***Lactobacillus plantarum* YS2 (yak yogurt *Lactobacillus*) exhibited an activity to attenuate activated carbon-induced constipation in male Kunming mice**

Xin Zhao,^{1,2,3,4} Yu Qian,^{1,2,3,4} Guijie Li,^{1,2,3,4} Ruokun Yi,^{1,2,3,4} Kun-Young Park,^{1,5*} and Jia-Le Song^{1,6*}

¹Chongqing Collaborative Innovation Center for Functional Food,

²Chongqing Engineering Research Center of Functional Food,

³Chongqing Engineering Laboratory for Research and Development of Functional Food, and

⁴College of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, P. R. China

⁵Department of Food Science and Biotechnology, Cha University, Seongnam, Gyeonggi 13488, Korea

⁶Department of Nutrition and Food Hygiene, School of Public Health, Guilin Medical University, Guilin, Guangxi 541004, P. R. China

ABSTRACT

The present study investigated the effects of *Lactobacillus plantarum* YS2 (LP-YS2) that was isolated from yak yogurt on activated carbon-induced constipation in Kunming (KM) mice. The KM mice were orally administered LP-YS2 and reference strain *Lactobacillus delbrueckii* ssp. *bulgaricus*. Administration of LP-YS2 [1.0×10^9 cfu/kg of body weight (BW)] promoted gastrointestinal peristalsis and reduced the first black stool defecation time (129 min), which clearly defines attenuation of the voiding difficulty in mice with constipation. The LP-YS2 treatment also increased the serum level of motilin (MTL; 178.2 pg/mL), gastrin (69.4 pg/mL), acetylcholine (Ach; 30.1 pg/mL), substance P (SP; 57.6 pg/mL), and vasoactive intestinal peptide (VIP; 53.2 pg/mL) and reduced the somatostatin (SS, 32.6 pg/mL) levels compared with the *L. delbrueckii* ssp. *bulgaricus* treatment (MTL, 139.7 pg/mL; gastrin, 43.1 pg/mL; Ach, 15.9 pg/mL; SP, 43.6 pg/mL; VIP, 32.3 pg/mL; SS, 55.1 pg/mL) and the control (MTL, 105.3 pg/mL; gastrin, 26.7 pg/mL; Ach, 9.7 pg/mL; SP, 30.2 pg/mL; VIP, 21.0 pg/mL; SS, 70.5 pg/mL). The LP-YS2 treatment significantly increased the colonic mRNA and protein expression of c-Kit (CD117, cluster of differentiation 117; 2.87 times mRNA expression of the control group), stem cell factor (30.40 times mRNA expression of the control group), and glial cell-derived neurotrophic factor (29.97 times mRNA expression of the control group) in mice with constipation. In addition, LP-YS2 reduced the expression of transient receptor potential vanilloid 1 (0.42 times mRNA expression of the control group) and nitric oxide synthase (0.49 times mRNA expression of the control group) in constipated mice. These results demonstrate that LP-YS2

was able to attenuate the activated carbon-induced constipation in KM mice.

Key words: *Lactobacillus fermentum* YS2, activated carbon, constipation, gastrointestinal transit

INTRODUCTION

Chronic constipation is a very common disease that is mainly observed in geriatric populations and children. The main reasons for constipation are unhealthy lifestyle (e.g., bad eating and defecation habits, low exercise), psychological factors, and some digestive diseases (Ueki and Otsuka, 2004; Basilisco and Coletta, 2013). The clinical treatment for constipation is administration of laxatives and prokinetic agents (5-HT modulators, motilin agonists, chloride-channel activators, and opioid antagonist; Shin et al., 2014). However, long-term intake of these anticonstipation drugs may cause drug dependence and be economically unfeasible in terms of both direct and indirect health care costs (Crowell et al., 2009). Constipation is generally not considered a disease; in most cases, it can be relieved by regulating diet and correcting some bad habits (Webster et al., 2016).

Nutritional therapy such as diet modulation has become a new idea for constipation treatment. Fiber-rich foods, including cereals, whole grains, vegetables, and fruits, help increase stool bulk and accelerate small intestine passage (Bae, 2014; Wang et al., 2015). Probiotics (e.g., bifidobacteria and lactobacilli strains) also help treat constipation by stimulating motility and peristalsis (Li et al., 2015; Wang et al., 2017). Some literature reviews have also suggested that probiotics (*Bifidobacterium* and *Lactobacillus*) improve whole-gut transit time, stool frequency, and stool consistency and reduce constipation simultaneously (Chmielewska and Szajewska, 2010; Mazlyn et al., 2013; Dimidi et al., 2014).

Yak yogurt is a natural fermented food that is very common in the minority areas of the Qinghai Tibet

Received June 13, 2018.

Accepted July 29, 2018.

*Corresponding authors: kunypark@cha.ac.kr and songjiale@glmc.edu.cn

plateau. Yak yogurt exhibits antioxidant activities, reduces cholesterol, and improves immunity (Wu et al., 2012b). Because of the special natural fermentation environment of Qinghai Tibet Plateau, fermented yak yogurt is produced the unique Tibetan way, including special microbial fermentation (Wu et al., 2012a). The *Lactobacillus* strains play very important roles, including promoting probiotic activity, acting as a leavening agent, and reducing harmful substances during food fermentation (Fei et al., 2014; Swain et al., 2014; Song et al., 2016). In our previous study, 2 kinds of important *Lactobacillus*—*L. fermentum* and *L. casei*, isolated from Ngawa Prefecture (Sichuan Province, China)—exhibited great activity to reduce activated carbon-induced constipation in mice (Suo et al., 2014; Qian et al., 2015; Zhao et al., 2015a,b). Recently, Li et al. (2015) reported that *Lactobacillus plantarum* NCU116 showed activity to attenuate loperamide-induced constipation in mice. However, the effect of *Lactobacillus plantarum* YS2 (LP-YS2) on activated carbon-induced constipation in mice has not been studied. *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) is one of the most widely used fermentation strains in industrial fermented yogurt (Suo et al., 2014). *Lactobacillus plantarum* YS2 was also isolated and identified from yak yogurt, and it could be used as a fermentation strain in industrial fermented yogurt. Compared with commercially available lactic acid bacteria of LB, LP-YS2 showed better survival in pH 3.0 artificial gastric juice and growth in bile salt; LP-YS2 could enter the intestines and colonize more effectively. Therefore, LP-YS2 might be used as a probiotic with better biological activity potential. Human clinical and animal studies showed that probiotic Minas Frescal cheese also had certain effects on hypertension and could reduce blood pressure (Lollo et al., 2015; Sperry et al., 2018) and that probiotic cheese could attenuate immune suppression induced by exhausting exercise in vivo (Lollo et al., 2012). These effects come from living probiotics—living microorganisms with biological activity. However, metabolic by-products, dead microorganisms, and other microbial-based, nonviable products do not fall under the probiotic construct (Hill et al., 2014). This study investigated the activity of living LP-YS2 on activated carbon-induced constipation in Kunming (KM) mice; LB was used as a comparison strain. The results provide scientific evidence that LP-YS2 helps prevent constipation and can be developed as a functional food.

