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Resistant gastric environment of Lactobacillus crispatus from stomach inhibits Helicobacter pylori colonization and attenuates gastric inflammation

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Abstract

Background Recent studies have shown that gastric-derived Lactobacillus can inhibit the colonization of H. pylori and attenuate gastric inflammation in conventional animals, but the resistant of Lactobacillus to gastric environment is still unknown. Here, we aimed to screen the candidate Lactobacillus that could adapt to the harsh gastric environment and inhibit the colonization of H. pylori. Results In vitro, the growth rate of seven Lactobacillus strains in different pH and bile salt concentration were tested, the size of inhibition zone and adhesion rate of H. pylori when Lactobacillus exist were measured. In gnotobiotic mice models, we examined the amount of colonization of L. crispatus and H. pylori by qRT-PCR and evaluated the inflammation in the gastric tissue by the content of MPO and H&E stain. In vitro experiments showed L. crispatus had a better growth rate than other six Lactobacilluses in pH 2.5 to 4.5; under the 0.2% bile salt concentration, other bacteria did not grow except for L. crispatus; L. crispatus yielded 24.2 mm of mean inhibitory zone diameters; the adhesion rate of H. pylori only reached 41.3% in H. pylori-L. crispatus group(HLG). In vivo, the amount of colonization of H. pylori in HLG is fifteen times less than that in H. pylori group (HG) ($p < 0.05$); the MPO value of HG was 1.4 times that of HLG; the gastric tissue inflammation of HLG was obviously lighter than HG. L. crispatus may be an adjunctive therapy for treating H. pylori- associated disease in clinic. Conclusions L. crispatus has resistance to low acid and high bile salts environment and it inhibits the growth of H. pylori and the subsequent inflammation H. pylori caused in gnotobiotic Kunming mice model, which suggest the potential of developing L. crispatus as clinical agents.

Background

Helicobacter pylori(*H. pylori*) is a pathogen, which may cause chronic gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma [1, 2]. *H. pylori* infection has a general prevalence from 11% in industrialized countries to 95% in developing countries [3]. Several strategies have been proposed to eradicate *H. pylori*, including the standard first-line treatment (such as proton pump inhibitors, two antibiotics), quadruple, sequential, and concomitant treatments [4]. However, the eradication rates for *H. pylori* infection have decreased to less than 80% in many countries [5], and the antibiotic resistance have become more serious [6]. Therefore, alternative treatments are of interest.

Microecology theory provide an excited idea to resist the infection of *H. pylori* [7]. *Lactobacilli* are one of human gastric microbiota, and can adhere to the stomach wall and grow under harsh acidic conditions[8], theyare also probiotics that have positive effects for the health of humans and animals [9]. It has been reported that *Lactobacillus* can inhibit the adhesion and growth of *H. pylori* in in vivo studies [10, 11]. In Mongolian gerbils model, *Lactobacillus* have an anti-infective effect against *H. pylori* [12]. According to Thiraworawong's reports, Gastric-derived *L. plantarum XB7* can modulate gastric mucosal inflammation in Sprague-Dawley rat model [13]. In some reports, *Lactobacillus* ingestion could even decrease the risk of some cancer and tumors [14, 15]. Meanwhile, *Lactobacillus* adjunct therapy have the potential to reduce severity of side effects related to *H. pylori* eradication therapy [13], increase the *H. pylori* eradication rate [16], and decrease the severity of host cell damage [17]. So *Lactobacillus* will be a

promising microbial ecological agent that functions as an adjunctive therapy for treating *H. pylori*-associated disease.

The stomach is a harsh environment, its pH is usually around 3.0 and the bile salt concentration is 0.03% to 0.3% [18]. *Lactobacillus* can colonize and live in the stomach which depend mainly on its acid and bile salt resistant [19, 20], acid and bile salt resistance are also important indicators for screening *Lactobacillus* as probiotics [21]. Although some studies have shown that *Lactobacillus* can inhibit the *H. pylori* growth and reduce inflammation, but they did not study the acid and bile salt resistant of *Lactobacillus*, which will be affected in further development of *Lactobacillus* as probiotics to play against *H. pylori* in human gastric.

In the present study, we chose six *Lactobacillus* strains from the stomach of 21 healthy person, and examined the ability of acid and bile salt resistant, the size of inhibition zone and the adhesion rate of *H. pylori* in vitro. Based on above results, the most promising candidate *L. crispatus* was chosen. We use the *L. crispatus* and *H. pylori* successfully constructed the model of mono- and di-associated gnotobiotic Kunming mice models. Meanwhile, the amount of bacteria in stomach were detected, the degree of gastric mucosal inflammation were evaluated by the content of MPO in stomach tissue and the HE staining of gastric biopsy.

Results

Acid and bile salt resistance of *Lactobacillus*

After four hours incubation, the growths of all the Lactobacillus strains were shown in Fig. 1. The net growth of *L. crispatus* reached more 170% at pH 2.5 and 3.0, the value of the *L. crispatus* were 1.5 times that of the L2 (p = 0.002, 0.000), meanwhile other strains were subject to different degrees of growth inhibition. At pH 4.0 and pH 4.5, *L. crispatus* also grow better than other strains, but the growth advantage was not obvious compared with pH 2.5 and 3.0. In general, *L. crispatus* has a stable resistance to acid under low pH relative to other strains.

