antioxidants to evaluate a potential antioxidant capacity of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (LPSP-YBJ01) and explore underlying mechanisms.

MATERIALS AND METHODS

Experimental Microorganism Strain

Strain LPSP-YBJ01 was isolated from fermented yogurt (Shangrila, Yunnan, China), and *Lactobacillus*

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bulgaricus (CCTCC AB 200048) was obtained from CCTCC (China Center for Type Culture Collection, Wuhan, Hubei, China). Pure cultures were identified according to Gram staining, cell morphology, catalase activity, and 16S rRNA sequencing. Pure cultures of LPSP-YBJ01 were stored at -80°C in 25% (vol/vol) sterile glycerol for later studies.

Determination of Stress Tolerance

In the stress tolerance assay, isolated LPSP-YBJ01 was first cultured using a De Man, Rogosa, and Sharpe (MRS) medium for 18 h at 37°C . Pure-cultured LPSP-YBJ01 and *L. bulgaricus* were centrifuged at $3,000 \times g$ for 10 min at 4°C . Collected pellets were washed twice with PBS buffer (pH 7.4) for later study. For the low-pH tolerance assay, LPSP-YBJ01 and *L. bulgaricus* cells were resuspended in normal saline and cell density was adjusted to 10^9 cfu/mL. The culture mix containing 9 mL of fresh artificial gastric fluid (pH 2.5, adjusted with HCl) and resuspended cells (1 mL) was incubated at 37°C for 0 and 3 h. Strain LPSP-YBJ01 and *L. bulgaricus* low-pH stress was assessed using a plate count method in triplicate. Incubated resuspensions were diluted with sterile deionized water, and diluents were plated on MRS agar plates (Hopebio Co., Qingdao, China). Colony-forming units on each plate were counted after incubating for 48 h at 37°C .

For the bile tolerance assay, collected pellets were resuspended in fresh artificial intestinal fluid [PBS solution containing 0, 0.2, 0.3, and 0.5% (wt/vol) bile salts, pH 8.0] and cell density was adjusted to 1×10^9 cfu/mL. Resuspensions were then incubated at 37°C for 24 h. Resistance to bile salts was assessed using a plate count method in triplicate.

For the osmotic stress tolerance assay, pure cultures of LPSP-YBJ01 and *L. bulgaricus* were inoculated at 2% (vol/vol) onto MRS agar plates containing NaCl at 3, 4, 5, 6, 7, and 8% (wt/vol). After incubation for 18 h at 37°C , viability of LPSP-YBJ01 and *L. bulgaricus* was measured using a plate count method for triplicate test.

In Vitro Antioxidant Activity Assay

In the in vitro antioxidant assay, LPSP-YBJ01 and *L. bulgaricus* cells were first cultured for 24 h at 37°C and then the pure culture was centrifuged at $4,000 \times g$ for 15 min at 4°C . Pellets were washed twice with PBS buffer (pH 7.4), and then resuspended in PBS solution (pH 7.4) to 1.0×10^9 cfu/mL. The prepared cell suspensions were divided into full-cell and intracellular cell-free extract groups. For intracellular cell-free

extract preparation, pure cultures of LPSP-YBJ01 and *L. bulgaricus* were treated with ultrasonic cell disruptor (JY92-IIDN, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, Zhejiang, China) in an ice bath. After removing cell debris by centrifugation ($10,000 \times g$, 15 min, 4°C), the resulting supernatant was obtained as an intracellular cell-free extract of LPSP-YBJ01.

For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, 2 mL of DPPH (200 $\mu\text{mol/L}$) reagent was mixed with 2 mL of samples (full-cell and intracellular cell-free extracts). After 30 min of incubation at room temperature, the reaction mixture was centrifuged at $3,000 \times g$ for 10 min at 4°C and absorbance was measured at 517 nm using an ELX808 microplate reader (BioTek Instruments, Winooski, VT).