MATERIALS AND METHODS

The present study was approved by the Animal Ethics Committee of Chongqing University of Education [Chongqing, China; SYXK (Yu) 2017-0001].

Experimental Microorganism Strains

Lactobacillus plantarum YS2 (M2016748), preserved in China Center for Type Culture Collection (Wuhan, China), was identified from yak yogurt produced in Yushu (Qinghai Province, China) by our previous unpublished work. *Lactobacillus delbrueckii* ssp. *bulgaricus* (AB200048), which is used in yogurt manufacture, was purchased from China Center for Type Culture Collection and used as a reference strain.

Acid and Bile Salt Tolerance Activity

The LP-YS2 and LB bacterial De Man, Rogosa and Sharpe medium (5 mL) was collected by centrifugation ($3,000 \times g$, 10 min, 25°C). The collected bacterial pellet was resuspended using sterile saline (5 mL), after which 1 mL of suspension was mixed with 9 mL of sterile artificial gastric juice (0.2% NaCl, 0.35% pepsin; pH 3.0) and incubated in a thermostatic oscillator at 37°C and 300 rpm. Subsequently, a sample (200 μ L) was mixed by pipetting thoroughly and incubated with a *Lactobacillus* De Man, Rogosa and Sharpe agar plate at 37°C for 48 h. After 48 h, the number of colony-forming units was counted and bacterial survival rate was calculated (Qian et al., 2015).

Experimental Constipation Model in KM Mice

A total of 50 KM mice (7 wk old, male, BW: 24.43 ± 0.39 g) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). The KM mice were maintained in a temperature ($25 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$) controlled facility with a 12-h light–dark cycle and had free access to a standard rodent AIN-93G chow diet (Jiangsu Medicine Ltd., Yangzhou, Jiangsu, China) and water. The constipation model in KM mice was as described in our previous study (Fei et al., 2014; Qian et al., 2015; Song et al., 2016). To induce the experimental constipation model, all mice were divided into 5 groups as follows. The normal and control groups received a normal diet for 17 d, whereas the LP-YS2 low-dose (LP-YS2-L; 1.0×10^8 cfu/kg of BW), LP-YS2 high-dose (LP-YS2-H; 1.0×10^9 cfu/kg of BW), and LB (1.0×10^9 cfu/kg of BW) groups received a normal diet and were orally administered LP-YS2 or LB at the same time. The control and treatment groups were orally administered 0.2 mL of 10% activated carbon (dissolved in 10% gum arabic) at 1800 h on a daily basis from d 14 to d 17 to induce constipation. Body weight, dietary and water intake, and stool weight and moisture were measured at 0900 h daily (Li et al., 2017).

Determination of Gastrointestinal Transit and Defecation Time

All mice were first fasted for 16 h with free access to water. The mice in the control and treatment groups received an oral administration of 10% activated carbon, whereas the mice in the normal group received an oral administration of 10% gum arabic. A total of 5 mice from each group were killed by CO₂ following 30 min of treatment, and their small intestines were collected carefully. The gastrointestinal transit of each mouse was calculated as the percentage of the distance the activated carbon meal traveled relative to the total length of the small intestine. The stools of mice were collected, and tool weight, particle counts of stool, and water content of stool of mice were further observed during the experiment. The gastrointestinal transit was calculated according to the following formula: distance the activated carbon traveled/total length of the small intestine × 100%. In addition, the remaining 5 mice from each group were used to measure the time of the first black stool defecation.

Measurement of Serum Gastrointestinal Motility-Related Biomarkers

All mice were killed using CO₂, and blood was collected from the central vein. The collected blood was centrifuged (3,000 × g, 10 min, 25°C) to prepare the serum. Next, serum levels of motilin (MTL), gastrin, substance P (SP), somatostatin (SS), vasoactive intestinal peptide (VIP), and acetylcholine (Ach) were determined by RIA kits (Beijing Puer Weiye Biotechnology Co. Ltd., Beijing, China) according to the manufacturer’s protocols.

Measurement of Antioxidant-Related Biomarkers in Small Intestine Tissue

The colon tissues were collected from all animals that were killed using CO₂. After washing with ice-cold saline, colon tissues (100 mg) were homogenized using PBS on ice. The colonic levels of myeloperoxidase (MPO), nitric oxide (NO), glutathione (GSH), and malondialdehyde (MDA) were determined by commercial kits (Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer’s protocols.

Total RNA Extraction and Quantitative Real-Time PCR Assay

The colon tissues were collected from all the treated animals and washed with normal saline. Then, total RNA was extracted using Trizol reagent (Invitrogen,

Carlsbad, CA) according to the manufacturer’s instructions. The extracted RNA (1 µg) was mixed with a cocktail reagent that contained oligo dT18 (1 µL), RNase (1 µL), deoxyribonucleotide triphosphates (1 µL), M-MLV (Moloney murine leukemia virus, Thermo Fisher Scientific, Waltham, MA) enzymes (1 µL), and 5× buffer (10 µL) to synthesize cDNA (37°C at 120 min, 99°C at 4 min, and 4°C at 3 min). The cDNA (2 µL) was mixed with total primer (10 µmol/L, 2 µL), 2× SYBR Premix Ex Taq II (10 µL), 50× ROX reference dye (0.4 µL; Thermo Fisher Scientific), and double distilled H₂O (5.6 µL), and PCR (Table 1) was performed in an automatic thermocycler (QuantStudio 6 Flex PCR, Life Technologies, Gaithersburg, MD) for 40 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 50 s, followed by 10 min at 75°C. The relative transcription levels of the mRNA were calculated according to the 2^{−ΔΔC_T} formula (Berstad et al., 2016).

Protein Extraction and Western Blot Analysis

The colon tissues were collected from all animals and washed with ice-cold normal saline. Total protein samples were extracted using a radioimmunoprecipitation assay buffer (Easybio, Beijing, China) on ice, centrifuged at 13,000 × g for 30 min at 4°C, and denaturation by boiling (98°C). The protein concentrations of extracted samples were determined using a Quartzzy Quick Start Bradford Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). Total protein (30 µg) was separated by 10 to 12% SDS-PAGE (Schleicher and Schuell, Keene, NH) for 4 h and transferred to nitrocellulose membranes. Membranes were sealed for 3 h in sealing fluid, washed 3 times with PBS containing 0.05% Tween 20 (PBS-T), and incubated with primary antibodies against CD117, cluster of differentia-

Table 1. Sequences of reverse-transcription PCR primers used in this study

Gene name ¹	Sequence
<i>cKit</i>	Forward: 5'-CATAGCCCAGGTAAAGCACAAT-3' Reverse: 5'-GAACACTCCAGAATCGTCAACTC-3'
<i>Scf</i>	Forward: 5'-TCAGGGACTACGCTGCGAAAG-3' Reverse: 5'-AAGAGCTGGCAGACCGACTCA-3'
<i>Trpv1</i>	Forward: 5'-CCGGCTTTTGGGAAGGT-3' Reverse: 5'-GAGACAGGTAGGTCCATCCAC-3'
<i>Gdnf</i>	Forward: 5'-GGGGTATGGAGAAGTTGGCTAG-3' Reverse: 5'-CTATGAGAATGCTGCCGAAAA-3'
<i>Nos</i>	Forward: 5'-CAGCGAACGACGGCAAGCA-3' Reverse: 5'-TGACACGACCAGCGGCAGGAT-3'
<i>Gapdh</i>	Forward: 5'-TGCACCACCAACTGCTTAG-3' Reverse: 5'-GATGCAGGGATGATGTTTC-3'

