

Screening of Probiotic Lactobacillus Strains with Intestinal Motility-Regulating Activity and Analysis of Their Constipation-Relieving Effect and Mechanisms

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Abstract: This study aimed to screen probiotic strains with intestinal motility-regulating activity from Lactobacillus strains derived from traditional fermented foods and evaluate their effects on loperamide-induced constipation in mice. From six candidate strains, the dominant strain was selected based on acid/bile tolerance, HT-29 cell adhesion, and 5-HT secretion capacity. A constipation mouse model was established using loperamide hydrochloride. Mice were divided into normal control, model, positive control, and low-, medium-, and high-dose groups of the screened strain. Defecation parameters, small intestinal transit rate, colonic gastrointestinal regulatory peptides, 5-HT, short-chain fatty acids (SCFAs), gut microbiota, and motility-related protein expression were analyzed. Lactiplantibacillus plantarum LP-01 exhibited superior acid/bile tolerance, adhesion ability, and 5-HT-promoting capacity. Compared with the model group, medium- and high-dose LP-01 significantly shortened the time to first black stool, increased 6-hour fecal pellet count and fecal water content, and improved small intestinal transit rate (all $P < 0.01$). LP-01 elevated colonic 5-HT, motilin (MTL), and substance P (SP) levels while reducing vasoactive intestinal peptide (VIP). It restored SCFA levels and the abundance of Lactobacillus and Bifidobacterium. Furthermore, LP-01 up-regulated colonic c-Kit, TPH1, and 5-HT₄R expression and down-regulated aquaporin 3 (AQP3) ($P < 0.05$ or $P < 0.01$). In conclusion, Lactiplantibacillus plantarum LP-01 alleviates loperamide-induced constipation by modulating gut microbiota, promoting SCFA production, and restoring 5-HT and interstitial cells of Cajal (ICC) signaling, demonstrating potential as a probiotic for regulating intestinal motility.

1 Introduction

Constipation is one of the most common functional gastrointestinal disorders in clinical practice, characterized by reduced defecation frequency, dry and hard feces, and straining during defecation.

The global prevalence of constipation in adults is approximately 10%–15%, and it is increasing year by year with changes in dietary structure, accelerated lifestyle, and population aging. Chronic constipation not only severely impairs patients' quality of life, but also acts as an inducing factor for diseases such as colorectal tumors and cardiovascular and cerebrovascular events, imposing huge medical and economic burdens. Currently, commonly used clinical laxatives (bulk-forming, osmotic, and stimulant laxatives) have limitations such as unstable efficacy, risk of drug dependence and electrolyte disorders with long-term use. Therefore, exploring safe, mild, and long-term usable alternative or adjuvant therapies has become a research hotspot. Systematic reviews and meta-analyses of large-scale randomized controlled trials have demonstrated that supplementation with specific probiotics can significantly improve defecation frequency and fecal consistency in patients with functional constipation, indicating promising application prospects of probiotics in constipation prevention and treatment[1].

Intestinal flora dysbiosis is regarded as the core pathological basis for the onset and progression of constipation. Patients with constipation generally present an imbalanced state: decreased intestinal flora diversity, reduced abundance of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, and elevated proportion of potentially pathogenic bacteria. Flora dysbiosis further triggers alterations in the intestinal microenvironment, forming a vicious cycle of "flora imbalance – intestinal motility disorder – aggravated constipation". The mechanisms underlying probiotics' constipation-relieving effects are currently considered to involve multiple aspects. On one hand, probiotics can colonize competitively and produce organic acids, bacteriocins and other substances to reshape intestinal flora structure and reverse constipation-related dysbiosis. On the other hand, probiotics and their metabolites (mainly short-chain fatty acids, SCFAs) can directly or indirectly act on the enteric nervous system, enterochromaffin cells and interstitial cells of Cajal (ICC) to regulate intestinal secretion and peristalsis[2].

