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Full Paper

Screening of the most efficacious lactic acid bacteria strain for myocardial infarction recovery and verification and exploration of its functions and mechanisms

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Screening efficient strains by cell platform is cost-effective, but to date, no screening experiments have been performed for targeted lactic acid bacteria with hypoxic/reoxygenation (H/R)-treated cardiomyocytes, and their effects on the phosphoinositide 3-kinase (PI3K)/protein kinase b (Akt)/endothelial nitric oxide synthase (eNOS) pathway in myocardial infarction (MI) are unclear. Here we activated 102 strains of lactic acid bacteria and inoculated them into MRS medium for fermentation. The fermentation supernatants of the lactic acid bacteria were incubated with an H/R model of H9C2 cells. We found that *Bifidobacterium longum* ZL0210 had the greatest potential for inhibiting the apoptosis of H/R-induced H9C2 cells. Furthermore, it significantly increased the expression of heme oxygenase-1 (HO-1) and quinone oxidoreductase 1 (NQO1) in H9C2 cardiomyocytes, as well as the Bcl-2/Bax protein ratio, protecting damaged myocardial cells via an anti-apoptotic pathway. Intragastric administration of *B. longum* ZL0210 to mice for one week before and after establishment of an MI model drastically attenuated the myocardial cell hypertrophy and fibrosis of the MI mice. Meanwhile, *B. longum* ZL0210 significantly reduced the secretion of myocardial enzymes, increased the activity of antioxidant enzymes, and inhibited lipid-oxidative malondialdehyde (MDA) levels. Moreover, it upregulated the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein and the phosphorylation levels of PI3K, Akt, and eNOS, resulting in increased NO contents. In summary, we screened 102 strains of lactic acid bacteria with a cell platform and determined that *B. longum* ZL0210 was a favorable candidate for protecting the myocardium. We are the first to reveal the protective effects of *B. longum* ZL0210 for MI via activation of the PI3K/Akt/eNOS pathway through TRAIL.

Key words: lactic acid bacteria, myocardial infarction, phosphoinositide 3-kinase (PI3K)/protein kinase b (Akt)/endothelial nitric oxide synthase (eNOS) pathway, oxidative stress, *Bifidobacterium longum*

INTRODUCTION

Cardiovascular disease is the leading cause of mortality, morbidity, and disability worldwide, causing one third of the world's deaths [1]. In addition, many people currently possess high-risk factors for cardiovascular diseases, such as hypertension, hyperlipidemia, and diabetes. Myocardial infarction (MI) is a fatal cardiovascular event that is induced by coronary artery stenosis, plaque rupture, and thrombosis, with subsequent acute coronary occlusion and continuous reduction of the myocardial blood supply, ultimately leading to myocardial ischemia and necrosis. It has become the leading cause of human death, according to the World Health Organization [2]. Antithrombotic drugs, percutaneous coronary intervention, and bypass surgery are usually used for MI treatment. Although these treatments can reduce the severity, they cannot restore the contractility of the infarcted myocardium [3]. Moreover, complications such as arrhythmia, enlargement of the infarct size, and reduction of cardiac diastolic function often occur after myocardial ischemia-reperfusion [4]. Therefore, actively looking to the extent possible for other therapies to reduce myocardial cell death and the infarction size, as well as to improve myocardial remodeling, has great benefits for the prognosis of MI.

Accumulating literature proves thatthere are close relationships between microorganisms and cardiovascular diseases. For example, bacteria from the mouth and intestine were found in

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atherosclerotic plaques [5], and *Lactobacillus* was correlated with lipid markers in patients with atherosclerosis [6]. Furthermore, the loss of intestinal flora can change the host immune system and impair cardiac repair after MI [7], suggesting that the intestinal flora is closely related to the occurrence and development of MI. A clinical study showed that probiotics can improve the left ventricular ejection fraction and reduce the left atrial diameter in patients with chronic systolic heart failure [8]. Likewise, animal experiments showed that pretreatment with *Bifidobacterium* B420 or *Limosilactobacillus reuteri* attenuated myocardial ischemia-reperfusion injury [9, 10] and decreased the levels of inflammatory markers [9] and the myocardial infarction area in mice [9, 10]. It can be seen that probiotics are ofvalue in the prevention and control of MI.

So methods of screening increasingly efficient probiotics to prevent and treat MI are important, and screening with an *in vitro* cell platform is an effective method. Screening efficient strains using a cell platform can reducecosts, avoid unnecessary waste, improve experimental efficiency, and provide data support for subsequent *in vivo* experiments, which is widely employed in basic experiments. For example, Hu *et al.* screened 87 strains of probiotics based on H_2O_2-induced oxidative stress damage in the Caco-2 colon cell line and determined that *Lacticaseibacillus rhamnosus* FLRH93 had the best effects on body weight loss and intestinal injury repair, which was also confirmed in related *in vivo* experiments [11]. However, to date, there have been no screening experiments for targeted lactic acid bacteria with hypoxic/reoxygenation (H/R) cardiomyocytes. The phosphatidylinositol-3 kinase/protein kinase B/endothelial nitric oxide synthase (PI3K/Akt/eNOS) signaling pathway plays a role in endothelial cell and myocardial cell injuries induced by ischemia and hypoxia, and activation of this pathway can promote cell survival [12, 13]. However, there have been no mechanistic studies on the effects of *Lactobacillus* on MI via regulation of the PI3K/Akt/eNOS pathway. Therefore, we aimed to use an H/R-induced H9C2 cardiomyocyte model to screen strains of *Lactobacillus* and, based on the results, observe the effect of pretreatment with a functional strain of *Lactobacillus* on MI mice and its effect on the PI3K/Akt/eNOS pathway in the myocardium We hoped to further clarify the biological mechanisms of *Lactobacillus* in MI prevention and treatment in order to provide new information for clinical research and the development of new probiotics.

MATERIALS AND METHODS

Bacterial isolation and fermentation

A total of 102 strains of lactic acid bacteria were isolated, 31 of which were from local cheese in Qinghai, 23 of which were from Tibetan mushrooms, 22 of which were from local yogurt in Inner Mongolia, and 26 of which were from pickles in Shandong. According to the experimental method by Alcine Chan *et al.* [14], each strain of lactic acid bacteria was individually inoculated into MRS liquid medium (Solarbio, Beijing, China) and fermented at 37°C for 48 hr, and the supernatant was collected after centrifugation (8,000 r/min, 15 min) and filtered through a 0.22 μm sterile filter membrane (Table 1).

Establishment of an H/R injury model in H9C2 cardiomyocytes for screening lactic acid bacteria

An H/R injury model was established according to the experimental method of Park *et al.* [15]. Briefly, commercially available rat H9C2 cardiomyocytes bought from the Cell Resource Center, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, were transferred into 96-well cell culture plates and cultured in Dulbecco's Modified Eagle Medium (DMEM; Solarbio, Beijing, China) containing 10% fetal bovine serum (FBS) and 1% streptomycin/penicillin (Solarbio, Beijing, China) at 37°C with 5% CO_2 for 24 hr to the logarithmic phase of growth, The supernatants of the fermented and filtered lactic acid bacteria (102 strains) were then individually added to the DMEM in each well at a ratio of 5% (v/v). Each fermentation broth was prepared in five replicate wells, and the blank medium without bacteria supernatants was used for the normal group and H/R model group. When 90% cell density was reached, the cells were washed twice with phosphate buffered saline (PBS) buffer, followed by culture in DMEM containing 10% FBS. Additionally, the cells cultured with the 5% lactic acid bacteria fermentation supernatants were used for the experimental groups (named according to strain), and those cultured with the blank medium without bacteria supernatants were used for normal and H/R model groups. We put the cell culture plates of H/R model group and experimental groups into a sealed hypoxic chamber, which was infused with a mixture of 95% N_2 and 5% CO_2 gas, and then placed them in a 5% CO_2 cell culture box at 37°C for 1 hr. Thereafter, the DMEM in the wells of the cell culture plates was replaced with sugar-free serum-free DMEM, and the cell culture plates were placed in a hypoxic chamber at 37°C for 8 hr . Next, the cells were subjected to reperfusion in DMEM containing 10% FBS and placed in an incubator for 4 hr to simulate an H/R-induced H9C2 myocardial cell injury model.

