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Lactobacillus plantarum CQPC05 Isolated from Pickled Vegetables Inhibits Constipation in Mice

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Abstract: This study mainly investigated the influences of *Lactobacillus plantarum* CQPC05 (CQPC05) isolated from pickled vegetables on mouse constipation caused by activated carbon water. We used 16S rDNA technology to identify the microorganism, and activated carbon to establish a mouse constipation model. After the mice received *L. plantarum* (10^9 CFU/mL) by gavage, small intestine tissue sections were collected. The serum indices and small intestine-related mRNA expression were obtained. A strain of *L. plantarum* was identified, and named CQPC05. The body weight and activated carbon progradation rate in the mice of the CQPC05 treatment groups were significantly higher than those in the control group, and the excretion time of the first tarry stool was earlier than that of the control group. The results of serum indices indicated that serum gastrin (Gas), endothelin (ET), and acetylcholinesterase (AchE) levels in the CQPC05 treatment groups were significantly higher than those in the control group, while the somatostatin (SS) level was significantly lower. Compared to the constipation control group, the result of q-PCR demonstrated that CQPC05 could up-regulate the mRNA expression of c-Kit (stem cell factor receptor), SCF (stem cell factor), and GDNF (glial cell-derived neurotrophic factor) genes, and down-regulate the expression of TRPV1 (transient receptor potential cation channel subfamily V member 1) and iNOS (inducible nitric oxide synthase). In conclusion, *L. plantarum* CQPC05 can effectively alleviate constipation, and has good probiotic potential and application value.

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

1. Introduction

Traditional Szechwan pickled vegetables are a type of food that is obtained through lactic acid fermentation, combined with acetic acid and alcohol fermentation, using salt water at a specific concentration [1]. The underlying fermentation principle is that in a saline solution at a concentration of 5–10%, and with the help of beneficial microorganisms (mainly *Lactobacillus*) on the surface of vegetables, acids are generated by fermentation, which decreases the pH [2,3]. Furthermore, benefitting

from the high osmotic pressure of salt, the growth of harmful microorganisms is inhibited. In salt water, *Lactobacillus* can use sugars and nitrogen substances for reproduction and the generation of acidic substances. *Lactobacillus* can also metabolize flavor components and generate good flavor in pickled vegetables. A large amount of *Lactobacillus* has been found in pickled vegetables, playing central roles for both the flavor and quality of the food [4,5]. *Lactobacillus* isolated from fermented food (such as pickled vegetables) has been reported to have multiple benefits for health, including the prevention of constipation, colonitis, liver injury, and diabetes [6–9]. Therefore, these microorganisms are used as probiotics. It has been proven that *Lactobacillus* possesses several functions. For example, *Lactobacillus* LF31 and *Lactobacillus coryniformis* have antioxidant effects [10], and *Lactobacillus plantarum* YS4 can prevent constipation in mice [11]. Our team investigated Szechwan-style pickled vegetables, and isolated and identified the microorganisms that they contain. One of the stains was named *Lactobacillus plantarum* CQPC05, which was comprehensively explored in this study.

A person with fewer than three defecation times per week is considered to be in a state of constipation. A time below one is classified as severe constipation [12]. It is a complicated symptom threatening human health, and greatly influences colon health. Generally, constipation is not considered a disease, and can be regulated by daily diet and dietary habit [13]. Under normal conditions, probiotics and harmful bacteria in the human intestinal tract are in a state of equilibrium. Probiotics participate in digestion and absorption, and eliminate harmful substances. However, harmful bacteria generate harmful substances, and damage the health of the intestinal tract. Disturbing the equilibrium between them will lead to dyspepsia, intestinal dysfunction, or even malignant diseases, such as severe tumors [14,15]. *Lactobacillus* can consolidate the endogenous defense barrier in the intestinal tract, improve the non-immune defense barrier function, and activate the endogenous bacterial metabolism. In addition, several *Lactobacillus* species can generate functional organic acids, promote intestinal tract repair, reduce the pH of the intestinal tract, regulate the neuromuscular motility of the intestinal tract, and improve peristalsis, as well as the digestion and absorption of the intestinal tract [16]. *Lactobacillus* can effectively inhibit the reproduction of putrefying bacteria in the intestinal tract as a probiotic, improve intestinal tract environment, soften feces, facilitate excretion, and prevent constipation. During constipation, the changes of intestinal microbiota include increased levels of aerobic bacteria, fungi, and *Escherichia coli*, as well as decreased levels of anaerobic bacteria, *Bacteroides*, and *Bifidobacterium* [17]. Probiotics plays an important role in the maintenance of intestinal ecological balance, the regulation of constipation, and the prevention of other diseases.

