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Preventive Effects of *Lactobacillus Plantarum* YS4 on Constipation Induced by Activated Carbon in Mice

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Abstract: This study was designed to analyze the effects of *Lactobacillus plantarum* YS4 (LP-YS4) on activated carbon-induced constipation in ICR (Institute of Cancer Research) mice. The mice were fed on YS4 and LB (*Lactobacillus bulgaricus*), followed by inducing constipation. The results of the experiment suggested that anti-gastric acid and bile salt activities of LP-YS4 were more effective than LB. It was conclusive that LP-YS4 could inhibit the weight loss induced by constipation and had an effect on fecal weight, particle number and further decrease in water content initiated by constipation. At the same time, LP-YS4 could increase gastrointestinal (GI) transit rate and limit the time of the first black stool defecation. It could also raise the motilin (MTL), endothelin (ET), acetylcholinesterase (AChE), substance P (SP), and vasoactive intestinal peptide (VIP) serum levels and reduce the somatostatin (SS) level in constipated mice as compared to the mice in control group. LP-YS4 could reduce myeloperoxidase (MPO), nitric oxide (NO), and malondialdehyde (MDA) levels in small intestinal tissue of mice and raise glutathione (GSH) levels as compared to the control group mice. By H&E (hematoxylin-eosin) assay, we determined that LP-YS4 could reduce the small intestinal tissue injury by activated carbon. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) experiment data demonstrated that LP-YS4 has the capability to increase c-Kit, stem cell factor (SCF), glial cellline-derived neurotrophic factor (GDNF) mRNA (messenger RNA) expressions and decrease transient receptor potential vanilloid 1 (TRPV1), nitric oxide synthase (NOS) expressions in small intestine tissue of constipated mice. High concentration of LP-YS4 exhibited much better effects than that of LB. From these results, LP-YS4 could be considered as an effective substance that actively inhibits constipation.

Keywords: *Lactobacillus plantarum*; activated carbon; constipation; expression; mRNA

1. Introduction

Kurut is a kind of natural fermented dairy product thatis abundant in nutrition and commonly found in Qinghai Tibet plateau areas. It is an antioxidant and can lower the cholesterol levels as well as regulate immunity [1]. The quality of kurut is quite special due to its natural fermentation technique, purity of the raw milk, fermentation temperature, time taken, and the vessels and microorganism used. In addition, its distinctive flavor is usually unavailable in commonly found fermented yogurt [2]. In this study, our research teamcollected natural fermented yak yogurt from Yushu Tibetan Autonomous Prefecture in Qinghai, and then their microbes were detected and isolated. One of them was named as *Lactobacillus plantarum* YS4 (LP-YS4), and its functional effects were studied.

If a person is defecating less than three times a week, then it can be defined as a condition of constipation. If the situation aggravates further to less than one time a week, it is considered a serious constipation state, which can cause harm to human health, especially colon health [3]. However, constipation is usually not considered a disease, and, in most cases, it can be relieved by balanced diet and changing lifestyle [4].

Lactic acid bacteria can consolidate the intestinal endogenous defense barrier, improve the intestinal non-immune defense barrier function, and activate endogenous bacterial metabolism. On the other hand, it can eliminate intestinal harmful bacteria and maintain intestinal microecological balance by improving intestinal immune defense barrier [5]. Some lactic acid bacteria can produce functional organic acids; thus, these lactic acid bacteria can promote intestinal repair, reduce the pH value of intestinal tract, regulate intestinal neuromuscular activity, enhance intestinal peristalsis and promote intestinal digestion and absorption. These lactic acid bacteria can be used as probiotics to effectively inhibit the reproduction of intestinal spoilage bacteria, improve the intestinal environment, and make the stool soft and convenient for excretion to prevent constipation [6]. During the state of constipation, intestinal microflora changes; aerobic bacteria, fungi and *Escherichia coli* levels are increased while anaerobic bacteria, *Bacteroides*, and bifidobacteria are reduced [7]. Probiotics play an important role in maintaining the ecological balance of the intestinal tract, regulating constipation and preventing other diseases.

The establishment of animal model of constipation can shedsome light on the physiological processes that happen to food in the intestine during constipation. Mice were given activated carbon using lavage to make the activated carbon attach to gastrointestinal mucous membrane surface. which leads to decreased water and digestive juices in digestive tract. These processes could decrease peristalsis and induce constipation [8]. High doses of activated carbon in the digestive tract can even cause obstructive bowel movements. As lactic acid bacteria have preventive effects on constipation, the thorough study of this group has confirmed that the first gain of black stool defecation time, as well as motilin (MTL), endothelin (ET), acetylcholinesterase (AChE), somatostatin (SS), substance P (SP) and vasoactive intestinal peptide (VIP) levels can be used as evaluation indexes to know about the preventive effects of lactic acid bacteria on constipation [9]. Moreover, precise molecular biological experiments can efficiently detect the expression of related mRNA in colonic tissues to confirm the physiological effect of *lactobacillus*.

Nowadays, there are many commercialized lactobacillus, but their bioactivities are not strong enough to enter the intestinal tract, and not many studies have proven that these commercialized lactobacilli have anti-constipation effects. Lactic acid bacteria used as probiotics in stomach and large intestine could have physiological effects based on their colonization and physiological activities [10]. Therefore, it is a hot topic to discover high-quality lactic acid bacteria with high bioactivity. At the same time, the analysis of the active mechanism of high quality lactic acid bacteria by means of molecular biology is beneficial to the better understanding of its role. This study takes LP-YS4 as the research object and common *Lactobacillus bulgaricus* (LB) as reference strain. First, we compared the tolerance of LP-YS4 and LB on artificial gastric juice as well as bile salt through in vitro experiments which gave us the preliminarily effects and physiological activity of LP-YS4 in gastrointestinal tract. This study will verify the preventive effects of LP-YS4 on activated carbon-induced constipation. The mechanism of

LP-YS4's effects on constipation are also studied. The results lay a theoretical foundation to further identify the development of LP-YS4, so that LP-YS4 can be further applied to the food industry.