For the hydroxyl radical scavenging assay, 0.2 mL of samples were mixed with 1.2 mL of reaction solutions that contained deoxyribose (6 mmol/L), H_2O_2 (3 mmol/L), $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$ buffer (20 mmol/L, pH 7.4), FeCl_3 (400 $\mu\text{mol/L}$), EDTA (400 $\mu\text{mol/L}$), and ascorbic acid (400 $\mu\text{mol/L}$); samples were then incubated at 37°C for 1 h. One milliliter of thiobarbituric acid (TBA)-trichloroacetic acid (TCA) solution was then added to the mixture and heated at 90°C for 30 min. Absorbance of the reaction mixture was measured at 532 nm using a UV-2450S spectrophotometer (Shimadzu Corp., Kyoto, Japan).

For the superoxide anion-scavenging assay, a total reaction system included samples (0.5 mL), 1 mL of Tris-HCl (150 mmol/L, pH 8.2), 1 mL of diethylenetriaminepentaacetic acid (1% wt/vol), and 1 mL of pyrogallol (1.2 mmol/L) and was incubated for 10 min at 25°C . After 10 min, the absorbance of the mixture was measured at 325 nm using a UV-2450S spectrophotometer (Shimadzu).

For the reducing power assay, samples (0.5 mL) were mixed with 0.5 mL of PBS (200 mmol/L, pH 6.6) and 0.5 mL of potassium ferricyanide (1% wt/vol). The mixture was incubated for 20 min at 50°C and then rapidly cooled to 20°C ; subsequently, 0.5 mL of TCA (10% wt/vol) was added. The mixture was centrifuged at $4,000 \times g$ for 10 min at 4°C . The upper layer (1 mL) was mixed with 1 mL of ferric chloride (0.1% wt/vol) and 1 mL of deionized water. After 10 min, the absorbance of the mixture was measured at 700 nm using a UV-2450S spectrophotometer (Shimadzu).

For the lipid peroxidation assay, 0.5 mL of sample was mixed with 0.5 mL of PBS (pH 7.4), 1 mL of linoleic acid, and 1 mL of FeSO_4 (1% wt/vol) and incubated for 90 min at 37°C . The reaction mixture was then mixed with 0.2 mL of TCA (4% wt/vol) and 2 mL of TBA (0.8% wt/vol), incubated for 30 min at 100°C , and centrifuged ($4,000 \times g$ for 15 min at 25°C). The

absorbance of the mixture was measured at 532 nm using a UV-2450S spectrophotometer (Shimadzu).

Animal Study and Grouping

A total of 60 Kunming (KM) mice (7 wk of age, male, 20–25 g) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China), animal permit number: SCXK (Yu) 2012–0001. All experimental KM mice were maintained in a temperature- and humidity-controlled (temperature = $25 \pm 2^\circ\text{C}$, relative humidity = $50 \pm 5\%$) facility with a 12-h light/dark cycle and free access to a standard rodent AIN-93G chow diet and water. Following 1 wk of adaption to the laboratory environment, all mice were randomized into 6 groups ($n = 10/\text{group}$): normal, oxidation model, vitamin C, and LPSP-YBJ01 cell-treated groups [low dose = 5×10^8 cfu/kg (L); middle dose = 5×10^9 cfu/kg (M); high dose: 5×10^{10} cfu/kg (H)]. Except for normal mice, other mice were treated with an intraperitoneal injection of D-galactose (125 mg/kg) once daily for 6 wk. Normal mice were given normal saline at the same dose and frequency. The vitamin C group was treated with oral gavage (200 mg/kg) once daily for 6 wk. The LPSP-YBJ01 cell-treated mice were treated via oral gavage (0.2 mL of LPSP-YBJ01 cell solution) once daily for 6 wk. All mice were given the same diet during the experiments. The present study was approved by the Animal Ethics Committee of Southwest University.

Blood Sampling and Organ Collection

All mice were euthanized using CO_2 , and blood was collected from the central vein at the end of the experiment. The collected blood was centrifuged ($3,000 \times g$, 10 min, 25°C) to prepare serum. The serum samples were stored at -80°C for further study. The main organs, including the heart, liver, spleen, and kidney, were removed from each mouse, weighed quickly, frozen using liquid nitrogen, and stored at -80°C for further study.