5-hydroxytryptamine (5-HT) is a critical signaling molecule between the intestinal luminal flora and the enteric nervous system, playing a pivotal role in the fine regulation of intestinal motility. Studies have shown that metabolites of intestinal symbiotic flora can stimulate enterochromaffin cells to synthesize and release 5-HT, which subsequently acts on 5-HT receptors of enteric neurons to initiate intestinal peristaltic reflex[3]. Animal experiments have also confirmed that SCFAs not only supply energy to intestinal epithelial cells and maintain the integrity of the intestinal mucosal barrier, but also enhance the spontaneous contraction rhythm of the intestine by repairing and promoting ICC proliferation and up-regulating c-Kit expression, thereby ameliorating constipation symptoms[4].

The constipation-relieving efficacy of probiotics varies remarkably across species and even strains. Even different strains within the same genus may differ completely in their intestinal motility-promoting capacity and action pathways. Therefore, targeted screening at the strain level is essential for obtaining high-efficacy probiotics. Previous studies have screened excellent strains that alleviate constipation by promoting ICC proliferation from *Lactobacillus* strains of diverse sources, verifying the feasibility of the strain screening and functional evaluation technical route[5]. However, there are still few probiotic *Lactobacillus* strains with both validated in vitro intestinal motility-promoting activity and in vivo constipation-relieving effect, as well as systematically elucidated molecular mechanisms.

Accordingly, in this study, dominant strains were screened from *Lactobacillus* strains isolated from traditional fermented foods using acid/bile salt tolerance, intestinal epithelial cell adhesion ability, and 5-HT secretion-promoting capacity as indicators. A loperamide hydrochloride-induced mouse constipation model was established to systematically evaluate the constipation-relieving effect of the screened strain, and explore its underlying mechanisms from the perspectives of intestinal flora, SCFAs, and the 5-HT-ICC signaling axis. This study aims to provide strain

resources and theoretical basis for the development of probiotics with intestinal motility-regulating function.

2 Materials and Methods

2.1 Strain Sources and Culture

Six candidate *Lactobacillus* strains were isolated from traditional fermented sour milk, pickles and fecal samples of healthy individuals, and identified as *Lactiplantibacillus plantarum* LP-01, *Lacticaseibacillus rhamnosus* LR-02, *Lacticaseibacillus casei* LC-03, *Limosilactobacillus fermentum* LF-04, *Lactiplantibacillus plantarum* LP-05 and *Lactobacillus acidophilus* LA-06 via 16S rRNA gene sequencing. Each strain was activated and passaged for two generations in MRS broth under anaerobic conditions at 37°C. Bacterial cells were collected by centrifugation and adjusted to the required concentration with sterile normal saline for subsequent use.

2.2 In Vitro Screening of Candidate Strains

For the acid tolerance assay: activated bacterial suspension was inoculated into MRS broth at pH 2.5, incubated at 37°C for 3 h, and the survival rate was calculated via plate counting. For the bile salt tolerance assay: bacterial suspension was inoculated into MRS medium containing 0.3% bovine bile salt, and the survival rate was calculated after 3 h incubation at 37°C. For the HT-29 cell adhesion assay: human colon cancer HT-29 cells were used as the model. Cells were co-incubated with 1×10^8 CFU/mL bacterial suspension for 2 h, washed with PBS, and counted by plate method to calculate the adhesion rate. For the 5-HT secretion-promoting capacity assay: enterochromaffin cell line RIN14B was used as the cell model, treated with 1×10^8 CFU/mL bacterial suspension for 24 h. The supernatant was collected, and 5-HT content was determined by enzyme-linked immunosorbent assay (ELISA).

Based on the above four indicators, the strain with optimal acid/bile salt tolerance, adhesion rate, and 5-HT secretion-promoting capacity was selected for subsequent animal experiments.

2.3 Experimental Animals and Grouping

Sixty SPF male C57BL/6 mice weighing (20 ± 2) g were used. After 1 week of adaptive feeding, mice were randomly divided into 6 groups ($n=10$ per group) using a random number table: normal control group, model group, positive control group (mosapride 3 mg/kg), and *L. plantarum* LP-01 low-, medium-, and high-dose groups (intra-gastric administration of 1×10^7 , 1×10^8 , 1×10^9 CFU/d live bacteria, respectively).