2. Materials and Methods

2.1. Isolation and Purification of Lactic Acid Bacteria

The natural fermented yak yoghurt was adopted from herdsman family in Qinghai Tibet Plateau in Yushu, Qinghai, China. The solution we prepared contains 1 mL natural fermented yak yoghurt which was diluted to 10^{-7} in a sterile saline for a 10-fold gradient. The 10^{-5}, 10^{-6} and 10^{-7} natural fermented yak yoghurt diluents were added on culture dish of 15 mL MRS (de man, rogosa and sharpe) medium (containing 5% $CaCO_3$). It then cultured in a constant temperature incubator (Biochemical incubator BI-150A, STIK, Shanghai, China) for 72 h. After colony formation, the colonies of bacteria that had calcium dissolving ring were inoculated on skim milk medium for 48 h (30 °C), and then streak inoculated on MRS agar medium for 48 h (30 °C). These two steps were repeated thrice until pure colonies were obtained.

2.2. DNA of Lactic Acid Bacteria Extraction

The pure strain was streak cultured on MRS medium for 48 h (30 °C), the colony size and shape were observed and tested by many different ways such as by using a microscope (BX43, Olympus, Tokyo, Japan), the gram staining, catalase test, exercise test, hydrogen sulfide test, gelatin liquefaction test, nitrate reduction test, indole test, litmus milk test, methyl red test, V.P. test, 6.5% NaCl growth test, growth temperature test (10 °C, 15 °C, 45 °C and 60 °C for 30 min), pH gradient test and sugar alcohol fermentation test were all measured with high precautions.

2.3. DNA of Lactic Acid Bacteria Extraction

Liquid culture of lactic acid bacteria (2 mL) was centrifugated at 12,000 r/min for 1 min, the supernatant fluid was discarded and the microbial biomass was collected. The DNA of lactic acid bacteria was extracted using the DNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China).

2.4. DNA of Lactic Acid Bacteria Determination

The extracted DNA solution was subjected to agarose gel electrophoresis (gel concentration 0.8%, voltage 100 V, electrophoresis 60 min).The gel was observed using gel imaging system (6200, Tanon, Shanghai, China). The concentration of DNA extraction was determined using a micro ultraviolet spectrophotometer (Nano300, Allsheng, Hangzhou, China). The PCR gene was amplified by 27F (F), AGAGTTTGATCCTGGCTCAG and 1492R (R) GGCTACCTTGTTACGACTT, and, using the 16SrDNA sequence of Lactobacillus, it was amplified and sequenced (SimpliAmp Thermal Cycler, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.5. Experimental Strains

The microorganism strain used for this study was isolated and identified. We named the lactic acid bacteria as *Lactobacillus plantarum* YS4 (LP-YS4); this strain was preserved in China Center for Type Culture Collection (CCTCC NO: 2016750, Wuhan, China). *Lactobacillus bulgaricus* (LB, CCTCC AB 200048) was obtained from CCTCC.

2.6. Detecting Tolerance Capacity of Lactic Acid Bacteria in the Artificial Gastric Juice

NaCl and pepsin both were dissolved in distilled water at maintained pH 3.0 having 1 mol/L of HCl to reach to a mass ratio 0.2% NaCl, with 0.35% pepsin, after that vacuum filtration was used to remove bacteria. The activated LP-YS4 was cultured and 5 mL solution was extracted by centrifugalizing at 3000 r/min for 10 min to collect pure form of LP-YS4. Then, saline was addedto get 5 mL of the bacterial suspension. It was then mixed with artificial gastric juice at the volume ratio of

1.0 to 9.0;subsequently, it was shaken, followed by culture for 3 h under 37 °C which gave the number of viable LP-YS4 as measured at 0 and 3 h, which helped calculate the tolerating ability of lactic acid bacteria in artificial gastric juice (%) = viable count (CFU/mL)(colony-forming units per milliliter) at 3 h/viable count (CFU/mL) at 0 h × 100% [11].

2.7. Detection of Bile Salt Tolerance Using Lactic Acid Bacteria

The 2.0% of inoculum concentration of LP-YS4 was inoculated on MRS-THIO culture medium containing 0.0%, 0.3%, 0.5% and 1.0% ox bile salt, which was then cultured for 24 h at37 °C. The culture medium without LP-YS4 was used for control group. Then, the absorbance value was measured at 600 nm to get bile salt tolerance by lactic acid bacteria (%) = Bile salt culture medium OD_{600}/blank medium OD_{600} × 100 [10].

2.8. In Vivo Experiment

LP-YS4 was inoculated on liquid MRS medium for 48 h (30 °C), and the lactic acid bacteria of LP-YS4 was collected for the mice experiment. Seven-week-old female ICR mice were purchased from the experimental animal center of Chongqing Medical University (SYXK (Chongqing) 2012-0001). Mice were fed under the temperature of 25 ± 2 °C, relative humidity of 50 ± 5%, 12-h light/dark cycles, and with standard mouse feed and water. The 100 ICR mice were divided into 5 groups: normal group, control group (constipation induced), LB group (LB treatment), LP-YS4-L group (low concentration of LP-YS treatment) and LP-YS4-H group (high concentration of LP-YS treatment). The normal and control groups received normal free intake of diet and water for 2 weeks. LB group, LP-YS4-L group, and LP-YS4-H group were fed 2 mL of LB of 1.0×10^9 CFU/kg, 2 mL of LP-YS4 of 1×10^8 CFU/kg, and 2 mL of LP-YS4 of 1×10^9 CFU/kg by lavage for 2 weeks, respectively. After 2 weeks, except the normal group, all mice of the other 4 groups were intragastrically infused with 2 mL of 10% activated carbon water (10% activated carbon is mixed with 10% Arabia gum according to the mass ratio to make the suspension) for 3 days. Meanwhile, the lactic acid bacteria of LB and LP-YS4 were continued to feed to mice. Weight, dietary intake, water consumption, fecal weight and fecal humidity were collected and measured at 9:00 a.m. daily during the experiment. The protocol of this experiment was approved by the Animal Ethics Committee of Chongqing Medical University (SYXK (Yu) 2017-0001).

2.9. Determination of Small Intestinal Propulsive Rate and Excretion Time of Activated Carbon

On Day 17 of the experiment, after intragastric administration of activated carbon water, all mice including the normal group were fasted for 24 h, but drinking water was available during this time. After 24 h, 0.2 mL of glacial activated carbon water was administered to all mice. Then, after 30 min, 10 mice in every group were killed by neck snapping to get plasma. The small intestine was separated immediately to observe the rate of propagation of activated carbon in the small intestine of mice. The activated carbon propulsive rate (gastrointestinal transit capability) (%) = length of gastrointestinal transit (GI) transit (activated carbon propulsive distance)/length of small intestine × 100. The remaining 10 mice in each group wereused to observe the time of the first black stool.