¹*cKit* = CD117, cluster of differentiation 117; *Scf* = stem cell factor; *Trpv1* = transient receptor potential cation channel subfamily V member 1; *Gdnf* = glial cell-derived neurotrophic factor; *Nos* = nitric oxide synthase.

tion 117 (**c-Kit**; cat. no. ab62154; Abcam, Cambridge, MA), stem cell factor (**SCF**; cat. no. ab83866; Abcam), transient receptor potential vanilloid 1 (**TRPV1**; cat. no. ACC-030, Alomone, Beijing, China), glial cell-derived neurotrophic factor (**GDNF**; cat. no. ab18956; Abcam), NO synthase (**NOS**; cat. no. sc-49058; Santa Cruz Biotechnology, Dallas, TX), and β -actin (cat. no. ab8226; Abcam) for 2 h. After washing with PBS-T, protein blots were then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Cell Signaling Technology Inc., Danvers, MA) for 1 h at room temperature (Moura et al., 2016). Following washing 3 times with PBS-T, the blots were incubated with enhanced chemiluminescence horseradish peroxidase substrates (Millipore, Billerica, MA) and determined by GelDoc XR System (Bio-Rad). The densitometry analysis of bands was performed using an Image J program (NIH Image, Bethesda, MD).

Statistical Analysis

The experimental data were presented as mean $\pm$ standard deviation. Differences between the mean values for each group were assessed by 1-way ANOVA with Duncan's multiple range tests; $P < 0.05$ was considered an indication for a statistically significant difference. The statistical software package SAS version 9.1 (SAS Institute Inc., Cary, NC) was used for the analysis.

RESULTS

Acid and Bile Salt Resistance Activity

Following exposure to the artificial gastric juice, LP-YS2 exhibited higher ($P < 0.05$) gastrointestinal survival ability than did LB. In addition, LP-YS2 showed greater bile salt resistance activity than did LB (Table 2).

BW Changes of Mice During the Experiment

As shown in Figure 1, the BW of normal mice increased during the total experimental period. However,

Figure 1. Effect of *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB; 1.0×10^9 cfu/kg of BW) and *Lactobacillus plantarum* YS2 (LP-YS2; low concentration: 1.0×10^8 cfu/kg of BW; high concentration: 1.0×10^9 cfu/kg of BW) on BW changes in mice with activated carbon-induced constipation during the experiment ($n = 10/\text{group}$). Values presented are the mean $\pm$ SE.

BW was slightly decreased in the mice with activated carbon-induced constipation (control group). Following treatment with LP-YS2 and LB for 17 d, BW in the *Lactobacillus*-treated mice was increased compared with control mice. In addition, LP-YS2 showed great activity to inhibit ($P < 0.05$) the activated carbon-induced BW loss than LB.

Effect of LP-YS2 and LB on Stool Status in Mice with Constipation

The stool status of all animals was similar; the stool weight, particle counts, and water content in mice had no significant gross difference ($P < 0.05$) during the first 14 d (Table 3). After induction of constipation, the stool weight, particle counts, and water content of the control group mice were lower than that of other groups; LB, LP-YS2-L, and LP-YS2-H treatment increased stool weight, particle counts, and water content, and LP-YS2-H made stool weight, particle counts, and water content almost the same as that of the normal group mice.

Table 2. Acid and bile salt resistance activity of *Lactobacillus plantarum* YS2 (LP-YS2) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB)¹

Strain	Survival in pH 3.0 artificial gastric juice (%)	Growth in bile salt (%)		
		0.3%	0.5%	1.0%
LP-YS2	67.72 $\pm$ 7.39*	19.85 $\pm$ 2.26*	15.01 $\pm$ 1.77*	8.35 $\pm$ 0.52*
LB	30.55 $\pm$ 5.74	3.08 $\pm$ 0.25	1.87 $\pm$ 0.22	1.44 $\pm$ 0.20

¹Values presented are the mean $\pm$ SE ($n = 3/\text{group}$).

* $P < 0.05$ compared with LB.

Table 3. Stool status of mice treated with lactic acid bacteria during the experiment¹

Item ²	Group ³				
	Normal	Control	LB	LP-YS2-L	LP-YS2-H
Days 1–14					
Stool weight (g)	0.86 ± 0.04 ^a	0.91 ± 0.05 ^a	0.88 ± 0.04 ^a	0.91 ± 0.04 ^a	0.92 ± 0.04 ^a
Stool particle count (particle number)	36 ± 3 ^a	35 ± 4 ^a	36 ± 5 ^a	35 ± 3 ^a	36 ± 4 ^a
Stool water content (%)	47 ± 3 ^a	47 ± 4 ^a	47 ± 5 ^a	47 ± 4 ^a	48 ± 5 ^a
Days 15–17					
Stool weight (g)	0.95 ± 0.05 ^a	0.40 ± 0.05 ^d	0.51 ± 0.05 ^{cd}	0.58 ± 0.04 ^c	0.74 ± 0.04 ^b
Stool particle count (particle number)	40 ± 4 ^a	18 ± 3 ^d	24 ± 5 ^c	27 ± 4 ^{bc}	33 ± 4 ^b
Stool water content (%)	49 ± 5 ^a	13 ± 3 ^d	29 ± 4 ^c	31 ± 4 ^{bc}	40 ± 3 ^b

^{a–d}Means within a row with different superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range test.

¹Values presented are the mean ± SE (n = 10/group).

²Days 1–14 = lactic acid bacteria administration period but no induction of constipation; Days 15–17 = lactic acid bacteria administration period and induction of constipation.

³LB = *Lactobacillus delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *Lactobacillus plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

Gastrointestinal Transit Capability in LP-YS2- and LB-Treated Mice with Activated Carbon-Induced Constipation

Activated carbon-induced constipation resulted in a decrease in the length of the small intestine in mice. However, it had no statistical difference compared with normal and LP-YS2- or LB-treated mice (Table 4). The LP-YS2 and LB treatments increased ($P < 0.05$) gastrointestinal transit length and were also able to promote gastrointestinal peristalsis (exhibited a high activated carbon propulsive rate) and reduce ($P < 0.05$) the first black stool defecation time. Gastrointestinal peristalsis promotes activity of LP-YS2, which was stronger than that of LB.