An animal constipation model can be used to detect the physiological effects of specific on constipation. The gastrointestinal mucosal surface is adsorbed by activated carbon through activated carbon gavage, leading to reduced water content and digestive juice in the digestive tract, thus slowing down gastrointestinal motility and leading to constipation. In this study, *Lactobacillus plantarum* CQPC05 from pickled vegetables was used as the research subject, and *Lactobacillus bulgaricus* (LB) was used as the control strain. The excretion time of the first tarry stool, gas, and serum motilin (MTL) were measured to verify the recovery effect of CQPC05 on the intestinal function of mice with constipation. Moreover, gene expression in the small intestine was confirmed by qPCR and Western blot (WB), which further elucidated the molecular mechanism with which CQPC05 alleviates constipation.

2. Materials and Methods

2.1. Isolation and Identification of Lactobacillus

First, one mL of pickled vegetable water was collected, and conventional plate dilution was used to dilute the samples to 10^{-6} with physiological saline. The plates were coated with 100 μL of the above diluted solutions at three concentrations (10^{-4} CFU/mL, 10^{-5} CFU/mL, and 10^{-6} CFU/mL). After incubation at 37 °C for 38 h, the colony morphology was observed. Single colonies were picked for culture. The above steps were repeated three times to obtain a single colony. The pure strain was seeded in MRS (DeMan Rogosa-Sharpe) liquid culture medium (five mL) and incubated at 37 °C for 24 h. Then,

one mL of the culture medium was transferred to a sterile tube, and centrifuged (4000 r/min, 10 min) to discard the supernatant. The bacteria at the bottom were used for Gram staining. Moreover, a single colony was seeded in MRS liquid culture medium (five mL) and incubated at 37 °C for 24 h. Bacterial DNA was extracted following the instructions of the manufacturer (TIANGEN Biotech Co., Ltd., Beijing, China), and amplified by 16S rDNA amplification. At the same time, the strains were cultured on MRS medium, and the colony morphology was observed.

The amplification system included: one μL of upstream primer 27F (5′-AGA GTT TGA TCC TGGCTC AG-3′), one μL of down-stream primer 1495R (5′-CTA CGG CTA CCTTGT TAC GA-3′), 12.5 μL of 2×Taq plus Buffer, and one μL of and template DNA. The system was filled to 25 μL with sterile ddH_2O. Sterile ultrapure water was used to replace template DNA as the negative control. The amplification conditions were: 94 °C for five minutes; 94 °C for 30 s, 55 °C for 30 s, 72 °C for one minute, for 29 cycles, and followed by a final 72 °C extension for five minutes. Five μL of amplified product was used for agarose gel electrophoresis (agarose concentration 1.5%, electrophoresis 110 V, 45 min). The PCR products were sequenced by Tsingke Biological Technology. The successful sequences were analyzed by BLAST (Basic Local Alignment Search Tool) from NVBI [18].

2.2. Experimental Strain

Lactobacillus delbrueckii subsp. *bulgaricus* (LB, CGMCC No. 1.16075) was purchased from China General Microbiological Culture Collection Center (CGMCC, Beijing, China) as a comparative strain with CQPC05.

2.3. Animal Model

Kunming mice (female, n = 50) at the age of seven weeks were purchased from Chongqing Medical University. The mice were raised at 25 ± 2 °C with humidity 50 ± 5%, under a 12 h-light/dark cycle, and were allowed free access to food and water. The mice were divided into five groups: control group, normal group, high CQPC05 concentration treatment group (CQPC05-H, 1×10^9 CFU/kg), low CQPC05 concentration treatment group (CQPC05-L, 1×10^8 CFU/kg), and LB treatment group (1×10^9 CFU/kg).

The mice in each group were treated as follows: raised for 10 days, weighted daily, and food and drinking were recorded. In the normal group, the mice were normally raised for nine days, without any treatment. They were fasted for 24 h from Day 9, and dissected at Day 10. In the control group, the mice were normally raised for nine days, without any treatment. They were fasted for 24 h from Day 9, and received activated carbon 0.3 mL (10%, water and ice) with the experiment samples (CQPC05 and LB) by gavage from Day 7. Mice were dissected after the administration of activated carbon (10%, water and ice) by gavage at Day 10. In the low CQPC05 concentration group, CQPC05 was administrated by gavage every day (1×10^8 CFU/kg) in a volume of 0.2 mL for nine days. The mice simultaneously received activated carbon 0.3 mL (10%, water and ice) by gavage from Day 7, and were dissected after the administration of activated carbon (10%, water and ice) by gavage at Day 10. In the high CQPC05 concentration group, CQPC05 was administrated by gavage every day (1×10^9 CFU/kg) in a volume of 0.2 mL for nine days. The mice simultaneously received 0.3 mL of activated carbon (10%, water and ice) by gavage from Day 7, and were dissected after administration with activated carbon (10%, water and ice) by gavage at Day 10. In the LB group, LB was administrated by gavage for nine days (1×10^9 CFU/kg). The mice were simultaneously administrated by gavage from Day 7, and were dissected after administration with activated carbon (10%, water and ice) by gavage at Day 10. The protocol for these experiments was approved by the Animal Ethics Committee of Chongqing Collaborative Innovation Center for Functional Food (201802002B).