In Fig. 2, growth rate of all strains fell with the increase of concentration of bile salts. At the bile salt concentration 0.2%, only *L. crispatus* were in growth, other strains' germination was stopped. When the concentration of bile salt is 0.1%, *L. crispatus* were grown better than other strains ($p < 0.05$), the net growth is 100%. The L6 and L7 did not grow at the concentration of bile salt 0.05-0.2%. According to the above results, *L. crispatus* also has better bile salt tolerance than other six strains.

Lactobacillus inhibit the growth and adhesion of *H. pylori* in vitro

As shown in Table 1, all the *Lactobacillus* have a inhibitory effect on the growth of *H. pylori* by Oxford cup method in vitro, but there is a best effect that *L. crispatus* inhibited the growth of the *H. pylori*, the value of inhibition zone is 24.2mm, which is 1.8 fold that of L2 (p = 0.000), and 3.2 fold that of L7 (p = 0.000). There were statistically significant differences in the L3 compared with other strains at IZD (p = 0.000).

With the *H. pylori's* adhesion rate as the positive control, the adhesion rate is defined as 100%. In the interaction between *H. pylori* and *Lactobacillus*, all the *Lactobacillus* have a inhibitory effect on the adhesion of *H. pylori* to GES, at the same time, the adhesion rate of *H. pylori* in L-H group are higher than that in H-L group for all *Lactobacillus* (Table 2). In L-H and H-L group, the adhesion rate of *H. pylori* when L3 exsited are 51.2% and 41.3%, the adhesion rate of *H. pylori* are 10% lower than other groups (p = 0.000). In turn, the inhibition of *H. pylori* adhesion of L3 is strongest.

The quantitative of *H. pylori* and *L. crispatus* in stomach

The colonization amount of *H. pylori* and *L. crispatus* in different gnotobiotic Kunming mice is shown in Table 3. The colonization amount of *H. pylori* in HG is 2.008×10^7 copies/g, it is 5.2 times that of LHG and 14.8 times that of HLG, there were statistically significant differences between them (p = 0.000, 0.000). At the same time, the amount of *L. crispatus* in LHG and HLG are more than 1.0×10^6 copies/g. It shows that *L. crispatus* can suppresse the adhesion and colonization of *H. pylori* in vivo, the *L. crispatus* has more obvious inhibitory effect among orally administered *L. crispatus* after *H. pylori* infection, which is consistent with the results of adhesion experimental in vitro.

Evaluation of gastric inflammation

To estimate the degree of inflammation, the MPO experiment data of four groups are shown in Fig. 3. In each groups, the value of MPO is relatively concentrated, this suggests that the experiment has good uniformity. In HG, the value of MPO is 0.248 μg/g, and the HLG is only 0.173μg/g, the HG is 1.2 and 1.4 times of LHG and HLG, separately (p = 0.003, 0.000). It shows that *L. crispatus* obviously reduces the MPO level of gastric tissue and the degree of gastric tissue damage caused by MPO.

The gastric inflammation was also determined by the histopathological examination and histology scores. Histopathology in the LG was normal, there was moderate inflammation in the HG, while the LHG and HLG improved stomach inflammation (Figure 4). The histology scores for gastric inflammation are summarized in Table 4, the neutrophil and mononuclear cell infiltration was significantly increased in the HG when compared with the LHG and HLG (1.25±015-16.46 vs 0.50±0.53 and 0.37±0.52, p = 0.010, 0.003, respectively).

Discussion

Recent studies have revealed that *Lactobacilli* can inhibit the growth of *H. pylori* and reduce gastric inflammation, some of them are also derived human gastric, and these have been shown that *Lactobacill* will be a potential therapeutic agent for treating the infection of *H. pylori.* However, it has not been clairified that whether these strains can colonize the human stomach and survival. In this study, we examined the acid and bile salt resistance of seven *Lactobacilli* strains in vitro, however, only *L. crispatus* has a best acid and bile salt resistance. We then studied the growth and adhesion rate of *H. pylori* that inhibited by the seven *Lactobacilli* strains, all these strains have a resistance to *H. pylori* activity, but the activity of *L. crispatus* is outstanding. Combined with the above results, we further confirmed the anti-growth and anti-inflammatory properties of *L. crispatus* in the gnotobiotic Kunming mice models, the effect of anti-growth is consistent with the experimental results in vitro, furthermore, it significantly reduce the *H. pylori*-associated gastritis at the same time. These data strongly suggest *L. crispatus* will be a probiotics agent that can prevent the infection and improve the inflammation of *H. pylori* in human stomach.

Lactobacillus secretes some antibacterial metabolic products, such as lactic acid, bacteriocin, peroxide, proteinase, exopolysaccharide[22], lactic acid inhibits the urease activity and viability of *H. pylori* among these. According to Sunanliganon's report [10], low pH values are important for anti-*H. pylori* activity. At the same time, *H. pylori* infection and the subsequent increase of gastric pH value may result in a more permissive milieu for colonization with other bacteria, that their metabolic products associated with stomach related diseases[23]. In this study, the acid-resisting strain also produced a large amount of acid (data not shown), which is also very important that to maintain the normal environment of stomach and resist the *H. pylori* infection.