Serum Levels of MTL, Gastrin, SS, Ach, SP, and VIP in LB- and LP-YS2-Treated Mice with Activated Carbon-Induced Constipation

The serum levels of gastrointestinal motility-related factors such as MTL, gastrin, Ach, SP, and VIP were decreased ($P < 0.05$) in mice with activated carbon-

induced constipation (Table 5). The serum levels of SS in the constipation group were higher ($P < 0.05$) than those in normal mice. However, compared with control mice, administration of LP-YS2 and LB increased ($P < 0.05$) MTL, gastrin, Ach, SP, and VIP levels and reduced ($P < 0.05$) SS levels. We noted that a high dose of LP-YS2 modulated these serum biomarkers better than the same dose of LB.

Colon Tissue Levels of MPO, NO, GSH, and MDA in LP-YS2- and LB-Treated Mice with Activated Carbon-Induced Constipation

As shown in Table 6, activated carbon-induced constipation was caused by oxidative stress in the mouse colon. In control mice, constipation increased the inflammation-related factor MPO activity, hence causing the induction of NO and MDA generation in colon tissue, which was higher compared with normal mice. In addition, a decreased level of antioxidant factors such as GSH was observed in constipated mice. However, the LP-YS2 and LB treatments were able to attenuate the oxidative stress in the mouse colon. The

Table 4. Gastrointestinal transit capability and time to first black stool defecation in *Lactobacillus plantarum* YS2- and *Lactobacillus delbrueckii* ssp. *bulgaricus*-treated mice with activated carbon-induced constipation¹

Item	Group ²				
	Normal	Control	LB	LP-YS2-L	LP-YS2-H
Length of small intestine (cm)	41.3 ± 2.8 ^a	39.8 ± 3.5 ^a	40.8 ± 3.1 ^a	41.0 ± 3.3 ^a	41.2 ± 2.9 ^a
Length of gastrointestinal transit (cm)	40.5 ± 2.5 ^a	7.4 ± 1.7 ^e	15.3 ± 1.9 ^d	21.2 ± 2.0 ^c	30.6 ± 2.6 ^b
Activated carbon propulsive rate (%)	98.1 ± 0.0 ^a	18.6 ± 2.4 ^e	37.5 ± 2.2 ^d	51.7 ± 2.5 ^c	74.3 ± 2.9 ^b
Time to first black stool defecation (min)	93 ± 10 ^d	211 ± 29 ^a	162 ± 21 ^b	157 ± 18 ^b	129 ± 12 ^c

^{a–e}Means within a row with different superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range test.

¹Values presented are the mean ± SE (n = 10/group).

²LB = *L. delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *L. plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

Table 5. Serum levels (pg/mL) of gastrointestinal motility-related biomarkers in *Lactobacillus plantarum* YS2- and *Lactobacillus delbrueckii* ssp. *bulgaricus*-treated mice with activated carbon-induced constipation¹

Item	Group ²				
	Normal	Control	LB	LP-YS2-L	LP-YS2-H
Motilin	203.3 ± 21.2 ^a	105.3 ± 11.7 ^e	139.7 ± 12.5 ^d	151.2 ± 10.8 ^c	178.2 ± 13.6 ^b
Gastrin	105.3 ± 7.8 ^a	26.7 ± 2.6 ^e	43.1 ± 2.5 ^d	55.7 ± 2.2 ^c	69.4 ± 2.0 ^b
Somatostatin	22.9 ± 2.1 ^e	70.5 ± 3.0 ^a	55.1 ± 2.4 ^b	45.2 ± 1.9 ^c	32.6 ± 1.4 ^d
Acetylcholine	38.9 ± 1.5 ^a	9.7 ± 0.5 ^e	15.9 ± 0.6 ^d	23.3 ± 0.9 ^c	30.1 ± 1.3 ^b
Substance P	67.6 ± 2.5 ^a	30.2 ± 1.7 ^e	43.6 ± 1.4 ^d	52.8 ± 1.1 ^c	57.6 ± 1.8 ^b
Vasoactive intestinal peptide	65.6 ± 1.8 ^a	21.0 ± 1.7 ^e	32.3 ± 1.8 ^d	39.4 ± 1.4 ^c	53.2 ± 1.9 ^b

^{a-c}Means within a row with different superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range test.

¹Values presented are the mean ± SE (n = 10/group).

²LB = *L. delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *L. plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

high dose of LP-YS2 treatment effectively reduced the MPO activity and decreased NO and MDA generation in colon tissue.

Small Intestine and Colon Tissue Expression Levels of c-Kit and SCF in LP-YS2- and LB-Treated Mice with Activated Carbon-Induced Constipation

As shown in Figure 2A, the small intestine tissue protein levels in c-Kit and SCF were lower in constipated mice than in normal mice. Treatment with LB and LP-YS2 increased the small intestine tissue expression of c-Kit and SCF in mice with constipation. However, the high dose of LP-YS2 showed a better activity to increase the expression levels of c-Kit and SCF in the small intestine tissue of mice with carbon-induced constipation compared with LB-treated mice.

The quantitative real-time PCR results showed that the small intestine and colon tissues of mice in the normal group contained the strongest c-Kit and SCF mRNA expression. The expression in LP-YS2-H treatment mice were only weaker than normal group

mice; LP-YS2-L treatment mice showed stronger c-Kit and SCF mRNA expression than LB-treated mice and control group mice (Figure 2B).

Small Intestine and Colon Tissue Expression Levels of TRPV1, GDNF, and NOS in LP-YS2- and LB-Treated Mice with Activated Carbon-Induced Constipation

As shown in Figure 3A, both the colonic tissue protein and mRNA levels of TRPV1 and NOS were higher in mice with activated carbon-induced constipation than in normal mice. Treatment with LB and LP-YS2 reduced the colonic expression of TRPV1 and NOS in mice with activated carbon-induced constipation. However, compared with the LB treatment, the LP-YS2 treatment showed a higher activity to reduce both TRPV1 and NOS expression. In addition, the high dose of LP-YS2 exhibited a better activity to increase the expression level of GDNF in the colon tissue of mice with carbon-induced constipation compared with the LB treatment.

Table 6. Small intestine tissue levels of antioxidant-related biomarkers in *Lactobacillus plantarum* YS2- and *Lactobacillus delbrueckii* ssp. *bulgaricus*-treated mice with activated carbon-induced constipation¹

Group ²	Biomarker ³			
	MPO (mU/mg)	NO (μmol/g of protein)	MDA (nmol/mg)	GSH (μmol/mg)
Normal	6.27 ± 0.25 ^d	0.35 ± 0.05 ^d	0.43 ± 0.04 ^d	8.05 ± 0.32 ^a
Control	15.23 ± 0.78 ^a	1.68 ± 0.21 ^a	0.97 ± 0.11 ^a	5.67 ± 0.29 ^d
LB	12.14 ± 0.52 ^b	1.01 ± 0.16 ^b	0.67 ± 0.04 ^b	6.79 ± 0.25 ^c
LP-YS2-L	10.74 ± 0.36 ^b	0.78 ± 0.09 ^b	0.60 ± 0.03 ^b	7.26 ± 0.17 ^c
LP-YS2-H	8.36 ± 0.31 ^c	0.55 ± 0.06 ^c	0.51 ± 0.05 ^c	7.64 ± 0.12 ^b

^{a-d}Means within a column with different superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range test.