2.10. Determination of Level of Serum MTL, GAS, ET, SS, AChE, SP and VIP in Mice

The mice plasma was collected and centrifuged at 4500 r/min for 15 min to get the serum. The serum levels of motilin (MTL), gastrin (GAS), endothelin-1 (ET-1), substance P (SP), somatostatin (SS), vasoactive intestinal peptide (VIP) and acetylcholine enzyme (AchE) were determined as per the instruction on the kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.11. Determination of Levels of Small Intestine Tissue MPO, NO, GSH and MDA in Mice

Small intestinal tissue (100 mg) was homogenized using a phosphate buffer saline (PBS) in ice. The levels of myeloperoxidase (MPO), nitric oxide (NO), glutathione (GSH) and malondialdehyde (MDA) in small intestine were determined using appropriate step as provided by commercial kits (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocols.

2.12. Histopathological Findings

The small intestine was placed in 10% formalin solution for 24 h and 95% ethanol to dehydrate prior to the xylene treatment. The tissues were sectioned and stained with H&E for observation; all sections were carefully observed under microscope (BX43, Olympus, Tokyo, Japan) by Prof. Lihua Qiu (Chongqing Medical University).

2.13. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Assay

The total RNA from small intestine tissues was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was detected by micro-UV (ultraviolet) spectrophotometer (Nano 300, Aosheng, Hanzhou, Zhejiang, China) and the concentration of RNA was adjusted to 1 μg/mL, then 1 mL mRNA solution was reverse transcribed into cDNA. PCR reaction program: pre-denaturation for 3 min at 95 °C, denaturation for 10 s at 95 °C, annealing for 30 s at 57 °C, and extension for 15 s at 72 °C, 40 cycles. The primers in this study are shown in Table 1. The relative transcription levels of the mRNAs were calculated according to the $2^{-\Delta\Delta Cr}$ formula [12].

Table 1. Sequences of reverse transcription-polymerase chain reaction primers were used in this study.

Gene Name	Sequence
c-Kit	Forward: 5′-CATAGCCCAGGTAAAGCACAAT-3′ Reverse: 5′-GAACACTCCAGAATCGTCAACTC-3′
SCF	Forward: 5′-TCAGGGACTACGCTGCGAAAG-3′ Reverse: 5′-AAGAGCTGGCAGACCGACTCA-3′
TRPV1	Forward: 5′-CCGGCTTTTTGGGAAGGGT-3′ Reverse: 5′-GAGACAGGTAGGTCCATCCAC-3′
GDNF	Forward: 5′-GGGGTATGGAGAAGTTGGCTAG-3′ Reverse: 5′-CTATGAGAATGCTGCCGAAAA-3′
NOS	Forward: 5′-CAGCGAACGGACGGCAAGCA-3′ Reverse: 5′-TGACACGACCAGCGGCAGGAT-3′
GAPDH	Forward: 5′-TGCACCACCAACTGCTTAG-3′ Reverse: 5′-GATGCAGGGATGATGTTC-3′

SCF, stem cell factor; TRPV1, transient receptor potential cation channel subfamily V member 1; GDNF, glial cell-derived neurotrophic factor; NOS, nitric oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

2.14. Protein Extraction and Western Blot Analysis

The homogenate from the small intestine tissues was collected, and the lysate was used to lyse the hepatocytes for 30 min on ice. The BCA (bicinchoninic acid) method was used to determine the protein concentration. The sample was added to 5× loading buffer by volume and heated in a water bath at 100 °C for 5 min. The hepatocytes were sonicated by SJIALAB (Ningbo Yinzhou Sjialab Equipment Co., Ltd., Ningbo, Zhejiang, China) for 2 min (10% ultrasound intensity) and centrifuged for 10 min to obtain the supernatant. The extracted protein was subjected to polyacrylamide gel electrophoresis (80–120 V), transferred to a PVDF membrane, sealed and incubated overnight at 4 °C with the addition of primary antibody of c-Kit (cat no. ab62154, Abcam, Cambridge, MA, USA), SCF (cat no. ab83866; Abcam), TRPV1 (cat no. ACC-030, Alomone; Beijing, China), GDNF (cat no. ab18956; Abcam), NOS (cat no. sc-49058; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and β-actin (cat no. ab8226; Abcam).

The protein was incubated overnight at 37 °C with the addition of a second antibody (A21241) for 2 h, and color detection and chemiluminescence imaging analysis system (Tanon 6200, Shanghai Tanon Technology Co., Ltd., Shanghai, China) were used to collect images [12].

2.15. Statistical Analysis

Three parallel experiment results were averaged. Then, SAS software (9.1, SAS Institute Inc., North Tustin, CA, USA) was used to analyze whether the data of each group had significant difference at the $p < 0.05$ level using one-way analysis of variance (ANOVA, analysis of variance).

3. Results

3.1. Identification of Lactic Acid Bacteria

After performing MRS culture medium, the strain showed neat white, round, edge, and slightly raised center; a diameter was about 1.6 mm; and the surface was thick, opaque, and smooth. Through gram staining, the stain also showed gram positive, long rod-shaped, arranged in clusters or arranged in chains, with darker staining. The physiological and biochemical test results of *Lactobacillus* are shown in Table 2, by comparing the identification criteria of lactic acid bacteria (GB 4789.35-2010), the stain was preliminary identified as *Lactobacillus plantarum*. Then, the PCR amplification of 16sDNA gene results showed that negative control had no banding, the amplified had no pollution (Figure 1A). The lane was a length of about 2000 bp the specificity of the amplified fragment, expected amplified fragment length (Figure 1B). After sequence determination and matching gene sequence in Genbank, the sequencing result showed that the similarity of the sequence of the 16SrDNA gene fragment of the experimental strain and *Lactobacillus plantarum* strain CIP 103151 reached 98%, the isolation and identification of lactic acid bacteria was named *Lactobacillus plantarum* YS2 (LP-YS2).

Table 2. Physiological, biochemical and Sugar alcohol fermentation characteristicsresults of LP-YS4.