Histological Observations

Five samples of liver from each mouse were subjected to histological examination. The liver tissues were fixed in 10% (vol/vol) neutral buffered formalin, dehydrated in ethanol, and embedded in paraffin. Sections (4- μm thick) were then prepared and stained with hematoxylin and eosin. Images were acquired using a Zeiss Axioskop 2 Plus microscope (Carl Zeiss MicroImaging, Thornwood, NY) equipped with an AxioCam MRC5 CCD camera (Carl Zeiss).

Biochemical Assay

Serum superoxide dismutase, GSH-Px, total antioxidant capability (T-AOC), and malondialdehyde (MDA) were measured using a commercial assay kit (Cayman Chemical Co., Ann Arbor, MI) according to kit instructions. Serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (T-CHO) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) according to the manufacturer's instructions (Pan et al., 2018).

Protein Extraction and Western Blot Analysis

One hundred milligrams of liver or spleen tissue samples was washed with ice-cold PBS, homogenized with ice-cold radioimmunoprecipitation assay buffer, and then centrifuged at $12,000 \times g$ for 20 min at 4°C . Protein was measured using a Bio-Rad protein assay kit (Hercules, CA). For Western blot, aliquots of homogenate (50 μg of protein) were resolved with SDS-PAGE and transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). The blots were incubated with antibodies against catalase, Cu/Zn-SOD and Mn-SOD obtained from Cell Signaling Technologies (Beverly, MA) and then incubated with horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technologies) for 1 h at room temperature. The blots were washed 3 times with PBS containing 0.05% vol/vol Tween 20 (PBS-T), and antibody binding was visualized with ECL (GE Healthcare Life Sciences, Little Chalfont, UK). Protein expression was quantified using ImageJ software (NIH, Bethesda, MD; <http://rsb.info.nih.gov/ij/>).

Statistical Analysis

All data are presented as the means $\pm$ standard deviations. Differences between means for individual groups were assessed by one-way ANOVA and Duncan's multiple range tests. Differences were considered significant when $P < 0.05$. SAS v9.1 statistical software package (SAS Institute Inc., Cary, NC) was used to perform analyses.

RESULTS

Tolerance to Low pH, Bile Salts, and Sodium Chloride

Data regarding survival of LPSP-YBJ01 and *L. bulgaricus* in artificial gastric fluid and growth efficiency

of LPSP-YBJ01 and *L. bulgaricus* in artificial intestinal fluid show that (Figure 1) LPSP-YBJ01 is well tolerated in artificial intestinal fluid and the effect is better than *L. bulgaricus* (Figure 1A). Increased bile salt decreased growth efficiency, but growth occurred with 0.5% bile salt. Tolerance of LPSP-YBJ01 to NaCl (Figure 1B) and growth efficiency were also better than that of *L. bulgaricus*. Strain LPSP-YBJ01 and *L. bulgaricus* survivals at low pH are seen Figure 1C. At low pH (pH = 3.0), LPSP-YBJ01 cell counts were higher better than that of *L. bulgaricus*, which show the superiority of LPSP-YBJ01 in resisting the gastrointestinal environment effect compared to *L. bulgaricus*.

In Vitro Antioxidant Activity of LPSP-YBJ01

We used 5 antioxidant assays to evaluate antioxidant activity of whole LPSP-YBJ01 and *L. bulgaricus* cells and intracellular cell-free extracts of LPSP-YBJ01 and *L. bulgaricus*. Figure 2 shows whole cells had more ability to scavenge free radical (DPPH and hydroxyl radical) and superoxide anion than intracellular cell-free extracts of LPSP-YBJ01 and *L. bulgaricus*. In addition, whole cells had more antilipid peroxidation capacity than extracts. However, intracellular cell-free extracts of LPSP-YBJ01 had greater reducing power than LPSP-YBJ01 cells, and intracellular cell-free extracts of *L. bulgaricus* also had greater reducing power than *L. bulgaricus* cells; the antioxidant effects of LPSP-YBJ01 were better than *L. bulgaricus*.