Effect of lactic acid bacteria fermentation supernatant on heme oxygenase-1 (HO-1), quinone oxidoreductase 1 (NQO1), Bcl-2, and Bax expression of the H/R injury model of H9C2 cardiomyocytes

Western blotting procedures were performed with reference to Zhang *et al.* [16]. In brief, the total protein of cardiomyocytes was extracted with cold cell lysate buffer, and the total protein concentration was determined by Bradford protein assay (Bio-Rad, Hercules, CA, USA). Then, 25 μg of protein was

Table 1. Details of bacteria isolation and fermentation

Bacteria	Original foods	Place of production	Separation medium and method
31 strains	local cheese	Qinghai, China	MRS liquid medium (containing vancomycin), anaerobic culture and coating separation
23 strains	mushroom	Tibetan, China	MRS liquid medium (containing vancomycin), anaerobic culture and coating separation
22 strains	local yogurt	Inner Mongolia, China	MRS liquid medium (containing vancomycin), anaerobic culture and coating separation
26 strains	pickles	Shandong, China	MRS liquid medium (containing vancomycin), anaerobic culture and coating separation

separated on 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk and incubated overnight at 4°C with anti-HO-1 (rabbit, 1:1,000, Solarbio, Beijing, China), anti-NQO1 (rabbit, 1:1,000, Solarbio, Beijing, China), anti-Bcl-2 (rabbit, 1:1,000, Solarbio, Beijing, China), anti-Bax (rabbit, 1:1,000, Solarbio, Beijing, China), and anti-β-actin (rabbit, 1:2,000, Solarbio, Beijing, China) and then incubated with a horseradish peroxidase-conjugated secondary antibody (rabbit, 1:5,000, Solarbio, Beijing, China) at ambient temperature for 1 hr. An ECL kit (Pierce, Thermo Fisher Scientific, Waltham, MA, USA) was used for detection. Images were analyzed with the ImageJ 1.43 software. The gray value of the target protein was divided by the gray value of the internal reference β-actin to represent the relative expression of the target protein.

In vivo assays

Mouse feeding and model construction

The Ethics Committee of the Third Affiliated Hospital of Qiqihar Medical University approved our protocol. Thirty 10-week-old male SPF C57BL/6J mice weighing about 30 g/mouse were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were fed in a 12 hr/12 hr light-dark cycle environment with a room temperature of 23 ± 1°C and humidity of 40 to 50%. They had *ad libitum* access to a standard mouse food and purified water. After 3 days of acclimation, the mice were randomly divided into 3 groups of 10 mice each using random number table method: normal, model, and lactic acid bacteria groups (*Bifidobacterium longum* ZL0210 was used for the *in vivo* assays). Freshly cultured *B. longum* ZL0210 was inoculated into 100 mL MRS liquid medium at 1% (v/v). After anaerobic culture at 37°C for 24 hr, the bacteria were collected and centrifuged at 5,000 r/min for 10 min. After washing with normal saline three times, the bacteria were resuspended. Viable bacteria were counted, and the concentration was adjusted to 1×10^9 CFU/mL forthe subsequent assay. In the *B. longum* ZL0210 group, 0.1 mL of *B. longum* ZL0210 (10^9 CFU/mL) was intragastrically administered to each mouse every day for one week before model construction; the other groups were administered the same volume of normal saline. The mice in the model and *B. longum* ZL0210 groups were then subjected to MI modeling. According to the experimental method of Hsueh *et al.* [17], the mice were anesthetized with isoflurane, an oblique incision was made in the left anterior chest wall, and the muscle tissue of the anterior chest wall was bluntly separated. The chest was then gently squeezed at the fourth intercostal space, and the heart was placed outside the chest. The left anterior descending coronary artery was permanently ligated with silk thread at 2–3 mm distal to the left atrial appendage to establish an acute MI model. The *B. longum* ZL0210 group continued to be intragastrically administered *B. longum* ZL0210 for 1 week after the operation, and the other groups were intragastrically administered equal volumes of normal saline. Finally, the mice were sacrificed, blood was collected from the eyeball, and heart samples were taken for the subsequent assays.

Morphological evaluation of the heart

The heart tissue was fixed with 10% paraformaldehyde for 24 hr, embedded in paraffin after dehydration,, and then sectioned into 4 μm slices according to the hematoxylin and eosin (HE) method reported previously [11]. Subsequently, the heart slices were dewaxed, and HE staining was performed at ambient temperature. Morphological changes in heart tissues were evaluated under an optical microscope (magnification ×200, CKX41, Olympus).

Determination of serum myocardial enzymes

Serum was harvested by centrifugation of 2 mL of blood at 3,500 rpm for 10 min at ambient temperature. Then, the levels of myocardial enzymes, aspartate aminotransferase (AST), phosphocreatine kinase (CPK), and lactate dehydrogenase (LDH), in the sera of each group were determined according to the instructions of the assay kits (CUSABIO, Wuhan, China).

Determination of oxidoreductase in heart tissue

First, half a gram of homogenized heart tissue was treated with 2 mL PBS in an ice bath for 30 sec. It was then centrifuged at 3,500 rpm for 10 min (4°C), and the supernatant was subsequently collected. The activities of antioxidant enzymes, superoxide dismutase (SOD; CUSABIO, Wuhan, China), glutathione peroxidase (GSH-Px; CUSABIO, Wuhan, China), catalase (CAT; Cambridge, Abcam, UK), and malondialdehyde (MDA; Cambridge, Abcam, UK), in the supernatant were determined according to the procedures of their respective kits.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein expression in serum and the heart

The remaining centrifuged serum and myocardium supernatants were used to detect the expression of TRAIL with an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the procedures of the manufacturer. Absorbance values were determined at 450 nm.

Changes in PI3K, Akt, and eNOS protein expression and phosphorylation in cardiomyocytes

In accordance with the experimental method of Zhang *et al.* [16], PI3K, Akt, and eNOS proteins and related phosphorylation levels in cardiomyocytes were quantified by western blotting, according to the same process as described above. The primary antibodies were anti-PI3K (rabbit, 1:1,000, Solarbio, Beijing, China), anti-p-PI3K (rabbit, 1:1,000, Solarbio, Beijing, China), anti-Akt (rabbit, 1:1,000, Solarbio, Beijing, China), anti-p-Akt (rabbit, 1:1,000, Solarbio, Beijing, China), anti-eNOS (rabbit, 1:1000, Solarbio, Beijing, China), anti-p-eNOS (rabbit, 1:1,000, Solarbio, Beijing, China), and anti-β-actin (rabbit, 1:2,000, Solarbio, Beijing, China), and the secondary antibody was an appropriate horseradish peroxidase-conjugated secondary antibody. An ECL kit (Pierce, Thermo Fisher Scientific, Waltham, MA, USA) and the ImageJ 1.43 software were used for detection and analysis.

Nitric oxide (NO) content in cardiomyocytes

The NO level in the supernatant of cardiomyocytes was detected by means of nitrite and nitrate content calculation (Griess reaction NO assay kit, Calbiochem), according to the kit's instruction.

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Statistical analysis

All experiments were carried out at least three times, and the SPSS 13.0 statistical software was used for the data analysis. GraphPad Prism 5 was used to plot results. One-way ANOVA was used to compare the differences between the three groups. The LSD t-test was used for pairwise comparisons. $p<0.05$, $p<0.01$, and $p<0.001$ indicate different degrees of statistically significant differences.

RESULTS

Screening lactic acid bacteria by H/R injury model of H9C2 cardiomyocytes

Among the screened lactic acid bacteria, 8 strains (*L. rhamnosus* ZBRC003, *L. rhamnosus* ZBRC342, *L. rhamnosus* ZBRC3425, *L. rhamnosus* ZBRC3427, *Lactiplantibacillus plantarum* HIP 1031, *L. plantarum* HIP 1033, *B. longum* ZL0210, *Lacticaseibacillus paracasei* LBRC1587) had significant protective effects on the H/R model of H9C2 cardiomyocytes ($p<0.05$, $p<0.01$, or $p<0.001$; Fig. 1). Among them, *B. longum* ZL0210 resulted in the highest survival rate against H/R-induced injury in H9C2 cardiomyocytes, which reached 53.51 ± 6.98%. As shown in Fig. 1, *B. longum* ZL0210 may protect cardiomyocytes better than the other strains, helping cardiomyocytes cope with the stress response caused by H/R, so this strain was selected for the subsequent *in vitro* and *in vivo* studies.