To make *Lactobacillus* inhabit the complex and changeable stomach and play the role of probiotics to maintain the normal pH and bacteria flora of stomach, acid and bile tolerance of its own is very important[21]. In this study, L2 and L3 have a certain degree of resistance to acid and bile salt, but in the bile salt concentration 0.2%, the growth of L2 was inhibited. At the same time, although L1 and L5 have a certain degree of resistance to low the bile salt concentration, but didn't grow under pH 3.5; the growth of L4, L6 and L7 were inhibited in low acid and bile salts. Those suggest that strains of acid and bile salt resistant properties are inconsistencies. In addition, all *Lactobacillus* strains inhibit the growth and adhesion of *H. pylori* in vitro, combined with acid and bile salt resistance, which shows that *Lactobacillus* has an inhibition effect for *H. pylori*, but it doesn't adapt to the complex stomach environment, which will also be affected to against the infection and inflammation of *H. pylori* in human gastric. It also suggests that the importance of acid and bile salt resistant properties in screening of *Lactobacillus* as a probiotics agent.

In order to verify the anti-inflammatory effect of *Lactobacilli* in vivo, we chose the gnotobiotic Kunming mice model. Through establish the mono- and di-associated gnotobiotic models, it clearly revealed the interaction between *H. pylori* and *Lactobacillus* in vivo. The colonization amount of *H. pylori* in LHG and HLG are significantly lower than that in HG, which suggest that *L. crispatus* play a role in reducing the engraftment of *H. pylori* in mice. At the same time, the better inhibitor effect that *L. crispatus* inoculated

after *H. pylori* than *L. crispatus* inoculated before *H. pylori* in vitro and in vivo, is helpful for developing *L. crispatus* as probiotics to treat *H. pylori* infections. In addition, the amount of colonization of *Lactobacillus* is 1.12× 10^6 copies/g or more in LHG and HLG, it is consistent with the reports that the colonization amount of *Lactobacillus* must be more than 1.00× 10^6 CFU/mL which can play a role of probiotics[24].

In order to study the anti-inflammatory effect of *Lactobacilli* in gnotobiotic Kunming mice, we detected MPO and histopathology to evaluate the gastritis of stomach tissues. Myeloperoxidase(MPO), which is an oxidative enzyme present in phagocytes, is an essential part of the inflammatory regulation[25]. In some reports, the activity of MPO in mucosal biopsy specimens from patients with *H. pylori* infection is elevated[26, 27]. At the same time, *H. pylori* density were correlated with MPO level in gastric antral mucosa, the *H. pylori* water extract also increases the secretion of MPO from human neutrophils[28]. Unfortunately, MPO can directly promotes oxidative injury of host tissues at sites of inflammation and induce the expression of IL-1 and TNF-α which can reduce the secretion of stomach acid, and benefit the survival and infection of *H. pylori*[29]. So by testing the MPO levels of organization, we confirmed the inflammation was sig7nificantly lower in LHG and HLG than HG. At the same time the results of histopathology for each groups was consistent with that of MPO. These results clearly demonstrated that the inflammation was significantly reduced by *L. crispatus* in LHG and HLG.

Conclusions

In conclusion, six stomach-derived *Lactobacillus* have resistance to *H. pylori*, but only *L. crispatus* has resistance to low acid and high bile salts environment. Through the gnotobiotic Kunming mice model, we further verify the resistance of *L. crispatus* for inhibiting the growth and inflammation *H. pylori* caused in vivo. It is steps closer to clinical potential agents for develop *L. crispatus.*

Material And Methods

Bacterial strains and culture conditions

Six *Lactobacillus* strains were isolated from the gastric mucosa of 21 healthy volunteers who received gastroscope inspection at Chongqing Southwest Hospital, Chongqing, China. These strains are *Lactobacillus oris* (L1, L2)*, Lactobacillus crispatus* (L3)*, Lactobacillus salivarius* (L4)*, Lactobacillus gasseri* (L5)*, Lactobacillus delbrueckii* (L6). The standard strain is *Lactobacillus acidophilus* ATCC4356 (L7). They were cultured in Man-Rogosa-Sharpe (MRS) broth (BD, Shanghai, China), and incubated for 2 days at 37°C under 87% N_2, 5% O_2 and 8% CO_2 in three gas incubators (Thermo Scientific, Shanghai, China). After harvesting, the bacterial suspensions were prepared at a concentration of 1.0 × 10^9 CFU/mL.

The *H. pylori* strain M13, which was derived from a Chinese clinical isolate and adapted to colonize the gastric mucous layer (GML) of mice [30]. Itwas recovered and cultivated on Skirrow's medium, and then inoculated in brain heart infusion broth supplemented with 5% foetal bovine serum and 1% glucose with

shaking at 220 rpm and 37℃ under 87% N_2, 5% O_2 and 8% CO_2 in three gas incubators. After harvesting, the concentration of bacteria was regulated to 1.0×10^9 CFU/mL.