2.4. Measurement of Small Intestine Progradation Rate and Excretion Time of the First Tarry Stool

At Day 9, all of the mice were fasted but allowed free access to water for 24 h after 10% activated carbon administration by gavage. After 24 h, all of the mice were administrated 0.3 mL of ice-activated

carbon solution by gavage. Then, 30 min later, five mice per group (n = 5) were sacrificed by breaking their neck to collect blood. Furthermore, the abdominal cavity was opened. The part from the digestive tract pylorus to ileocecal valve was completely harvested, and paved on a steel plate. The total length of the small intestine and the progradation distance of the activated carbon in the small intestine were measured. The remaining five mice per group were observed, and the excretion times of the first tarry stool were recorded. The progradation rate was calculated as follows: progradation rate (%) = (progradation distance of activated carbon (cm))/total length of small intestine × 100% [19].

2.5. Detection of Serum Indices

The mice were anesthetized; then, both sides of the neck of the mice were compressed to make the eyes protrude and congest the posterior orbital venous plexus, and the blood was taken from the inner canthus of mice by means of capillary puncture. The mouse blood was placed in centrifuge tubes, and freeze-centrifuged at 4500 rpm/min to collect the serum. The MTL, serum gastrin (gas), endothelin (ET), somatostatin (SS), and acetylcholinesterase (AChE) levels were measured following the instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.6. Histopathological Assay

The small intestine of mouse from gastric pyloric entrance to ileocecal valve was collected. The luminal matter of the small intestine was washed with saline, and then the small intestine was immobilized in 10% formalin solution. Then, it was dehydrated in 95% ethanol for 24 h, and then treated with xylene. The small intestine of mice was directly transected for embedding. Then, the small intestine tissue was treated with H&E (haematoxylin and eosin) staining and the sections were observed. All of the sections were carefully observed under a microscope (BX43, Olympus, Tokyo, Japan).

2.7. Detection of mRNA Expression in Mouse Small Intestine by q-PCR

Total RNA from colon tissues was extracted by RNAzol (Trimo Fisher HealthInc., Waltham, MA, USA) after small intestine tissues were ground to a slurry. The purity and concentration of total RNA were measured by ultramicrospectrophotometer, and the RNA concentration was standardized to one μg/μL. OligodT18, RNase, dNTP (deoxy-ribonucleotide triphosphate), M-MLV (moloney murine leukemia virus) enzyme, and 10 μL 5×buffer (Thermo Fisher Scientific, Waltham, MA, USA) were added to the diluted total RNA extracting solution (two μL) in order, to synthesize cDNA under conditions of 65 °C for five minutes, 42 °C for six minutes, and 70 °C for five minutes. The mRNAs of c-Kit (stem cell factor receptor), SCF (stem cell factor), GDNF (glial cell line-derived neurotrophic factor), TRPV1 (transient receptor potential vanilloid 1), and iNOS (inducible nitric oxide synthase) in the small intestine tissues were amplified by q-PCR (primers shown in Table 1), and amplified using GAPDH as an internal reference under identical conditions. The relative expression levels of c-Kit, SCF, GDNF, TRPV1, and iNOS were calculated using the $2^{-\Delta\Delta CT}$ method based on the obtained Ct value (SteponePlus, Thermo Fisher Scientific, Waltham, MA, USA) [20].

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′

c-Kit, stem cell factor receptor; 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.

2.8. Statistical Analysis

Three parallel experimental results of all 10 mice in each group were averaged. In each index, the difference between the average and the value of each mouse was within 5%. SAS9.1 software was used for one-way ANOVA to analyze whether there were significant differences in the data of each group at the level of $p < 0.05$.

3. Results

3.1. Isolation and Identification of the Microorganism

Most of the colonies are round white or milky white, with clean margin, and a moist and smooth surface (Figure 1A). *Lactobacillus* was preliminarily identified by Gram staining. Under the microscope, the strain shows a long-rod and short-rod shape without spores (Figure 1B). Electrophoresis shows no band in the negative control group. Then, 1500 bp was estimated by DNA marker (molecular weight 2000) (Figure 1C), indicating that the PCR sample was not contaminated, and 16S rDNA sequencing could be performed. The sequencing results demonstrated that the strain had 99% homology with the known fermentative *Lactobacillus* in GenBank (GenBank No.: NC_004567.2). Consequently, the strain was a new *Lactobacillus* strain, and was named CQPC05, and reserved in the China General Microbiological Culture Collection Center (CGMCC No. 14494).

