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Construction and Characterization of an mCherry-Labeled *Lactiplantibacillus plantarum* L_3 Strain for Intestinal Distribution Studies

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Abstract:

Fluorescent labeling facilitates visualization of administered bacteria, but the intestinal distribution of labeled strains during prolonged dosing remains poorly characterized. We constructed an inducible mCherry-expressing *Lactiplantibacillus plantarum* L_3 strain and assessed fluorescence retention, growth, antimicrobial activity, and survival under simulated gastrointestinal conditions. The labeled strain was administered orally to C57BL/6J mice for 4 days or 8 weeks (six mice per group), followed by ex vivo intestinal fluorescence imaging. mCherry expression was confirmed by PCR, immunoblotting, and microscopy, and fluorescence remained detectable after 98 generations. Labeling did not visibly alter cell morphology or growth, but inhibition of *Staphylococcus aureus* decreased. After 4 days, fluorescence was most prominent in the duodenum, with signals in the cecum and colon; after 8 weeks, signals appeared more broadly distributed. These observations support endpoint localization during repeated administration, not evidence of stable intestinal colonization.

Key words: *Lactiplantibacillus plantarum* L_3; mCherry; fluorescent labeling; intestinal distribution; oral administration

1 INTRODUCTION

Lactiplantibacillus plantarum is a lactic acid bacterium widely distributed in fermented foods and the gastrointestinal tracts of humans and animals (Noemí et al. 2022). Several strains of this species have been investigated for their potential roles in maintaining intestinal microbial homeostasis, modulating immune responses, and improving host health (Hiraishi et al. 2019; Bao et al. 2022; Wang et al. 2022a). Interactions between administered bacteria and the gastrointestinal environment are important considerations when evaluating their potential probiotic functions (Phùng et al. 2024). Because microbial communities and local environmental conditions vary along the gastrointestinal tract (Donaldson et al. 2016), determining the spatial distribution of administered strains may provide useful information about their interactions with the host. However, distinguishing exogenously

administered *L. plantarum* from closely related endogenous bacteria remains challenging.

Several approaches have been used to investigate the gastrointestinal distribution of probiotic bacteria, including culture-based enumeration, fluorescence in situ hybridization, and quantitative polymerase chain reaction. Culture-based methods have been applied to monitor administered probiotic strains in mice (Dehghani et al. 2022), whereas fluorescence in situ hybridization has been used to investigate the gastrointestinal localization of *L. plantarum* in piglets (Mare et al. 2006). Quantitative polymerase chain reaction has also been employed to assess the abundance of lactic acid bacteria in different intestinal regions (Su et al. 2008). Although these approaches provide valuable information, their suitability depends on the specificity of strain identification, sample processing requirements, and experimental objectives. Fluorescent protein labeling offers an alternative strategy for visualizing administered microorganisms and examining their spatial distribution.

Fluorescent proteins, including green and red fluorescent proteins, have been used to label lactic acid bacteria for experimental tracking (Stojanov et al. 2021). Previous studies have demonstrated the feasibility of fluorescently labeling *L. plantarum* and examining its gastrointestinal distribution following administration (Van Zyl et al. 2015; Cui et al. 2021). Fluorescence imaging has also been applied to investigate the intestinal persistence and distribution of recombinant *Yarrowia lipolytica* (Madzak et al. 2023). However, many fluorescent tracking studies have focused on relatively short observation periods. Because the effects of probiotic administration may depend partly on treatment duration (Dixon et al. 2020; Kadja et al. 2021), comparing intestinal fluorescence patterns after different periods of repeated administration may provide additional information. Nevertheless, fluorescence detected during ongoing administration cannot independently establish long-term persistence or stable colonization.

Lactiplantibacillus plantarum L_3 was previously isolated from fermented cabbage and characterized for several potentially beneficial properties, including antimicrobial activity, cell surface hydrophobicity, autoaggregation, and biofilm formation (Gao et al. 2024; Qi et al. 2024). In the present study, we constructed an inducible mCherry-

expressing *L. plantarum* L_3 strain and characterized its fluorescence stability and selected physiological properties. We subsequently examined intestinal fluorescence distribution in mice following 4 days or 8 weeks of repeated oral administration. This study aimed to establish a fluorescently labeled L_3 strain suitable for experimental tracking and to describe its intestinal fluorescence patterns under different administration durations.

2 MATERIALS AND METHODS

2.1 Bacterial strains and culture conditions

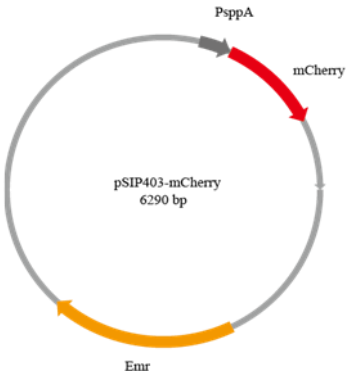
Lactiplantibacillus plantarum L_3(*L. plantarum* L_3) was stored at the College of Life Sciences, Heilongjiang University. The NCBI sequence number is MT781360, and the strain collection number is CGMCC No.28730.