Physiological test	10 °C, 30 min growth	+
	15 °C, 30 min growth	+
	45 °C, 30 min growth	+
	60 °C, 30 min growth	−
	pH4.5 growth	+
	pH 9.6 growth	+w
	Anaerobic growth	+w
	Motility	−
Biochemical test	Contact enzyme test	−
	Oxidase test	−
	Hydrogen sulfide test	−
	Gelatin liquefaction test	−
	Nitrate reduction test	−
	Indole test	−
	Glucose gas production test	−
	Benzidine test	−
	Litmus milk test-acid production	+
	Litmus milk test-decolorization	+
	Litmus milk test-freezing	+
	Litmus milk test-peptonization	−
	Arginine producing ammonia test	−
	Casein decomposition test	−

Table 2. *Cont.*

Physiological test	10 °C, 30 min growth	+
	Amygdalin	+
	Arabinose	+
	Cellobiose	+
	Aesculin	+
	Fructose	+
	Galactose	+
	Glucose	+
	Gluconate	+
Sugar alcohol fermentation test	Lactose	+
	Maltose	+
	Mannite	+
	Seminose	+
	Melizitose	−
	Melibiose	+
	Raffinose	+
	Rhamnose	−
	D-ribose	+
	Salicin	+
	Sorbierite	+
	Sucrose	+
	Fucose	+
	Xylose	−

+ means positive strain; − means negative strain; +w means weakly positive strain.

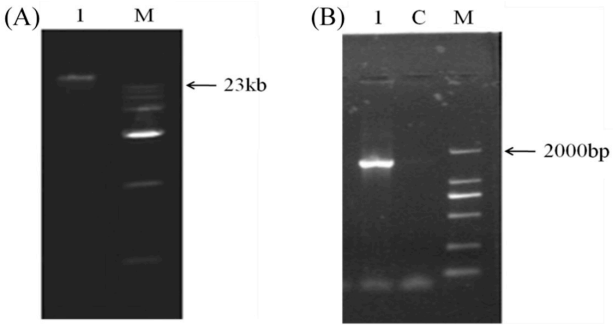

Figure 1. The 0.8% agarose gel electrophoresis map, M: λDNA/Hind III Marker; 1: *Lactobacillus plantarum* (**A**); and Polymerase Chain Reaction (PCR) amplification map of lactic acid bacteria 16SrDNA gene, M: DNA Marker; C: Invisible control; 1: *Lactobacillus plantarum* (**B**).

3.2. Acid and Bile Salt Resistant Activities

As shown in Table 3, the survival ability in artificial gastric juice (71.08%) of pH 3.0 of LP-YS4 was significantly ($p < 0.05$) stronger than those of LB (24.84%). The growth in 0.3%, 0.5% and 1.0% bile salt of LP-YS4 (20.08%, 17.62% and 11.25%, respectively) was significantly ($p < 0.05$) more than those of LB (2.22%, 1.32% and 1.06%, respectively).

Table 3. Acid and bile salt resistant activity of *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus*.

Strain	Survival in pH 3.0 Artificial Gastric Juice (%)	Growth in Bile Salt (%)		
		0.3%	0.5%	1.0%
LP-YS4	71.08 ± 7.17	20.08± 1.17	17.62± 2.31	11.25± 1.46
LB	24.84 ± 4.52	2.22± 0.41	1.32± 0.35	1.06± 0.23

LP-YS4: *Lactobacillus plantarum* YS4; LB, *Lactobacillus bulgaricus*. Values presented are the mean ± standard deviation.

3.3. Body Weight of Mice

As shown in Figure 2, after treatment with activated carbon, which was used to induce constipation, the body weight of all mice was significantly ($p < 0.05$) reduced, while LB and LP-YS4 could prevent further weight loss. It is noted that LP-YS4-H (high concentration of LP-YS4) showed the best ability to prevent weight loss. The body weights of mice in LP-YS4-H group were theclosest to the mice in normal group.

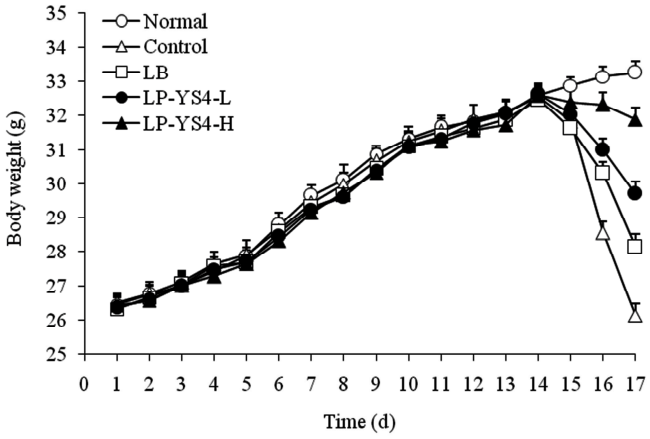

Figure 2. Effect of *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* on body weight changes in activated carbon-induced constipation mice during the experiment (N = 10/group). LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

3.4. Stool Status of Mice

From Day 1 to Day 14, weight, particle counts and water content of stool of mice in five groups were similar;there was no significant ($p > 0.05$) difference between these groups (Table 4). After inducing constipation by activated carbon, the stool weight, particle counts and water content werereduced;these indexes in control group were lowest.LB, LP-YS4-L and LP-YS4-H treatment show the ability to raise the stool weight, particle counts and water content. These indexes in LP-YS4-H treated mice were higher than LB and LP-YS4-L treated mice.

Table 4. Stool status of lactic acid bacteria treated mice during the experiment.

Groups	Normal	Control	LB	LP-YS4-L	LP-YS4-H
Days 1–14 (lactic acid bacteria administration period, but not inductionconstipation)					
Stool weight (g)	0.93 ± 0.04^{a}	0.92 ± 0.04^{a}	0.92 ± 0.05^{a}	0.93 ± 0.05^{a}	0.93 ± 0.04^{a}
Particle counts of stool	39 ± 3^{a}	38 ± 5^{a}	39 ± 4^{a}	40 ± 4^{a}	40 ± 4^{a}
Water content of stool (%)	48 ± 3^{a}	49 ± 4^{a}	49 ± 5^{a}	50 ± 5^{a}	50 ± 5^{a}
Days 15–17 (lactic acid bacteria administration period, inductionconstipation)					
Stool weight (g)	0.95 ± 0.05^{a}	0.35 ± 0.04^{e}	0.55 ± 0.05^{d}	0.67 ± 0.04^{c}	0.73 ± 0.05^{b}
Particle counts of stool	40 ± 4^{a}	17 ± 4^{e}	24 ± 4^{d}	$28 \pm {}^{b}{}^{c}$	35 ± 4^{b}
Water content of stool (%)	49 ± 4^{a}	15 ± 3^{d}	33 ± 4^{c}	34 ± 3^{c}	40 ± 4^{b}

Values presented are the mean ± standard deviation (N = 10/group). $^{a–e}$ Mean values with different letters in the same row are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

3.5. First Black Stool Defecation Time of Mice

The first black stool defecation time of mice in normal group was shortest which was calculated as 81 min, but in control group it was measured to be 202 min (Figure 3). This time in LB, LP-YS4-L and LP-YS4-H treated mice were 175 min, 155 min and 120 min, respectively.