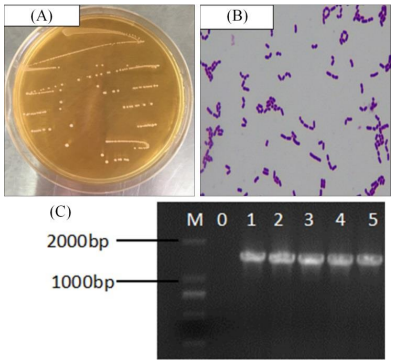

Figure 1. (**A**) Colony morphology, (**B**) Gram staining result, and (**C**) 16S rDNA agarose gel electrophoresis of PCR amplified product of *Lactobacillus plantarum* CQPC05. M: 2000 bp DNA Ladder; 0: negative control group; 5: *Lactobacillus plantarum* CQPC05.

3.2. Influence of CQPC05 on Body Weight of Constipated Mice

The body weight changes of each group are shown in Figure 2. The body weight of mice in each group during the first week showed a normal increase, and there was no significant difference between groups. After constipation was induced by the administration of activated carbon, the body weight in the normal group without activated carbon induction increased continuously, while that in other groups decreased to different degrees. The reduction in the constipation control group was the greatest, and the high CQPC05 concentration group was the lightest and closest to normal mice. The results of the above experiments indicated that CQPC05 exerted a good inhibitory effect on the decrease of the body weight caused by constipation.

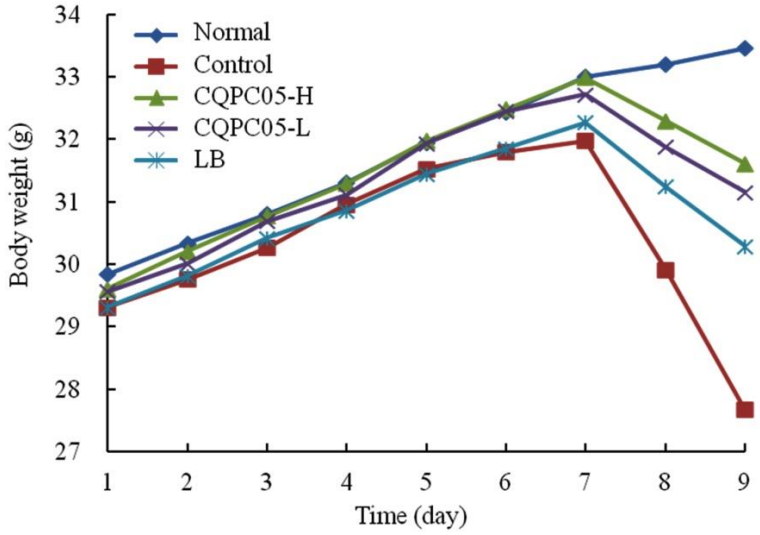

Figure 2. Weight changes of mice in each experimental group (average value of 10 mice in each group). LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw).

3.3. Influence of CQPC05 on Defecation Capability

As shown in Figure 3, the average excretion times of the first tarry stool in high CQPC05 concentration (56 ± four minutes), low CQPC05 concentration (67 ± five minutes), and LB (74 ± four minutes) groups were significantly shorter than that of the constipation control group (93 ± seven minutes). Constipation leads to slow intestinal motility, thus extending the residence time of feces in the intestinal tract, which lengthens the excretion time of the first tarry stool. Shorter excretion times indicate normal intestinal motility. In this study, compared to the constipation control group, CQPC05 could significantly decrease the excretion time of the first tarry stool, showing a good comparison alleviation effect.

In the small intestine of the control group, the progradation rate of activated carbon water was the lowest (28.3 ± 4.1%), and the progradation distance of normal group was the largest (100.0 ± 0.0%) (Figure 4). Thus, the calculated progradation rate of the high CQPC05 concentration (CQPC05-H) group (68.7 ± 3.2%) was significantly ($p < 0.05$) higher than that in the low CQPC05 concentration (CQPC05-L) group (59.3 ± 2.9%) and LB group (54.6 ± 3.3%). In this study, CQPC05 made the progradation length and rate of activated carbon in the small intestine longer and higher than those of LB, and the CQPC05 with high concentration achieved better effectiveness. This demonstrates that CQPC05 can alleviate constipation, but also indicates that the alleviation degree is related to dose.