L. plantarum L_3 was cultured in MRS Liquid medium at 37℃ standing. MCherry *Lactiplantibacillus plantarum* L_3(MCherry-L3) is the *L. plantarum* L_3 strain successfully labeled with *mCherry*, which emits red fluorescence. It was cultured in MRS-Erythromycin (MRS-Em) liquid medium containing erythromycin at a concentration of 10 μg/mL at 37℃.

2.2 Construction and verification of mCherry-L3

The transformation method described by Meng *et al.* was slightly modified (Meng et al. 2021). The schematic of pSIP403-mCherry recombinant plasmid is shown in Figure 1. To transform *L. plantarum* L_3, 5 μL of recombinant plasmid DNA (Concentration: 649.3 ng/μL) was mixed with 100 μL of competent cells and transferred to a precooled sterile electroporation cuvette placed in an ice bath for 10 min. The electroporation was performed using a Micro Pulser (BIO-RAD) with the following parameters: 2.2 KV, 4 ms pulse time. After electroporation, the cells were incubated at 37℃ for 2.5 h. The culture was then plated on MRS agar containing 10 μg/mL erythromycin and incubated at 37℃ in an inverted position. Single colonies were selected and verified by PCR using primers Pm-1 (ATGGTGAGCAAGGGCGAG) and Pm-2 (TCACTTGTACAGCTCGTCCATG). PCR analysis confirmed that the colonies were correct, and they were subsequently sequenced. The *L. plantarum* L_3 recombinant strain, with 100%

89 correct sequencing results, was stored at -80℃ and named mCherry-L3.



90

91 **Fig. 1** pSIP403-mCherry plasmid profile

92 **2.3 Induced expression and western-blot identification**

93 The recombinant plasmid utilized the inducible promoter PsppA, requiring the addition of the SppIP inducing
94 peptide to initiate expression of the *mcherry* gene. Upon culturing the strain to the second-generation bacterial
95 solution, 30 μL of the SppIP inducing peptide stock solution was added after incubating at 37℃ for 3 h. SppIP
96 Inducing Peptide (Sequence: MAGNSSNFIHKIKQIFTHR): Accurately weigh 5 mg of SppIP peptide powder, make
97 up the volume to 100 mL with ultrapure water, and store it at -20°C for later use. The working concentration in the
98 culture medium is 50 ng/mL. Following a 24 h incubation at 37℃, the strain was centrifuged (10,528×*g*, 10 min),
99 and the pellet was washed and resuspended in 500 μL of PBS buffer. This preparation resulted in *L. plantarum* L_3,
100 which was induced to express the fluorescent protein.

101 The expression level of the fluorescent protein was determined following the method outlined by Yang *et al.*
102 (Mo et al. 2021). The activated bacterial solution was sonicated and centrifuged at 4℃ (12,000×*g*, 10 min). Protein
103 concentration in the supernatant was measured using a BCA assay kit (KGP902, Biyuntian Biotechnology Co., LTD,
104 China). Proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The membrane was blocked
105 with a blocking solution for 1 h at room temperature. After removing the blocking solution, diluted primary antibodies
106 (1:2000 concentration) were incubated overnight at 4℃. The primary antibodies were washed three times with TBST
107 for 5 min each. Diluted secondary antibodies (1:5000 concentration) were added and incubated for 30 min at room

temperature, followed by four washes with TBST for 5 min each, on a shaker at room temperature. After thorough washing, the membrane was incubated with a chemiluminescence detection substrate prepared by mixing two developers at a 1:1 ratio for 10 s. Imaging was performed using a chemiluminescence instrument.

2.4 Fluorescence observation of mCherry-L3

To observe fluorescence, 10 μL of the 20-fold diluted induced bacterial solution was mixed with 10 μL of 1% agarose solution. A drop of this mixture was placed onto a slide, covered with a cover glass to prevent bubbles, and sealed with neutral resin. Fluorescence was observed using a laser confocal microscope (Nikon C2, Nikon, Japan).

2.5 Fluorescence activity and growth curve determination

Starting from the time after adding the SppIP-induced peptide, 500 μL of bacterial solution was sampled every 2 h from two tubes. One tube was used to measure the optical density (OD_{600}) after dilution, using an ultra-micro spectrophotometer (Nanodrop One, Thermo Fisher Scientific, USA). The other tube was centrifuged at 8000×*g* for 1 min. The supernatant was discarded, and the bacterial pellet was washed twice with PBS and resuspended in 500 μL of PBS. Measure the fluorescence value of each sample using a fluorescence microplate reader (Gemini XPS, Shanghai Meigo Molecular Instruments) with an excitation wavelength of 587 nm and an emission wavelength of 610 nm.

2.6 Stability of mCherry labeling

The expression was induced and cultured to the first-generation bacterial solution, with the recombinant *L. plantarum* L_3 being passaged every 24 h for a total of 98 generations. The genetic stability of red fluorescence in the recombinant *L. plantarum* L_3 at the first and 98th generations was observed using laser confocal microscopy. Additionally, plasmids were extracted from the bacterial culture every two weeks, and PCR verification was performed using primers Pm-1 and Pm-2 to detect whether the *mCherry* gene remained stable in the recombinant *L. plantarum* L_3.