Body Weight of D-Galactose-Treated Mice

As shown in Figure 3, the mice in normal group had BW of 49.0 ± 5.5 g, higher than other group, with mice in model group having the lowest BW (42.8 ± 0.5 g). The vitamin C, LPSP-YBJ01-L, LPSP-YBJ01-M, and LPSP-YBJ01-H mice had BW of 46.0 ± 5.3 , 43.9 ± 0.4 , 45.4 ± 4.5 , and 47.7 ± 1.6 g, respectively.

Effect of LPSP-YBJ01 on Serum SOD, GSH-Px, T-AOC and MDA in D-Galactose-Treated Mice

No mice died during the study and D-galactose treatment induced decreased organ weight, but this organ reduction was not significantly different from that of controls (Table 1). Compared with model mice, LPSP-YBJ01 treatment increased 4 kinds of main organ indices. Following treatment with a high dose (5×10^{10} cfu/kg per day) of LPSP-YBJ01, the organ index increased to 6.05 ± 0.82 mg/g in heart, 46.69 ± 3.13 mg/g in liver, 2.56 ± 0.45 mg/g in spleen, and 16.96 ± 0.91 mg/g in kidney, respectively.

Figure 1. Tolerance (means $\pm$ SD) of *Lactobacillus paracasei* ssp. *paracasei* (LPSP) YBJ01 and *Lactobacillus bulgaricus* in bile salt (A), with NaCl (B), and low pH (C).

Strain LPSP-YBJ01 effects on antioxidant capacity data for D-galactose-treated mice appear in Figure 4. D-Galactose treatment significantly reduced serum SOD, GSH-Px, and T-AOC ($P < 0.05$) compared with controls. Compared with D-galactose-treated mice, serum SOD of LPSP-YBJ01-treated groups were significantly increased in a dose-dependent manner ($P < 0.05$). The LPSP-YBJ01 treatment increased serum T-AOC com-

Figure 2. Antioxidant activity (means $\pm$ SD) of *Lactobacillus paracasei* ssp. *paracasei* (LPSP) YBJ01 and *Lactobacillus bulgaricus* in vitro. Bars with different letters (a–d) are significantly different ($P < 0.05$) according to Duncan's multiple range test. DPPH = 2,2-diphenyl-1-picrylhydrazyl.

pared oxidation control mice ($P < 0.05$); in addition, LPSP-YBJ01 reduced serum MDA in oxidation mice but we found no significant difference between LPSP-

YBJ01 strain and vitamin C-treated mice ($P < 0.05$). Finally, LPSP-YBJ01 treatment did not change serum GSH-Px in D-galactose-treated mice.

Figure 3. Body weight (means $\pm$ SD) of mice on the last day of the experiment. Bars with different letters (a,b) are significantly different ($P < 0.05$) according to Duncan's multiple range test. VitC = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection (125 mg/kg per day); LPSP-YBJ = mice treated with increasing doses (L, M, H) of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

Effect of LPSP-YBJ01 on Serum TG, T-CHO, HDL-C and LDL-C in D-Galactose-Treated Mice

Table 2 shows that D-galactose treatment significantly increased serum TG in serum oxidation mice. In the LPSP-YBJ01-treated group, TG in LPSP-YBJ01-L was lower than that in LPSP-YBJ01-M and LPSP-YBJ01-H ($P < 0.05$); D-galactose also increased serum T-CHO compared with controls. In LPSP-YBJ01 strain and with vitamin C, serum T-CHO decreased but we found no difference between groups ($P < 0.05$). In oxidation mice, HDL-C was the lowest of all groups. Strain LPSP-YBJ01 had higher HDL-C ($P < 0.05$) than that of the control group, but oxidation mice treated with vitamin C had the greatest ($P < 0.05$) HDL-C. Finally, LPSP-YBJ01 and vitamin C did not significantly influence serum LDL-C ($P < 0.05$).