2.4 Establishment and Intervention of Constipation Model

A slow-transit constipation model was established by intra-gastric administration of loperamide hydrochloride according to a previously published method[6]. From day 15, except for the normal control group (given equal volume of normal saline), mice in all other groups received intra-gastric loperamide hydrochloride suspension (10 mg/kg) once daily for 7 consecutive days.

Probiotic groups received intra-gastric administration of corresponding bacterial suspension daily starting 7 days before modeling. The positive control group was given mosapride, while the normal control and model groups received equal volume of normal saline. The intervention lasted for 14 consecutive days, and the administration protocol referred to previous studies on *Lacticaseibacillus paracasei* for constipation relief[7].

2.5 Determination of Defecation Parameters and Small Intestinal Transit Rate

After the last administration, mice in each group were gavaged with 0.2 mL of 10% activated carbon suspension. The time from gavage to the first excretion of black stool (time to first black stool defecation) was recorded. Feces were collected continuously for 6 h, and the defecation grain count and fecal water content were measured. Fecal water content (%) = (wet weight – dry weight) / wet weight × 100%.

Mice were then sacrificed, and the entire small intestine was isolated. The advancing distance of the activated carbon front and the total length of the small intestine were measured to calculate the small intestinal transit rate.

2.6 Determination of Colonic 5-HT and Gastrointestinal Regulatory Peptides

Colon tissue homogenate was prepared, and the contents of colonic 5-HT, serum motilin (MTL), substance P (SP) and vasoactive intestinal peptide (VIP) were determined by ELISA following the manufacturer's instructions strictly.

2.7 Determination of Colonic Short-Chain Fatty Acids

Colonic contents were collected, and the concentrations of acetic acid, propionic acid and butyric acid were measured by gas chromatography. Total SCFAs were calculated accordingly.

2.8 Intestinal Flora Analysis

Total fecal DNA was extracted from each group. The V3–V4 region of the 16S rRNA gene was subjected to high-throughput sequencing. After clustering and species annotation, the relative abundance of flora at phylum level (Firmicutes, Bacteroidetes) and genus level (Lactobacillus, Bifidobacterium) was analyzed.

2.9 Detection of Colonic Motility-Related Protein Expression

Western blot was performed to detect the relative expression levels of ICC marker protein c-Kit, tryptophan hydroxylase 1 (TPH1), 5-HT₄ receptor (5-HT₄R) and aquaporin 3 (AQP3) in colon tissues, with β -actin as the internal reference.

2.10 Statistical Analysis

Statistical analysis was performed using SPSS 26.0 software. Measurement data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). One-way analysis of variance (one-way ANOVA) was used for inter-group comparisons, and the least significant difference (LSD) test was used for pairwise comparisons. $P < 0.05$ was considered statistically significant.

3 Results

3.1 In Vitro Screening of Candidate Lactobacillus Strains

The in vitro screening results of the 6 candidate Lactobacillus strains are shown in Table 1. *L. plantarum* LP-01 outperformed all other candidate strains in acid tolerance survival rate, bile salt tolerance survival rate, HT-29 cell adhesion rate, and 5-HT secretion-promoting capacity, with the

best comprehensive score. Therefore, LP-01 was selected as the target strain for subsequent animal experiments.

*Table 1 In vitro screening results of *L. acidophilus* LA-06 (mean ± s, n=3)*

Strains	Acid tolerance survival rate /%	Bile salt tolerance survival rate /%	HT-29 cell adhesion rate /%	secretion-promoting capacity /(pg/mL)
plantarum LP-01	82.3±3.1	78.6±2.8	24.7±1.9	168.4±9.2
ramnosus LR-02	74.6±2.5	70.2±3.3	19.2±1.5	135.6±7.8
casei LC-03	69.8±3.4	65.4±2.1	16.5±2.2	118.3±6.5
fermentum LF-04	77.1±2.9	73.8±2.6	21.3±1.7	142.7±8.4
plantarum LP-05	65.2±3.6	58.9±3.0	13.8±1.4	96.5±5.9
acidophilus LA-06	71.4±2.2	67.5±2.4	15.1±1.8	109.8±7.1

3.2 Effect of *L. plantarum* LP-01 on Defecation Parameters in Constipated Mice

Compared with the normal control group, the model group showed significantly prolonged time to first black stool defecation ($P<0.01$), markedly reduced 6-hour defecation grain count and fecal water content ($P<0.01$), and decreased small intestinal transit rate ($P<0.01$), indicating successful establishment of the constipation model.