¹Values presented are the mean ± SE (n = 10/group).

²LB = *L. delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *L. plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

³MPO = myeloperoxidase; NO = nitric oxide; MDA = malondialdehyde; GSH = glutathione.

The small intestine and colon tissue of mice in the control group had the strongest *TRPV1* and *NOS* mRNA expression and the weakest *GDNF* expression (Figure 3B). Mice in the LP-YS2-H group had weaker *TRPV1* and *NOS* mRNA expression but stronger *GDNF* mRNA expression in the small intestine and colon tissues than mice in the LB, LP-YS2-L, and control groups. The mRNA expression of mice in the LP-YS2-H treatment was closest to that of mice in the normal group.

DISCUSSION

Lactic acid bacteria is a commonly known probiotic that has been reported to exhibit many benefits in human health and disease prevention (Suo et al., 2014). Probiotics with good resistance to gastric acid and bile salt can enter the intestinal tract with more viable bacteria and play a probiotic role (Champagne et al., 2018); LP-YS2 has such potential. In this study, we found that LP-YS2 exhibits better acid and bile salt tolerance ability than LB. These results were similar to those for *L. fermentum* and *L. casei* in our previous study (Suo et al., 2014; Qian et al., 2015; Zhao et al.,

2015a,b). A high tolerance to acid and bile salt means that the lactic acid bacteria can easily pass the stomach and are colonized in the intestinal tract (Bustos et al., 2012). Some studies have reported that probiotics may act as an enhancer to prevent or cure constipation in humans and animals (Indrio et al., 2014; Choi and Chang, 2015; Kim et al., 2015; Tabbers and Benninga, 2015). The live probiotics increased the colonic levels of short-chain fatty acids, which showed a laxative effect by affecting the enteral mucosa (Hu et al., 2013). Acetic acid, which is a main compound in short-chain fatty acids, has been reported to promote motility and defecation (Niwa et al., 2002).

Patients with constipation experienced serious symptoms such as gastrointestinal peristalsis and voiding difficulty in clinical observation (Suo et al., 2014). In an animal study, activated carbon-induced constipation decreased activated carbon propulsive rate and resulted in prolonged time to the first black stool defecation; the stool weight, particle counts, and water content were lower in patients with constipation compared with healthy people (Dimidi et al., 2017). In this study, we observed that administration of LP-YS2 improved

Figure 2. Small intestine tissue levels of protein and mRNA expression of c-Kit (CD117, cluster of differentiation 117) and stem cell factor (SCF) in *Lactobacillus plantarum* YS2- and *Lactobacillus delbrueckii* ssp. *bulgaricus*-treated mice with activated carbon-induced constipation. (A) Protein expression by Western blot; (B) semiquantitative analysis of protein expression; and (C) mRNA expression by qPCR. Values presented are the mean $\pm$ SE ($n = 7$ /group). Fold ratio: gene expression/GAPDH $\times$ control numerical value (control fold ratio: 1). Mean values with different letters (a–e) are significantly different ($P < 0.05$) according to Duncan's multiple range test. LB = *L. delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *L. plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

gastrointestinal peristalsis (e.g., increased the activated carbon propulsive rate; Table 4) and attenuated constipation (i.e., shortened the first black stool defecation time; Table 4) in mice. These results were similar to our previous studies that suggested the *Lactobacillus* strains isolated from Ngawa Prefecture all showed anticonstipation effects (Sichuan, China; Suo et al., 2014; Qian et al., 2015; Zhao et al., 2015a,b).

In normal physiological conditions, gastrointestinal tract hormones such as MTL, gastrin, ET, ACh, SP, and VIP participate in regulating gastrointestinal peristalsis. Some studies have reported abnormal serum levels of these hormones in patients with constipation (Sjölund et al., 1986, 1997; Silkoff et al., 1988; el-Salhy and Norrgard, 1998). In the present study, we observed that the LP-YS2 treatment helped increase the serum levels of MTL and gastrin in constipated mice. Both MTL and gastrin are a type of hormone that regulates the contraction of smooth muscle and promotes gastrointestinal peristalsis (Sjölund et al., 1986; Dimidi et al., 2017). Administration of LP-YS2 increased the serum

levels of ACh more than the administration of LB in constipated mice. In addition, ACh induced intestinal contraction and promoted defecation to attenuate constipation (Iwanaga et al., 1996).

Vasoactive intestinal peptide is a peptide hormone that acts as a neuromodulator to stimulate colonic motility through its vasoactive activity in the intestine (Nassif et al., 1995). Probiotics enter the intestinal tract after colonization, form a biological barrier, break down glucose, produce lactic acid, improve intestinal motility, and promote the production of VIP. Treatment with LP-YS2 increases the serum levels of VIP in mice with constipation. Clinical studies have also proven that decreased colonic levels of VIP may be associated with diminution of inhibitory innervations of colonic circular smooth muscle in patients with chronic constipation (Koch et al., 1988; King et al., 2010). We also observed that administration of LP-YS2 increased SP levels in constipated mice. Substance P is a neuropeptide that exhibits an excitatory activity to stimulate gastrointestinal peristalsis and reduce rectal sensory

Figure 3. Small intestine tissue levels of protein expression of transient receptor potential vanilloid 1 (TRPV1), glial cell-derived neurotrophic factor (GDNF), and nitric oxide synthase (NOS) in *Lactobacillus plantarum* YS2- and *Lactobacillus delbrueckii* ssp. *bulgaricus*-treated mice with activated carbon-induced constipation. (A) Protein expression by Western blot; (B) semiquantitative analysis of protein expression; and (C) mRNA expression by qPCR. Values presented are the mean $\pm$ SE ($n = 7$ /group). Fold ratio: gene expression/GAPDH $\times$ control numerical value (control fold ratio: 1). Mean values with different letters (a–e) are significantly different ($P < 0.05$) according to Duncan's multiple range test. LB = *L. delbrueckii* ssp. *bulgaricus* (1.0×10^9 cfu/kg of BW); LP-YS2-L = low dose of *L. plantarum* YS2 (1.0×10^8 cfu/kg of BW); LP-YS2-H = high dose of *L. plantarum* YS2 (1.0×10^9 cfu/kg of BW).

dysfunction, which induces constipation (King et al., 2010). However, the release SP was associated with the activation of transient receptor potential cation channel subfamily V member 1 (TRPV1), which is a member of the TRPV group of transient receptor potential family of ion channels (Peng and Li, 2010). Administration of LP-YS2 reduced the mRNA and protein levels of TRPV1 in small intestine and colon tissue of mice with constipation (Figure 3).

We also found that constipated mice administrated LP-YS2 and LB showed lower levels of SS than non-treated control mice. Somatostatin inhibits gastrin secretion, reduces the contractions of smooth muscle, and prompts constipation (Saffouri et al., 1979). The circulated SS also exhibit an activity to regulate gastric juice secretion and control islet cell function to influence the normal function of the gastrointestinal system (Colturi et al., 1984).