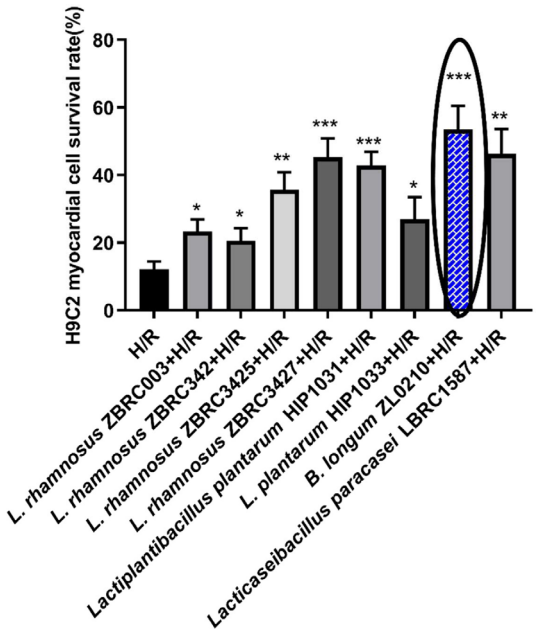

Fig. 1. Functional probiotics were screened according to the survival rate of cardiomyocytes. The figure shows the hypoxia/reoxygenation (H/R)-induced injury model of H9C2 cardiomyocytes in normal medium and 8 other groups selected from among the lactic acid bacteria treatment groups that showed significant protective effects. Among the 8 groups, the *B. longum* ZL0210 group had the best effect. Data are expressed as $\bar{\chi} \pm$ SD (n=3). *$p<0.05$ vs. H/R. **$p<0.01$ vs. H/R. ***$p<0.001$ vs. H/R.

B. longum *ZL0210 inhibits H/R-induced oxidative stress injury in H9C2 cardiomyocytes*

The results of western blotting after the extraction of total protein of cardiomyocytes are shown in Figs. 2 and 3. The relative expression of HO-1 protein in the H/R model group was 0.41 ± 0.11 and slightly increased compared with the normal group (0.25 ± 0.08), but the difference was not statistically significant ($p>0.05$). The relative expression of NQO1 protein in the H/R group was 0.52 ± 0.09, which was higher than that in normal group (0.43 ± 0.12), but there was no significant difference between the H/R and normal groups ($p>0.05$). We attributed the above changes to the response of H9C2 cardiomyocytes to the H/R condition.

After treatment of the H9C2 cardiomyocytes with the fermentation supernatant of *B. longum* ZL0210, the expression of HO-1 protein showed a significant increase, reaching 1.23 ± 0.09, relative to that in the H/R group ($p<0.001$). Similarly, the level of NQO1 expression in the *B. longum* ZL0210 fermentation supernatant treatment group also increased, reaching 1.66 ± 0.22, and it was significantly different from that in the H/R group ($p<0.01$).

There were only small amounts of Bcl-2 and Bax expression in the normal group, with their relative expression levels being 0.31 ± 0.08 and 0.13 ± 0.08, respectively (Fig. 3). The Bcl-2 expression level of cardiomyocytes in the H/R group was increased compared with that in the normal group, which was 0.46 ± 0.13, but there was no significant difference ($p>0.05$). The Bax expression level in the H/R group was significantly increased compared with that in the normal group, which was 0.42 ± 0.09 ($p<0.05$). After treatment with the fermentation supernatant of *B. longum* ZL0210, the expression of Bcl-2 in cardiomyocytes was significantly higher than that in the H/R group ($p<0.01$), which was 0.95 ± 0.05, while the expression of Bax was significantly lower than that in the H/R group ($p<0.05$). The Bcl-2/Bax ratio in the H/R group was higher than that in the normal group ($p<0.05$). After treatment with the fermentation supernatant of *B. longum* ZL0210, the Bcl-2/Bax ratio was significantly higher than that in the H/R group ($p<0.001$), as shown in Fig. 3. The effect of *B. longum* ZL0210 on the expression of HO-1, NQO1, Bcl-2, and Bax indicated that the fermentation supernatant of *B. longum* ZL0210 increased the antioxidant capacity of H9C2 cardiomyocytes under the H/R conditions.

Effect of **B. longum** *ZL0210 on the morphological changes in heart tissues of MI mice*

In our experiment, a high concentration of live bacteria (10^9 CFU/mL) was specifically selected for intragastric administration in the hope that *B. longum* ZL0210 could survive, ferment, and reproduce like other symbiotic bacteria in the body after reaching the intestinal tract, so that the metabolites of the bacteria could play a role in the animal's body. There are indeed differences between *in vivo* and *in vitro* experiments, so further validation in animal models was conducted after the cell line assay.

Our in vivo study showed that one week after acute MI, the hearts of the mice in the model group showed significant hypertrophy, scar formation and collapse in the infarct area, hypertrophy, and disorder of myocardial cells at the sites far from the infarct area, along with proliferation of extracellular matrix fibers and capillary congestion and expansion (Fig. 4). Compared with the model group, the myocardial hypertrophy

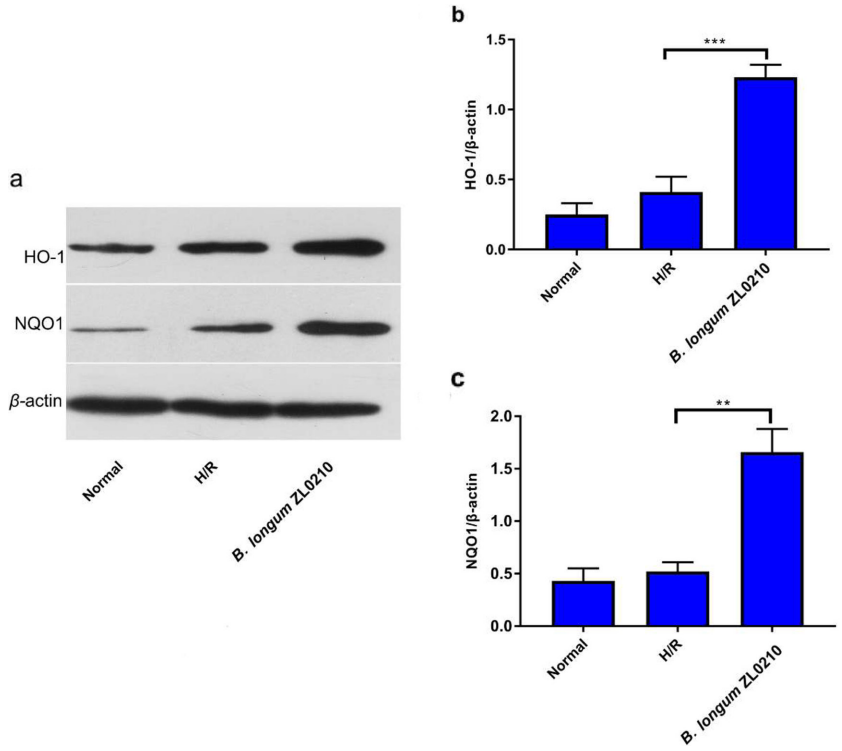

Fig. 2. Western blotting was used to measure the expression of HO-1 and NQO1 in H9C2 cardiomyocytes. a, Western blotting of HO-1 and NQO1 protein; b, relative expression of HO-1/β-actin quantified from western blotting; c, relative expression of NQO1/β-actin quantified from western blotting. Normal, normal H9C2 cardiomyocytes group; H/R, H/R-induced H9C2 cardiomyocytes injury model group; *B. longum* ZL0210, same as H/R but treated with the fermentation supernatant of *B. longum* ZL0210. Data are expressed as $\bar{\chi} \pm$ SD (n=3). *p<0.05, **p<0.01, ***p<0.001.