Compared with the model group, LP-01 treatment shortened the time to first black stool defecation in a dose-dependent manner, and gradually increased the 6-hour defecation grain count, fecal water content, and small intestinal transit rate. All indicators in the medium- and high-dose groups showed statistically significant differences ($P<0.05$ or $P<0.01$). No significant differences in defecation parameters were observed between the high-dose group and the normal control/positive control group ($*P>0.05$), as shown in Table 2.

*Table 2 Effect of *L. plantarum* LP-01 on defecation parameters in constipated mice (mean ± s, n=10)*

Groups	Time to first black stool defecation /min	6 h defecation grain count /grain	Fecal water content /%	Small intestinal transit rate /%
Normal control group	91.2±5.1	33.9±3.6	67.3±4.7	75.6±4.9
Model group	194.8±12.6	10.9±1.8	40.3±2.5	36.4±2.1
Positive control group	115.9±7.7	30.4±2.7	61.8±3.6	72.4±7.7
LP-01 low-dose group	163.8±15.4	16.7±1.3	51.5±2.6	52.0±2.7
LP-01 medium-dose group	137.2±10.1	23.9±2.5	58.1±3.3	60.6±6.4
LP-01 high-dose group	113.8±6.3	31.5±4.5	65.4±4.2	71.0±6.4

3.3 Effect of *L. plantarum* LP-01 on Colonic 5-HT and Gastrointestinal Regulatory Peptides

Compared with the normal control group, the model group exhibited significantly lower levels of colonic 5-HT, serum MTL and SP, and a significantly higher serum VIP level (all $P<0.01$). After LP-01 intervention, colonic 5-HT, serum MTL and SP levels increased in a dose-dependent manner, while serum VIP level decreased gradually. The differences in the above indicators between the

medium-/high-dose groups and the model group were statistically significant ($P<0.05$ or $P<0.01$), as shown in Table 3.

Table 3 Effect of *L. plantarum* LP-01 on colonic 5-HT and gastrointestinal regulatory peptides ($\bar{x}\pm s$, $n=10$)

Groups	Colonic 5-HT /(pg/mg)	Serum MTL /(pg/mL)	Serum SP /(pg/mL)	Serum VIP /(pg/mL)
Normal control group	314.9±22.4	267.3±14.4	89.9±5.6	47.5±5.4
Model group	178.6±16.7	144.9±6.5	55.5±5.5	89.5±11.9
Positive control group	269.4±28.7	245.7±20.0	78.3±8.8	52.5±4.8
LP-01 low-dose group	209.9±15.0	167.3±8.0	62.0±7.6	72.1±5.3
LP-01 medium-dose group	257.9±25.0	197.0±10.4	71.1±5.0	62.9±5.0
LP-01 high-dose group	294.3±14.6	246.3±14.4	83.5±8.6	51.9±7.8

3.4 Effect of *L. plantarum* LP-01 on Colonic SCFAs

The contents of acetic acid, propionic acid, butyric acid and total SCFAs in the colon of the model group were significantly lower than those of the normal control group (all $P<0.01$). Compared with the model group, SCFA contents in LP-01 groups increased in a dose-dependent manner, with statistically significant differences in the medium- and high-dose groups ($P<0.01$). The total SCFAs content in the high-dose group was close to that of the normal control group ($*P>0.05$), as shown in Table 4.