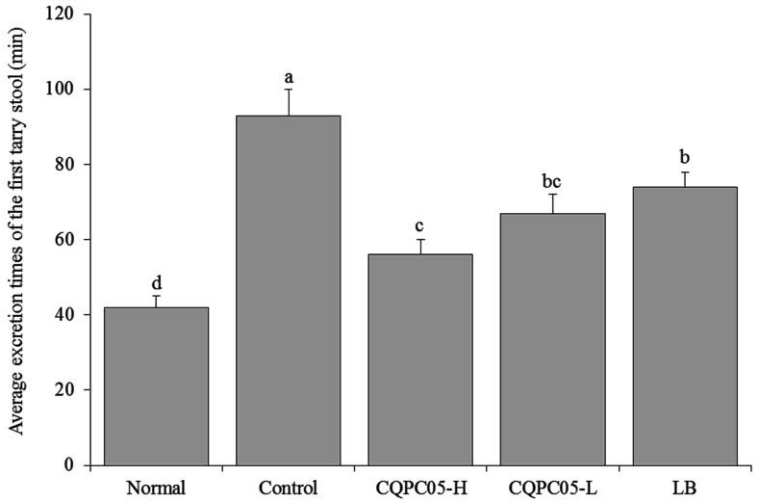

Figure 3. Average excretion times of the first tarry stool of the *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbrueckii* subsp. *bulgaricus* treated activated carbon-induced constipation mice (average value of five mice in each group). $^{a–d}$ The different letters mean that there are significant differences ($p < 0.05$) between every two groups according to Duncan's multiple range test. LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw).

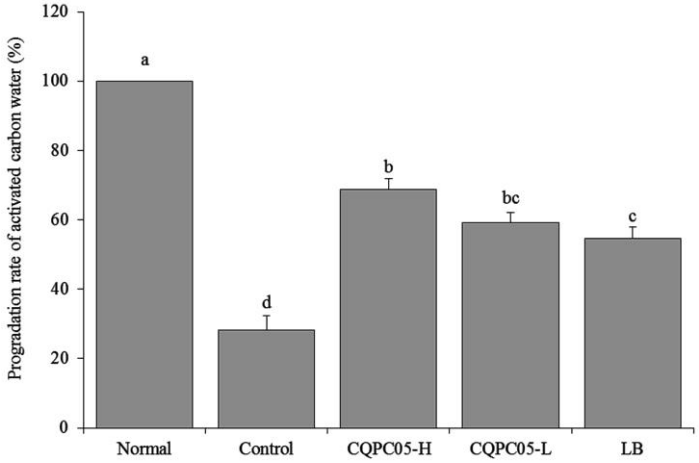

Figure 4. Average excretion times of the first tarry stool of the *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbrueckii* subsp. *bulgaricus* treated activated carbon-induced constipation mice (average value of five mice in each group). $^{a–d}$ The different letters mean that there are significant differences ($p < 0.05$) between every two groups according to Duncan's multiple range test. LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw).

3.4. Influences of CQPC05 on Serum MTL, Gas, ET, SS, and AChE Levels

As shown in Table 2, serum MTL, Gas, ET, and AChE levels in the normal group were highest, while the SS level was the lowest. However, the mice in the constipation control group had opposite results. The serum ET and AChE levels in the mice that were administered with CQPC05 were higher than those in the constipation control group, and their serum SS level was lower than that in the control group. Furthermore, the serum level in the high CQPC05 concentration group was closest to the normal group, followed by the low CQPC05 concentration group and the LB group.

Table 2. Serum MTL, Gas, ET, SS, and AChE levels of *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbruechii* subsp. *bulgaricus* treated activated carbon-induced constipation mice.

Treatment	Normal	Control	CQPC05-H	CQPC05-L	LB
MTL	12.47 ± 0.08^{a}	8.47 ± 0.03^{d}	10.84 ± 0.13^{b}	9.88 ± 0.04^{c}	$9.84 \pm 0.03^{c,d}$
Gas	13.54 ± 0.08^{a}	6.91 ± 0.04^{e}	11.32 ± 0.04^{b}	9.37 ± 0.03^{c}	9.00 ± 0.04^{d}
ET	10.56 ± 0.03^{a}	6.43 ± 0.05^{e}	8.91 ± 0.03^{b}	8.01 ± 0.02^{c}	7.56 ± 0.04^{d}
SS	8.04 ± 0.06^{e}	12.03 ± 0.07^{a}	8.56 ± 0.10^{d}	10.24 ± 0.08^{c}	10.79 ± 0.09^{b}
AChE	11.14 ± 0.09^{a}	7.37 ± 0.05^{e}	10.01 ± 0.06^{b}	8.90 ± 0.10^{c}	8.70 ± 0.08^{d}

Values presented are the mean ± standard deviation (average value of 10 mice in each group). $^{a-e}$ The different letters in the same row mean that there are significant differences ($p < 0.05$) between every two groups according to Duncan's multiple range test. LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw). MTL, motilin; Gas, gastrin; ET, endothelin; SS, somatostatin; AchE, acetylcholinesterase.