Acid and bile salt resistance of *Lactobacillus* strains in vitro

For the acid resistance experiment, the MRS broth's pH value was adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, they were shaked at 220 rpm and 37℃ for 4h. In the bile salt resistance experiment, the bile salt concentration of MRS broth is 0.05%, 0.1% and 0.2%, the medium were shaked at 220 rpm and 37℃ for 4h, 8h and 12h, respectively. All the initial concentration is 1×10^8 CFU/mL, the total volume is 20 mL. Then the bacterium solution were centrifuged and then resuspended in 1mL, each of them was added to 96-well plates, 200μL per well for two wells, MRS medium as blank control. All the wells tested the value of OD600nm by iMark™ Microplate Absorbance Reader (BioRad, Hercules, USA).

Inhibition and adhesion of *H. pylori* in vitro

According to Oxford cup method[31], The concentration of *H. pylori* and *Lactobacillus* strains suspensions was separately regulated to 1.0×10^7 and 1.0×10^8 CFU/mL. 200 μL of *H. pylori* were plated on Skirrow's medium evenly, and waiting for no obvious water droplets, the sterile Oxford cup (Lu Si Precision Instrument, Shanghai, China) was placed inside the tablet for equidistant, 240 μL of *Lactobacillus* strains suspensions were added to the Oxford cup, MRS broth as negative control, and the agar plates were incubated for 48h at 37℃ under 87% N_2, 5% O_2 and 8% CO_2 in three gas incubators. Then measure the size of inhibitory zone diameters (IZD).

For the adhesion assay, the experiment were divided into three groups: the control group, the *H. pylori* M13 were inoculated in microtiter plates and incubated for 2h; the H-L group, the *H. pylori* M13 were inoculated in microtiter plates and incubated for 60 minutes, then the *Lactobacillus* strains were added, followed cultivation for 1h; the L-H group, the *Lactobacillus* strains were added to wells first, and inoculated the *H. pylori* M13 in microtiter plates later on, then cultivated for 1 h, respectively. In these groups, gastric mucosal epithelial cells (GES-1) were firstly grown on microtiter plates to form a confluent monolayer after 1 day of culturing[32]. The microtiter plates were washed three times with normal saline, then the *H. pylori* and *Lactobacillus* strains suspensions (100 μL) was added to each well, their concentration were 1.0×10^7 and 1.0×10^8 CFU/mL, separately. After 120 minutes' incubation, nonadherent bacteria were washed off by normal saline for three times. The level of adherent bacteria was estimated by the urease assay[33], 100μL of urease test solution (7 mM phosphate buffer pH 6.8, 110 mM urea, 10 mg/L phenol red) was added into each well of microtiter plate, after a reaction time of 30 min at 37℃, absorbance values at 540nmline were recorded with a iMark™ Microplate Absorbance Reader. The adhesion level was calculated by the absorbance value of the sample divided by the absorbance value of control group.

Mono- and di-associated gnotobiotic Kunming mice models

Six-week-old male gnotobiotic Kunming mice (weight, 20±1g) were purchased from Department of Laboratory Animal Science, College of Basic Medical Sciences, the Third Military Medical University (Chongqing, China) and were maintained under standard laboratory conditions (constant temperature, 23 ± 2℃; relative humidity, 55 ± 5%; 12-h light: 12-h dark cycle) with free access to an autoclaved pellet diet and sterile water. After a 1-week equilibration period, these mice were used in the experiments. All animal experiments were approved by the Animal Ethical and Experimental Committee of Third Military Medical University.

The mice were divided randomizedly into four groups (eight animals per group): *H. pylori* M13 gnotobiotic group (HG), which received only 0.4 mL of *H. pylori* suspension orally by gavage; *L. crispatus* gnotobiotic group (LG), which was orally given 0.4 mL *L. crispatus*; *L. crispatus* and *H. pylori* M13gnotobiotic group (LHG), which were successively given oral administration of *L. crispatus* (0.4 mL) and *H. pylori* (0.4 mL); *H. pylori* M13and *L. crispatus* gnotobiotic group (HLG), which were successively given oral administration of *H. pylori* (0.4 mL) and *L. crispatus* (0.4 mL). Both of the concentration of *H. pylori* and *L. crispatus* is 1.0×10^9 CFU/mL, they were inoculated twice a day for two days. In LHG and HLG, after four weeks the *L. crispatus* or *H. pylori* M13 were successively given oral administration, *H. pylori* M13 or *L. crispatus line*were given oral administration again. All mice were housed for 8 weeks under above described conditions.

Detection of *H. pylori* and *L. crispatus*

Infection with *H. pylori* was assessed using bacterial culture, urease test and PCR. Viable colonies for each stomach were identified as *H. pylori* on the agar plates, which were positive for mice. Both the urease test and PCR were positive, which were defined as positive for *H. pylori*. According to Bergey's Manual of Determinative Bacteriology*, L. crispatus* were identified by colony morphology, gram staining and microscopic examination. The viable colonies were white colony on MRS agars, gram positive, rod-shaped under microscope, which were positive for *L. crispatus*.