130 **2.7 Antimicrobial activity assay**

131 The inhibitory effect of strain supernatants against several common indicator bacteria was evaluated using the
132 Oxford cup method (Muhammad et al. 2019). *L. plantarum* L_3, both before and after labeling, was activated and
133 centrifuged at 10,000×*g* for 10 min. The supernatant was then filtered through a 0.22μm filter membrane and collected.
134 The following bacteria were used: *Bacillus subtilis* ATCC 26633, *Staphylococcus aureus* ATCC 25923, *Listeria*
135 *monocytogenes* CMCC 54001, and *Escherichia coli* ATCC 25922. The bacterial solution was appropriately diluted
136 (10^8 CFU/mL) and evenly spread on the surface of LB solid medium. A sterilized Oxford cup was gently placed in
137 the freshly coated bacterial plate using sterile forceps. Subsequently, 200 μL of the filtered *L. plantarum* L_3
138 supernatant (before and after labeling) was added to the Oxford cup. The plate was then incubated at 4℃ for 4 h to
139 allow diffusion. After diffusion, the plate was transferred to a constant temperature incubator at 37℃ for 24 h, and
140 the diameter of the inhibition zone was measured using a vernier caliper.

141 **2.8 Simulated gastrointestinal digestion in vitro**

142 According to the method outlined by Charoenrak *et al.* (Charoenrak et al. 2023), *L. plantarum* L_3 and mCherry-
143 L3 were activated, centrifuged, and the bacteria collected. They were then washed and resuspended, and the initial
144 viable bacterial count was determined using the plate count method. The bacteria were suspended in simulated saliva
145 and shaken at 37℃ for 5 min. After centrifugation, the supernatant was discarded. The bacteria were then resuspended
146 in artificial stomach fluid, shaken at 37℃ for 2 h, followed by resuspension in artificial bile, shaken at 37℃ for 20
147 min, and finally resuspended in artificial small intestine fluid and shaken at 37℃ for 2 h. Bacteria were collected by
148 centrifugation after each step, followed by colony counting.

149 **2.9 Animal experiments and intestinal fluorescence imaging**

150 Twelve 7-8 week-old C57BL/6J mice were randomly divided into two groups after one week of adaptive feeding.
151 The experimental mice were housed at the SPF-level. Environmental parameters were strictly controlled, with

temperature set at 20±2℃, relative humidity at 50±5%, and a 12 h light/dark cycle maintained by an automatic lighting control system. During the experiment, strict animal welfare standards were followed, including daily monitoring of food intake with precise measurements and records. Cage cleaning and bedding replacement were performed twice a week. All animal procedures in this study were conducted in strict accordance with the guidelines of the Animal Ethics Committee of Heilongjiang University (Ethical Review No. 20220523006). There are two groups of mice in total. In the first group, there are six mice. Each mouse is intragastrically administered with 0.2 mL of normal saline containing mCherry-L3 per day for four days. In the second group, there are also six mice. Each mouse is intragastrically administered with 0.2 mL of normal saline containing mCherry-L3 per day for eight weeks. One group was sacrificed under deep anesthesia after 4 days of gavage, while the other group was sacrificed after 8 weeks of gavage. The whole intestinal tracts were dissected from mice. Fluorescence imaging was performed with the Tanon 5200 Multi fluorescence imaging system to achieve qualitative visualization of intestinal distribution following consecutive drug administration. The bacterial concentrations in all treatment groups reached 10^9 cfu/mL. The animal experiment was approved by the Ethics Review Committee with approval number 20220523006.

2.10 Data Analysis

The data significance analysis and graph drawing were performed using SPSS 27.0 and Origin 2019 scientific research drawing software. Quantitative data are presented as the mean ± standard deviation (SD). The results were represented by different letters to indicate significant differences ($P < 0.05$).

3 RESULTS

3.1 Results of colony PCR identification of mCherry-L3 strain

The *mCherry* gene fragment was amplified from the transformed single colony using specific primers and verified via 1% agarose gel electrophoresis (Fig. 2). No target band was observed in the negative control group, while the recombinant strain exhibited a clear target band at approximately 700 bp. The *mCherry* gene fragment was

confirmed to be 711 bp, and the band corresponded to the expected target fragment. These results confirmed that *L. plantarum* L_3 had been successfully labeled with red fluorescent protein. Subsequently, the positive strain containing the *mCherry* gene, as identified by colony PCR, was sent to Shanghai Bioengineering Co., Ltd. for DNA sequencing, which yielded a 100% match.