Effect of LPSP-YBJ01 on Hepatic Histological Observation in D-Galactose-Treated Mice

Histology was used to evaluate the effect of LPSP-YBJ01 strain on D-galactose-induced liver damage in

Table 1. Effect of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 on organ indices in D-galactose-treated mice¹

Group	Heart (mg/g)	Liver (mg/g)	Spleen (mg/g)	Kidney (mg/g)
Normal	5.95 ± 0.60	46.53 ± 2.43	2.66 ± 0.45	16.55 ± 2.44
Model	4.85 ± 0.41	41.85 ± 1.55	2.49 ± 0.88	13.79 ± 0.85
LPSP-YBJ01-L	6.06 ± 1.36	45.08 ± 2.70	2.49 ± 0.42	15.15 ± 2.05
LPSP-YBJ01-M	5.56 ± 0.65	46.10 ± 3.07	2.89 ± 0.75	17.08 ± 0.98
LPSP-YBJ01-H	6.05 ± 0.82	46.69 ± 3.13	2.56 ± 0.45	16.96 ± 0.91
Vitamin C	5.76 ± 1.36	44.31 ± 2.44	2.40 ± 0.25	16.25 ± 1.65

¹Data are means ± SD. Normal = normal mice; Model = mice treated with D-galactose injection; Vitamin C = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection (125 mg/kg per day); LPSP-YBJ = mice treated with increasing doses (L, M, H) of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

Figure 4. Effects (means ± SD) of *Lactobacillus paracasei* ssp. *paracasei* (LPSP) YBJ01 on serum superoxide dismutase (SOD; A), glutathione peroxidase (GSH-Px; B), total antioxidant capacity (T-AOC; C), and malondialdehyde (MDA; D) in D-galactose-treated mice. Bars with different letters (a–d) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Normal = normal mice; Model = mice treated with D-galactose injection; Vitamin C = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection (125 mg/kg per day); LPSP-YBJ = mice treated with increasing doses (L, M, H) of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

Table 2. Effect of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 on serum measurements in D-galactose (125 mg/kg per day)-treated mice¹

Group	TG (mmol/L)	T-CHO (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Normal	0.88 ± 0.06 ^c	2.53 ± 0.85	2.88 ± 0.24 ^{ab}	0.28 ± 0.09
Model	1.59 ± 0.35 ^a	3.18 ± 0.23	2.27 ± 0.61 ^b	0.33 ± 0.04
LPSP-YBJ01-L	0.97 ± 0.10 ^c	2.95 ± 1.14	2.57 ± 0.72 ^{ab}	0.31 ± 0.15
LPSP-YBJ01-M	1.07 ± 0.13 ^{bc}	3.02 ± 0.09	2.59 ± 0.49 ^{ab}	0.31 ± 0.11
LPSP-YBJ01-H	1.28 ± 0.14 ^b	2.83 ± 0.65	2.80 ± 0.04 ^{ab}	0.28 ± 0.03
Vitamin C	1.28 ± 0.09 ^b	3.04 ± 1.23	3.24 ± 0.53 ^a	0.29 ± 0.02

^{a-c}Columns with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

¹Data are means ± SD. TG = triglyceride; T-CHO = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Normal = normal mice; Model = mice treated with D-galactose injection; Vitamin C = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection (125 mg/kg per day); LPSP-YBJ = mice treated with increasing doses (L, M, H) of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

oxidation mice, and we found no significant difference in liver appearance between LPSP-YBJ01 and vitamin C groups and controls. Figure 5 shows that daily injection of D-galactose (125 mg/kg per day) significantly induced visible structural damage in mice livers, and both LPSP-YBJ01 treatments reduced this chemical damage. In the vitamin C-treated group, liver damage was less severe than in controls (only D-galactose treatment) and in LPSP-YBJ01-L mice.