Eight weeks after inoculation, mice were sacrificed by cervical dislocation. The stomachs were removed from the mice and cut into four parts. One part of the stomach was serial plated on Skirrow's medium and MRS agars, separately. The Skirrow agar plates were incubated for 2 days at 37℃ under N_2 87%, 5% O_2 and 8% CO_2 in three gas incubators; the MRS agars were incubated for 24h under the same conditions. One part of the stomach was homogenised with normal saline, the mixture was identified using the urease test. In addition, the mixture was also centrifuged for 5 min at 13000 × g, and the precipitate was collected for PCR and qRT-PCR. PCR and qRT-PCR analysis of the samples was performed using a previously reported method [34]. The DNA was isolated from the precipitate using the Microbial Genomic DNA Extraction Kit [35]. The PCR primers used to amplify the ureC (glmM) gene of *H. pylori* were F: 5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3' and R: 5'-AAGCTTACTTTCTAACACTAACGC-3'. The assay was run

on an S-1000 (BioRad). After a denaturation step at 94℃ for 3 min; 35 cycles were performed for PCR amplification, where the cycle parameters were 94℃for 45 sec, 55℃, for 30 sec, 72℃ for 30 sec; followed by an extension step at 72℃ for 5 min. The PCR product was separated by electrophoresis with a 2% gel containing ethidium bromide and visualised with a UV light source.

The colonisation of *H. pylori* and *L.crispatus* was quantified by amplifying the 16S rRNA. For 16S rRNA, the primers used were *H. pylori*-F: 5'-TTTGTTAGAGAAGATAATGACGGTATCTAAC-3' and R: 5'-CATAGGATTTCACACCTGACTGACTATC-3'; *L. crispatus*-F: 5'-TGGAAACAGATGCTAATACCG-3' and R: 5'-CGTCCATTGTGGTAGATTCCCT-3'; the TaqMan probe was *H. pylori*-P: 5'-FAM-CGTGCCAGCAGCCGCGGT-TAMRA-3' and *L. crispatus*-P: 5'-FAM-CTGAGACACGGCCCAAACTCCTACGG-ECLIPSE-3'. The assay was run on a CFXTM Real-Time System (BioRad). After a denaturation step at 95℃ for 2 min, 40 cycles were performed for PCR amplification. The cycle parameters were 95℃ for 5 sec and 60℃ for 30 sec.

Determination of gastric tissue MPO level

One part of the stomach tissue was homogenised with normal saline, the mixture were also centrifuged at 20000 × g for 15 min at 4℃, then the supernatant was collected. MPO level was measured using a Mouse MPO Elisa Kit (R&D Systems, Minneapolis, USA).

Histopathology analysis of gastric tissue by H&E staining

One part of the stomach tissue were fixed by standard methods for histopathology (10% formaldehyde, 0.2 M sodium phosphate buffer, pH 7.4), fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin (Absin, Shanghai, China) for evaluation of gastric inflammation. Inflammation was scored using the updated Sydney System[36]. The pathologic characteristics were scored for the degree of neutrophil and mononuclear cell infiltration in the gastric mucosa: score 0, normal; score 1, mild; score 2, moderate; score 3, marked inflammatory changes.

Statistical analysis

The data from three independent experiments and are expressed as means ± standard deviations (SD). The statistical analyses were performed using IBM SPSS Statistics version 19 software (IBM China Inc., Beijing, China), for statistical comparisons, the independent-sample t test and χ^2 test or Fisher's exact test were performed, statistically significant differences between groups were defined as a P value of < 0.05.

List Of Abbreviations

Helicobacter pylori (*H. pylori*), gastric mucous layer (GML), *H. pylori* M13 gnotobiotic group (HG), *L. crispatus* gnotobiotic group (LG), *L. crispatus* and *H. pylori* M13gnotobiotic group (LHG), *H. pylori* M13and *L. crispatus* gnotobiotic group (HLG).

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Southwest Hospital of Third Military Medical University. Written informed consent was obtained from all individual participants included in the study. The breeding of animals and protocol of animal experiment were approved by the Animal Ethics Committee of Third Military Medical University, Chongqing, China.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GG and YL designed the study. NW and FM contributed equally to this work, and carried out the experimental work together. NW drafted the manuscript. WH and HK provided technical support. YS and ZY participated in its design. QZ coordinated the study. All authors read and approved the final manuscript.

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Tables

Table 1 Inhibition zone diameters (mm) of all *Lactobacillus* suspensions

strain	L1	L2	L3	L4	L5	L6	L7	MRS
IZM (mm)	15.7±0.3	17.0±0.1	24.2±0.1	15.0±0.2	14.2±0.1	14.1±0.1	13.0±0.1	8.0±0.1
p value a	0.000	0.000		0.000	0.000	0.000	0.000	

a L3 versus other strains.

Table 2 The adhesion rate of *H. pylori* in the presence of *Lactobacillus*

	L-H group(%)	H-L group(%)	*p* value
L1	54.0	49.6	<0.01a, b
L2	56.3	52.2	<0.01a, b
L3	51.2	41.3	<0.01c
L4	61.2	53.6	<0.01a, b
L5	62.4	55.4	<0.01a, b
L6	66.8	56.9	<0.01a, b
L7	66.0	52.3	<0.01a, b

a L3 versus other strains in L-H group.

b L3 versus other strains in H-L group.

c L3 in L-H group versus that in H-L group.

