Original Article

Generation of epidermal growth factor and green fluorescent protein coexpressing Lactobacillus acidophilus and its effect on intestinal histology improvement in mature LPS-challenged mice

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Abstract

The aim of the present study was to construct a recombinant strain by using the probiotics Lactobacillus acidophilus expressing the epidermal growth factor (EGF), which can improve intestinal morphology, and to test its effect on the intestinal histology in LPS (lipopolysaccharide)-challenged mice. The EGF gene was cloned into the shuttle vector pSET4s and then transformed into the *L. acidophilus* MG6243 strain by electroporation. Two recombinant strains were confirmed by SDS-PAGE and named ΔMG6243-1 and ΔMG6243-2. Sixty mice were randomly assigned to six treatments: 1) non-challenged control, 2) LPS-challenged control, 3) LPS-challenged + 200 µl MG6243, 4) LPS-challenged + 200 µl ΔMG6243-1, 5) LPS-challenged + 200 µl ΔMG6243-1 + inducer IPTG, and 6) LPS-challenged + 200 µl ΔMG6243-2. There were ten replicates for each treatment, and each replicate had one mouse. On d 15, the LPS-challenged mice were injected intraperitoneally with LPS at 10 mg/kg BW, while the non-challenged mice were injected with 0.9% NaCl solution. Three hours after the challenge, all mice were killed. Results showed that both ΔMG6243-1 and ΔMG6243-2 could increase villus height in the duodenum, ileum and jejunum ($P<0.05$). In addition, the recombinant *L. acidophilus* decreased the crypt depth in the duodenum and ileum ($P<0.05$). These results indicated that the recombinant *L. acidophilus* could effectively improve the intestinal histology in LPS-challenged mice.

Keywords: epidermal growth factor, *Lactobacillus acidophilus*, intestinal histology, LPS-challenged mice

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Introduction

Epidermal growth factor (EGF) is a small polypeptide with 6045 Da molecular mass. EGF can stimulate all epithelial cell type proliferation in the gastrointestinal tract (Carpenter and Cohen, 1990; Currie et al., 2001), and modulate enterocyte differentiation (Cheung et al., 2009). Previous studies reported that oral administration of EGF could increase lactase and sucrase activities in the jejunum (Jaeger et al., 1990; James et al., 1987), inhibit diarrhea induced by the enteropathogenic *E. coli* (Buret et al., 1998), and stimulate intestine recovery in rotavirus-infected piglets (Donovan et al., 1994).

Lactic acid bacteria (LAB), as a large group of beneficial bacteria, usually used in fermented food, have been found in animal digestive systems. *Lactobacillus acidophilus* is one of the LAB and can adhere to the gastrointestinal tract in animal naturally. Huang et al. (2015) reported that *L. acidophilus* could attenuate Salmonella-induced intestinal inflammation. Imani et al. (2015) reported that *L. acidophilus* could motivate immune responses in mice, such as stimulating the production of IFN-γ and inhibiting the production IL-4 and IL-10. Brisbin et al. (2015) also found that *L. acidophilus* could have immunomodulatory effect on chicken macrophages.

Previous studies used *Lactococcus Lactis* to express the active cytokines, EGF, staphylococcal nuclease (Bermúdez-Humarán et al., 2003a), ovine omega interferon, interleukin-12 (Bermúdez-Humarán et al., 2003b), and membrane proteins (Kunji et al., 2003). However, these bacteria could not colonize in the intestine. The expressing vectors they used were also antibiotic resistant, which was harmful to the host animals. Zhao et al. (2015) constructed the recombinant *L. acidophilus* expressing bovine viral diarrhea virus E0 gene and found it could improve the immune response in mice. Similarly, Park et al. (2015) used *L. acidophilus* to produce linoleate 13-hydratase. Ma et al. (2013) thought that *L. acidophilus* would be attractive delivery vehicles.

The intestines are very important for digestion and absorption, therefore, injured intestine has a harmful effect on the body health. LPS challenge could cause intestinal inflammation (Eunok et al., 2012). Therefore, this study was conducted to construct the recombinant *Lactobacillus acidophilus* expressing EGF and to study the effect of the recombinant *Lactobacillus acidophilus* expressing EGF on the intestines in LPS-challenged mice.