The interstitial cells of Cajal (ICC) are mesenchymal cells that are distributed in the gastrointestinal system (Yu et al., 2002) and play an important role in promoting intestinal motility (He et al., 2000). Abnormal colonic motility may be associated with low levels of ICC in the gastrointestinal tract (Yu et al., 2002; Li et al., 2010), which has been observed in patients with constipation (He et al., 2000; Sabri et al., 2003). It is well known that c-Kit is a factor for maintaining ICC proliferation and growth in normal conditions (Lee et al., 2005). We found low mRNA and protein levels of c-Kit in small intestine and colon tissue of constipated mice (Figure 2). However, administration of LP-YS2 increased the mRNA and protein expression of c-Kit in mice with constipation. In addition, LP-YS2 treatment effectively improved the mRNA and protein levels of stem cell factor (SCF) in small intestine and colon tissue of constipation mice. Stem cell factor is a ligand for c-Kit that is necessary for appropriate development and survival of interstitial cells of ICC in the intestine (Wedel et al., 2002). In animal and human studies of constipation, the expression levels of c-Kit and SCF in mice or patients with constipation were both reduced compared with those in normal mice or healthy people (Wedel et al., 2002; Sabri et al., 2003; Lee et al., 2005; Li et al., 2010).

Glial cell-derived neurotrophic factor, a protein that is distributed in the gastrointestinal tract, plays an important role in modulating the growth and development of nerve cells and protects and repairs damaged nerves (Allen et al., 2013). Glial cell-derived neurotrophic factor also showed an activity to promote intestinal epithelial cell proliferation and inhibit apoptosis in these cells (Sidorova and Saarma, 2016). Administration of LP-YS2 increased GDNF expression levels in small in-

testine and colon tissue of constipated mice (Figure 3). In addition, exogenous GDNF significantly attenuated the constipation (Xu et al., 2010).

Many studies have reported that the *L. plantarum* strains exhibited great antioxidant activity and anti-inflammation capability in vitro (Zhang et al., 2005; Chiu et al., 2013; Das and Goyal, 2015). We found a high level of oxidative stress in mice with activated carbon-induced constipation. The intestinal levels of MPO and NO in constipated mice were higher than those in normal mice. Administration of LP-YS2 and LB decreased the levels of MPO and NO and helped reduce MDA generation in constipated mice. Constipated mice treated with LP-YS2 also showed a higher level of GSH than LB-treated mice and control mice. In addition, patients with constipation have abnormal levels of Nos in intestinal tissue (King et al., 2010). We also found that LP-YS2 decreased the mRNA and protein of Nos in small intestine and colon tissue of constipated mice.

Lactobacillus delbrueckii ssp. *bulgaricus* is the most popular fermented strain for yogurt. It is a good food starter, but its activities of survival in pH 3.0 artificial gastric juice, hydrophobic property, and growth in bile salt are weaker than that of some lactic acid bacteria isolated from natural fermented foods. The functional role of *L. delbrueckii* ssp. *bulgaricus* is not strong, and its effects against constipation and gastric injury are weaker than those of some other lactic acid bacteria isolated from natural fermented foods (Gruter et al., 1993; Suo et al., 2014; Qian et al., 2015; Zhao et al., 2015a; Suo et al., 2016). *Lactobacillus plantarum* can be used not only as food starter but also for many functional effects (Swain et al., 2014). *Lactobacillus plantarum* is used more widely than *L. delbrueckii* ssp. *bulgaricus*. In this study, the results also showed that LP-YS2 had a stronger functional effect than LB.

The aim of this study was to investigate whether LP-YS2 showed an activity to attenuate the activated carbon-induced constipation in male KM mice. *Lactobacillus plantarum* YS2 was found to have better acid resistance and bile salt tolerance than LB. Administration of LP-YS2 reduced the first black stool defecation and promoted gastrointestinal peristalsis in constipated mice. In addition, LP-YS2 showed greater activity to increase the serum levels of MTL, gastrin, ACh, SP, and VIP compared with LB-treated mice. In the same way, LP-YS2-treated mice exhibited lower levels of SS compared with LB-treated mice. The LP-YS2-treated mice showed higher mRNA and protein expression levels of c-Kit, SCF, and GDNF and lower levels of TRPV1 and NOS than LB-treated mice and control mice. These results suggest that LP-YS2 attenuated

the activated carbon-induced constipation in KM mice effectively and that it can be the preventive as well as curing measure for constipation.

ACKNOWLEDGMENTS

The present research was supported by the Program for Innovation Team Building at Institutions of Higher Education in Chongqing (CXTDX201601040), Chongqing Research Program of Basic Research and Frontier Technology (cstc2016jcyjA0339), and Research Project of Chongqing University of Education (KY2015TBZC), Chongqing, China.