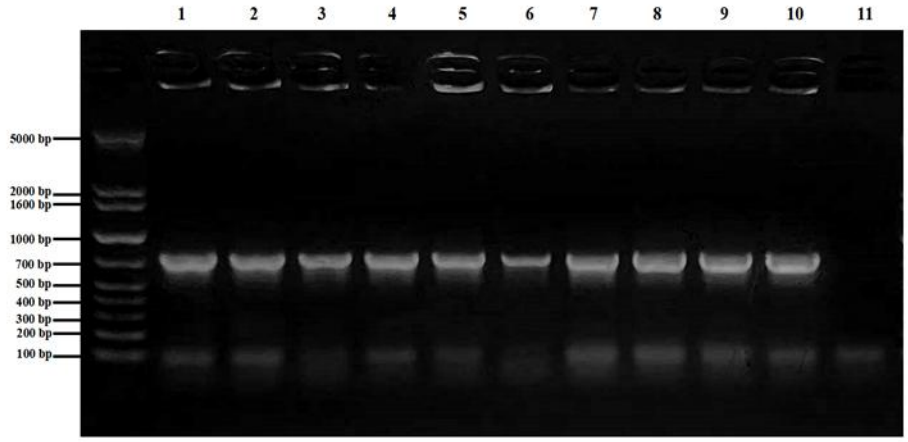


Fig. 2 Results of PCR electrophoresis of colonies. (M:-marker；1-10-labeled strains；11-wild strains)

3.2 Induced expression results of mCherry-L3

The expression of the *mCherry* protein in the recombinant *L. plantarum* L_3 was detected by Western blot (Fig. 3). Compared to the wild-type and non-induced bacterial cultures, a protein band with a size of approximately 27 kDa was clearly observed in the induced bacterial culture. This protein band matched the expected target band, confirming that the *mCherry* fluorescent protein was successfully expressed in *L. plantarum* L_3.

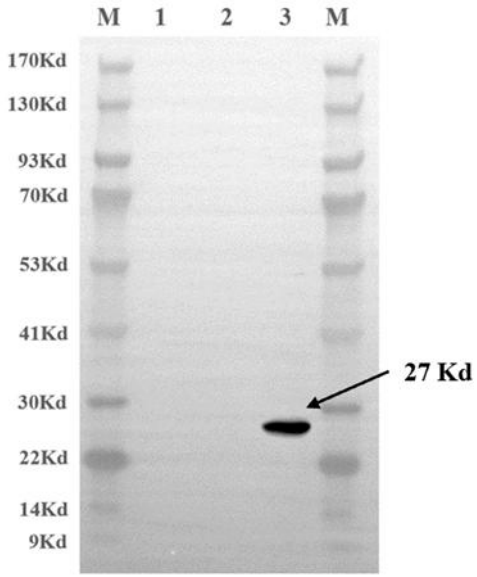


Fig. 3 Western-Blot identification results of recombinant proteins

No. M: Protein Marker; 1: *L. plantarum* L_3; 2: mCherry-L3 without inducer induction; 3: mCherry-L3 after induction.

3.3 Laser confocal microscopy observation

The results of laser confocal microscopy of the strains before and after labeling are shown in Figure 4. It is evident that the morphology of the bacteria remained unchanged before and after labeling, with both displaying a rod-shaped form (Fig. 4a-b). The labeled strain exhibited clear red fluorescence compared to the control group, with strong fluorescence intensity and low background. This result is consistent with the Western blot findings described above, further confirming the successful expression of RFP in *L. plantarum* L_3.

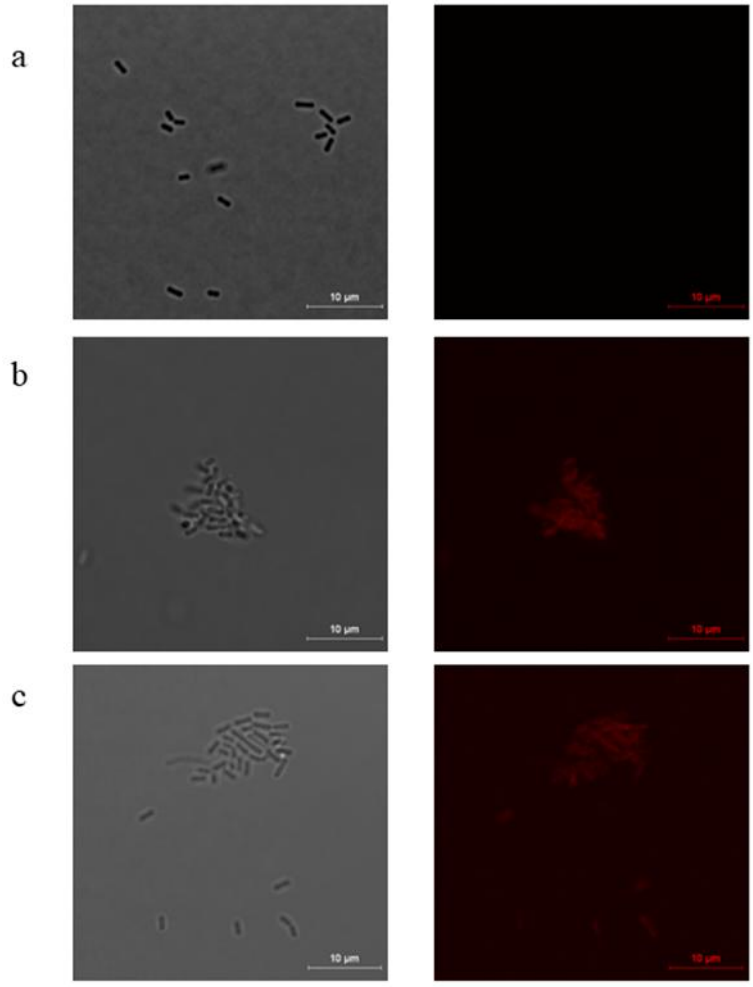


Fig. 4 Laser scanning confocal microscope of wild strains and labeled strains. (The left side was the bright-field visual field. The fluorescence field is shown on the right，a，*L. plantarum* L_3；b, mCherry-L3；c, mCherry-L3 at 98th generation)