Materials and Methods

Construction of the recombinant strain: EGF gene was amplified according to the methods of a previous study (Cheung et al., 2009) from piglet mandibular salivary gland, except the annealing temperature which was 66℃ in our study. Green fluorescent protein (GFP) came from the plasmid pMD18-T-GFP, which was a present from the Academy of Military Medical Sciences (Beijing, China). The polymerase chain reaction (PCR) conditions were at 95℃ (5 min) [94℃ (30 sec), 54.2℃ (45 sec), and 72℃ (2 min)], for 35 cycles, and at 72℃ (10 min) for the final extension. In order to avoid antibiotic resistance, in this study, a homologous arm (P1, P2, P3, and P4, Table 1) between the target genes was designed according to the total *Lactobacillus acidophilus* (*L. acidophilus*) gene sequence, which could help the expressing vector integrate into the *L. acidophilus* genome. The PCR conditions were at 95℃ (5 min) [94℃ (1 min), 56℃ (1 min), and 72℃ (1 min)], for 35 cycles, and at 72℃ (10 min) for the final extension for P1. For P3, the PCR conditions were at 95℃ (5 min) [94℃ (1 min), 63.5℃ (1 min), and 72℃ (1 min)], for 35 cycles, and at 72℃ (10 min) for the final extension. For P2 and P4, the PCR conditions were at 95℃ (5 min) [94℃ (1 min), 64.2℃ (1 min), and 72℃ (1 min)], for 35 cycles, and at 72℃ (10 min) for the final extension. The PCR products were gel-purified by using a Gel Extraction Kit (Omega, American), and ligated into pMD18-T plasmid to generate pMD18-T-P1, pMD18-T-P2, pMD18-T-P3, pMD18-T-P4, and pMD18-T-EGF-GCF, which were then digested with Pst I and Sph I for pMD18-T-P1 and pMD18-T-P3, and BamH I and Sac I for pMD18-T-P2 and pMD18-T-P4, and Pst I and BamH I for pMD18-T-EGF+GFP. Then, all the digested bands were gel-purified and ligated with the plasmid pSET4s, to obtain pSET4s-P1-EGF+GFP-P2 and pSET4s-P3-EGF+GFP-P4, which were then electrically transformed into *Lactobacillus acidophilus* MG6243 (2.5 KV, 2.4-3.0 ms), to generate ΔMG6243-1 and ΔMG6243-2, respectively. The recombinant bacterium was selected by spectinomycin resistance, and confirmed by PCR. All the primer sequences are shown in Table 1. Difference between these two recombinant bacteria was the integrated locations, the location of ΔMG6243-1 was an inducible location selected downstream of lacZ, encoding a β-galactosidase, while the locations of ΔMG6243-2 was selected in a low-expression region. The vector maps of pSET4s-P1-EGF+GFP-P2 and pSET4s-P3-EGF+GFP-P4 are shown in figure 1.

Southern blot analysis: The genome of recombinant *L. Acidophilus* was extracted and confirmed by southern blot by using oligonucleotide probes. The probes were prepared as follows. About 500 bp DNA band based on the EGF+GFP gene sequence (the oligonucleotide probes used are listed in Table 1) was obtained by PCR. Then, the probe was diluted by ddH_2O (final concentration of 0.05-0.2 μg/μL). The diluted probe (15 μL) was cultured in water bath in boiling water for 10 min, then was added 4 μL DIG-High prime mixture and cultured overnight at 37℃. The mixed solution was added 2 μL 0.2 mol/L EDTA (pH 8.0) to terminate the reaction, and added 2.5 μL 4.0 mol/L LiCl and 75 μL 100% alcohol. The mixture was centrifuged at 12000×g for 10 min (4℃), and the pellet was diluted with 50 μL TE (pH 8.0) and stored at -20℃. The genome of recombinant *L. Acidophilus* digested with *Sau*III was subjected to electrophoresis for 5 h at 50 v. The DNA bands were transferred into the NC membrane, which was cultured with the probe solution overnight at 70℃. The membrane was washed two times with 2×SSC、0.1% SDS and two times with 0.1×SSC、0.1% SDS, then soaked in Malic acid for 1-5 min, blocked for 30 min, and cultured with monoclonal antibody (1:5000, Sangon Biotech) for 30 min. The blot result was detected with a camera (canon, 1800 mega pixels).

Western blot analysis: Western blot was used to investigate whether the recombinant *L. acidophilus* could produce EGF. The samples (0.1 mL) were homogenized in 1 mL of lysis buffer using a Polytron homogenizer and centrifuged at 12,000 g for 15 min at 4°C. The supernatant fluid was aliquoted into micro-centrifuge tubes, to which 2 × SDS sample buffer (2 mL of 0.5 mol/L Tris, pH 6.8, 2 mL glycerol, 2 mL of 10% SDS, 0.2 mL of b-mercaptoethanol, 0.4 mL of a 4% solution of bromophenol blue and 1.4 mL of water) was added in a 1:1 ratio. The samples were boiled and cooled on ice before use for western blotting. Proteins were separated by electrophoresis on a 10% polyacrylamide gel, and then electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane. Skim-milk powder in TBST buffer (1 × Tris-buffered saline including 0.1% Tween 20) was used to blot the membrane for 1 h at room temperature. Membranes were incubated overnight at 4°C with one of the primary antibodies: β-actin and GFP (1:750, Sangon Biotech). Thereafter, the membranes were washed with TBS-T and incubated for 1 h at room temperature with secondary antibody: mouse (1:3000, Sangon Biotech). After being washed with TBST, blots on the membrane were developed using an Enhanced Chemiluminescence Western Blotting Kit (ECL-plus, AmershamBiosciences, Sweden), visualized, and quantified using an imaging system (Alpha Innotech FluorChem FC2, CA, USA). Abundances of all proteins of interest were normalized to those for β-actin.