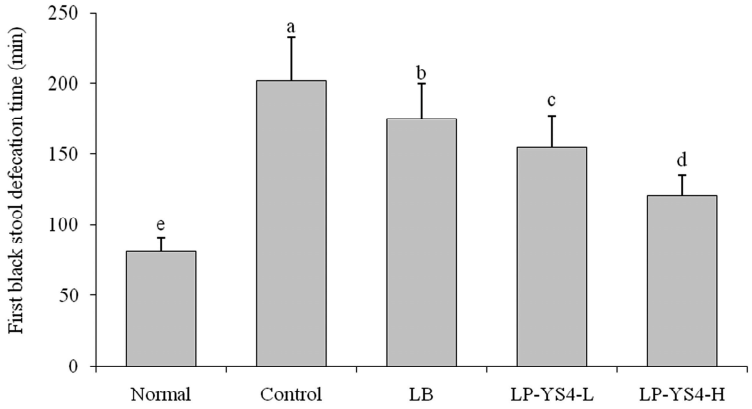

Figure 3. The first black stool defecation time in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice. [a–e] Mean values with different letters over the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test. LB: *Lactobacillus bulgaricus* [1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)]; LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

3.6. Gastrointestinal (GI) Transit Capability of Mice

The length of small intestine in different group had no significant ($p > 0.05$) difference (Table 5). The length of GI transit of mice in normal group was longest (39.6 cm), but the length of GI transit of mice in control group was shortest (7.1 cm). LB, LY-PS4-L and LP-YS4-H could increase the length of GI transits, and LP-YS4-H group had a length of GI transit only shorter than normal group. Therefore, activated carbon propulsive rate in normal, control, LB, LP-YS4-L and LP-YS4-H groups were 96.6%, 17.5%, 55.3%, 65.8% and 75.3%, respectively.

Table 5. The gastrointestinal (GI) transit capability and first black stool defection time in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice.

Groups	Length of Small Intestine (cm)	Length of GI Transit (cm)	Activated Carbon Propulsive Rate(%)
Normal	41.0 ± 3.4^{a}	39.6 ± 2.9^{a}	96.6 ± 1.5^{a}
Control	40.5 ± 3.1^{a}	7.1 ± 1.5^{e}	17.5 ± 2.0^{e}
LB	40.7 ± 3.2^{a}	22.5 ± 2.3^{d}	55.3 ± 2.2^{d}
LP-YS4-L	40.9 ± 3.1^{a}	26.9 ± 1.7^{c}	65.8 ± 2.4^{c}
LP-YS4-H	40.9 ± 2.8^{a}	30.8 ± 2.4^{b}	75.3 ± 2.6^{b}

Values presented are the mean ± standard deviation (N = 10/group). [a–e] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

3.7. Serum Levels of Mice

The MTL, GAS, ET, AchE, SP and VIP serum levels of mice in control group were lowest, and the SS level was highest (Table 6). LB, LP-YS4-L and LP-YS4-H all could raise the MTL, GAS, ET, AchE, SP and VIP serum levels but reduce the SS level more than as compared to control group. Meanwhile, these levels of mice in LP-YS4-H group were only slightly different from the mice in normal group.

Table 6. The serum levels of MTL, GAS, ET, SS, AchE, SP and VIP in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice.

Levels (pg/mL)	Normal	Control	LB	LP-YS4-L	LP-YS4-H
MTL	223.6 ± 21.3 ^{a}	91.3 ± 8.1 ^{e}	139.7 ± 13.5 ^{d}	166.3 ± 15.1 ^{c}	190.3 ± 7.6 ^{b}
GAS	102.3 ± 6.6 ^{a}	29.3 ± 3.7 ^{e}	50.3 ± 3.2 ^{d}	61.8 ± 2.7 ^{c}	77.9 ± 3.3 ^{b}
ET	21.3 ± 2.1 ^{a}	4.5 ± 0.7 ^{e}	7.5 ± 0.6 ^{d}	10.5 ± 0.9 ^{c}	15.2 ± 1.1 ^{b}
SS	28.7 ± 2.2 ^{e}	81.2 ± 3.3 ^{a}	64.3 ± 2.5 ^{b}	51.8 ± 2.6 ^{c}	31.2 ± 1.6 ^{d}
AchE	36.7 ± 1.6 ^{a}	7.6 ± 0.5 ^{e}	15.1 ± 0.7 ^{d}	22.1 ± 0.6 ^{c}	30.8 ± 1.7 ^{b}
SP	79.3 ± 2.5 ^{a}	22.0 ± 2.5 ^{e}	41.2 ± 2.3	55.2 ± 2.1 ^{c}	63.9 ± 2.0 ^{b}
VIP	69.2 ± 2.3 ^{a}	19.5 ± 2.1 ^{e}	38.7 ± 2.2 ^{d}	45.6 ± 2.8 ^{c}	60.2 ± 2.1 ^{b}

Values presented are the mean ± standard deviation (N = 10/group). $^{a–e}$ Mean values with different letters in the same row are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw). MTL, motilin; GAS, gastrin, ET-1, endothelin; SP, substance P; SS, somatostatin; VIP, vasoactive intestinal peptide.

3.8. Small Intestine Tissue Levels of Mice

The MPO, NO, and MDA small intestine tissue levels of mice in normal group were weakest, and GSH level was strongest (Table 7). On the contrary, the MPO, NO, and MDA levels of mice in control group were strongest, and GSH level was weakest. The MPO, NO, MDA levels of mice in LP-YS4-H group were higher than those in LB and LP-YS3-L groups, but MDA was lower than LB and LP-YS3-L groups.

Table 7. The small intestine tissue levels of MPO, NO, MDA and GSH in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice.