3.4 Growth curves and determination of fluorescence intensity at different growth stages

The growth curve of *L. plantarum* L_3 after labeling was nearly identical to that of the labeled strain before labeling, with the OD_{600} reaching approximately 1.01 at the end of the experiment (Fig. 5a). This suggests that the *mCherry* gene marker had no significant impact on the growth of *L. plantarum* L_3. The fluorescence intensity of labeled *L. plantarum* L_3 was significantly higher than that of the unlabeled strain (Fig. 5b). The fluorescence intensity increased over time, peaking at 30,949 U at 16 h after the addition of the inducing agent.

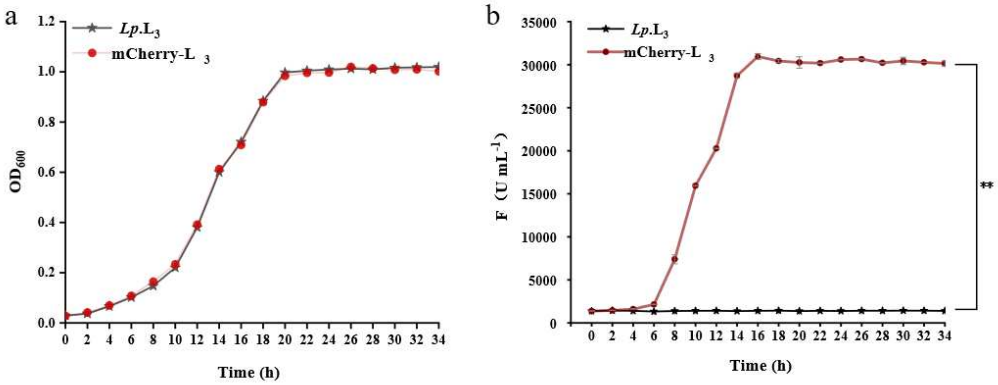


Fig. 5 Growth curves and fluorescence intensity changes of wild strains and labeled strains.

3.5 Genetic stability

MCherry-L3 was used as the first generation for 24 h, and the fluorescence of the first generation and the 98th generation of induced bacterial solution were observed (Fig. 4c). The morphology of the bacteria did not change after 98 generations of passage, and clear red fluorescence was still visible, with a strong fluorescence effect. Colony PCR was performed every two weeks, and the results were verified by 1% agarose gel electrophoresis (Fig. 6). From the gel image, it can be seen that each colony PCR yielded a distinct target band around 711 bp, confirming that the *mCherry* gene stably exists in the recombinant bacteria. This indicates that the recombinant bacteria has good stability and can be used for subsequent *in vivo* experiments.