Effects of LPSP-YBJ01 on Hepatic Catalase, Cu/Zn-SOD, and Mn-SOD Protein in Mice

D-Galactose treatment significantly reduced hepatic catalase, Cu/Zn-SOD, and Mn-SOD protein in mice ($P < 0.05$). Treatment with LPSP-YBJ01 increased Cu/Zn-SOD and Mn-SOD protein expression in livers of oxidation mice (Figure 6). However, catalase after the middle dose of LPSP-YBJ01 (LPSP-YBJ01-M) treat-

Figure 5. Effect of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 on morphological changes of liver tissue in D-galactose-treated mice. Normal = liver sections from controls; Model = mice treated with D-galactose (125 mg/kg per day); VitC = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection; LPSP-YBJ01 = mice treated with increasing doses (L, M, H) of *L. paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection. Original magnification 100×. Color version available online.

ment was lower than in the higher and lower treatment groups. In the vitamin C-treated group, hepatic Cu/Zn-SOD expression was higher than in the LPSP-YBJ01-treated group. However, Mn-SOD protein in the vitamin C-treated group was lower in LPSP-YBJ01-treated mice.

Effects of LPSP-YBJ01 on Splenic Catalase, Cu/Zn-SOD and Mn-SOD Protein in Mice

Figure 7 shows that D-galactose treatment significantly reduced catalase, Cu/Zn-SOD, and Mn-SOD protein in mouse spleens. Strain LPSP-YBJ01 increased splenic protein expression in oxidation mice, but Mn-SOD in LPSP-YBJ01-M was lower than in LPSP-YBJ01-L and LPSP-YBJ01-H groups. Splenic catalase, Cu/Zn-SOD, and Mn-SOD protein in vitamin C-treated groups were

greater than those in LPSP-YBJ01-treated oxidation mice.

DISCUSSION

Lactic acid bacteria are key to food manufacturing and maintaining human or animal health (Laiño et al., 2015; Landete et al., 2017), and LAB strains isolated from fermented foods may have antioxidant activity (Das and Goyal, 2015; Leite et al., 2015). Thus, we focused on studying antioxidant activity of LPSP-YBJ01 strain. First, we observed that LPSP-YBJ01 was highly tolerant to low pH and bile salts in vitro as compared with common *L. bulgaricus* used as reference strain. These data agreed with previous studies, which suggested that *L. paracasei* strains had acid and bile salt stress tolerance (Desmond et al., 2004). In addition,

Figure 6. Effects of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 on protein expression of catalase, Cu/Zn-superoxide dismutase (SOD) and Mn-SOD in the liver of Kuming (KM) mice treated with D-galactose (125 mg/kg per day). Proteins were quantified and normalized relative to β -actin (internal control). Data are expressed as means $\pm$ SD. Bars with different letters (a–d, A–D) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Normal = normal mice; Model = mice treated with D-galactose injection (125 mg/kg per day); VitC = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection; LPSP-YBJ01 = mice treated with increasing doses (L, M, H) of *L. paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

LPSP-YBJ01 could grow in a high-NaCl environment, indicating that LPSP-YBJ01 strain has tolerance to osmotic stress.

A D-galactose-induced oxidation mice model has been used to evaluate antioxidation strategies in vivo (Li et al., 2012). D-Galactose is a reducing sugar and naturally occurring substance in the body. During normal physiology, accumulation of D-galactose was completely metabolized to aldose, hydrogen peroxide, and galactose oxidase and generated oxygen-derived free radicals, superoxide anion, and other ROS. D-Galactose-induced oxidation-related changes included increased generation of protein carbonyls and MDA, DNA fragmentation, antioxidant enzyme inactivation, inflammation, and apoptosis (Li et al., 2014; Nita and Grzybowski, 2016).

After the oxidative senescence of the body, a weak body could lead to weight loss (Kong et al., 2018). This

was seen our study, as mice in the model group showed lower BW than that of mice in the normal group, and LPSP-YBJ01 could inhibit BW loss.