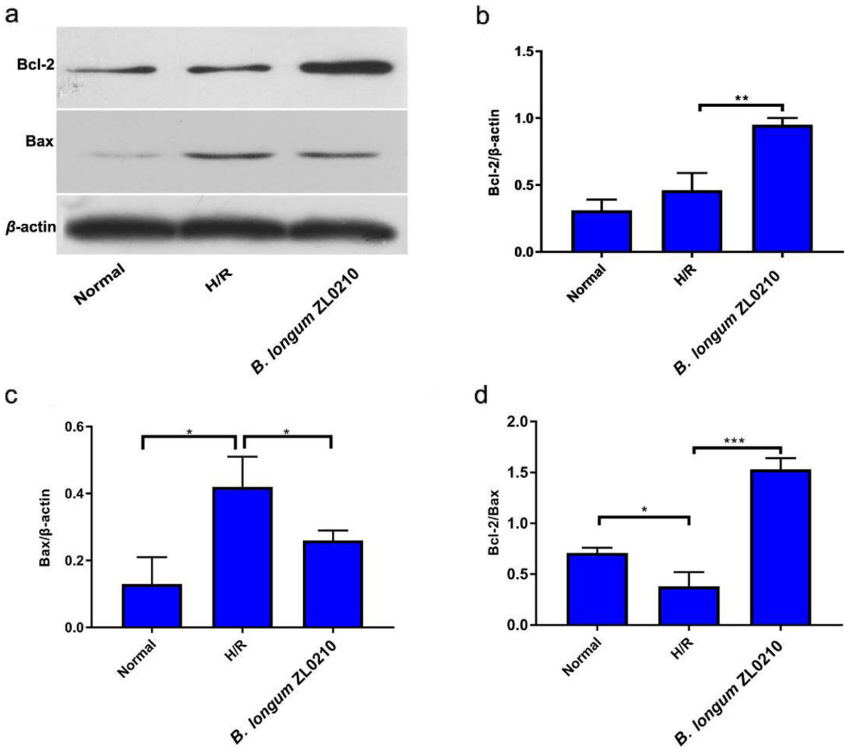

Fig. 3. Effects on Bcl-2, Bax protein, and Bcl-2/Bax in H9C2 cardiomyocytes treated with H/R and *B. longum* ZL0210. a, Western blotting of Bcl-2 and Bax protein; b, relative expression of Bcl-2/β-actin quantified from western blotting; c, relative expression of Bax/β-actin quantified from western blotting; d, relative expression of Bcl-2/Bax quantified from western blotting. Normal, normal H9C2 cardiomyocytes group; H/R, H/R-induced H9C2 cardiomyocytes injury model group; *B. longum* ZL0210, same as H/R but treated with the fermentation supernatant of *B. longum* ZL0210. Data are expressed as $\bar{\chi} \pm$ SD (n=3). *p<0.05, **p<0.01, ***p<0.001.

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and myocardial fibrosis of mice in the *B. longum* ZL0210 group were significantly reduced, indicating that the screened *B. longum* ZL0210 had a positive effect on the prevention and treatment of MI, as shown in Fig. 4.

Effect of B. longum *ZL0210 on myocardial enzymes in MI mice*

The activities of AST, CPK, and LDH in the serum of the model group were significantly higher than those of the normal group (all $p<0.001$), whereas the *B. longum* ZL0210 group showed significantly reduced contents of the three myocardial enzymes (all $p<0.001$). The results showed that *B. longum* ZL0210 could protect cardiomyocytes from rupture, as shown in Fig. 5.

Effect of B. longum *ZL0210 on the antioxidant system of cardiomyocytes in MI mice*

Compared with the normal group, the SOD, GSH-Px and CAT activities in the cardiomyocytes of the model group were significantly decreased ($p<0.001$, Fig. 6), and the MDA content was significantly increased ($p<0.001$, Fig. 6). After intragastric administration of *B. longum* ZL0210 for one week before and after surgery, the SOD, GSH-Px, and CAT activities in cardiomyocytes were significantly higher than those of the model group, and the MDA content was significantly decreased ($p<0.001$, Fig. 6). The results showed that *B. longum* ZL0210 could protect cardiomyocytes from oxidative damage and contribute to cardiac repair.

Intragastric administration of B. longum *ZL0210 significantly increased TRAIL protein expression in MI mice*

The level of TRAIL protein expression in the serum of normal mice detected by ELISA was 52.31 ± 11.22 pg/mL (Fig. 7). After the MI model was established, the level of TRAIL protein expression was 32.12 ± 10.19 pg/mL, which was significantly lower than that in the normal group ($p<0.001$). After intragastric administration of *B. longum* ZL0210 for one week before and after surgery, the level of TRAIL protein expression in the MI mice was significantly upregulated, reaching 46.61 ± 9.54 pg/mL, relative to that in the MI model group ($p<0.01$). The differential expression trend of TRAIL protein in cardiomyocytes among 3 groups was similar. The expression of TRAIL protein in the MI model group was significantly lower than that in the normal group ($p<0.001$). The expression of TRAIL protein in the *B. longum* ZL0210 group was significantly upregulated compared with that the in model group ($p<0.001$; Fig. 7).

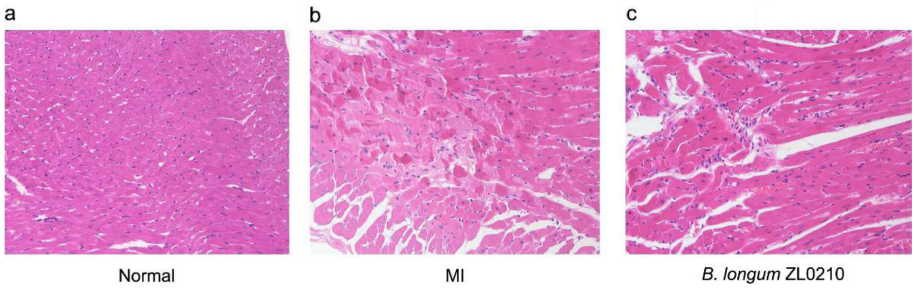

Fig. 4. Effects of *B. longum* ZL0210 on cardiac morphological changes of MI mice by HE staining (magnification ×200). a, Normal; b, MI; c, *B. longum* ZL0210 group. Normal, normal group; MI, myocardial infarction model group; *B. longum* ZL0210, mice intragastrically administered *B. longum* ZL0210 one week before and after MI modeling.

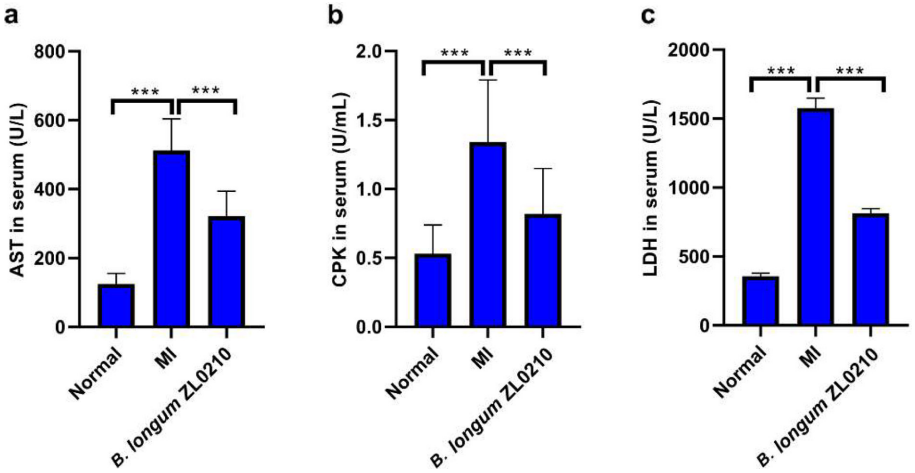

Fig. 5. Effects of *B. longum* ZL0210 on serum myocardial enzymes AST (a), CPK (b), and LDH (c) in mice with MI. Normal, normal group; MI, myocardial infarction model group; *B. longum* ZL0210, mice intragastrically administered *B. longum* ZL0210 one week before and after MI modeling. Data are expressed as $\bar{\chi} \pm$ SD, with 10 mice per group. ***$p<0.001$.