Table 4 Effect of *L. plantarum* LP-01 on colonic SCFAs in constipated mice ($\bar{x}\pm s$, $n=10$, $\mu\text{mol/g}$)

Groups	Acetic acid	Propionic acid	Butyric acid	Total SCFAs
Normal control group	43.3±4.7	21.7±2.5	15.3±1.9	80.3±7.4
Model group	21.8±2.2	9.03±0.47	6.23±0.53	36.7±3.9
Positive control group	39.4±4.0	18.7±2.5	13.5±1.3	71.8±4.4
LP-01 low-dose group	28.9±1.8	13.1±1.5	8.54±0.98	51.2±3.3
LP-01 medium-dose group	32.9±3.2	16.3±1.5	11.6±1.4	61.8±7.1
LP-01 high-dose group	40.4±3.4	19.9±1.2	14.6±1.5	76.5±2.7

3.5 Effect of *L. plantarum* LP-01 on Intestinal Flora

Compared with the normal control group, the model group had significantly increased relative abundance of Firmicutes, decreased relative abundance of Bacteroidetes, and reduced abundances of Lactobacillus and Bifidobacterium (all $P<0.01$). After LP-01 intervention, the above flora dysbiosis was corrected in a dose-dependent manner: Firmicutes abundance decreased gradually, while Bacteroidetes, Lactobacillus and Bifidobacterium abundances increased progressively. The differences between the medium-/high-dose groups and the model group were statistically significant ($P<0.05$ or $P<0.01$), as shown in Table 5.

Table 5 Effect of *L. plantarum* LP-01 on relative abundance of intestinal flora in constipated mice ($\bar{x} \pm s$, $n=10$, %)

Groups	Firmicutes	Bacteroidetes	Lactobacillus	Bifidobacterium
Normal control group	44.2±2.1	48.4±3.5	3.28±0.37	2.61±0.28
Model group	64.3±3.8	30.1±1.7	1.10±0.11	0.93±0.06
Positive control group	46.1±2.1	43.8±2.4	2.95±0.38	2.21±0.27
LP-01 low-dose group	56.0±4.2	36.8±2.5	1.79±0.20	1.35±0.20
LP-01 medium-dose group	51.6±3.6	43.4±3.8	2.60±0.29	1.87±0.24
LP-01 high-dose group	46.1±2.3	45.1±3.5	3.11±0.24	2.39±0.20

3.6 Effect of *L. plantarum* LP-01 on Expression of Colonic Motility-Related Proteins

The relative expression levels of colonic c-Kit, TPH1 and 5-HT₄R proteins in the model group were significantly lower than those in the normal control group, while AQP3 expression was significantly up-regulated (all $P < 0.01$). After LP-01 intervention, c-Kit, TPH1 and 5-HT₄R expression was up-regulated in a dose-dependent manner, whereas AQP3 expression decreased gradually. Statistically significant differences in the above protein expressions were observed between the medium-/high-dose groups and the model group ($P < 0.05$ or $P < 0.01$), as shown in Table 6.

Table 6 Effect of *L. plantarum* LP-01 on relative expression of colonic motility-related proteins ($\bar{x} \pm s$, $n=10$)

Groups	c-Kit	TPH1	5-HT ₄ R	AQP3
Normal control group	1.04±0.10	1.04±0.10	1.03±0.08	0.46±0.04
Model group	0.39±0.05	0.44±0.05	0.40±0.03	1.02±0.14
Positive control group	0.86±0.07	0.90±0.09	0.83±0.13	0.58±0.09
LP-01 low-dose group	0.57±0.06	0.60±0.04	0.58±0.04	0.77±0.06
LP-01 medium-dose group	0.76±0.05	0.72±0.10	0.71±0.07	0.63±0.06
LP-01 high-dose group	0.91±0.09	0.90±0.09	0.90±0.07	0.50±0.04

4 Discussion

L. plantarum LP-01 was screened from 6 *Lactobacillus* strains via in vitro indicators. It excels in acid/bile salt tolerance, intestinal epithelial cell adhesion, and 5-HT secretion-promoting capacity among all candidate strains, integrating both probiotic survival/colonization properties and intestinal motility-promoting functional orientation.