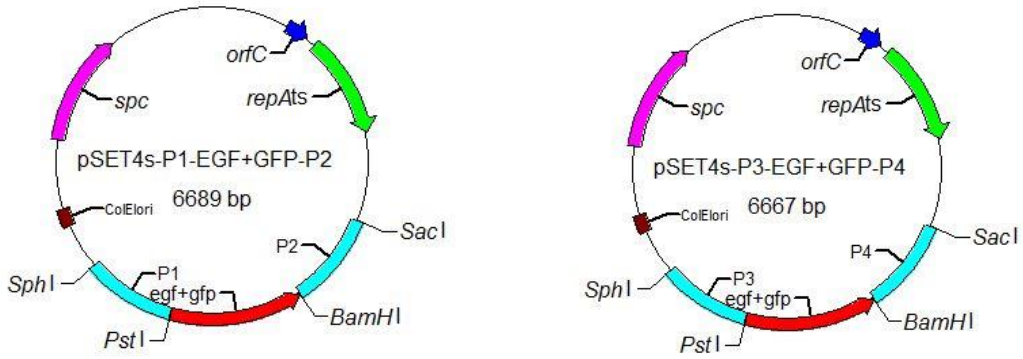

Figure 1 The vector maps of pSET4s-P1-EGF+GFP-P2 and pSET4s-P3-EGF+GFP-P4

Table 1 Specific primer sequences for pigs used for real-time PCR

item	Primer sequence(5′-3′)	application
P1	(F)GGCATGCATAGCGATTGTATTAGTAAC (R)GCTGCAGTTAACCGAGTACAATTTCAA	625 bp upstream homology arm P1
P2	(F)GGGATCCAAATTAATCGTGGTCAACAT (R)GGAGCTCAAGGCTTTACACGAATCAAT	642 bp downstream homology arm P2
P3	(F)TGCATGCGATGTTGCTCAGGTAGATAG (R)GCTGCAGAATCACTAAAGAGGTCGAGA	626 bp upstream homology arm P3
P4	(F)GGGATCCTATATGGTATTTTTATGTGC (R)GGAGCTCATGAGTAAACATCGAGAAAT	619 bp downstream homology arm P4
P5	(F)CGAGCTGTACAAGTAAGAATTATGAATAGTTACTCTGAATGC (R)CGAGCTGTACAAGTAAGAATTTTAGCGCAGCTCCCACCATTT	159 bp EGFgene
P6	(F)CGAGCTGTACAAGTAAGAATTATGAATAGTTACTCTGAATGC (R)CGAGCTGTACAAGTAAGAATTTTAGCGCAGCTCCCACCATTT	757 bp GFP gene
P7	(F)GCTGCAGGCCACCATGGTGAGCAAGGG (R)GCGGATCCATGAATAGTTACTCTGAAT	901 bp EGF+GFP gene
P8	(F)GAAAAAATCAAAATAGGGTGGATG (R)GAAACTCTTGAAACTATCCGTATGT	ΔMG6243-1
P9	(F)ATTTTTGTGCATCTTTTCGACGACG (R)GAACAAACAATGAAAGTGCCTGAT	ΔMG6243-2

ELISA analysis: The recombinant EGF expressed by *L. acidophilus* was detected with ELISA by using commercially available ^{125}I RIA kit (Sangon Biotech, Shanghai, China). Human EGF antibody was used as the standard. The intra- and inter-assay coefficients of variation were <5 and <10%, respectively. The detection limit was 0.1 μg/L. Concentrations of the culture was determined by OD at 450 nm. The assays were carried out in triplicate.

Stability of the recombinant L. acidophilus: The recombinant *L. acidophilus* was cultured continuously for 10 generations, and the GFP protein for each generation was detected with an inverted fluorescence microscope (Olympus, Japan). Briefly, the recombinant fluorescence protein (ΔMG6243 (GFP)) and wild type fluorescence protein (MG6243) was cultured in LB at 37°C for 12-16 h, respectively. Then, the culture (1.5 ml) was removed and centrifuged at 4000 r/min for 10 min. The pellet was resoluted in 100 µL PBS. Then, the solution was mixed with glycerol (20%) and observed with the inverted fluorescence microscope.

Growth characteristics of the recombinant L. acidophilus: The stored bacterium (1.5 ml) was cultured overnight and centrifuged at 4000 r/min for 10 min. The pellet was washed with PBS three times, and diluted with the same OD600 (0.2), and cultured in MRS at 37°C. Growth of the bacteria was studied at different times for 14 h. Concentrations of the culture was measured at OD600 by spectrophotometry.

Acid and bile salt resistance test of the recombinant L. acidophilus: The cultured bacterium was cultured in PBS at 37°C for 3 h. pH values were 2.0, 2.5, 3.0, and 4.0 for pH resistance test. For the bile salt resistance experiment, the bacteria was cultured in PBS, which contained 0.2%, 0.5%, 0.8%, and 1.0% bile salt. Active cells were quantified with MRS plate, and 0 h was the control.