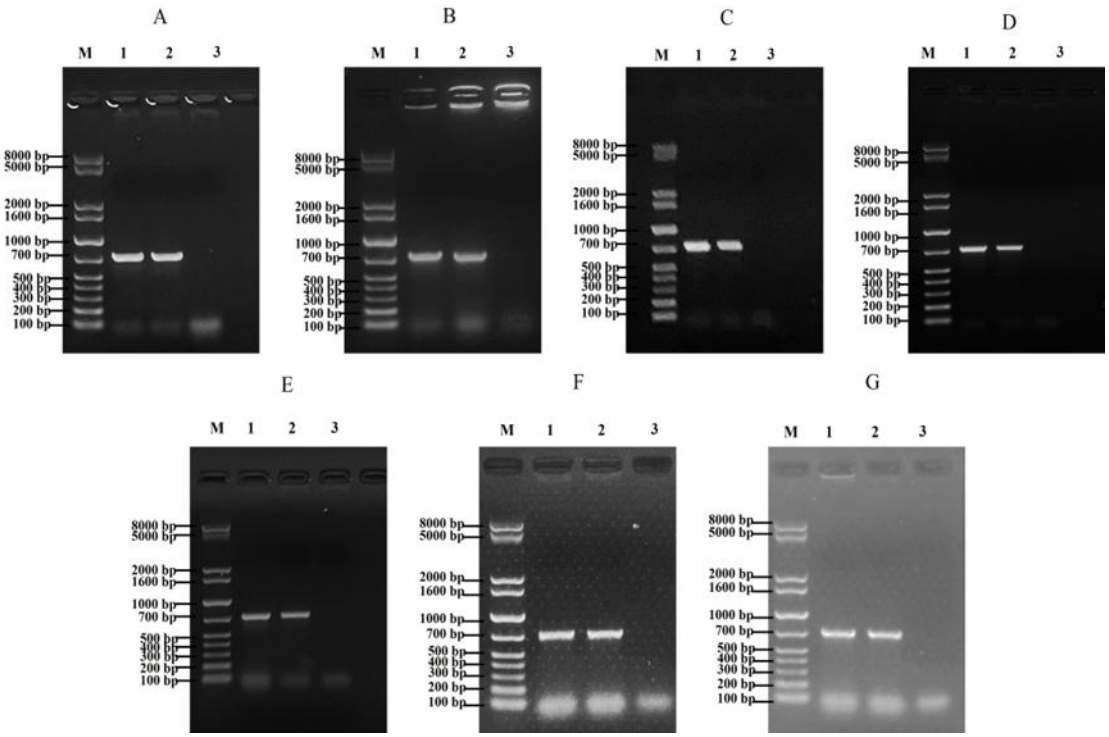


Fig. 6 Genetic stability of the recombinant *L. plantarum* L_3

No. A-G: mCherry-L3 PCR gel plot of colonies that were subcultured at two-week intervals and electrophoresed on 1% agarose gels.

3.6 Antimicrobial activity assay

The antibacterial activity of *L. plantarum* L_3 against four common indicator bacteria before and after labeling is shown in Table 1 and Figure 7. After labeling, the antibacterial activity against *Staphylococcus aureus* was significantly reduced ($P<0.05$), while the antibacterial activity against other bacteria remained unaffected. *L. plantarum* L_3 exhibited a strong inhibitory effect on *S. aureus*, with an inhibition zone diameter greater than 16 mm. The labeled strain showed slightly better antibacterial activity against *Escherichia coli* ATCC 25922 (inhibition zone diameter 13.29 mm–13.93 mm) and *Bacillus subtilis* ATCC 26633 (inhibition zone diameter 13.20 mm–14.58 mm) compared with the unlabeled strain, but the difference was not statistically significant ($P>0.05$).

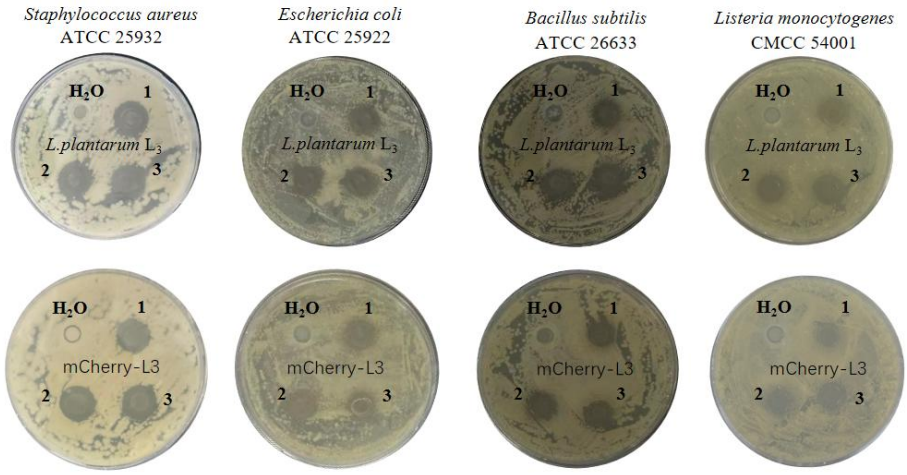


Fig. 7 Antibacterial effect of *L. plantarum* L_3 before and after labeling

3.7 Results of *in vitro* continuous simulation of gastrointestinal tract digestibility measurement

It can be observed that there was no significant difference in the number of viable bacteria between the labeled and unlabeled strains within the same group after treatment with simulated saliva, gastric juice, bile, and small intestinal fluid. This suggests that labeling did not affect the strains' ability to resist gastrointestinal environmental stress *in vitro* (Fig. 8). However, it also indicates that the strains exhibited poor tolerance to the harsh conditions of gastric juice and bile, as the number of viable bacteria decreased significantly before and after labeling.

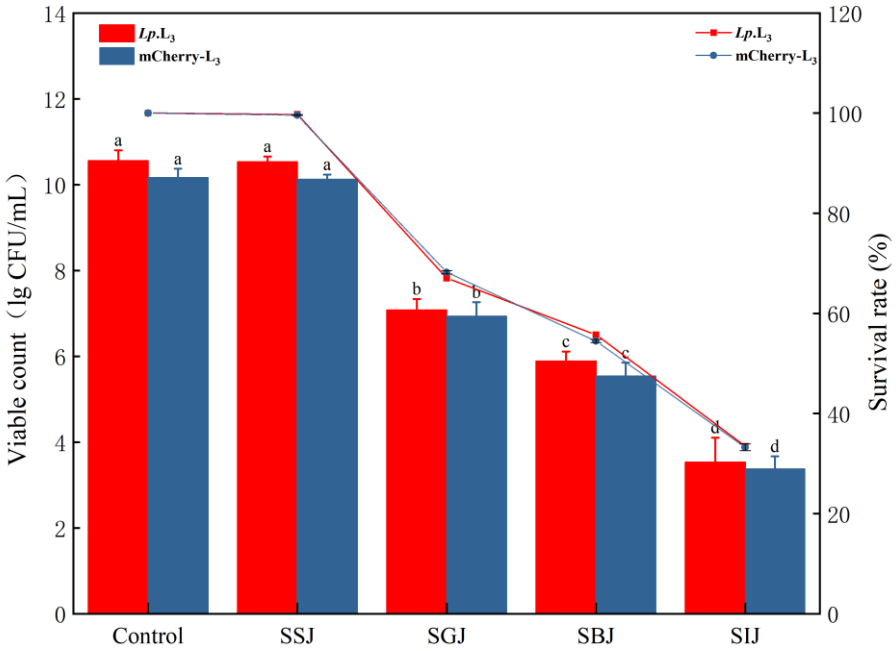


Fig. 8 Simulated gastrointestinal tolerance of *L. plantarum* L_3 before and after labeling