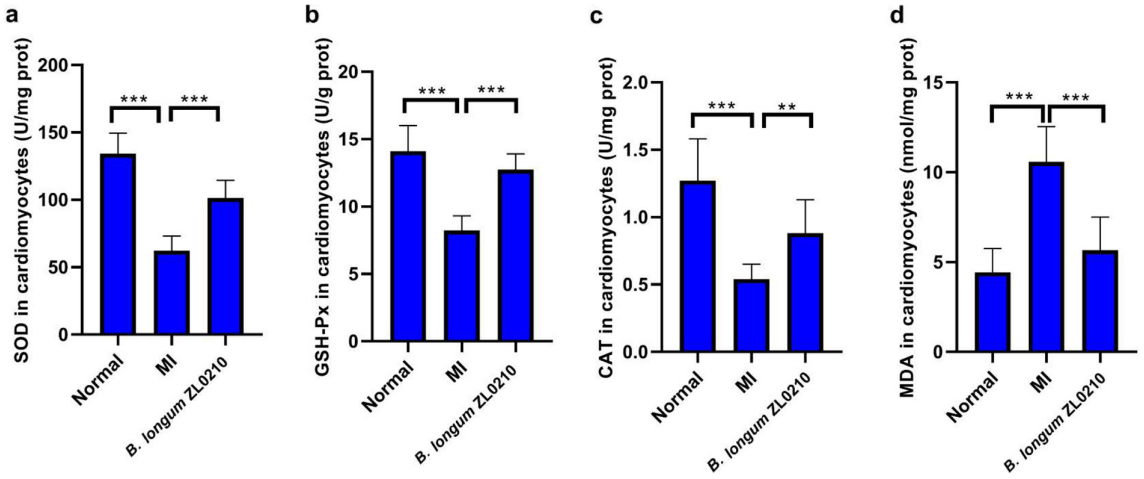

Fig. 6. Effects of *B. longum* ZL0210 on oxidoreductase SOD (a), GSH-Px (b), CAT (c), and MDA (d) in cardiomyocytes of MI model mice. Normal, normal group; MI, MI model group; *B. longum* ZL0210, MI mice receiving gavage administration of *B. longum* ZL0210 one week before and after MI modeling. Data are expressed as $\bar{\chi} \pm$ SD, with 10 mice in each group. **p<0.01, ***p<0.001.

B. longum *ZL0210 can activate the PI3K/Akt/eNOS pathway in cardiomyocytes*

The results of western blotting showed that there were no significant differences in the expression of PI3K, Akt, and eNOS total proteins among the three groups, but phosphorylation was significantly different. The relative expression of p-PI3K/t-PI3K in the normal group was 0.92 ± 0.13, and phosphorylation was significantly lower in the model group than in the normal group (p<0.01, Fig. 8). The relative expression of p-PI3K/t-PI3K was 0.71 ± 0.12. After intragastric administration of *B. longum* ZL0210, the relative expression of p-PI3K/t-PI3K was upregulated to 0.80 ± 0.09, which was not statistically different from the model group (p>0.05, Fig. 8). The relative expression levels of p-Akt/t-Akt in the normal and model groups were 0.84 ± 0.09 and 0.68 ± 0.11, respectively, and the difference between them was remarkable and statistically significant (p<0.01, Fig. 8). The relative expression of p-Akt/t-Akt in the *B. longum* ZL0210 group was significantly upregulated compared with that in the model group, which was 0.78 ± 0.05 (p<0.05, Fig. 8). The relative expression of p-eNOS/t-eNOS in the normal group was 0.72 ± 0.07, and the phosphorylation of eNOS in the model group was downregulated. The relative expression of p-eNOS/t-eNOS was 0.53 ± 0.09 in the model group, and the difference between the normal and model groups was extremely significant (p<0.001). Compared with the model group, the relative expression of p-eNOS/t-eNOS in the *B. longum* ZL0210 group was significantly increased, reaching 0.62 ± 0.08 (p<0.05, Fig. 8).

The NO contents of cardiomyocytes in the normal and model groups were 180.12 ± 11.23 umol/L and 96.21 ± 9.87 umol/L, respectively, and there was a significant difference between the two groups (p<0.001, Fig. 8). After intragastric administration of *B. longum* ZL0210, the NO content in the cardiomyocytes of the MI mice was significantly increased, reaching 135.27 ± 12.26 umol/L (p<0.001, Fig. 8). This indicated that intragastric administration of *B. longum* ZL0210 could activate the PI3K/Akt/eNOS pathway by promoting phosphorylation, ultimately resulting in the release of NO to protect cardiomyocytes.

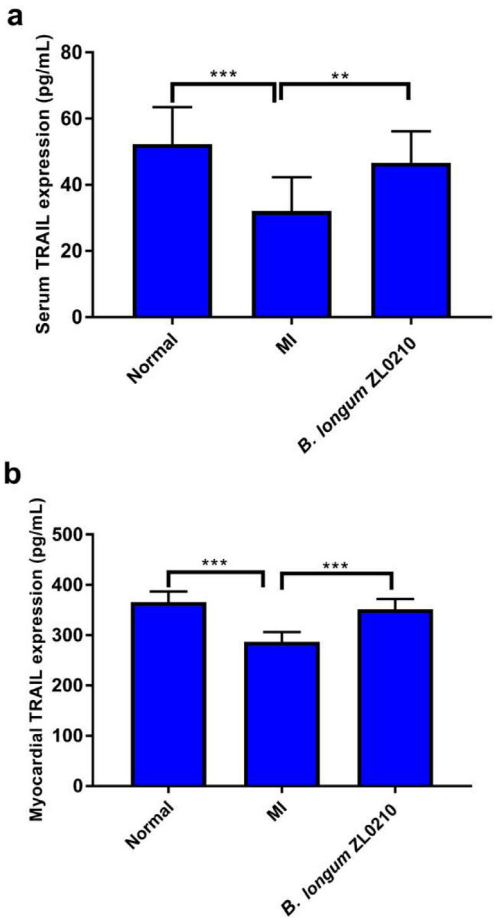

Fig. 7. Effect of *B. longum* ZL0210 on TRAIL expression in serum (a) and cardiomyocytes (b) of MI mice. Normal, normal group; MI, MI model group; *B. longum* ZL0210, group receiving gavage administration of *B. longum* ZL0210 one week before and after MI modeling. Data are expressed as $\bar{\chi} \pm$ SD, with 10 mice in each group. **p<0.01, ***p<0.001.

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DISCUSSION

Acute MI is a malignant cardiovascular disease that is a direct threat to life. As a result of changes in lifestyle and increases in work-related stress, the incidence of acute MI is increasing year by year. The prevention and treatment of acute MI has been a major challenge to global public health. Disorder of the internal environment caused by internal and external stimuli lead to the accumulation of free radicals. Free radicals produced in the body are eliminated by the antioxidant system in order to maintain as much as possible of the normal function of the body. Imbalance of the antioxidant system and severe oxidative stress lead to incomplete scavenging of free radicals, causing cell damage, such as cardiomyocyte and vascular endothelial damage. Accumulating evidence suggests that oxidative stress is closely related to the occurrence and development of acute MI and its