Effect of oral administration of the recombinant Lactobacillus acidophilus on the intestine in LPS-challenged mice: Sixty mice (6-8 weeks, 20 g) were randomly assigned to six treatments, 1) non-challenged control (saline-treated + oral administration 200 µL PBS), 2) LPS-challenged control (LPS-challenged + oral administration 200 µL PBS), 3) LPS-challenged + oral administration 200 µL wild *L. acidophilus* MG6243, 4) LPS-challenged + oral administration 200 µl recombinant *L. acidophilus* ΔMG6243-1, 5) LPS-challenged + oral administration 200 µL recombinant *L. acidophilus* ΔMG6243-1 + inducer (IPTG), and 6) LPS-challenged + oral administration 200 µL recombinant *L. acidophilus* ΔMG6243-2. There were ten replicates for each treatment, and each replicate had one mouse. On d 15, the LPS-challenged mice were injected intraperitoneally with *Escherichia coli* LPS at 10 mg/kg BW, and the saline-treated mice were injected with the same volume of 0.9% NaCl solution. Three hours after the LPS challenge or saline treatment, all mice were killed by cervical dislocation. The small intestine (duodenum, jejunum, and ileum) was removed as soon as possible, and approximately 1-2 cm of each part was fixed overnight with 4% formalin, then embedded, and stained with hematoxylin and eosin (H&E). Villus height and crypt depth were measured with HPIAS-1000 software.

Statistics: Data were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The experimental unit for histology was ten mice. Differences among treatment means were identified by the Duncan's multiple range test. All data were expressed as mean ± SD. Statistical significance level for all analyses was set at $P<0.05$, and $0.05 < P < 0.10$ were discussed as trends.

Results

Generation of EGF-expressing recombinant L. acidophilus: EGF-GPF cDNA was inserted into *L. acidophilus* MG6243, to generate ΔMG6243-1 and ΔMG6243-2, respectively, and the target sequence was confirmed by southern blot, as shown in figure 2, in which lanes 1 and 3 were ΔMG6243-1 and ΔMG6243-2, respectively. Western blot analysis was used to investigate whether the recombinant *L. acidophilus* could produce EGF. As shown in figure 3, EGF protein was detected, which suggests that the recombinant bacteria could produce the EGF protein, and the amount of EGF in bacterial culture was respectively 3.2, 3.7 and 3.9 ng/mL (Table 2). The growth curve is shown in figure 4. During the 14 h culturing period, the growth of bacteria entered a comparatively smooth developing period at 10-12 h, and the OD 600 was 2.4 and 2.35 for ΔMG6243-1 and ΔMG6243-2, respectively.

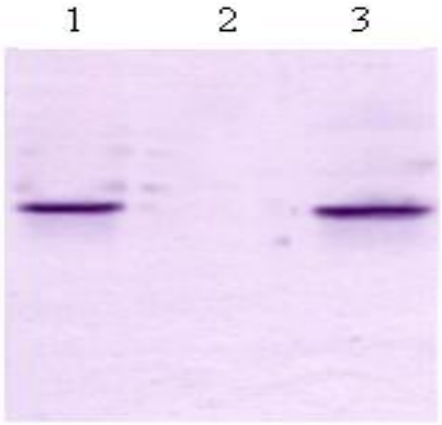

Figure 2 Southern blot analysis for *L. acidophilus*
Lane 1: ΔMG6243-1; lane 2: MG6243; lane3: ΔMG6243-2

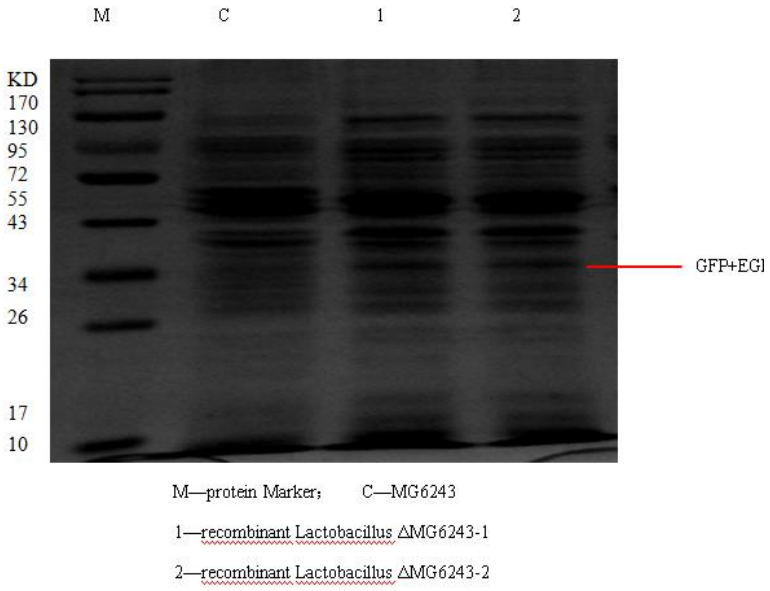

Figure 3 SDS-PAGE analysis for wild *L. acidophilus* and recombinant *L. acidophilus*
There was difference at 26-34kD between the wild *L. acidophilus* and the recombinant *L. acidophilus.*