Table 3 The colonization amount of *H. pylori* and *L. crispatus* in different gnotobiotic Kunming mice.

Group (n=8)	qRT-PCR (copies/g)		*p* value
	H. pylori	*L. crispatus*	
HG	2.008E+07±7.27E+06	–	
LG	–	1.650E+08±4.26E+07	
LHG	3.85E+06±2.53E+06	1.12E+06±8.9E+05	<0.01a, b
HLG	1.35E+06±6.5E+05	5.32E+06±2.14E+06	<0.01a

a HG versus LHG and HLG about the colonization amount of *H. pylori*.

b HLG versus LHG about the colonization amount of *L. crispatus*.

Table 4 Summary of gastric inflammation scores in all groups

Group (n=8)	Gastric inflammation				*p* value
	0	1	2	3	
LG	8	-	-	-	
HG	-	6	2	-	
LHG	4	4	-	-	0.010a
HLG	5	3	-	-	0.003a

a HG versus LHG and HLG.

Figures

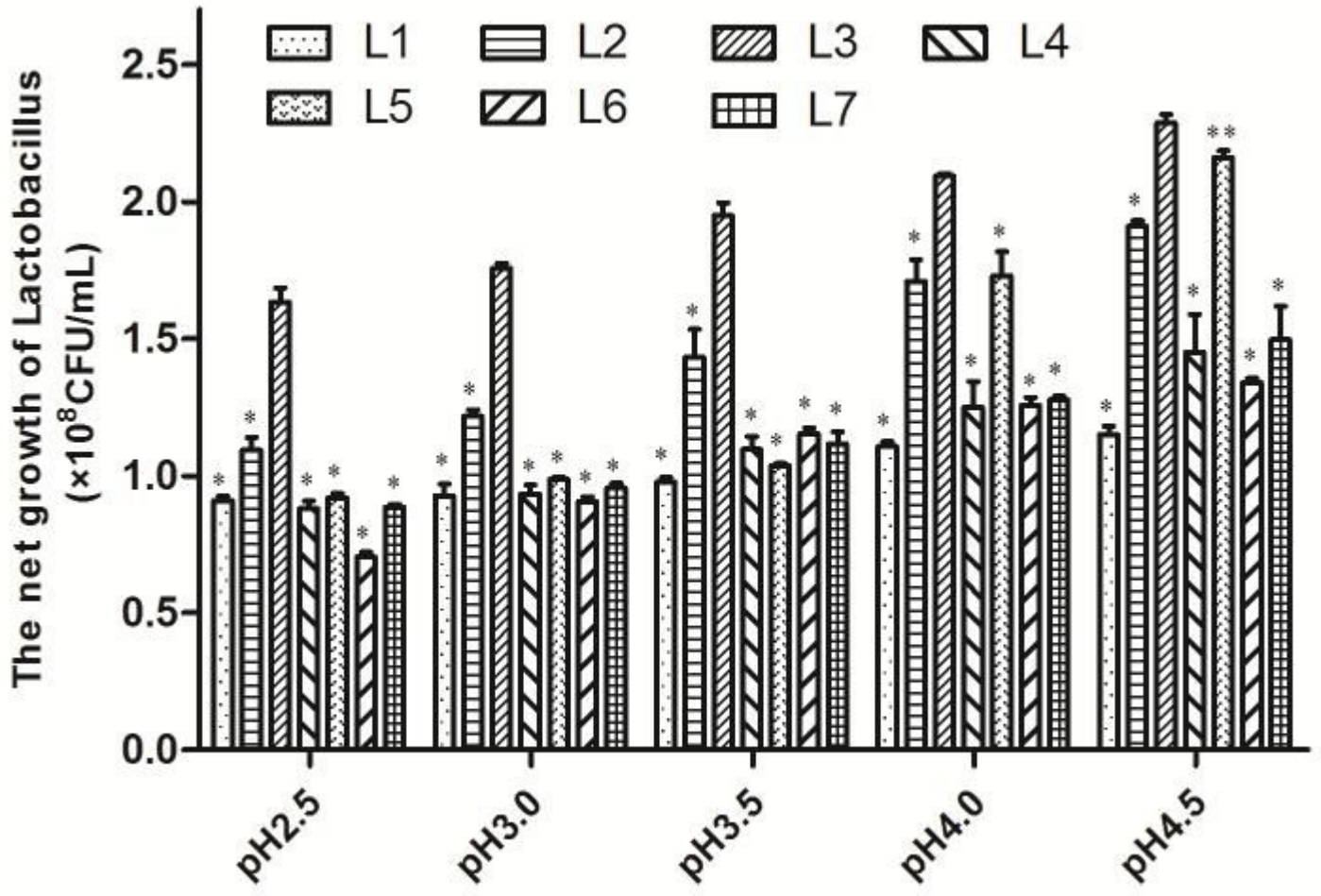

Figure 1

Comparison of net growth of seven Lactobacillus strains in different pH MRS broth after four hours incubation. The initial concentration of cultivation is 1×108 CFU/mL, the results are expressed as the mean ± SD. The net growth were statistically significant differences in the L3 compared with the other strains at the same pH (*p < 0.01, **p < 0.05).