3.5. Pathological Observation of Small Intestine

As shown in Figure 5, the intestinal villi of mice in the normal group were neat, and the intestinal wall was intact. The intestinal villi of mice in the control group were severely broken, and some damage occurred to the intestinal wall. Compared with the control group, the breakage of small intestinal villi and injury of the intestinal inner wall were alleviated after the administration of CQPC05 and LB, and the effect of CQPC05-H was better than that of CQPC05-L and LB.

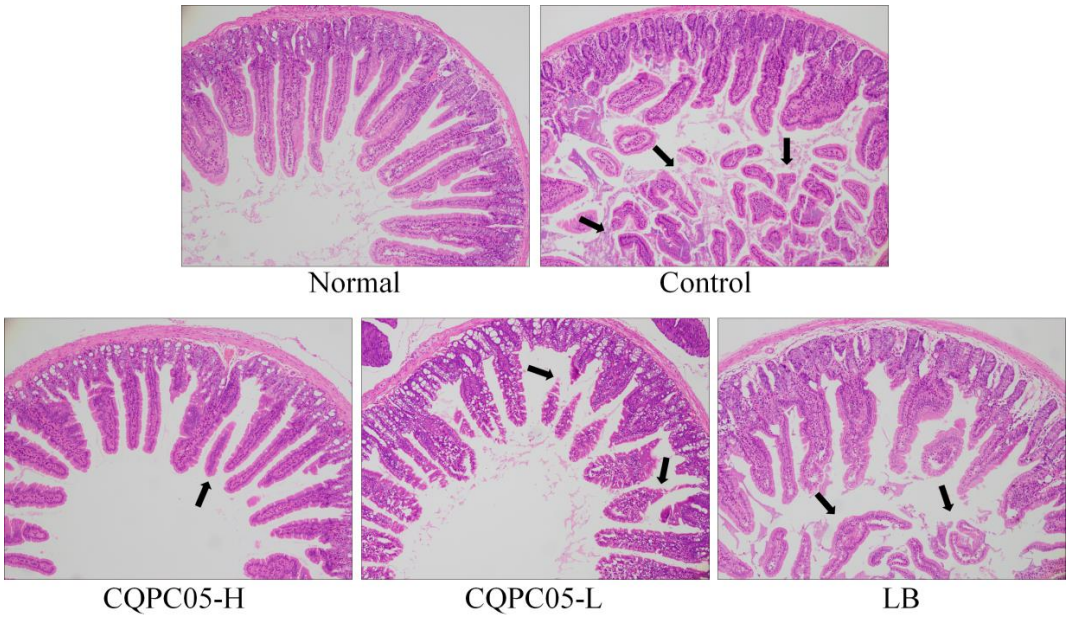

Figure 5. Pathological observation of small intestinal tissue in the *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbrueckii* subsp. *bulgaricus* treated activated carbon-induced constipation mice (40×). LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw).

3.6. Influences of CQPC05 on c-Kit and SCF mRNA Expression in the Small Intestine

As shown in Figure 6, c-Kit and SCF mRNA expression in small intestine in the normal group were the highest (8.21 and 7.62 times the control group), while those in the control group were the lowest. Compared to the control group, CQPC05 and LB could increase the mRNA expression of c-Kit and SCF in the small intestine of constipated mice, and high-concentration CQPC05 (CQPC05-H) could lead to the highest increase (6.93 and 3.84 times the control group).

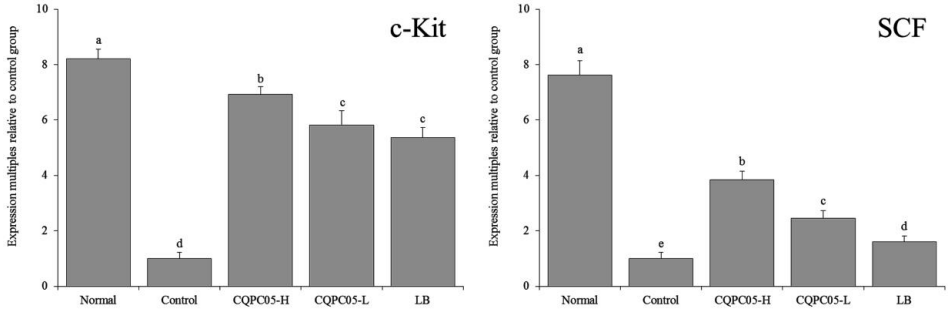

Figure 6. The small intestine tissue levels of mRNA expression of c-Kit and SCF in the *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbrueckii* subsp. *bulgaricus* treated activated carbon-induced constipation mice. $^{a-e}$ The different letters mean that there are significant differences ($p < 0.05$) between every two groups according to Duncan's multiple range test. LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw). SCF: stem cell factor.