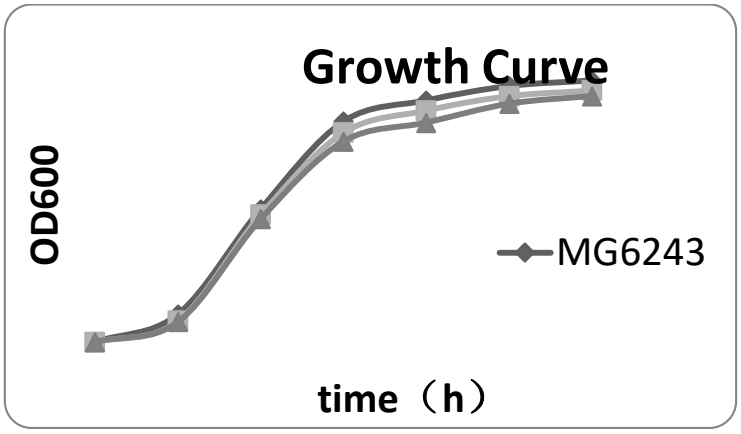

Figure 4 Fermentation of epidermal growth factor-expressing *L. Acidophilus*
Comparison of the *in vitro* growth of wild and recombinant *L. lactis* [ΔMG6243-1, MG6243 and ΔMG6243-2 strains, respectively]. Optical densities at 600 nm (OD600) show that these strains achieved similar growth characteristics in a 12-h fermentation study.

Table 2 Results of EGF in recombinant *L. acidophilus*[1]

item	OD450nm
Lactobacillus acidophilus 6243	0.9685±0.1460[a]
ΔMG6243-1 group	3.7177±0.2129[b]
ΔMG6243-1 (induced) group	3.9176±0.1234[b]
ΔMG6243-2 group	3.1851±0.2377[b]

[1]Values are presented as means ± SD;
Means in a row with different letters differ significantly (P<0.05).

Characteristics of EGF-expressing recombinant L. acidophilus: The stability of the recombinant *L. acidophilus* was examined by the fluorescence protein microscope. As shown in figure 5, there was a large amount of green fluorescence protein after 10 generations, which indicates that this recombinant *L. Acidophilus* has a good stability. In addition, as shown in figure 6, there was no difference among these three bacteria when the pH was 2.0, 2.5, and 4.0. However, the live bacteria ΔMG6243-2 was decreased when the pH value was 3.0, which indicates that the tolerance of ΔMG6243-1 to acid was superior to ΔMG6243-2 (P<0.05). For the bile salt resistance test (Fig. 7), compared to ΔMG6243-2, ΔMG6243-1 had higher bile salt resistance under 1.0 bile salt concentration (P<0.05).

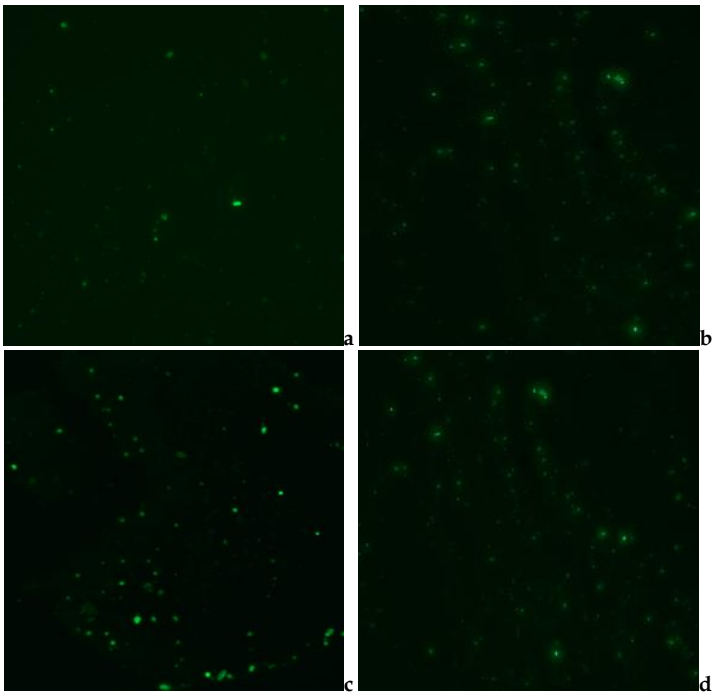

Figure 5 Recombinant *L. acidophilus* imaged by fluorescence microscope
(a) the first generation of the recombinant *L. acidophilus*; (b) the third generation of the recombinant *L. acidophilus*; (c) the sixth generation of the recombinant *L. acidophilus*; (d) the tenth generation of the recombinant *L. acidophilus*.
The recombinant cells expressing GFP were detected among these generations.