Group	MPO (mU/mg)	NO (μmol/gprot)	MDA (nmol/mg)	GSH (μmol/mg)
Normal	5.39 ± 0.32 ^{e}	0.36 ± 0.06 ^{e}	0.44 ± 0.05 ^{e}	8.96 ± 0.54 ^{a}
Control	15.38 ± 0.49 ^{a}	2.03 ± 0.33 ^{a}	1.17 ± 0.20 ^{a}	4.77 ± 0.36 ^{e}
LB	11.88 ± 0.37 ^{b}	1.46 ± 0.23 ^{b}	0.82 ± 0.13 ^{b}	6.31 ± 0.23 ^{d}
LP-YS4-L	10.03 ± 0.35 ^{c}	1.19 ± 0.24 ^{c}	0.69 ± 0.07 ^{c}	6.83 ± 0.18 ^{c}
LP-YS4-H	7.31 ± 0.33 ^{d}	0.72 ± 0.14 ^{d}	0.53 ± 0.04 ^{d}	7.26 ± 0.16 ^{b}

Values presented are the mean ± standard deviation (N = 10/group). $^{a–e}$ Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw). MPO, myeloperoxidase; NO, nitric oxide; GSH, glutathione; MDA, malondialdehyde.

3.9. Pathological Observation of Small Intestine

As shown in Figure 4, the wall thickness of small intestine and the morphology of small intestinal villi in mice of normal group were both intact. However, the thickness of the small intestinal wall of mice in control group was thinning, and the villi of the small intestine were broken and the shape was very incomplete. LB, LP-YS4-L and LP-YS4-H could inhibit the injury of small intestinal wall and villi in constipated mice, and LP-YS4-H had the best effects.

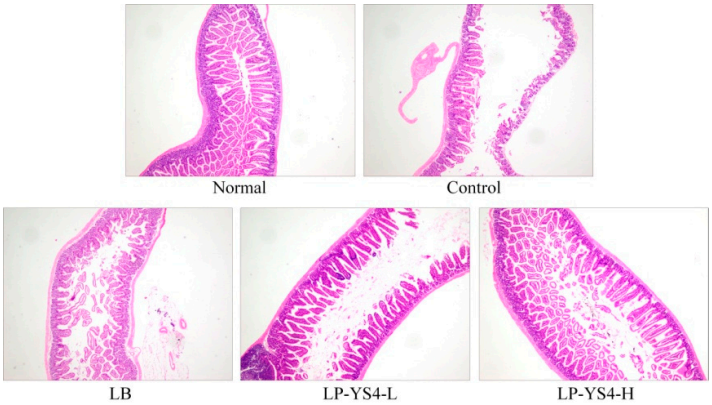

Figure 4. Pathological observation of small intestinal tissue in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

3.10. c-Kit and SCF mRNA Expression in Small Intestine Tissue

The small intestine tissue of mice in normal group had the strongest c-Kit and SCF mRNA and protein expressions (Figure 5 and Tables 8 and 9), but the mice in control group had the weakest expressions. LB, LP-YS4-L and LP-YS4-H could increase these expressions as compared to the control group, but the c-Kit and SCF expressions of LP-YS4-H were both higher than LB and LP-YS4-L groups.

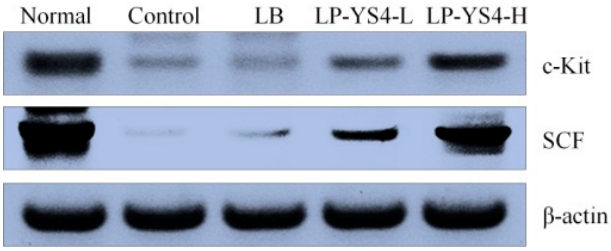

Figure 5. The small intestine tissue levels of protein expression of c-Kit and SCF in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

Table 8. The small intestine tissue levels of mRNA expression of c-Kit and SCF in *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice.

Group	GAPDH	c-Kit		SCF	
	Ct Value	Ct Value	Relative Expression	Ct Value	Relative Expression
Normal	19.21 ± 1.12 ^{a}	28.37 ± 1.52 ^{a}	5.90 ± 0.42 ^{a}	27.65 ± 1.69 ^{a}	7.36 ± 0.66 ^{a}
Control	18.92 ± 1.16 ^{a}	31.22 ± 1.71 ^{a}	1.00 ± 0.09 ^{e}	30.82 ± 1.48 ^{a}	1.00 ± 0.12 ^{e}
LB	19.11 ± 1.12 ^{a}	30.19 ± 1.63 ^{a}	1.91 ± 0.23 ^{d}	29.33 ± 1.86 ^{a}	2.62 ± 0.32 ^{d}
LP-YS4-L	19.05 ± 1.21 ^{a}	29.77 ± 1.48 ^{a}	2.44 ± 0.15 ^{c}	28.71 ± 1.62 ^{a}	3.86 ± 0.30 ^{c}
LP-YS4-H	19.08 ± 1.18 ^{a}	28.94 ± 1.51 ^{a}	4.44 ± 0.27 ^{b}	28.25 ± 1.77 ^{a}	5.43 ± 0.54 ^{b}

Values presented are the mean ± standard deviation (N = 10/group). $^{a–e}$ Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw). Ct, cycle threshold.

Table 9. Semi-quantitative analysis of c-Kit and SCF protein of *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* on small intestine tissue of mice (folds of control group).

Group	c-Kit Expression	SCF Expression
Normal	3.12 ± 0.32^{a}	81.99 ± 4.75^{a}
Control	1.00 ± 0.04^{e}	1.00 ± 0.09^{e}
LB	1.14 ± 0.07^{d}	4.36 ± 0.62^{d}
LP-YS4-L	1.32 ± 0.11^{c}	20.51 ± 3.88^{c}
LP-YS4-H	2.20 ± 0.22^{b}	59.42 ± 5.12^{b}

Values presented are the mean ± standard deviation (N = 10/group). Fold-ratio: gene (or protein) expression/β-actin × control numerical value (control fold ratio: 1). $^{a–e}$ Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* [1.0×10^{9} colony-forming unit (CFU)/kg body weight (bw)]; LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^{8} CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^{9} CFU/kg bw).

3.11. TRPV1, GDNF and NOS mRNA Expression in Small Intestine Tissue

The TRPV1 and NOS mRNA expressions of mice in control group were highest, but GDNF expression was lowest (Figure 6 and Tables 10 and 11). LP-YS4-H could reduce the TRPV1 and NOS expressions and raise GDNF expression as compared to the control group, and make these expressions closer to the normal group.