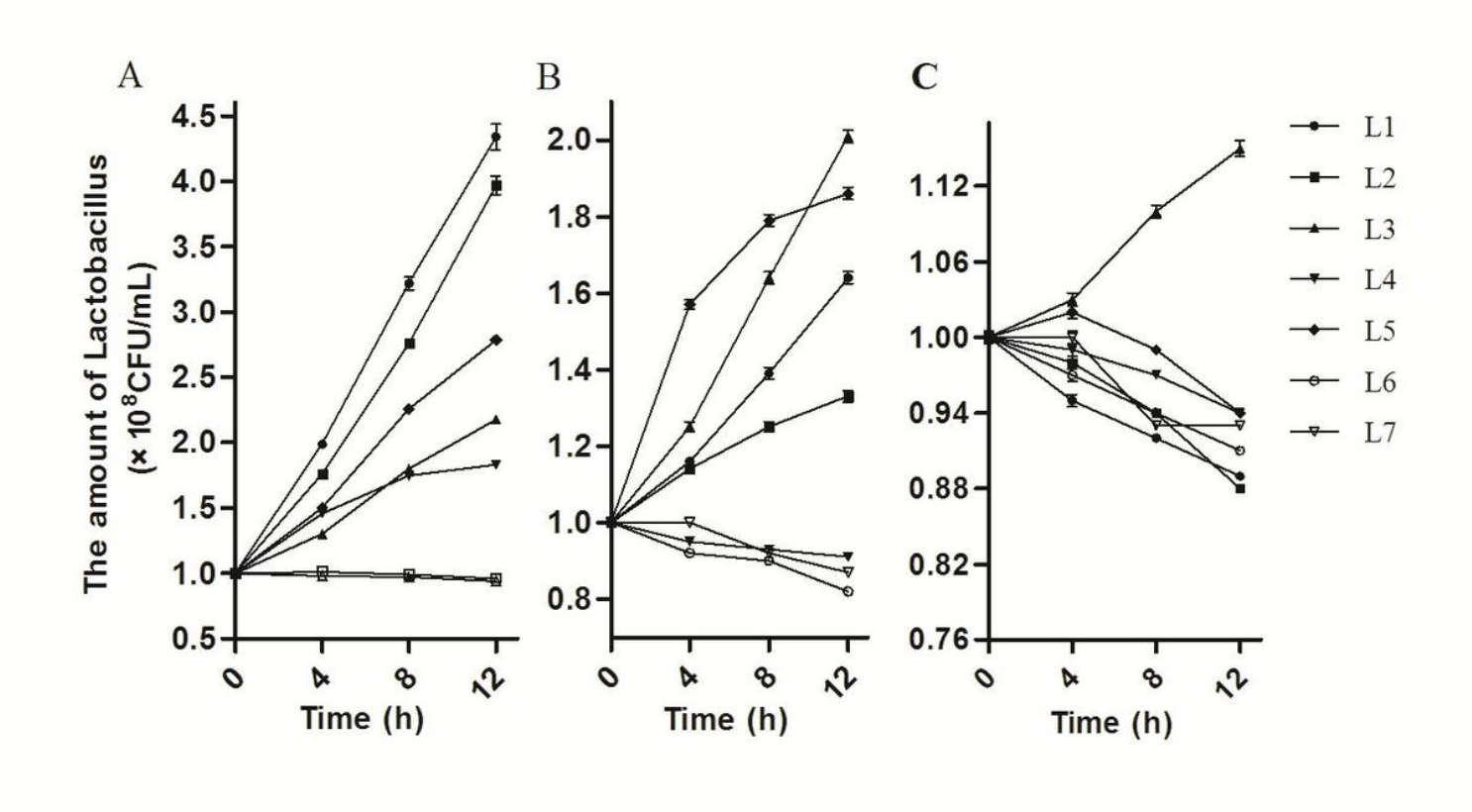

Figure 2

The growth rate of strains at different concentrations of bile salts. (A) The bile salt concentration of MRS broth is 0.05%. (B) The bile salt concentration of MRS broth is 0.1%. (C) The bile salt concentration of MRS broth is 0.2%. All the initial concentration of cultivation is 1×108 CFU/mL, the results are expressed as the mean ± SD.