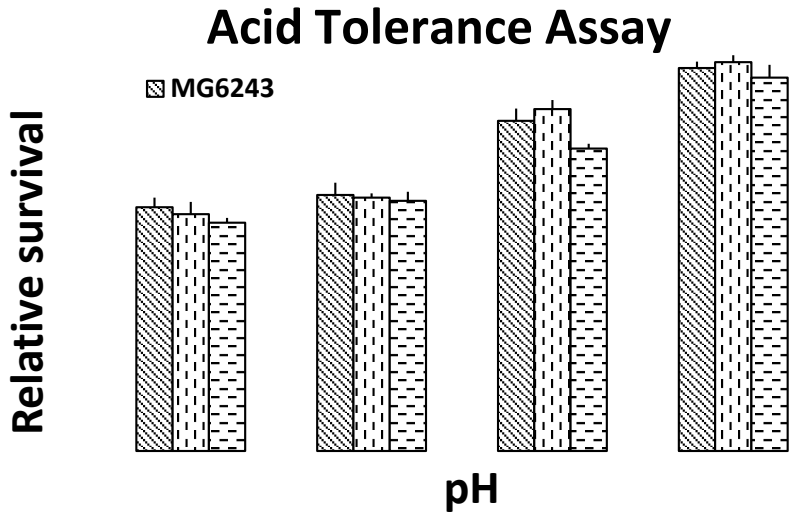

Figure 6 Acid tolerance assay
There was no difference among these three bacteria when the pH values were 2.0, 2.5, and 4.0. However, the live bacteria ΔMG6243-2 decreased when the pH value was 3.0 ($P<0.05$).

EGF expressing L. Acidophilus improved intestinal development in LPS-challenged mice: The results of intestinal morphology are shown in Table 3. From results of villus height, one index of the intestinal morphology was shorter in the LPS-challenged mice than the control group, which means LPS can cause damage to the villus. However, when the mice challenged by LPS were fed *L. acidophilus* either the wide type strain or the recombinant strain at the same time, the intestinal villus damaged by LPS could be restored, especially the recombinant strain in which EGF was expressed. Also, the repaired effect was more obvious with the increase in EGF amount. For the results of crypt depth, one index of the intestinal morphology was higher in the LPS-challenged mice than in the control group. When the mice challenged by LPS were fed *L. acidophilus* either the wide type strain or the recombinant strain at the same time, however, the intestinal crypt depth damaged by LPS could be restored, especially the recombinant strain in which EGF was expressed. For the results of ratio of villus height to crypt depth, one index of the intestinal morphology was smaller in the LPS-challenged mice than in the control group. However, when the mice challenged by LPS were fed *L. acidophilus* either the wide type strain or the recombinant strain at the same time, the index could be restored, especially the recombinant strain in which EGF was expressed. From

the changes in the index of the intestinal morphology, one conclusion can be drawn that the LPS challenge can cause damage to the intestine, but when the mice challenged by LPS were fed *L. acidophilus*, the intestinal damaged by LPS can be repaired, especially the recombinant strain in which EGF is expressed. The repaired effect was more obvious in the ΔMG6243-1 + inducer group than the other two recombinant strain groups (ΔMG6243-1 and ΔMG6243-2). The main reason was the EGF; ΔMG6243-1 with inducer can express higher level EGF than ΔMG6243-2 and ΔMG6243-1 without inducer.

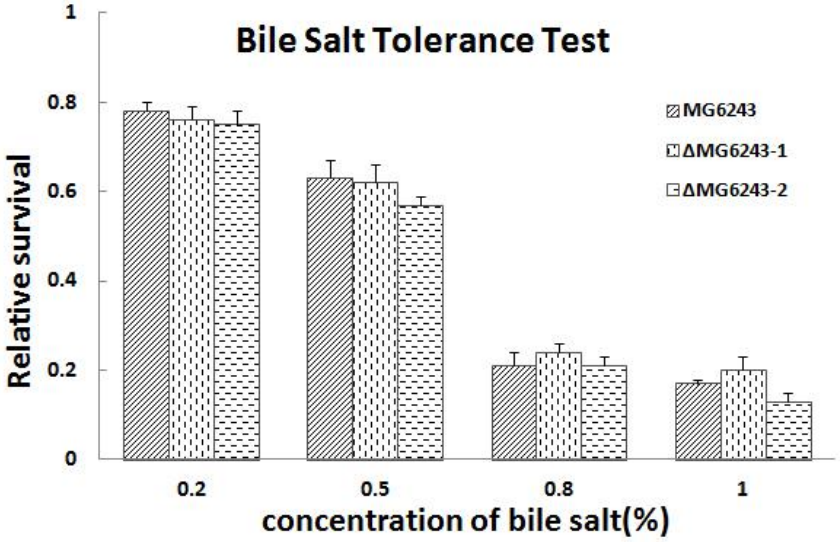

Figure 7 Bile salt tolerance assay
There was no difference among these three bacteria when the bile salt concentrations were 2.0, 0.5, and 0.8%. However, the live bacteria ΔMG6243-2 decreased when the bile salt concentration was 1.0% (P<0.05).