Control, *L. plantarum* L_3 and mCherry-L3 before simulated gastrointestinal treatment; SSJ, simulated salivary juice; SGJ, simulated gastric juice; SBJ, simulated bile juice; SIJ, simulated intestinal juice

3.8 Results of *in vivo* fluorescence observation

After 4 days of gavage, fluorescence responses were observed in all parts of the intestinal tract, with the strongest fluorescence detected in the duodenum. The fluorescence response was also notably strong in the colon, and a certain level of fluorescence was observed in the cecum. This indicated that mCherry-L3 had successfully reached the intestinal tract of the mice and was abundant in the duodenum, colon, and cecum. After 8 weeks of gavage, a stronger and more widespread fluorescence signal was visually observed throughout the intestinal tract compared with that observed after 4 days. Additionally, the fluorescence response became more evenly distributed throughout the intestinal tract, suggesting that a large number of mCherry-L3 strains were present in the entire intestinal tract after 8 weeks of continuous gavage.

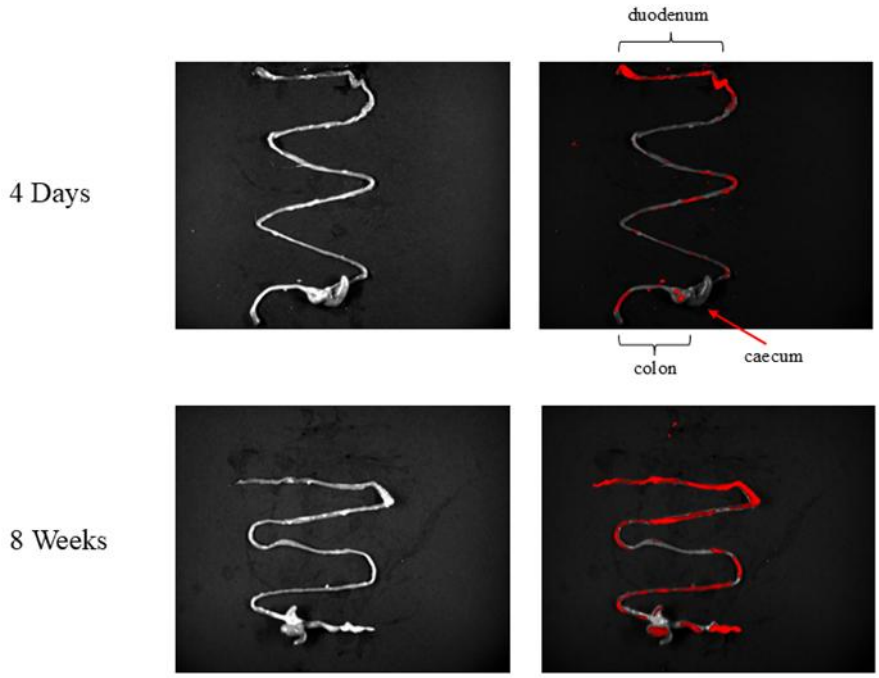


Fig. 9 The location of the distribution of mCherry-L3 in the gut

4 DISCUSSION

Fluorescent protein labeling provides a useful approach for investigating the behavior and spatial distribution of microorganisms under experimental conditions. In the present study, an inducible mCherry-expressing *L. plantarum* L_3 strain was successfully constructed and characterized. The recombinant strain exhibited detectable red fluorescence, retained the mCherry sequence during serial passage, and showed growth characteristics comparable to those of the parental strain. Moreover, intestinal fluorescence patterns differed between mice receiving the labeled strain for 4 days and those receiving it for 8 weeks. These findings demonstrate the feasibility of using mCherry-labeled L_3 for fluorescence-based investigations of intestinal distribution during repeated administration.

The successful expression of mCherry in *L. plantarum* L_3 is consistent with previous studies demonstrating the applicability of fluorescent proteins in lactic acid bacteria. Langa et al. constructed fluorescently labeled lactic acid bacteria, including *L. plantarum*, and demonstrated their suitability for fluorescence-based detection (Langa et al. 2021). Similarly, Mohedano et al. employed mCherry labeling in *L. plantarum* M5MA1 for experimental investigations (Mohedano et al. 2019). In the present study, mCherry expression was verified by molecular and protein analyses, and red fluorescence remained detectable after 98 generations of serial propagation. These observations support the stability of detectable labeling under the conditions examined. However, further assessment under nonselective culture conditions would be required to establish plasmid maintenance in the absence of antibiotic selection.