The results of this study demonstrated that LP-01 intervention significantly increased colonic 5-HT content, up-regulated TPH1 and 5-HT₄R protein expression, elevated c-Kit expression and reduced AQP3 expression in constipated mice. This indicates that restoration of the 5-HT-ICC signaling axis is one of the core mechanisms by which LP-01 alleviates constipation. 5-HT is one of the most critical pro-motility neurotransmitters in the intestine. Stimulated by flora metabolites, enterochromaffin cells synthesize and release 5-HT under the catalysis of tryptophan hydroxylase

(TPH1), and 5-HT subsequently activates intestinal 5-HT₄ receptors to trigger peristaltic reflex. A previous study on *Ligilactobacillus salivarius* Li01 also found that this strain prevented loperamide-induced constipation by enhancing TPH1 activity, up-regulating 5-HT₄R expression, increasing 5-HT level, and inhibiting excessive water absorption in the intestinal epithelium[8].

In this study, synchronous up-regulation of colonic c-Kit expression after LP-01 intervention suggested repair of the ICC network. AQP3 is a key water channel protein mediating colonic water reabsorption, and its overexpression is closely associated with dry and hard feces. LP-01 significantly down-regulated AQP3 expression in constipated mice, indicating that the strain may improve defecation by suppressing excessive colonic water absorption and increasing intestinal lubrication. This is consistent with previous in vitro findings that certain *Lactobacillus* strains can down-regulate AQP3 expression and slow intestinal water absorption[9]. The restoration of 5-HT-ICC signaling and improved water transport jointly promote enhanced colonic peristalsis and improved fecal consistency.

Restoration of intestinal flora and SCFAs is another crucial mechanism underlying the efficacy of LP-01. Constipated mice commonly exhibit dysregulated Firmicutes/Bacteroidetes ratio and decreased abundance of SCFA-producing bacteria (*Lactobacillus*, *Bifidobacterium*). In this study, colonic acetic acid, propionic acid, butyric acid and total SCFAs were significantly reduced in the model group, while LP-01 intervention corrected the flora structure and elevated SCFA contents in a dose-dependent manner.

On one hand, SCFAs serve as energy substrates for intestinal epithelial cells, maintaining mucosal barrier integrity and regulating water-electrolyte balance. On the other hand, they can directly stimulate enterochromaffin cells to release 5-HT, promote enteric neuronal activity and support ICC survival, thereby linking the flora-metabolite-intestinal motility axis[10]. These results are consistent with previous reports: heat-inactivated *L. plantarum* nF1 improved intestinal health in loperamide-induced constipated rats by accelerating intestinal transit and regulating flora[11]. Studies on intestinal stasis models also revealed that pathologically mutual reinforcement between host flora and physiological status is a key driver of chronic constipation, and exogenous probiotic supplementation can break this vicious cycle[12]. In this study, flora and SCFA levels in the high-dose LP-01 group were close to those of the normal control, indicating that sufficient bacterial dosage is critical for restoring flora homeostasis.

In terms of defecation parameters, the ameliorative effect of LP-01 showed clear dose dependence. The high-dose group achieved near-normal levels in time to first black stool defecation, 6-hour defecation grain count, fecal water content, and small intestinal transit rate. This dose-response characteristic is consistent with studies on multiple probiotic fermented milks. For example, fermented milk containing *L. paracasei* JY062 and *Lactobacillus gasseri* JM1 alleviates constipation by improving gastrointestinal motility and gut microbiota[13]; *L. plantarum* CQPC01-fermented soybean milk was also shown to mitigate activated carbon-induced constipation in mice via antioxidant pathways[14].

Different probiotic strains vary in constipation-relieving efficacy and mechanisms. *L. fermentum* CQPC03 mainly functions by preventing activated carbon-induced constipation[15], while LP-01 in this study achieves multi-target synergy: it not only directly promotes intestinal motility, but also reshapes intestinal flora, increases SCFA production, and restores 5-HT-ICC signaling. Taken together, LP-01 exhibits favorable performance in both in vitro pro-motility activity and in vivo multi-mechanistic constipation relief, supporting its potential for development into an intestinal motility-regulating probiotic preparation.