Table 3 Effect of the recombinant *L. acidophilus* expressing EGF on intestinal histology in LPS-challenged mice[1]

Item	Control	LPS	Wild MG6243+LPS	ΔMG6243-1+LPS	ΔMG6243-1 +inducer+LPS	ΔMG6243-2+LPS
Villus height (μm)						
Duodenum	132.428±10.22^{b}	113.222±3.10^{a}	116.796±5.52^{a}	121.223±3.94^{a}	138.732±7.31^{b}	120.370±10.00^{a}
Jejunum	100.262±4.44^{c}	86.950±2.02^{a}	90.320±1.28^{ab}	88.170±2.18^{a}	105.252±5.78^{c}	94.567±6.81^{b}
Ileum	93.374±16.42^{ab}	85.320±3.94^{a}	87.374±8.19^{a}	93.733±7.23^{ab}	106.540±10.43^{b}	91.780±12.60^{ab}
crypt depth (μm)						
Duodenum	60.860±6.74	68.146±5.08	66.106±6.95	62.212±3.25	64.856±3.32	65.812±10.90
Jejunum	47.576±9.57^{a}	56.280±2.42^{ab}	54.182±8.39^{ab}	60.582±11.79^{b}	52.608±5.80^{ab}	45.762±5.28^{a}
Ileum	48.938±7.90^{ab}	53.152±6.07^{b}	46.520±4.20^{ab}	52.067±8.66^{ab}	50.970±6.46^{ab}	44.032±4.60^{a}
Ratio of villus height to crypt depth						
Duodenum	2.1842±0.14^{c}	1.6704±0.14^{a}	1.7757±0.13^{ab}	1.9523±0.11^{bc}	2.1417±0.15^{c}	1.8610±0.28^{ab}
Jejunum	2.1660±0.37^{b}	1.5465±0.54^{a}	1.6946±0.22^{a}	1.4949±0.26^{a}	2.0166±0.20^{b}	2.0746±0.10^{b}
Ileum	1.9128±0.223^{b}	1.6184±0.16^{a}	1.8823±0.15^{b}	1.9116±0.13^{b}	2.1045±0.18^{b}	2.0842±0.19^{b}

[1]Values are presented as means ± SD;
Means in a row with different letters differ significantly (P<0.05).

Discussion and Conclusion

L. acidophilus can colonize in the gastrointestinal tract of animals (Zhang et al., 2008), and inhibit the growth of harmful microorganisms in the gut by producing lactic acid. Many studies have found that *L. acidophilus* could relieve Salmonella-induced intestinal inflammation, stimulate immune response, and protect periodontal tissues (Zhao et al., 2011; Zhao et al., 2012; Brisbin et al., 2015; Huang et al., 2015; Imani et al., 2015). Previous studies used *L. acidophilus* to express exogenous proteins to improve immunity, produce hydroxy fatty acids, which could be used in medicine, and prevent periodontal infection (Ma et al., 2013; Park et al., 2015; Zhao et al., 2015). The pSET-4s is a thermosensitive (Ts) suicide vector; Takamatsu et al. (2001) reported that this vector might be an effective carrier to allelic exchange target genes via homologous recombination. In the current study, the recombinant *L. acidophilus* expressing EGF was successfully structured through pSET4s, evidenced by the southern blot, western blot and ELISA.

In the challenge experiment the ΔMG6243-1 + inducer (IPTG) + LPS increased the duodenum- and jejunum-villus heights, similar to those of the control mice. It is known that EGF can promote proliferation of the intestinal crypt cells and restore injured intestinal mucosa caused by LPS challenge. ΔMG6243-1 and ΔMG6243-2 were all the

same recombinant strain Lactobacillus acidophilus MG6243, but the integrated locations of the EGF gene were different; the integrated location in ΔMG6243-1 was inserted after an inducible location was selected downstream of lacZ encoding a β-glucuronidase, but the integrated location in ΔMG6243-1 was selected in a low-expression region in ΔMG6243-2. The expression level of the integrated gene in different integrated locations was different, which is in accord with the results of Grace et al. (2011). Therefore, when the inducer (IPTG) was added, the expression of EGF in the culture of ΔMG6243-1 was higher than in the culture of ΔMG6243-1without IPTG and in ΔMG6243-2 as the results of expression of EGF. As a result, in the challenge experience the IPTG can help improve the intestinal histology.