3.7. Influences of CQPC05 on TRPV1, GDNF, and iNOS mRNA Expression on the Small Intestine

Figure 7 showed that the GDNF expression (52.31 times the control group) level was the highest in the normal group, and the expression levels of TRPV1 (0.08 times the control group) and iNOS (0.19 times the control group) were the lowest, which was in contrast to the control group. Compared to the control group, LB and CQPC05 could both up-regulate GDNF expression and down-regulate TRPV1 and iNOS expression, and the effect of high-concentration CQPC05 (43.15, 0.12 and 0.32 times the control group) was the greatest.

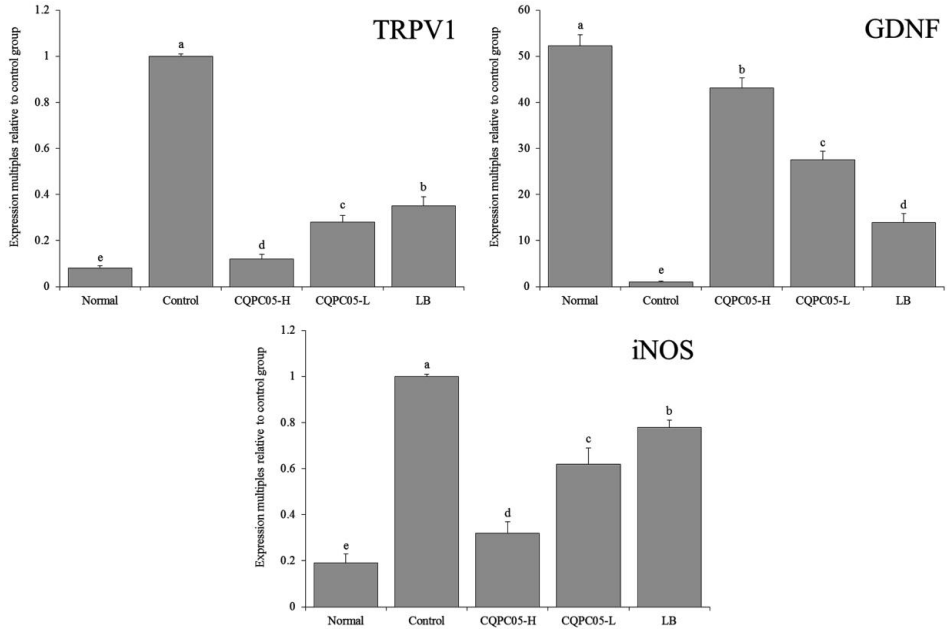

Figure 7. The small intestine tissue levels of mRNA expression of TRPV1, GDNF, and iNOS in the *Lactobacillus plantarum* CQPC05 and *Lactobacillus delbrueckii* subsp. *bulgaricus* treated activated carbon-induced constipation mice. $^{a-e}$ The different letters mean that there are significant differences ($p < 0.05$) between every two groups according to Duncan's multiple range test. LB: *Lactobacillus delbrueckii* subsp. *bulgaricus* (1.0×10^9 colony-forming unit (CFU)/kg body weight (bw)); CQPC05-L: *Lactobacillus plantarum* CQPC05 low dose (1.0×10^8 CFU/kg bw); CQPC05-H: *Lactobacillus plantarum* CQPC05 high dose (1.0×10^9 CFU/kg bw). GDNF: glial cell-derived neurotrophic factor; TRPV1: transient receptor potential cation channel subfamily V member 1; iNOS: inducible nitric oxide synthase.

4. Discussion

Constipation decreases the quality of life. Long-term constipation not only influences normal life, but also causes other intestinal tract diseases or even malignant diseases. Constipation may cause abdominal distension and loss of appetite, leading to physical deterioration, and decreased body weight [6,21]. Animal studies have indicated that constipation can lead to decreased body weight, lack of defecation, and dry feces [22]. The change of body weight is an important index for constipation. It has been shown that the body weight induced by activated carbon in constipation mice is lower than that of normal mice, which is corroborated by our study [23]. Moreover, the body weight of constipated mice induced by loperamide hydrochloride has been reported to be decreased [24]. Thus, constipation can lead to a slow increase in body weight. LB CQPC05 can alleviate this decrease in body weight, the lower defecation times, dry feces, and constipation. Furthermore, the most significant symptom of constipation is defecation difficulty. Thus, in this study, the excretion time of the first tarry stool after activated carbon administration was also considered as an important index for the evaluation of constipation. Constipation can slow down intestine peristalsis, keep the activated carbon in the intestinal tract, and extend the excretion time of tarry stool. Here, the excretion times of the first tarry stool in constipated mice were longer than that of normal mice. CQPC05 could significantly shorten this time, indicating an alleviation of constipation.