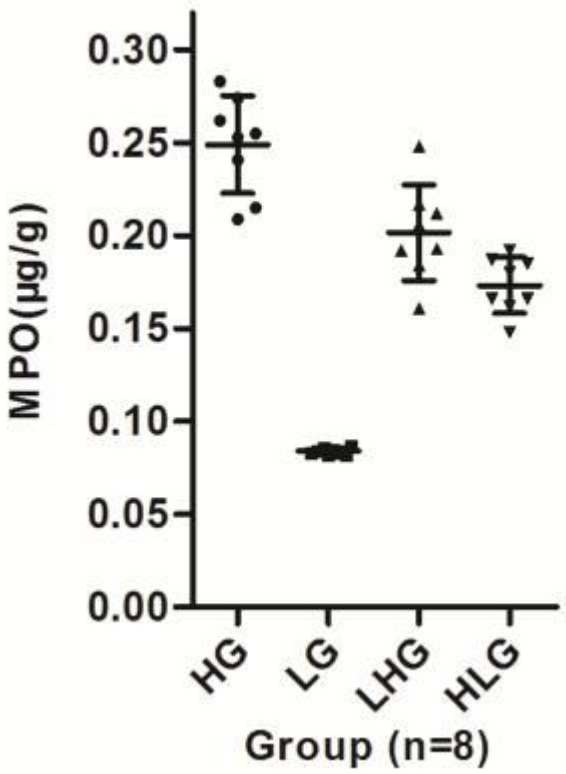

Figure 3

MPO content of gastric tissue in different gnotibiotic Kunming mice. Medians (long horizontal lines) and SD (error bars) are shown. The level of MPO had significant difference between any two of those four groups ($p < 0.05$).

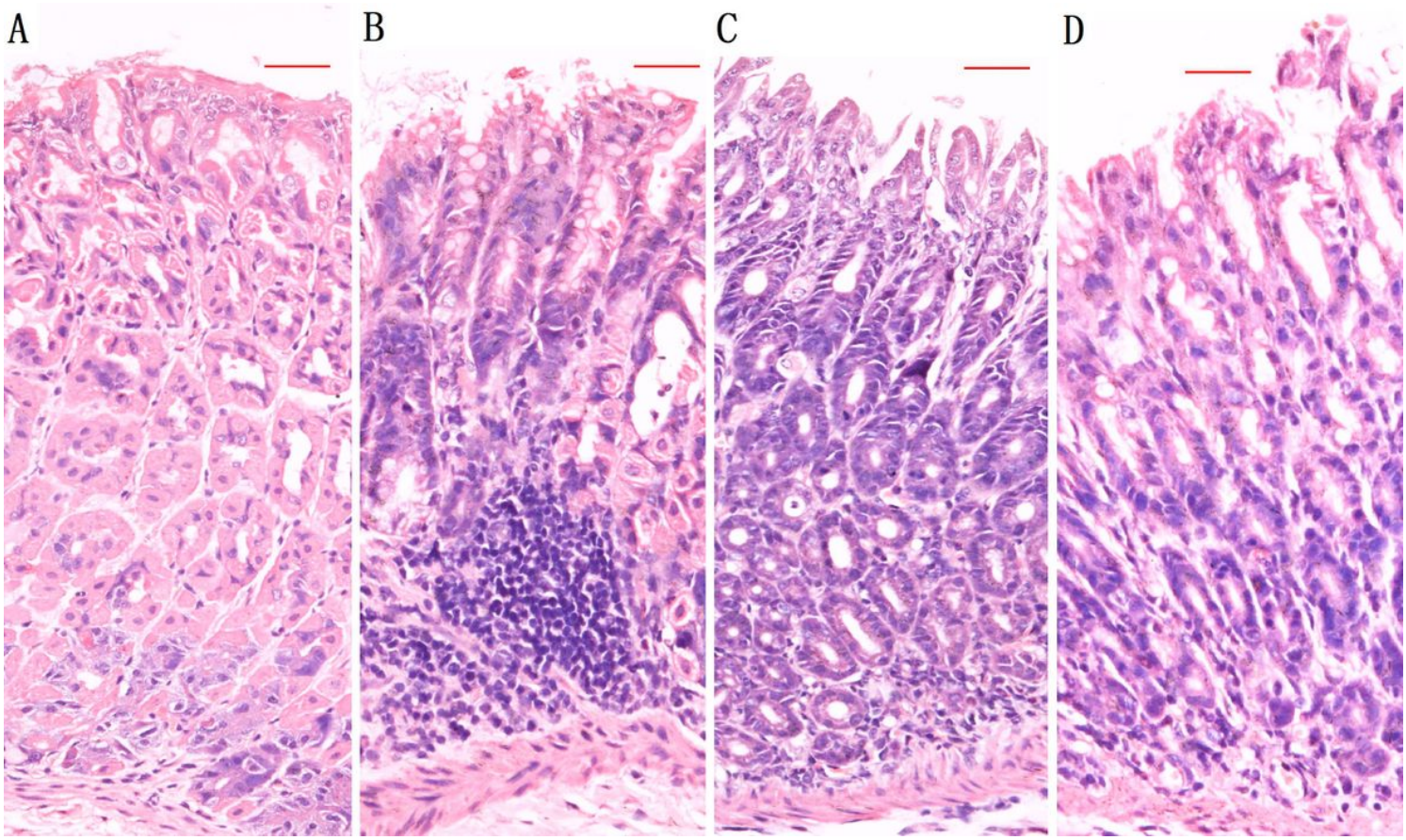

Figure 4

Histopathological examination of stomachs of gnotobiotic mice by H&E stained. a LG showed no gastric inflammation; b HG showed a amount of neutrophil and mononuclear cell infiltration and the destroyed glands structure (arrows); c, d LHG and HLG showed small number of neutrophil and mononuclear cell infiltration (arrows). Original magnification, ×200; bars = 25μm.

Supplementary Files

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- NC3RsARRIVEGuidelinesChecklist.PDF