In the upper intestine, there are high concentrations of bile acid, and low pH, which could be harmful to some kinds of *L. acidophilus*. In this study, it was found that these recombinant bacteria could be resistant to the higher bile salt and the lower pH, which indicates that these recombinant bacteria could live actively in the small intestine, which in consistent with our intestinal morphology results. Li et al. (2006) reported that LPS challenge could injure the intestine of rats (Li et al., 2006). A previous study used LPS to build an animal model of intestinal injury (Liu et al., 2008). In the current study, the villus height decreasing after LPS was injected was also found. EGF belongs to the growth factor family, and its target cells include epithelial cells, endothelial cells, and macrophages in the intestine (Dignass and Sturm, 2001). Transamniotic administration of EGF could stimulate enzyme activity and growth intestine in rabbits (Buchmiller et al., 1993). Moreover, previous studies have reported that EGF could improve intestinal morphology, including increasing the villus height, protecting the digestive mucosa, and stimulating the mucus production (Égéa et al., 2002). In this study, it was found that the recombinant *L. acidophilus* ΔMG6243-1 + inducer could improve the injured intestinal morphology of the LPS-challenged mice. Compared to the mild *L. acidophilus*, ΔMG6243-1 + inducer could increase the villus height significantly, which indicates that this effect on the intestinal villus height resulted from the EGF secreted from the recombinant *L. acidophilus*. Cheung et al. (2009) also found that the recombinant *L. latics* expressing EGF had a positive effect on intestinal development.

In this study, the recombinants *L. acidophilus* pSET4s-EGF+GFP-P2 (ΔMG6243-1) and pSET4s-P3-EGF+GFP-P4 (ΔMG6243-2) were confirmed by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Both recombinants could effectively improve the intestinal histology of LPS-challenged mice.

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Conflict of interest statement: We declare that we have no conflict of interest.

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บทคัดย่อ

การสร้างอิพิเดอร์มอลโกรทแฟคเตอร์และกรีนฟลูออเรสเซนโปรตีนของแลคโตบาซิลลัส อะซิโดฟิลลัส และผลต่อการฟื้นตัวทางเนื้อเยื่อวิทยาของลำไส้ในแอลพีเอส ไมซ์

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การศึกษานี้มีเป้าหมายในการสร้างเรคคอมบิแนนต์สเตรนโดยใช้โปรไบโอติกส์คือ แลคโตบาซิลลัส อะซิโดฟิลลัส ในการแสดงออกของอิพิเดอร์มอลโกรทแฟคเตอร์ (EGF) ที่ช่วยการฟื้นตัวทางสันฐานวิทยาของลำไส้และผลต่อเนื้อเยื่อวิทยาของลำไส้ในแอลพีเอสไมซ์ EGF ที่ถูกโคลนในเวคเตอร์คือ $pSET_{45}$ จากนั้นนำเข้าสู่แลคโตบาซิลลัส สเตรน MG6243 ด้วยวิธีการทำให้เกิดรูด้วยไฟฟ้า เรคคอมบิแนนต์สเตรน 2 ตัว เรียกว่า MG 6243-1 และ MG6243-2 ถูกยืนยันด้วย SDS-PAGE นำหนูไมซ์ 60 ตัวแบ่งกลุ่มการทดลองดังนี้ 1)กลุ่มควบคุม 2) กลุ่มควบคุมแอลพีเอส 3) กลุ่มที่ใช้แอลพีเอสและ MG6243 200 ไมโครลิตร 4)กลุ่มที่ใช้แอลพีเอส และ MG 6243-1 200 ไมโครลิตร 5) กลุ่มที่ใช้แอลพีเอสและ MG6243-1 และ IPTG กลุ่ม 6) กลุ่มที่ให้แอลพีเอส และ MG6243-2 ทำการทดลอง 10 ซ้ำโดยใช้หนูทดลอง 1 ตัวในแต่ละซ้ำ ในวันที่ 15 ฉีดแอลพีเอส 10 มก./กก.น้ำหนักตัว เข้าทางช่องท้องของหนูที่ได้รับแอลพีเอส ส่วนหนูกลุ่มที่ไม่ได้รับแอลพีเอสถูกฉีดด้วย 0.5**%** นอร์มัลซาไลด์ จากนั้น 3 ชั่วโมงทำการการุณฆาตหนูทุกตัวเพื่อนำมาวิเคราะห์ผลการทดลองพบว่า MG 6243-1 และ MG 6243-2 สามารถเพิ่มความสูงของวิลไลในดูโอดีนั่ม ไอเลียม และเจจูนั่ม ($P < 0.05$) นอกจากนี้พบว่าเรคคอมบิแนนต์ แอล อะซิโดฟิลลัส ช่วยลดการตายของคริปท์ในดูโอดินั่มและ ไอเลียม ($P < 0.05$) ผลการศึกษาบ่งชี้ว่าเรคคอมบิแนนต์ แอล. อะซิโดฟิลรัสสามารถทำให้เนื้อเยื่อวิทยาของลำไส้ดีขึ้นในแอลพีเอสไมซ์

คำสำคัญ: อิพิเดอร์มอลโกรทแฟคเตอร์ แลคโตบาซิลลัส อาซิโดฟิลลัส วิทยาฮิสโตของลำไส้ แอลพีเอสไมซ์

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