An important consideration when constructing fluorescently labeled bacteria is whether the labeling procedure alters their physiological characteristics. In this study, the growth curves and morphological characteristics of mCherry-L3 were comparable to those of the parental strain. Similar observations have been reported for fluorescently labeled *L. plantarum* strains (Yang et al. 2021), although changes in bacterial growth following fluorescent labeling have also been described in other lactic acid bacteria (Laure et al. 2001). The labeled L_3 strain

also showed a gastrointestinal stress tolerance pattern similar to that of the parental strain under simulated conditions. Nevertheless, its inhibitory activity against *Staphylococcus aureus* was significantly reduced. This finding indicates that fluorescent labeling may influence specific physiological characteristics even when overall growth remains unchanged. Therefore, the biological properties of labeled strains should be evaluated before they are used as experimental substitutes for their parental strains.

Intestinal fluorescence imaging revealed distinct distribution patterns after different durations of oral administration. Following 4 days of administration, the strongest fluorescence was observed in the duodenum, with additional signals in the cecum and colon. Previous fluorescence-based studies have reported the intestinal localization of administered *L. plantarum* strains in experimental animals (Van Zyl et al. 2015; Coelho-Rocha et al. 2018). The intestinal environment varies considerably among anatomical regions, particularly in microbial density and community composition (Xu et al. 2021), which may influence the distribution and survival of administered bacteria. After 8 weeks of repeated administration, fluorescence signals appeared more widespread throughout the intestinal tract. Previous studies have suggested that the physiological effects of probiotic supplementation may depend partly on administration duration (Goldin 1998; Alvi et al. 2017; Kim et al. 2020; Wang et al. 2022; Colletti et al. 2023). However, the present study did not assess host physiological outcomes or probiotic efficacy. Therefore, the broader fluorescence distribution observed after 8 weeks should not be interpreted as evidence of enhanced probiotic activity.

Several limitations should be considered when interpreting these findings. First, intestinal fluorescence was evaluated at the experimental endpoints during repeated administration, without a post-administration washout period. Consequently, the observed fluorescence patterns cannot establish whether the labeled strain persisted independently of continued dosing or achieved stable intestinal colonization. Second, the intestinal distribution was assessed qualitatively, without quantitative enumeration of the labeled strain in individual intestinal segments. Third,

fluorescence detection alone does not establish the viability of all fluorescent material detected in intestinal samples. In addition, the absence of explicitly documented intestinal imaging controls limits the interpretation of background fluorescence and signal specificity. Future studies should incorporate appropriate controls, post-administration sampling, strain-specific molecular detection, and viable bacterial enumeration to investigate intestinal persistence more rigorously. Assessment of intestinal microbial communities and host physiological responses would further clarify the biological relevance of the observed distribution patterns.

Overall, this study establishes an inducible mCherry-labeled *L. plantarum* L_3 strain with detectable fluorescence after serial passage and largely preserved growth and gastrointestinal tolerance characteristics. The strain provides a potential experimental tool for examining intestinal fluorescence distribution, while the differences observed between administration durations offer a basis for future investigations of bacterial persistence and host–microorganism interactions.

5 CONCLUSION

An inducible mCherry-labeled *L. plantarum* L_3 strain was successfully constructed and characterized. The recombinant strain retained detectable fluorescence after 98 generations and exhibited growth and simulated gastrointestinal tolerance characteristics comparable to those of the parental strain, although antimicrobial activity against *Staphylococcus aureus* was reduced. Intestinal fluorescence imaging revealed distinct distribution patterns following 4 days and 8 weeks of repeated oral administration, with more widespread signals observed after prolonged administration. These findings demonstrate the potential of mCherry-L3 as a fluorescence-based tool for investigating intestinal distribution. Further studies incorporating quantitative detection and post-administration sampling are required to determine bacterial persistence and colonization.

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Ethical approval and consent to participate

The animal experiments were in accordance with the requirements of the Experimental Animal Ethics Committee of Heilongjiang University, and the ethical review acceptance number of animal experiments is 20220523006. All efforts were made to minimize animal suffering.

CRediT authorship contribution statement

Huiwen Zhang: Writing - Original Draft, Validation, Formal analysis, Visualization, Software; **Xinze Guo**: Validation, Data Curation, Methodology, Software; **Xinrui Yang**: Validation, Formal analysis, Visualization, Software; **Mengyang Li**: Validation, investigation, Methodology, Formal analysis; **Yang Yu**: Validation, Formal analysis, Visualization, Software; **Xiuliang Li**: Resources, Conceptualization, Methodology, Writing - Review &Editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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425 Table 1 Determination of antibacterial activity of strains against pathogen indicator bacteria before and after labeling

Strain	Diameter of inhibition zone /mm			
	Staphylococcus aureus ATCC 25923	*Escherichia coli* ATCC 25922	*Bacillus subtilis* ATCC 26633	*Listeria monocytogenes* CMCC 54001
L. plantarum L_3	16.06±0.28[a]	13.36±0.71[a]	12.51±0.25[a]	12.73±0.40[a]
MCherry-L3	14.80±0.36[b]	13.61±0.32[a]	13.89±0.69[a]	13.02±0.37[a]

426 Note: Different lowercase letters within the same column indicate significant differences between strains ($P < 0.05$).