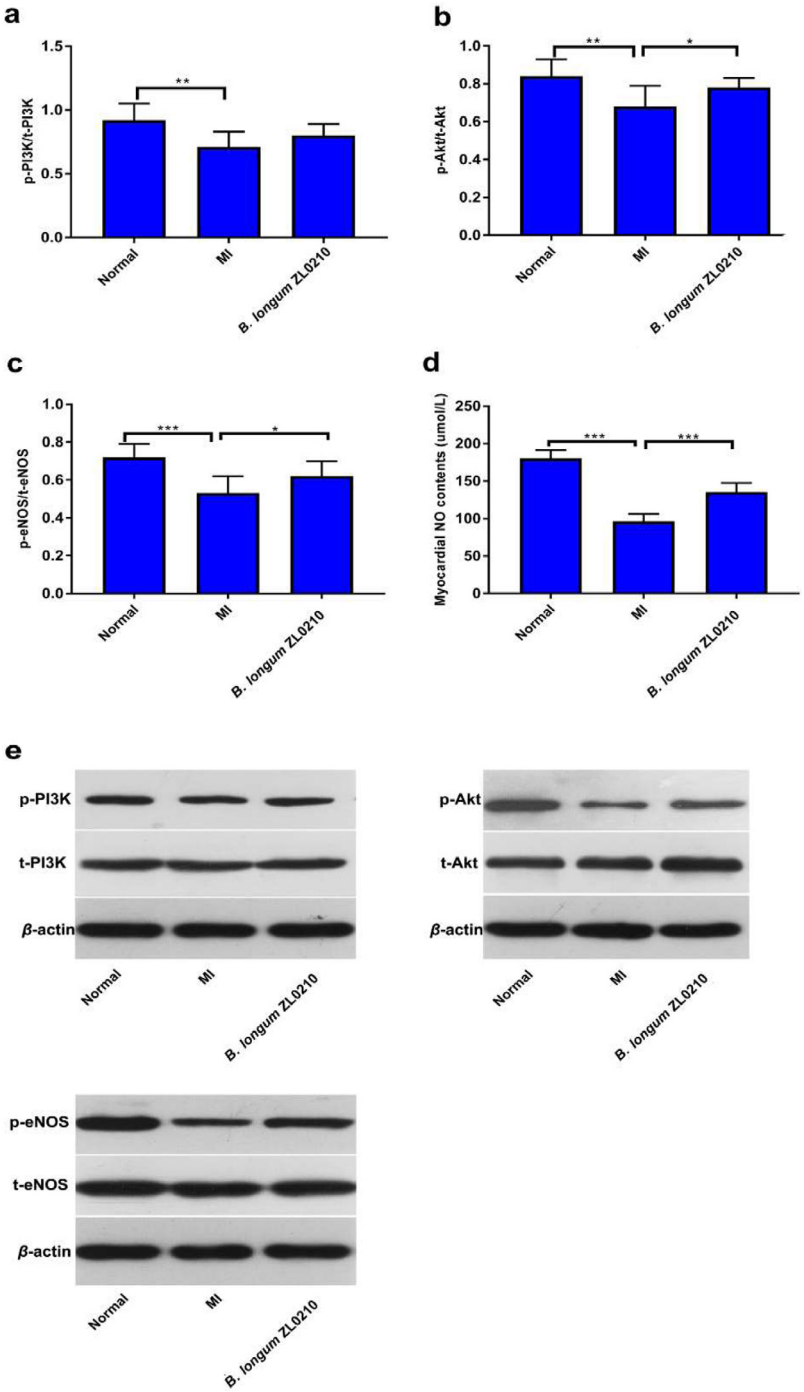

Fig. 8. Effect of *B. longum* ZL0210 on the PI3K/Akt/eNOS pathway in cardiomyocytes of MI model mice. a–d, expression levels of p-PI3K/t-PI3K, p-Akt/t-Akt, p-eNOS/t-eNOS, and NO, respectively, quantified from western blotting; e, western blotting results. p-, phosphorylated; t-, total; normal, normal group; MI, MI model group; *B. longum* ZL0210, group receiving gavage administration of *B. longum* ZL0210 treatment one week before and after MI modeling. Data are expressed as $\bar{\chi} \pm$ SD, with 10 mice in each group. *p<0.05, **p<0.01, ***p<0.001.

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complications [18, 19]. Therefore, regulating the balance between cell regeneration, proliferation, and antioxidative stress is of great significance in improving the prognosis of MI.

Probiotics can improve the automatic control of cardiovascular function and reduce the generation of reactive oxygen species and oxidative stress [20]. Therefore, we used *in vitro* cell assays to screen functional lactic acid bacteria that inhibit myocardial ischemia and hypoxia injury. Heme oxygenase-1 (HO-1) is an induced stress response protein with antioxidant and anti-apoptotic effects. It can improve cardiac function and reduce cardiomyocyte aging caused by ischemic injury [21]. NAD(P)H quinone oxidoreductase (NQO1) is a phase II metabolic enzyme that together with other phase I and II metabolic enzymes constitutes the metabolic network of toxic substances *in vivo* and *in vitro* and plays an important role in detoxification metabolism in the body. Regulating the levels of detoxifying enzyme and antioxidant enzyme genes and maintaining the redox balance *in vivo* are helpful to resist myocardial oxidative damage. Ulinastatin is an adjuvant drug for the rescue of acute circulatory failure. Studies have shown that ulinastatin treatment can significantly reduce the accumulation of reactive oxygen species in rats with acute MI, upregulate HO-1 and NQO1, combat myocardial cell apoptosis, and slow down myocardial cell injury [22]. After H_2O_2-induced oxidative damage in H9C2 cardiomyocytes, azafrin (a natural carotenoid) can increase the protein expressions of HO-1 and NQO1 in the cytoplasm and improve cell viability [23]. This is consistent with our *in vitro* cell experimental results. Our *in vitro* experiments showed that the expression of HO-1 and NQO1 in H/R-treated H9C2 cardiomyocytes was significantly increased after treatment with the fermentation supernatant of *B. longum* ZL0210, indicating that the fermentation supernatant of *B. longum* ZL0210 improved the antioxidant capacity of H/R-treated H9C2 cardiomyocytes. Bcl_2 is a classic anti-apoptotic molecule, and Bax is a classic pro-apoptotic molecule. Previous cell experiments have shown that breviscapine can significantly reduce the release of LDH and increase the level of Bcl-2 in hypoxic-ischemic H9C2 cardiomyocytes but that Bax is not affected. Breviscapine can improve the viability of hypoxic-ischemic H9C2 cardiomyocytes and inhibit apoptosis [24]. Our *in vitro* experiments also confirmed that the fermentation supernatant of *B. longum* ZL0210 significantly increased the expression of Bcl-2 and the ratio of Bcl-2/Bax in H/R-treated H9C2 cardiomyocytes. The difference was that the fermentation supernatant of *B. longum* ZL0210 also decreased the expression of Bax. Altogether, our *in vitro* assays proved the anti-apoptotic and antioxidant effects of *B. longum* ZL0210 on H/R-treated H9C2 cardiomyocytes.

A complete oxidative stress reaction system includes enzyme (such as SOD, CAT, GSH-Px, and lipid peroxides like MDA) and non-enzyme systems (such as vitamins, amino acids, and metal proteins). Improving antioxidant capacity and inhibiting lipid peroxidation are conducive to restoring cell activity and protecting cell membrane integrity. A previous study showed that pretreatment with viable probiotics significantly reduces lipid peroxidation in MI rats, reduces MDA content in cardiac tissue, and improves antioxidant capacity and cardiac function, while inactivated probiotics have no such effects [25]. However, another study showed that pretreatment with inactivated lactic acid bacteria by intravenous administration could reduce the MI area and endothelial apoptosis, decrease the serum CPK, LDH and MDA contents, increase serum SOD activity, and inhibit myocardial NF-κB signaling and expression, ultimately attenuating myocardial ischemia-reperfusion injury in rats [26]. In a clinical study, patients undergoing percutaneous coronary interventions after MI were randomly assigned to receive L. rhamnosus capsules containing 1.6×10^9 CFU or capsules containing maltodextrin for 12 weeks. It was found that the depressive symptoms of patients in the *L. rhamnosus* capsule group were significantly reduced, with a significant improvement in the quality of life, and the total antioxidant capacity of serum was improved, indicating that *L. rhamnosus* can significantly improve depressive symptoms, oxidative stress, and inflammatory markers in patients with MI [27]. This is similar to our *in vivo* results. In this study, the mice were continuously intragastrically administered active *B. longum* ZL0210 for one week before and after MI modeling. We found that the activities of SOD, GSH-Px, and CAT in cardiomyocytes were significantly increased, while the content of MDA in cardiomyocytes as well as the activities of AST, CPK, and LDH in serum were decreased, indicating that *B. longum* ZL0210 could protect cardiomyocytes from oxidative damage in MI mice.

TRAIL is mainly produced by immune cells (such as natural killer T cells and mononuclear macrophages), and it can also be expressed in a variety of tissues and organs, like smooth muscle cells and endothelial cells in blood vessels [28] and cardiomyocytes, which has been proven by our experiment here and by other reports [29, 30]. The expression products of TRAIL and its receptors DR4, DR5, DcR1, and DcR2 are mainly distributed in the cytoplasm and also expressed in the cell membrane [28]. TRAIL has been increasingly recognized as a protective agent in atherosclerosis. In patients with coronary heart disease, the plasma TRAIL level is significantly lower than that of healthy individuals. TRAIL can inhibit endothelial dysfunction by controlling the generation of endothelial cell free radicals [31]. A low TRAIL level is related to a worse left ventricular ejection fraction in the acute phase of ST elevation MI and after one month. TRAIL may represent a protective mediator of injury after MI [32]. This is consistent with the decrease in TRAIL expression in the serum and myocardium of MI mice in our study.

The PI3K/Akt/eNOS signaling pathway plays an important role in inhibiting apoptosis. Endothelial NOS is an important downstream target of Akt, and activation of Akt can induce eNOS phosphorylation and activation [33]. Upregulation of eNOS can prevent excessive infiltration of white blood cells by inhibiting the expression of vascular cell adhesion molecules, thereby slowing the myocardial ischemia-reperfusion injury [34], promoting activation of the PI3K/Akt/eNOS pathway, and helping to reduce the hypoxia-induced injury of endothelial cells and cardiomyocytes [12]. It has also been shown that exercise can promote myocardial angiogenesis by activating the PI3K/AKT/eNOS pathway, ultimately improving the heart function of rats with MI [35]. *In vitro* experiments have shown that TRAIL can protect vascular endothelial cells by activating the PI3K/AKT/eNOS signaling pathway. In unstimulated human umbilical vein endothelial cells, eNOS is mostly located on the cell membrane. TRAIL mediates the subcellular distribution of eNOS through modification of the cytoskeleton and Golgi complex, induces increases in eNOS cytoplasmic localization, and promotes NO production [36]. At the same time, TRAIL can inhibit the production of reactive oxygen species and apoptosis

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of human umbilical vein endothelial cells induced by high glucose. The anti-apoptotic effect of TRAIL is accompanied by the phosphorylation of Akt and eNOS and an increase in NO production [37]. However, there is no research on the prevention and treatment of MI with lactic acid bacteria by regulating the PI3K/AKT/eNOS signaling pathway via TRAIL.