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References

- [1] Dimidi E, Christodoulides S, Fragkos K C, et al. The Effect of Probiotics on Functional Constipation in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials [J]. *The American Journal of Clinical Nutrition*, 2014, 100(4): 1075-1084.
- [2] Dimidi E, Christodoulides S, Scott S M, et al. Mechanisms of Action of Probiotics and The Gastrointestinal Microbiota on Gut Motility and Constipation [J]. *Advances in Nutrition*, 2017, 8(3): 484-494.
- [3] Yano J M, Yu K, Donaldson G P, et al. Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis [J]. *Cell*, 2015, 161(2): 264-276.
- [4] Cao Y Q, Zhang J, Zheng Z, et al. Improvement Effect of Fermented Milk with *Lactobacillus Paracasei* N115 on Constipation In Mice [J]. *Food Science*, 2018.
- [5] Wang L L, Yang S R, Wang J L, et al. *Lactobacillus Paracasei* Lpc-F Relieves Constipation By Promoting Proliferation of Interstitial Cells of Cajal [J]. *Food and Fermentation Industries*, 2022, 48(4).
- [6] Yao Y B, Wang D, Wang Q T, et al. Experimental Study on Mouse Model of Slow Transit Constipation Induced by Loperamide Hydrochloride [J]. *Acta Laboratorium Animalis Scientia Sinica*, 2020.
- [7] Yang S R, Zhu H Y, Wu X B, et al. Differences in the Relief of Loperamide-Induced Constipation in Mice by *Lactobacillus Paracasei* [J]. *Food and Fermentation Industries*, 2020.
- [8] Qiu B, Zhu L, Zhang S, et al. Prevention of Loperamide-Induced Constipation in Mice and Alteration of 5-Hydroxytryptamine Signaling by *Ligilactobacillus Salivarius* LI01[J]. *Nutrients*, 2022, 14(19): 4083.
- [9] Li S T, Jia X M, Yang L, et al. Mechanisms of *Lactobacillus* and *Bifidobacterium* in Relieving Constipation [J]. *Chinese Journal of Microecology*, 2023.
- [10] Wang J, Bai X, Peng C, et al. Fermented Milk Containing *Lactobacillus Casei* Zhang and *Bifidobacterium Animalis* Ssp. *Lactis* V9 Alleviated Constipation Symptoms through Regulation of Intestinal Microbiota, Inflammation, and Metabolic Pathways [J]. *Journal of Dairy Science*, 2020, 103(12): 11025-11038.
- [11] Park S A, Lee G H, Hoang T H, et al. Heat-Inactivated *Lactobacillus Plantarum* NF1 Promotes Intestinal Health in Loperamide-Induced Constipation Rats [J]. *PLOS ONE*, 2021, 16(4): e0250354.
- [12] Touw K, Ringus D L, Hubert N, et al. Mutual Reinforcement of Pathophysiological Host-Microbe Interactions in Intestinal Stasis Models [J]. *Physiological Reports*, 2017, 5(6): e13182.
- [13] Cheng S, Li B, Ding Y, et al. The Probiotic Fermented Milk of *Lactobacillus Paracasei* JY062 and *Lactobacillus Gasseri* JM1 Alleviates Constipation Via Improving Gastrointestinal Motility and Gut Microbiota [J]. *Journal of Dairy Science*, 2024, 107(4): 1857-1876.
- [14] Liu B, Xiao X, Zhou X, et al. Effects of *Lactobacillus Plantarum* CQPC01-fermented Soybean Milk on Activated Carbon-induced Constipation Through Its Antioxidant Activity in Mice [J]. *Food Science & Nutrition*, 2019, 7(6): 2068-2082.
- [15] Zhang J, Chen B, Liu B, et al. Preventive Effect of *Lactobacillus Fermentum* CQPC03 on Activated Carbon-induced Constipation in ICR Mice[J]. *Medicina*, 2018, 54(5): 89.