Gastrointestinal hormones, such as MTL and gas, exert direct influences on constipation, and the measurement of these indices can evaluate the degree of constipation [25,26]. MTL can improve stomach peristalsis, leading to the secretion of hydrochloric acid, pancreatic juice, and bile, which reflects gastrointestinal motility to a certain degree. Gas can regulate the digestive system, improve gastric peristalsis, and slow down gastric emptying [27]. ET can maintain the stability of vascular tension and the cardiovascular system [28]. SS can inhibit the release of gastrointestinal hormones, and decrease the contraction of smooth muscle. AchE is a hydrolase of acetylcholine (AcH), and its activity shows a positive correlation with the Ach level [29]. Acetylcholine is an important neurotransmitter, which promotes gastrointestinal motility. Constipation leads to decreased levels of ET, AchE, and gas, and increased level of SS. The results of this study show that CQPC05 can significantly inhibit these changes, leading to a return of these indices in constipation mice to normal levels. Moreover, the effectiveness was superior to that of commonly used LB.

Cajal cells (ICCs) are interstitial cells related to intestinal tract health. Their main function is to act as a pacemaker, generate and transduce electric slow waves, lead to the contraction of smooth muscle, and determine the direction, frequency, and speed of gastrointestinal peristaltic wave propagation [30]. Abnormal intestinal ICC levels and structure can slow down peristalsis, thus causing intestinal dysfunction and constipation. However, c-kit plays a very important role in maintaining ICC function, which is a specific marker for ICC [31]. SCF is a ligand of the c-Kit receptor, and the increase in SCF levels can also promote the recovery of the intestinal tract [32]. The SCF level is very important for ICC reproduction, because ICC cannot grow without SCF. Our study showed that CQPC05 could effectively alleviate the influence of constipation on c-Kit and SCF expression, and keep the expression level close to normal. It further stabilized the ICC level in vivo, and alleviated constipation.

TRPV1 can promote the release of neurotransmitters and lead to intestinal motility disorder and difficulties with defecation. It has been reported that the expression of TRPV1 is increased in constipation patients with vulnerable intestines [33]. GDNF plays a regulatory role in nerves, repairs damage to the intestinal tract, increases intestinal motility, and alleviates constipation [34]. In this study, CQPC05 could inhibit constipation by regulating TRPV1 and GDNF expression levels. iNOS has been reported to weaken the gastrointestinal motility, resulting in constipation [35]. This study showed that LF-CQPC03 alleviated constipation by down-regulating the iNOS expression. The regulation of TRPV1, GDNF, and iNOS expression can influence intestinal motility and alleviate constipation, which is the main mechanism with which *Lactobacillus* inhibits constipation.

CQPC05 is a newly discovered strain. Through this study, it was observed that the bacterium has a good inhibitory effect on constipation in the laboratory, which proves that it is a probiotic

microorganism. The results of this study are similar to those of previous studies [6,11,13,23], which suggest that *Lactobacillus* can inhibit constipation and be used as intestinal microorganisms. At the same time, the results of molecular biology further show that CQPC05 can regulate constipation by regulating the intensity of gene expression in the small intestine. CQPC05 is a beneficial microorganism with molecular regulation.

5. Conclusions

Here, the constipation inhibitory effect of *Lactobacillus* CQPC05 isolated from pickled vegetables was investigated. *Lactobacillus* CQPC05 could effectively alleviate the decrease in body weight caused by constipation, improve the progradation rate of activated carbon in the small intestine, and shorten the excretion time of the first tarry stool. The results of serum detection also indicated that CQPC05 could lead to close levels of MTL, ET, gas, SS, and AChE in constipation mice compared to those of normal mice. The result of q-PCR indicated that CQPC05 up-regulated the mRNA expression of c-Kit, SCF, and GDNF genes, and down-regulated the expression of both TRPV1 and iNOS. The results of these experiments demonstrated that CQPC05 could alleviate constipation, achieving an effectiveness that is superior to the commonly used commercial strain (LB) at the same dose. Therefore, CQPC05 has good probiotic potential, and is promising for application in the medical and food industries.

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