A previous study showed that compared with a placebo group, pre-administration of *B. longum* R0175 for two weeks had no significant effect on MI size in rats with myocardial ischemia-reperfusion [38]. However, the rats in its *B. longum* R0175 group could socialize better, learn a passive avoidance test faster, and were more active in a forced swimming test. *B. longum* R0175 could reduce the activity of caspase-3 and plasma C-reactive protein in the lateral amygdala, medial amygdala, and dentate gyrus of rats, indicating that it can effectively improve depression induced by MI [38]. The combination of *B. longum* R0175 and *Lactobacillus helveticus* R0052 can significantly attenuate the depressive symptoms after MI in rats and restore intestinal permeability [39]. This shows that *B. longum* is beneficial to the prognosis of MI. After intragastric administration of *B. longum* ZL0210 to MI mice in the present study, we found for the first time that the expression of TRAIL protein in cardiomyocytes was significantly upregulated. In the model group, the relative expressions of p-PI3K/t-PI3K, p-Akt/t-Akt, and p-eNOS/t-eNOS were all significantly lower than those in the normal group, and intragastric administration of *B. longum* ZL0210 significantly improved the phosphorylation of pathway proteins, restored the activity of the PI3K/Akt/eNOS pathway, increased the generation of NO, and ultimately inhibited cardiomyocyte injury and improved myocardial remodeling. A previous study showed that compared with a placebo, the Bax/Bcl-2 ratio and caspase-3 activity of the amygdala (lateral and medial) and dentate gyrus were decreased by the combination of *B. longum* R0175 and *L. helveticus* R0052, whereas the Akt activity was increased [40]. This is somewhat different from our findings. In this study, *B. longum* ZL0210 had little effect on total Akt activity but significantly increased its phosphorylation level; meanwhile, the anti-apoptotic effect was similar. This may be related to the different sampling sites or strains. Here we studied cardiomyocytes, not the brain tissue used in the previous study. Based on our results, we tend to believe that the metabolites of *B. Longum* ZL0210 stimulate the epithelial cells and immune cells in the intestinal tract to produce TRAIL and that the newly generated TRAIL acts on the heart along with the blood. Liu *et al.* [37] reported that TRAIL activated the PI3K/AKT/eNOS/NO pathway, and the same phenomenon was observed in our experiment. So, we presumed the metabolites of *B. longum* ZL0210, such as soluble proteins and polysaccharides, might stimulate the intestinal tract to produce TRAIL and ultimately attenuate the deterioration of cardiac function by acting on the PI3K/AKT/eNOS signaling pathway of cardiomyocytes via the circulation, leading to the promotion of myogenic differentiation, improved angiogenesis, and reduced oxidative stress [41, 42]. In the future, we will attempt to further study the specific functional components. Regarding the *in vitro* system in the present study, we thought that the NO pathways would likely not be remarkably induced by TRAIL because the expression of TRAIL would be low in cardiomyoblast cells, so we only observed the HO-1 antioxidant pathways in the *in vitro* assay here. We will attempt to study the impact of lactic acid bacteria on PI3K/AKT/eNOS/NO antioxidant pathways in an *in vitro* system in the future.

In summary, our study indicated that intragastric administration of the strain of lactic acid bacteria found to be the most efficacious for myocardial protection in screening via a cell platform, *B. longum* ZL0210, could upregulate the PI3K/Akt/eNOS pathway via TRAIL and increase the NO content and antioxidant capacity, resulting in attenuation of myocardial injury and improvement of cardiac remodeling. Herein, we provide data support for future screening of new microecological preparations based on a cell line platform, as well as theoretical support for lactic acid bacteria interventions for MI. One limitation of this study was the lack of use of a pathway inhibitor for evaluation of its impact on the effects of *B. longum* ZL0210 on MI, which deserves future exploration.

ETHICS APPROVAL

The Ethics Committee of the Third Affiliated Hospital of Qiqihar Medical University approved our protocol.

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CONFLICTS OF INTEREST

There is no potential conflict of interest to declare.

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REFERENCES

1. Akoudad H, El Khorb N, Sekkali N, Mechrafi A, Zakari N, Ouaha L, Lahlou I. 2015. [Acute myocardial infarction in Morocco: FES-AMI registry data]. Ann Cardiol Angeiol (Paris) 64: 434–438 (in French). [Medline] [CrossRef]
2. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. 2006. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 367: 1747–1757. [Medline] [CrossRef]
3. Yeh RW, Kereiakes DJ, Steg PG, Windecker S, Rinaldi MJ, Gershlick AH, Cutlip DE, Cohen DJ, Tanguay JF, Jacobs A, Wiviott SD, Massaro JM, Iancu AC, Mauri L, DAPT Study Investigators. 2015. Benefits and risks of extended duration dual antiplatelet therapy after PCI in patients with and without acute myocardial infarction. J Am Coll Cardiol 65: 2211–2221. [Medline] [CrossRef]
4. Mather AN, Fairbairn TA, Ball SG, Greenwood JP, Plein S. 2011. Reperfusion haemorrhage as determined by cardiovascular MRI is a predictor of adverse left ventricular remodelling and markers of late arrhythmic risk. Heart 97: 453–459. [Medline] [CrossRef]
5. Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Tremaroli V, Behre CJ, Knight R, Fagerberg B, Ley RE, Bäckhed F. 2011. Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci USA 108 Suppl 1: 4592–4598. [Medline] [CrossRef]
6. Fåk F, Tremaroli V, Bergström G, Bäckhed F. 2015. Oral microbiota in patients with atherosclerosis. Atherosclerosis 243: 573–578. [Medline] [CrossRef]
7. Tang TWH, Chen HC, Chen CY, Yen CYT, Lin CJ, Prajnamitra RP, Chen LL, Ruan SC, Lin JH, Lin PJ, Lu HH, Kuo CW, Chang CM, Hall AD, Vivas EI, Shui JW, Chen P, Hacker TA, Rey FE, Kamp TJ, Hsieh PCH. 2019. Loss of gut microbiota alters immune system composition and cripples postinfarction cardiac repair. Circulation 139: 647–659. [Medline] [CrossRef]
8. Costanza AC, Moscavitch SD, Faria Neto HC, Mesquita ET. 2015. Probiotic therapy with *Saccharomyces boulardii* for heart failure patients: a randomized, double-blind, placebo-controlled pilot trial. Int J Cardiol 179: 348–350. [Medline] [CrossRef]
9. Danilo CA, Constantopoulos E, McKee LA, Chen H, Regan JA, Lipovka Y, Lahtinen S, Stenman LK, Nguyen TV, Doyle KP, Slepian MJ, Khalpey ZI, Konhilas JP. 2017.