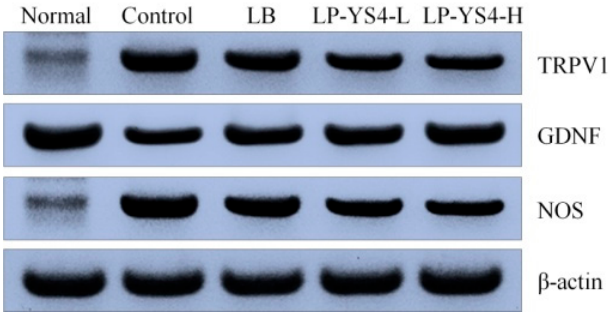

Figure 6. The small intestine tissue levels of protein expression of TRPV1, GDNF and NOS in the *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice. LB: *Lactobacillus bulgaricus* (1.0×10^{9} colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^{8} CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^{9} CFU/kg bw).

Table 10. The small intestine tissue levels of mRNA expression of TRPV1, GDNF and NOS in *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* treated activated carbon-induced constipation mice.

Group	GAPDH	TRPV1		GDNF		NOS	
	Ct Value	Ct Value	Relative Expression	Ct Value	Relative Expression	Ct Value	Relative Expression
Normal	19.21 ± 1.12^{a}	31.02 ± 1.62^{a}	0.23 ± 0.02^{e}	26.62 ± 1.44^{a}	8.88 ± 0.71^{a}	31.81 ± 1.87^{a}	0.19 ± 0.03^{e}
Control	18.92 ± 1.16^{a}	29.17 ± 2.01^{a}	1.00 ± 0.04^{a}	30.06 ± 1.51^{a}	1.00 ± 0.10^{e}	29.69 ± 1.82^{a}	1.00 ± 0.11^{a}
LB	19.11 ± 1.12^{a}	29.44 ± 1.55^{a}	0.77 ± 0.05^{b}	28.71 ± 1.40^{a}	2.38 ± 0.41^{d}	30.32 ± 1.76^{a}	0.60 ± 0.05^{b}
LP-YS4-L	19.05 ± 1.21^{a}	29.77 ± 1.23^{a}	0.59 ± 0.04^{c}	27.76 ± 1.45^{a}	4.41 ± 0.52^{c}	30.76 ± 1.74^{a}	0.43 ± 0.04^{c}
LP-YS4-H	19.08 ± 1.18^{a}	30.31 ± 1.48^{a}	0.41 ± 0.04^{d}	27.23 ± 1.57^{a}	6.50 ± 0.69^{b}	31.33 ± 1.79^{a}	0.29 ± 0.03^{d}

Values presented are the mean ± standard deviation (N = 10/group). $^{a–e}$ Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* [1.0×10^{9} colony-forming unit (CFU)/kg body weight (bw)]; LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^{8} CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^{9} CFU/kg bw). Ct, cycle threshold.

Table 11. Semi-quantitative analysis of TRPV1, GDNF and NOS protein of *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* on small intestine tissue of mice (folds of control group).

Group	TRPV1 Expression	GDNF Expression	NOS Expression
Normal	0.47 ± 0.03^{e}	1.61 ± 0.08^{a}	0.19 ± 0.03^{e}
Control	1.00 ± 0.05^{a}	1.00 ± 0.05^{e}	1.00 ± 0.07^{a}
LB	0.89 ± 0.03^{b}	1.21 ± 0.04^{d}	0.87 ± 0.02^{b}
LP-YS4-L	0.74 ± 0.04^{c}	1.29 ± 0.03^{c}	0.74 ± 0.04^{c}
LP-YS4-H	0.67 ± 0.04^{d}	1.39 ± 0.06^{b}	0.62 ± 0.04^{d}

Values presented are the mean ± standard deviation (N = 10/group). Fold-ratio: gene (or protein) expression/β-actin × control numerical value (control fold ratio: 1). $^{a–e}$ Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple-range test. LB: *Lactobacillus bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); LP-YS4-L: *Lactobacillus plantarum* YS4 low dose (1.0×10^8 CFU/kg bw); LP-YS4-H: *Lactobacillus plantarum* YS4 high dose (1.0×10^9 CFU/kg bw).

4. Discussion

Lactic acid bacteria can act as probiotics only under high concentration of acid in stomach and large intestine; reaching the destination (usually in the large intestine), they colonize and there start their physiological function [10]. Therefore, to study the potential probiotic function of *lactobacillus*, a virtual model in vitro was established to detect its resistance capability in gastric acid and its tolerance capability of cholate salt [13]. In this study, LP-YS4 was better than LB in terms of ability to resist artificial gastric acid and cholate salt. In addition, it exhibited better physiological activities. LP-YS4 is gram-positive bacteria. The decarboxylation of amino acid decarboxylase of LP-YS4 might be able to control the pH environment through the consumption value of H^+ bacteria. Lysine, arginine or GAD can transform extracellular amino acids into extracellular products, while depleting intracellular protons, resulting an increase in intracellular pH, thereby obtaining the effect of anti-gastric acid [4,14]. Bile salt hydrolase (BSHs) has the ability to change the characteristics of the cell membrane, removing poison from the bile, and stay in the gastrointestinal tract, which is typically endoenzyme. The optimum pH value is 5–6. LP-YS4 might be able to produce BSHs enzyme, the enzyme degradation cross linking of bile salts. Conjugated bile acid is decomposed into free bile acid low solubility, and precipitates; this process reduces the toxicity of bile salts, and improves the bile salt tolerance ability of LP-YS4 [8,14].

The change in body mass is an important indicator of constipation in mice. Studies have confirmed that mice with activated carbon-induced constipation were lighter than that of normal mice [15]. It was also confirmed by the result of this study. Rats became constipated after inducing by lalpidipine hydrochloride, which can also lead to significantly lower body mass of the rats with constipation than that of normal group [16]. Thus, it can be concluded that, in animals, constipation can lead to slower growth in body mass. The results of various procedures from above experiment showed that the LP-YS4 has a better inhibitory effect on the degradation of mice body mass which was initiated by constipation.