REFERENCES

- Allen, S. J., J. J. Watson, D. K. Shoemark, N. U. Barua, and N. K. Patel. 2013. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol. Ther.* 138:155–175.
- Bae, S. H. 2014. Diets for constipation. *Pediatr. Gastroenterol. Hepatol. Nutr.* 17:203–208.
- Basilisco, G., and M. Coletta. 2013. Chronic constipation: A critical review. *Dig. Liver Dis.* 45:886–893.
- Berstad, A., J. Raa, T. Midtvedt, and J. Valeur. 2016. Probiotic lactic acid bacteria—The fledgling cuckoos of the gut? *Microb. Ecol. Health Dis.* 27:31557.
- Bustos, A. Y., L. Saavedra, G. F. de Valdez, R. R. Raya, and M. P. Taranto. 2012. Relationship between bile salt hydrolase activity, changes in the internal pH and tolerance to bile acids in lactic acid bacteria. *Biotechnol. Lett.* 34:1511–1518.
- Champagne, C. P., A. G. Cruz, and M. Daga. 2018. Strategies to improve the functionality of probiotics in supplements and foods. *Curr. Opin. Food Sci.* 22:160–166.
- Chiu, Y. H., Y. C. Lu, C. C. Ou, S. L. Lin, C. C. Tsai, C. T. Huang, and M. Y. Lin. 2013. *Lactobacillus plantarum* MYL26 induces endotoxin tolerance phenotype in Caco-2 cells. *BMC Microbiol.* 13:190.
- Chmielewska, A., and H. Szajewska. 2010. Systematic review of randomised controlled trials: Probiotics for functional constipation. *World J. Gastroenterol.* 16:69–75.
- Choi, C. H., and S. K. Chang. 2015. Alteration of gut microbiota and efficacy of probiotics in functional constipation. *J. Neurogastroenterol. Motil.* 21:4–7.
- Colteri, T. J., R. H. Unger, and M. Feldman. 1984. Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release, and islet cell function. Studies in healthy subjects and duodenal ulcer patients. *J. Clin. Invest.* 74:417–423.
- Crowell, M. D., L. A. Harris, T. N. Lunsford, and J. K. Dibaise. 2009. Emerging drugs for chronic constipation. *Expert Opin. Emerg. Drugs* 14:493–504.
- Das, D., and A. Goyal. 2015. Antioxidant activity and γ -aminobutyric acid (GABA) producing ability of probiotic *Lactobacillus plantarum* DM5 isolated from Marcha of Sikkim. *Lebensm. Wiss. Technol.* 61:263–268.
- Dimidi, E., S. Christodoulides, K. C. Fragkos, S. M. Scott, and K. Whelan. 2014. The effect of probiotics on functional constipation in adults: A systematic review and meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* 100:1075–1084.
- Dimidi, E., S. Christodoulides, S. M. Scott, and K. Whelan. 2017. Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. *Adv. Nutr.* 8:484–494.
- el-Salhy, M., and O. Norrgard. 1998. Colonic neuroendocrine peptide levels in patients with chronic idiopathic slow transit constipation. *Ups. J. Med. Sci.* 103:223–230.
- Fei, Y. T., D. M. Liu, T. H. Luo, G. Chen, H. Wu, L. Li, and Y. G. Yu. 2014. Molecular characterization of *Lactobacillus plantarum* DMDL 9010, a strain with efficient nitrite degradation capacity. *PLoS One* 9:e113792.
- Gruter, M., B. R. Leeflang, J. Kuiper, J. P. Kamerling, and J. F. Vliegthart. 1993. Structural characterisation of the exopolysaccharide produced by *Lactobacillus delbrückii* subspecies *bulgaricus* rr grown in skimmed milk. *Carbohydr. Res.* 239:209–226.
- He, C. L., L. Burgart, L. Wang, J. Pemberton, T. Young-Fadok, J. Szurszewski, and G. Farrugia. 2000. Decreased interstitial cell of Cajal volume in patients with slow-transit constipation. *Gastroenterology* 118:14–21.
- Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder, and M. E. Sanders. 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11:506–514.
- Hu, J. L., S. P. Nie, and M. Y. Xie. 2013. High pressure homogenization increases antioxidant capacity and short-chain fatty acid yield of polysaccharide from seeds of *Plantago asiatica* L. *Food Chem.* 138:2338–2345.
- Indrio, F., A. Di Mauro, G. Riezzo, E. Civardi, C. Intini, L. Corvaglia, E. Ballardini, M. Bisceglia, M. Cinquetti, E. Brazzoduro, A. Del Vecchio, S. Tafuri, and R. Francavilla. 2014. Prophylactic use of a probiotic in the prevention of colic, regurgitation, and functional constipation: A randomized clinical trial. *JAMA Pediatr.* 168:228–233.
- Iwanaga, Y., N. Miyashita, T. Saito, K. Morikawa, and Z. Itoh. 1996. Gastropromkinetic effect of a new benzamide derivative itopride and its action mechanisms in conscious dogs. *Jpn. J. Pharmacol.* 71:129–137.
- Kim, S. E., S. C. Choi, K. S. Park, M. I. Park, J. E. Shin, T. H. Lee, K. W. Jung, H. S. Koo, and S. J. Myung. 2015. Change of fecal flora and effectiveness of the short-term VSL#3 probiotic treatment in patients with functional constipation. *J. Neurogastroenterol. Motil.* 21:111–120.
- King, S. K., J. R. Sutcliffe, S. Y. Ong, M. Lee, T. L. Koh, S. Q. Wong, P. J. Farmer, C. J. Peck, M. P. Stanton, J. Keck, D. J. Cook, C. W. Chow, J. M. Hutson, and B. R. Southwell. 2010. Substance P and vasoactive intestinal peptide are reduced in right transverse colon in pediatric slow-transit constipation. *Neurogastroenterol. Motil.* 22:883–892.
- Koch, T. R., J. A. Carney, L. Go, and V. L. Go. 1988. Idiopathic chronic constipation is associated with decreased colonic vasoactive intestinal peptide. *Gastroenterology* 94:300–310.
- Lee, J. I., H. Park, M. A. Kamm, and I. C. Talbot. 2005. Decreased density of interstitial cells of Cajal and neuronal cells in patients with slow-transit constipation and acquired megacolon. *J. Gastroenterol. Hepatol.* 20:1292–1298.
- Li, C., S. P. Nie, K. X. Zhu, T. Xiong, C. Li, J. Gong, and M. Y. Xie. 2015. Effect of *Lactobacillus plantarum* NCU116 on loperamide-induced constipation in mice. *Int. J. Food Sci. Nutr.* 66:533–538.
- Li, F., L. Zhang, C. Li, B. Ni, Y. Wu, Y. Huang, G. Zhang, L. Wang, A. Zhang, Y. He, T. Fu, W. Tong, and B. Liu. 2010. Adenovirus-mediated stem cell leukemia gene transfer induces rescue of interstitial cells of Cajal in ICC-loss mice. *Int. J. Colorectal Dis.* 25:557–566.
- Li, G., X. C. Zou, G. Kuang, Y. R. Ren, C. F. Deng, Q. Lin, X. Zhao, S. Xu, and J. L. Song. 2017. Preventative effects of fermented *Chimonobambusa quadrangularis* shoot on activated carbon-induced constipation. *Exp. Ther. Med.* 13:1093–1100.
- Lollo, P. C. B., A. G. Cruz, P. N. Morato, C. S. Moura, L. B. Carvalho-Silva, C. A. F. Oliveira, J. A. F. Faria, and J. Amaya-Farfan. 2012. Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. *J. Dairy Sci.* 95:3549–3558.
- Lollo, P. C. B., P. N. Morato, C. S. Moura, C. N. Almada, T. L. Felicio, E. A. Esmerino, M. E. Barros, J. Amaya-Farfan, A. S. Sant'Ana, R. R. S. Raices, M. C. Silva, and A. G. Cruz. 2015. Hypertension parameters are attenuated by the continuous consumption of probiotic Minas cheese. *Food Res. Int.* 76:611–617.
- Mazlyn, M. M., L. H. Nagarajah, A. Fatimah, A. K. Norimah, and K. L. Goh. 2013. Effects of a probiotic fermented milk on function-