Bifidobacterium animalis subsp. *lactis* 420 mitigates the pathological impact of myocardial infarction in the mouse. Benef Microbes 8: 257–269. [Medline] [CrossRef]
10. Koppinger MP, Lopez-Pier MA, Skaria R, Harris PR, Konhilas JP. 2020. *Lactobacillus reuteri* attenuates cardiac injury without lowering cholesterol in low-density lipoprotein receptor-deficient mice fed standard chow. Am J Physiol Heart Circ Physiol 319: H32–H41. [Medline] [CrossRef]
11. Hu M, Wu X, Luo M, Wei H, Xu D, Xu F. 2020. *Lactobacillus rhamnosus* FLRH93 protects against intestinal damage in mice induced by 5-fluorouracil. J Dairy Sci 103: 5003–5018. [Medline] [CrossRef]
12. Ning W, Li S, Yang W, Yang B, Xin C, Ping X, Huang C, Gu Y, Guo L. 2021. Blocking exosomal miRNA-153-3p derived from bone marrow mesenchymal stem cells ameliorates hypoxia-induced myocardial and microvascular damage by targeting the ANGPT1-mediated VEGF/PI3k/Akt/eNOS pathway. Cell Signal 77: 109812. [Medline] [CrossRef]
13. Sheng Z, Yao Y, Li Y, Yan F, Huang J, Ma G. 2013. Bradykinin preconditioning improves therapeutic potential of human endothelial progenitor cells in infarcted myocardium. PLoS One 8: e81505. [Medline] [CrossRef]
14. Alcine Chan MZ, Chua JY, Toh M, Liu SQ. 2019. Survival of probiotic strain *Lactobacillus paracasei* L26 during co-fermentation with *S. cerevisiae* for the development of a novel beer beverage. Food Microbiol 82: 541–550. [Medline] [CrossRef]
15. Park M, Youn B, Zheng XL, Wu D, Xu A, Sweeney G. 2011. Globular adiponectin, acting via AdipoR1/APPL1, protects H9c2 cells from hypoxia/reoxygenation-induced apoptosis. PLoS One 6: e19143. [Medline] [CrossRef]
16. Zhang J, Xiao F, Zhang L, Wang X, Lai X, Shen Y, Zhang M, Zhou B, Lang H, Yu P, Hua F. 2018. Alpha-Lipoic acid preconditioning and ischaemic postconditioning synergistically protect rats from cerebral injury induced by ischemia and reperfusion partly via inhibition TLR4/MyD88/NF-κB signaling pathway. Cell Physiol Biochem 51: 1448–1460. [Medline] [CrossRef]
17. Hsueh YC, Wu JM, Yu CK, Wu KK, Hsieh PC. 2014. Prostaglandin E_2 promotes post-infarction cardiomyocyte replenishment by endogenous stem cells. EMBO Mol Med 6: 496–503. [Medline] [CrossRef]
18. Chen H, Xu Y, Wang J, Zhao W, Ruan H. 2015. Baicalin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation and oxidative stress in rat. Int J Clin Exp Pathol 8: 10139–10147. [Medline]
19. Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D. 2015. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr Vasc Pharmacol 13: 26–36. [Medline] [CrossRef]
20. Vasquez EC, Pereira TMC, Peotta VA, Baldo MP, Campos-Toimil M. 2019. Probiotics as beneficial dietary supplements to prevent and treat cardiovascular diseases: uncovering their impact on oxidative stress. Oxid Med Cell Longev 2019: 3086270. [Medline] [CrossRef]
21. Shan H, Li T, Zhang L, Yang R, Li Y, Zhang M, Dong Y, Zhou Y, Xu C, Yang B, Liang H, Gao X, Shan H. 2019. Heme oxygenase-1 prevents heart against myocardial infarction by attenuating ischemic injury-induced cardiomyocytes senescence. EBioMedicine 39: 59–68. [Medline] [CrossRef]
22. Wang S, Cheng ZY, Chen XJ, Xue HZ. 2018. Ulinastatin protects rats with myocardial infarction by activating Nrf2/NOS pathway. Eur Rev Med Pharmacol Sci 22: 8990–8998. [Medline]
23. Yang S, Chou G, Li Q. 2018. Cardioprotective role of azafrin in against myocardial injury in rats via activation of the Nrf2-ARE pathway. Phytomedicine 47: 12–22. [Medline] [CrossRef]
24. Wang J, Ji SY, Liu SZ, Jing R, Lou WJ. 2015. Cardioprotective effect of breviscapine: inhibition of apoptosis in H9c2 cardiomyocytes via the PI3K/Akt/eNOS pathway following simulated ischemia/reperfusion injury. Pharmazie 70: 593–597. [Medline]
25. Sadeghzadeh J, Vakili A, Sameni HR, Shadnoush M, Bandegi AR, Zahedi Khorasani M. 2017. The effect of oral consumption of probiotics in prevention of heart injury in a rat myocardial infarction model: a histopathological, hemodynamic and biochemical evaluation. Iran Biomed J 21: 174–181. [Medline] [CrossRef]
26. Wang N, Song G, Yang Y, Yuan W, Qi M. 2017. Inactivated *Lactobacillus* promotes protection against myocardial ischemia-reperfusion injury through NF-κB pathway. Biosci Rep 37: BSR20171025.
27. Moludi J, Alizadeh M, Mohammadzad MHS, Davari M. 2019. The effect of probiotic supplementation on depressive symptoms and quality of life in patients after myocardial infarction: results of a preliminary double-blind clinical trial. Psychosom Med 81: 770–777. [Medline] [CrossRef]
28. Suliman A, Lam A, Datta R, Srivastava RK. 2001. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. Oncogene 20: 2122–2133. [Medline] [CrossRef]
29. Samra YA, Amin MN, Said E. 2021. Cardio-protective impact of gabapentin against doxorubicin-induced myocardial toxicity in rats; emphasis on modulation of inflammatory-apoptotic signaling. Int Immunopharmacol 90: 107125. [Medline] [CrossRef]
30. Tanner MA, Grisanti LA. 2021. A dual role for death receptor 5 in regulating cardiac fibroblast function. Front Cardiovasc Med 8: 699102. [Medline] [CrossRef]
31. Manuneedhi Cholan P, Cartland SP, Dang L, Rayner BS, Patel S, Thomas SR, Kavurma MM. 2018. TRAIL protects against endothelial dysfunction in vivo and inhibits angiotensin-II-induced oxidative stress in vascular endothelial cells in vitro. Free Radic Biol Med 126: 341–349. [Medline] [CrossRef]
32. Teringova E, Kozel M, Knot J, Kocka V, Benesova K, Tousek P. 2018. Relationship between TRAIL and left ventricular ejection fraction in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. BioMed Res Int 2018: 3709084. [Medline] [CrossRef]
33. Bell RM, Yellon DM. 2003. Bradykinin limits infarction when administered as an adjunct to reperfusion in mouse heart: the role of PI3K, Akt and eNOS. J Mol Cell Cardiol 35: 185–193. [Medline] [CrossRef]
34. Han Y, Zhang W, Tang Y, Bai W, Yang F, Xie L, Li X, Zhou S, Pan S, Chen Q, Ferro A, Ji Y. 2012. l-Tetrahydropalmatine, an active component of *Corydalis yanhusuo W.T. Wang*, protects against myocardial ischaemia-reperfusion injury in rats. PLoS One 7: e38627. [Medline] [CrossRef]
35. Song W, Liang Q, Cai M, Tian Z. 2020. HIF-1α-induced up-regulation of microRNA-126 contributes to the effectiveness of exercise training on myocardial angiogenesis in myocardial infarction rats. J Cell Mol Med 24: 12970–12979. [Medline] [CrossRef]
36. Di Pietro R, Mariggiò MA, Guarnieri S, Sancilio S, Giardinelli A, Di Silvestre S, Consoli A, Zauli G, Pandolfi A. 2006. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) regulates endothelial nitric oxide synthase (eNOS) activity and its localization within the human vein endothelial cells (HUVEC) in culture. J Cell Biochem 97: 782–794. [Medline] [CrossRef]
37. Liu M, Xiang G, Lu J, Xiang L, Dong J, Mei W. 2014. TRAIL protects against endothelium injury in diabetes via Akt-eNOS signaling. Atherosclerosis 237: 718–724. [Medline] [CrossRef]
38. Trudeau F, Gilbert K, Tremblay A, Tompkins TA, Godbout R, Rousseau G. 2019. *Bifidobacterium longum* R0175 attenuates post-myocardial infarction depressive-like behaviour in rats. PLoS One 14: e0215101. [Medline] [CrossRef]
39. Arseneault-Bréard J, Rondeau I, Gilbert K, Girard SA, Tompkins TA, Godbout R, Rousseau G. 2012. Combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 reduces post-myocardial infarction depression symptoms and restores intestinal permeability in a rat model. Br J Nutr 107: 1793–1799. [Medline] [CrossRef]
40. Girard SA, Bah TM, Kaloustian S, Lada-Moldovan L, Rondeau I, Tompkins TA, Godbout R, Rousseau G. 2009. *Lactobacillus helveticus* and *Bifidobacterium longum* taken in combination reduce the apoptosis propensity in the limbic system after myocardial infarction in a rat model. Br J Nutr 102: 1420–1425. [Medline] [CrossRef]
41. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. 2007. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 132: 562–575. [Medline] [CrossRef]
42. Lam V, Su J, Hsu A, Gross GJ, Salzman NH, Baker JE. 2016. Intestinal microbial metabolites are linked to severity of myocardial infarction in rats. PLoS One 11: e0160840. [Medline] [CrossRef]