The liver is important for detoxification and health maintenance, and it is affected by ROS-induced oxidative stress. In this study, D-galactose significantly damaged hepatic structures in oxidation mice. However, when given with LPSP-YBJ01 strain, D-galactose-induced histological changes were reduced. Accumulation of ROS and free radicals is a trait of oxidation (Rein-salo et al., 2015), and both can induce dysfunction of the endogenous antioxidant system, and indirectly cause oxidative damage. Reduction of ROS and free radical accumulation may prevent and treat age-related disorders (Reiter et al., 2000). We found that D-galactose injection did not affect animal organ weight organs (heart, liver, spleen, and kidney), but it decreased SOD, GSH-Px, and T-AOC in sera of oxidation mice.

Figure 7. Effects of *Lactobacillus paracasei* ssp. *paracasei* YBJ01 on protein expression of catalase, Cu/Zn-superoxide dismutase (SOD) and Mn-SOD in the spleen of Kuming (KM) mice treated with D-galactose (125 mg/kg per day). Proteins were quantified and normalized relative to β -actin (internal control). Data are means $\pm$ SD. Bars with different letters (a–d, a–e, A–E) are significantly different ($P < 0.05$) according to Duncan's multiple range test. Normal = normal mice; Model = mice treated with D-galactose injection (125 mg/kg per day); VitC = mice treated with vitamin C (200 mg/kg per day) after D-galactose injection; LPSP-YBJ01 = mice treated with increasing doses (L, M, H) of *L. paracasei* ssp. *paracasei* YBJ01 (5×10^8 , 5×10^9 , and 5×10^{10} cfu/kg per day) after D-galactose injection.

Strain LPSP-YBJ01 significantly increased serum SOD and T-AOC in oxidation mice. Wang et al. (2016) reported that *Lactobacillus paracasei* FM-LP-4 was able to increase the SOD and GSH-Px levels in main organs (liver and kidney) of D-galactose-treated mice. In addition, *Lactobacillus casei* Zhang treatment also increased the hepatic levels of SOD in D-galactosamine-treated rats (Wang et al., 2013). Endogenous antioxidants, such as SOD, GSH-Px, and other antioxidant enzymes (catalase, glutathione S-transferases), reduced over-generated ROS and free radicals (Sakiyama et al., 2016); however, LPSP-YBJ01 did not change serum GSH-Px in oxidation mice. In addition, LPSP-YBJ01 strain treatment significantly reduced serum MDA in oxidation mice, which is a biomarker of membrane lipid oxidation (Sarniak et al., 2016). Our data were similar to those from other probiotic strain studies (Speakman and Selman, 2011; Wang et al., 2013, 2016; Solieri et al., 2014; Van Raamsdonk et al., 2017).

We also observed that hepatic and splenic catalase was increased in LPSP-YBJ01-treated oxidation mice. Catalase decomposes H_2O_2 to H_2O and O_2 in all living organisms and exhibits a protective effect against oxidative injury (Foussal et al., 2010). Increasing activity of catalase attenuated the oxidative stress-induced liver damage in mice (Lai et al., 2008). In addition, LPSP-YBJ01 strain increased protein expression of antioxidant enzymes Cu/Zn-SOD and Mn-SOD in liver and spleen of oxidation mice, respectively. Both Cu/Zn-SOD and Mn-SOD are antioxidant gatekeepers and scavenge superoxide anion and other radicals (Wouters et al., 2013). Activation of Cu/Zn-SOD reduced oxidative stress induced accumulation of hepatic collagen (Ye et al., 2014), and oxidative damage and inflammation in mice (Zhang et al., 2007). Thus, LPSP-YBJ01 strain can reduce D-galactose-induced oxidative damage in livers of oxidation mice.

CONCLUSIONS

We found that LPSP-YBJ01 strain with antioxidant activity was protective against D-galactose-induced liver damage in male KM mice, and this may be due to reduced oxidative stress. Data may suggest novel insights into mechanisms underlying hepatoprotective effects of LPSP-YBJ01. More work is needed to learn whether LPSP-YBJ01 is useful for age-related disease prevention.

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