- al constipation: A randomized, double-blind, placebo-controlled study. *J. Gastroenterol. Hepatol.* 28:1141–1147.
- Moura, C. S., P. C. B. Lollo, P. N. Morato, E. A. Esmerino, L. P. Margallo, V. A. Santos-Junior, P. T. Coimbra, L. P. Cappato, M. C. Silva, A. S. Garcia-Gomes, D. Granato, H. M. A. Bolini, A. S. Sant'Ana, A. G. Cruz, and J. Amaya-Farfan. 2016. Assessment of antioxidant activity, lipid profile, general biochemical and immune system responses of Wistar rats fed with dairy dessert containing *Lactobacillus acidophilus* La-5. *Food Res. Int.* 90:275–280.
- Nassif, A., W. E. Longo, R. Sexe, M. Stratton, J. Standeven, A. M. Vernava, and D. L. Kaminski. 1995. *Clostridium difficile* suppresses colonic vasoactive intestinal peptide associated with altered motility. *Mediators Inflamm.* 4:452–453.
- Niwa, T., M. Nakao, S. Hoshi, K. Yamada, K. Inagaki, M. Nishida, and T. Nabeshima. 2002. Effect of dietary fiber on morphine-induced constipation in rats. *Biosci. Biotechnol. Biochem.* 66:1233–1240.
- Peng, J., and Y. J. Li. 2010. The vanilloid receptor TRPV1: Role in cardiovascular and gastrointestinal protection. *Eur. J. Pharmacol.* 627:1–7.
- Qian, Y., H. Suo, M. Du, X. Zhao, J. Li, G. J. Li, J. L. Song, and Z. Liu. 2015. Preventive effect of *Lactobacillus fermentum* Lee on activated carbon-induced constipation in mice. *Exp. Ther. Med.* 9:272–278.
- Sabri, M., E. Barksdale, and C. Di Lorenzo. 2003. Constipation and lack of colonic interstitial cells of Cajal. *Dig. Dis. Sci.* 48:849–853.
- Saffouri, B., G. Weir, K. Bitar, and G. Makhoul. 1979. Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. *Life Sci.* 25:1749–1753.
- Shin, A., M. Camilleri, G. Kolar, P. Erwin, C. P. West, and M. H. Murad. 2014. Systematic review with meta-analysis: Highly selective 5-HT₄ agonists (prucalopride, velusetrag or naronapride) in chronic constipation. *Aliment. Pharmacol. Ther.* 39:239–253.
- Sidorova, Y. A., and M. Saarna. 2016. Glial cell line-derived neurotrophic factor family ligands and their therapeutic potential. *Mol. Biol. (Mosk.)* 50:589–598.
- Silkoff, P., F. Karmeli, E. Goldin, A. Ewenson, C. Gilon, M. Chorev, R. Laufer, Z. Selinger, and D. Rachmilewitz. 1988. Effect of substance P on rat gastrointestinal transit. *Dig. Dis. Sci.* 33:74–77.
- Sjölund, K., R. Ekman, F. Akre, and P. Lindner. 1986. Motilin in chronic idiopathic constipation. *Scand. J. Gastroenterol.* 21:914–918.
- Sjölund, K., S. Fasth, R. Ekman, L. Hultén, H. Jiborn, S. Nordgren, and F. Sundler. 1997. Neuropeptides in idiopathic chronic constipation (slow transit constipation). *Neurogastroenterol. Motil.* 9:143–150.
- Song, Y., Z. H. Sun, C. Y. Guo, Y. R. Wu, W. J. Liu, J. Yu, B. Menghe, R. F. Yang, and H. P. Zhang. 2016. Genetic diversity and population structure of *Lactobacillus delbrueckii* subspecies *bulgaricus* isolated from naturally fermented dairy foods. *Sci. Rep.* 6:22704.
- Sperry, M. F., H. L. A. Silva, C. F. Balthazar, E. A. Esmerino, S. Verruck, E. S. Prudencio, R. P. C. Neto, M. I. B. Tavares, J. C. Peixoto, F. Nazzaro, R. S. Rocha, J. Moraes, A. S. G. Gomes, R. S. L. Raices, M. C. Silva, D. Granato, T. C. Pimentel, M. Q. Freitas, and A. G. Cruz. 2018. Probiotic Minas Frescal cheese added with *L. casei* 01: Physicochemical and bioactivity characterization and effects on hematological/biochemical parameters of hypertensive overweighted women—A randomized double-blind pilot trial. *J. Funct. Foods* 45:435–443.
- Suo, H., X. Zhao, Y. Qian, G. Li, Z. Liu, J. Xie, and J. Li. 2014. Therapeutic effect of activated carbon-induced constipation mice with *Lactobacillus fermentum* Suo on treatment. *Int. J. Mol. Sci.* 15:21875–21895.
- Suo, H., X. Zhao, Y. Qian, P. Sun, K. Zhu, J. Li, and B. Z. Sun. 2016. *Lactobacillus fermentum* Suo attenuates HCl/ethanol induced gastric injury in mice through its antioxidant effects. *Nutrients* 8:155.
- Swain, M. R., M. Anandharaj, R. C. Ray, and R. Parveen Rani. 2014. Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnol. Res. Int.* 2014:250424.
- Tabbers, M. M., and M. A. Benninga. 2015. Constipation in children: Fibre and probiotics. *BMJ Clin. Evid.* 2015:0303.
- Ueki, A., and M. Otsuka. 2004. Life style risks of Parkinson's disease: Association between decreased water intake and constipation. *J. Neurol.* 251(Suppl. 7):18–23.
- Wang, L., L. Hu, Q. Xu, B. Yin, D. Fang, G. Wang, J. Zhao, H. Zhang, and W. Chen. 2017. *Bifidobacterium adolescentis* exerts strain-specific effects on constipation induced by loperamide in balb/c mice. *Int. J. Mol. Sci.* 18:E318.
- Wang, R., P. Sun, Y. Zhou, and X. Zhao. 2015. Preventive effect of *Dendrobium candidum* Wall. ex Lindl. on activated carbon-induced constipation in mice. *Exp. Ther. Med.* 9:563–568.
- Webster, L., R. Tummala, U. Diva, and J. Lappalainen. 2016. A 12-week extension study to assess the safety and tolerability of naloxegol in patients with noncancer pain and opioid-induced constipation. *J. Opioid. Manag.* 12:405–419.
- Wedel, T., J. Spiegler, S. Soellner, U. J. Roblick, T. H. Schiedeck, H. P. Bruch, and H. J. Krammer. 2002. Enteric nerves and interstitial cells of cajal are altered in patients with slow-transit constipation and megacolon. *Gastroenterology* 123:1459–1467.
- Wu, C. S., J. Li, Y. Qian, and H. Y. Suo. 2012a. Research progress of yak milk and their nutritional value. *Dairy Sci. Technol.* 35:43–46.
- Wu, C. S., C. Shu, J. Li, Y. Qian, and H. Y. Suo. 2012b. The research progress and prospect of yak yogurt lactic acid bacteria. *Food Ind.* 33:129–133.
- Xu, G. P., Y. H. Fan, and B. Lv. 2010. Advances in understanding the role of neurotrophins in physiological and pathological processes in the intestinal tract. *World Chin. J. Digestology* 18:2884–2888.
- Yu, S., M. D. Crowell, T. Tihan, and B. E. Lacy. 2002. Distribution of interstitial cells of cajal in the whole colon from patients with slow-transit constipation. *Am. J. Gastroenterol.* 97:S118.
- Zhang, L., N. Li, R. Caicedo, and J. Neu. 2005. Alive and dead *Lactobacillus rhamnosus* GG decrease tumor necrosis factor-alpha-induced interleukin-8 production in Caco-2 cells. *J. Nutr.* 135:1752–1756.
- Zhao, X., Y. Qian, H. Suo, M. Y. Du, G. J. Li, Z. H. Liu, and J. Li. 2015a. Preventive effect of *Lactobacillus fermentum* Zhao on activated carbon-induced constipation in mice. *J. Nutr. Sci. Vitaminol. (Tokyo)* 61:131–137.
- Zhao, X., H. Y. Suo, Y. Qian, G. J. Li, Z. H. Liu, and J. Li. 2015b. Therapeutic effects of *Lactobacillus casei* Qian treatment in activated carbon-induced constipated mice. *Mol. Med. Rep.* 12:3191–3199.