Defecation material properties is the most obvious reflection on the degree of constipation. The weight, number and water content of feces in constipated state are important indicators of feces status. The reduction in the above indicators shows the aggravation in constipation [15]. Studies have shown that, when rats and mice were given *lactobacillus* by lavage after inducing constipation, which either helps relieves or further aggravates constipation by inhibiting further detouring of the number of fecal particles and water content caused by the constipation, it can finally inhibit the effects of constipation on animal body [16,17]. In this study, lactobacillus LP-YS4 could also relieve the constipation by increasing the defecation weight, frequency and water content of constipated mice.

Constipation reduces intestinal peristalsis and makes the excrement stay longer in bowel, causing harmful bacteria to continuously reproduce by feeding on the feces. As a result, it can cause harm to the intestinal health and further aggravate symptoms that can lead to many acute or chronic bowel diseases [18]. After mice were induced constipation by activated carbon, its propulsive length and propulsive rate in small intestine can be used as an index to evaluate the activity of small intestine and

the degree of constipation [9]. In this study, LP-YS4 made the propulsive length and propulsive rate of activated carbon in the small intestine more than that of LB. High-concentration LP-YS1 had more obvious effects.

According to the theory of traditional Chinese medicine, the accumulation of cold in the stomach will lead to stagnation of the circulation of vital energy. Ice activated carbon solution can cause difficulty in defecation and induce constipation in mice; this process is similar to the theory of traditional Chinese Medicine [19]. Constipation slows down bowel movements and makes the excrement stuck longer in the intestines, which delays the defecation of first black grain. Shorter time of defecation of first black grain means better bowel movement [9]. In this study, as compared with constipation control group mice, LP-YS4 could significantly reduce the defecation time of first black grain and have good constipation relief function.

MTL can stimulate the secretion of pepsin and promote bowel movements [20]. GAS plays a very significant role in stomach and intestine, which can promote gastric secretion, bowel movements and can relax pylorus, thus relieving constipation [9]. ET plays an important role in stabilizing vascular tension and maintaining basic cardiovascular system [21]. SS has been used to stimulate bowel movements [22]. They can help alleviate constipation. AChE can regulate muscle contraction and mucus secretion, which can relax the muscles to push the stool out [23]. SP is another substance that helps intestinal peristalsis [24]. It can keep the normal contents of VIP in intestinal walls, which is also an important factor in stabilizing intestinal function [25]. The result of this study also shows that LP-YS1 could keep the serum levels as normal as possible and can relieve constipation to a great extent.

ICC (Interstitial cells of Cajal) is the pacemaker cell of intestinal slow wave and also plays an important role in the transmission of intestinal nerve signals, which affects the gastrointestinal function [26]. Studies have shown that ICC density in intestine of patients with constipation is lower than normal people; it reduces the postsynaptic response between ICC and neurotransmitter which makes ICC create spontaneous rhythmic activity of slow wave. This leads to irregular colon movement, affecting intestinal function [26–28]. c-Kit is the specific marker of ICC and the key to the proliferation of ICC [29]. The concentration of SCF is very important in the reproduction of ICC, because ICC cannot grow without SCF. Animal experiments also have showed that there was less ICC in colon tissues of constipated mice, and the expression level of c-kit also decreased [30]. Study has shown that *lactobacillus* could effectively improve the content of c-kit in intestine of constipated mice, which shows an increase in the content of ICC, promoting intestinal peristalsis and relieving constipation [31]. In this experiment, constipation can reduce the expression of c-Kit and SCF in mice small intestine and LP-YS4 could effectively up-regulate the expression of c-Kit and SCF ($p < 0.05$), which increases ICC in intestine of constipated mice, thus inhibiting constipation.

TRPV1 has been confirmed to be closely associated to defecation, so the activation of TRPV1 can trigger the release of neurotransmitters, which can lead to bowel movement disorders in small intestine. The increased expression of TRPV1 is a significant indication of intestinal injury. Gastrointestinal disorders can cause intestinal injury, which leads to higher expression of TRPV1 in constipated patients [32]. GDNF can regulate ganglion cells, which can help in the healing of damaged intestinal track and prevent constipation. Constipation is somehow related to enteric nervous system. NO is a major type of inhibitory neurotransmitter of enteric nervous system, which can relax smooth muscles and slow down bowel movements. The increase of NOS (nitric oxide synthase) positive fibers will increase the content of NO, thus affecting intestinal function and causing constipation [33,34]. NOS plays an important role in regulating gastrointestinal movement [35]. The increase of NO can exacerbate colonic dynamic disturbance. Thus, controlling NOS can reduce the content of NO, which is a feasible way to control constipation [36]. One of the mechanisms for the inhibitory effect of *lactobacillus* is to regulate and maintain the expression levels of TRPV1, GDNF and NOS at appropriate levels to relieve constipation.

Yak is a special species living in the Tibetan Plateau of China. Because of the special environment in the plateau, yak milk contains rich nutrients, such as immunoglobulin, calcium, iron, and conjugated

linoleic acid [2]. Meanwhile, because the plateau environment is special, temperature, humidity and oxygen content are all different from other areas [1]. The microbial diversity of yak yogurt is also different from that of commonly fermented dairy products [2].There are few studies on lactic acid bacteria in Yak yoghurt now. Only a few studies have been made on the diversity of lactic acid bacteria [1,2,11] and on the functional effect of yak yoghurt *Lactobacillus* [4]. The yak yoghurt *Lactobacillus* has better intestinal colonization than general lactic acid bacteria;its functional effects may also be stronger [9]. This study has proven that yak yogurt *Lactobacillus* has a good constipation inhibitory effect. Its mechanism is also analyzed, which helps to better understand yak yogurt *Lactobacillus*.

5. Conclusions

LP-YS4 separated from kurut in Tibetan area had good resistance capabilities in gastric acid and bile salt, which benefit effectively by relieving weight loss and decreasing defecation weight, grain number and water content caused by constipation in mice. It improves the propulsive rate of active carbon in small intestine and shorten the time of discharge of the first black grain. The serum levels results showed that LP-YS4 could improve the content of MTL, ET, SS, GAS, AChE, SP and VIP and lower the level of SS in the constipated mice. RT-PCR experiments further showed that LP-YS4 could up-regulate the mRNA expression of c-Kit, SCF and GDNF genes, and down-regulate the expression of TRPV1 and NOS in constipated mice. These results showed that LP-YS1 could effectively relieve constipation and had better effect than the commonly-used LB (*Lactobacillus bulgaricus*). It is notable that its effects were better with the increase of concentration.

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Conflicts of Interest: The authors declare no conflict of interest.

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