Lactobacillus fermentum JX306 Restrain D-galactose-induced Oxidative Stress of Mice through its Antioxidant Activity

DI ZHANG[&], CHUANG LI[&], RUIRUI SHI, FENGCHUN ZHAO[*] and ZHENGYOU YANG[*]

Department of Microbiology, College of Life Science, Key Laboratory for Agriculture Microbiology,
Shandong Agricultural University, Taian, China

Abstract

Oxidative stress-induced series of related degenerative diseases have received widespread attention. To screen new lactic acid bacteria (LAB) strains to resist oxidative stress, traditional Chinese fermented vegetables were used as a resource library to screen of LAB. The *Lactobacillus fermentum* JX306 strain, which showed high scavenging activity of DPPH free radical and hydrogen radical, and a strong lipid peroxidation inhibition rate *in vitro* was selected. *L. fermentum* JX306 was also examined for its antioxidant capacity in D-galactose-induced aging mice. The results showed that *L. fermentum* JX306 could significantly decrease malondialdehyde (MDA) levels and improve the activity of glutathione peroxidase (GSH-Px), and total antioxygenic capacity (TOC) in the serum, kidney, and liver. Meanwhile, the strain could remarkably upregulate the transcriptional level of the antioxidant-related enzyme genes, such as peroxiredoxin1 (Prdx1), glutathione reductase (Gsr), glutathione peroxidase (Gpx1), and thioredoxin reductase (TR3) encoding genes in the liver. Besides, histopathological observation proves that this probiotic strain could effectively inhibit oxidative damage to the liver and kidney in aging mice. Therefore, this unique antioxidant strain may have a high application value in the functional food industry and medicine industry.

K e y   w o r d s: lactic acid bacteria, antioxidant activity, *Lactobacillus fermentum*, traditional Chinese fermented vegetables, D-galactose-induced aging mice

Introduction

Oxidative stress refers to an imbalance, which is caused by high levels of reactive oxygen species (ROS) and low levels of antioxidant activity (Preiser 2012). The excessive ROS can damage enzymes, fatty acids, proteins, nucleic acids, and other physiological substances of cells, which leads to structure and function disorders (Dizdaroglu et al. 1992; Wu et al. 2014). The oxidative stress can cause various diseases, including amyotrophic lateral sclerosis, asthma, allergies, and diabetes as well as further accelerate aging (Nyström 2003; Kurien et al. 2006; Lin and Beal 2006). When the entire antioxidant defense system cannot protect all biological macromolecules from the effects of oxidative damage, it is necessary to increase the defense capacity of the antioxidant system in order to protect human health (Wang et al. 2017). In recent years, LAB have received increasing attention because of their long history of safe use and their potential health benefits, such as improving stool consistency, immune modulation, and antagonism towards the pathogens. Another attractive feature of the LAB is their antioxidant capacity. Increasing experimental evidence indicates that probiotic LAB exerts beneficial antioxidative effects by ROS scavenging, chelating transition metal ions, and activating certain enzyme activities. Therefore, using LAB to scavenge the excess of free radicals, inhibit oxidative damage, and prevent the related restrictive diseases can be a potential treatment option (Mishra et al. 2015; Wang et al. 2017; Lin et al. 2018a).

The most studied probiotics are *Lactobacillus* and *Bifidobacterium*. Compared with other probiotic strains such as *L. rhamnosus*, *L. casei*, and *L. plantarum*, the

[&] These authors contributed equally to this work.

[*] Corresponding authors: Z.Y. Yang, Department of Microbiology, College of Life Science, Key Laboratory for Agriculture Microbiology, Shandong Agricultural University, Taian, China; **e-mail: zhyou21cn@sdau.edu.cn**
    F.C. Zhao, Department of Microbiology, College of Life Science, Key Laboratory for Agriculture Microbiology, Shandong Agricultural University, Taian, China; **e-mail: zhaofengchun@126.com**

study on *L. fermentum* is one of the less-studied potential probiotic strain and still in a developing stage (Lin et al. 2017). *L. fermentum* has beneficial effects on the cholesterol level, effectiveness of the immune response, and reduction of the gastrointestinal and upper respiratory tract infections in infants (Wang et al. 2009; Pan et al. 2011; Maldonado et al. 2012; Russo et al. 2015). It is worth noting that the antioxidant properties of *L. fermentum* have received extensive attention. Using the oxygen radical absorbance capacity (ORAC) method, a significant *in vitro* antioxidant capacity of *L. fermentum* LF31 has been shown (Persichetti et al. 2014). *L. fermentum* ME-3, as a well-known anti-oxidant probiotic strain, inhibited oxidative damage to the body and reduced the risk of intestinal infection in patients (Mikelsaar and Zilmer 2009). *L. fermentum* Suo could eliminate the chain reaction of oxygen free radicals and lipid peroxidation as well as inhibit HCl/ethanol-induced oxidative damage in the gastric tissue (Suo et al. 2016). *L. fermentum* MTCC589 could improve antioxidant enzyme activity, resist the reinfection of *Escherichia coli*, and reduce the immune aging of mice (Sharma et al. 2014).

In this study, 481 of LAB strains from Chinese traditional fermented vegetables were screened to isolate the probiotic strains with antioxidant activity. After the characterization of the antioxidant properties of the LAB strains *in vitro*, *L. fermentum* strain JX306 with high antioxidant activity was selected, and these properties were further studied *in vivo* using a D-galactose-induced aging mice model.

Experimental

Materials and Methods

Bacterial strains and culture conditions. A total of 481 isolates (Table SI) were used in this study, which were obtained from 35 Chinese traditional fermented vegetable samples collected from different areas of China. They were identified using the methods described earlier (Wu et al. 2009). All the strains were stored at –80°C in MRS broth with 20% glycerol. For all subsequent experiments, the strains were incubated in MRS broth at 37°C for 18 h. The intact cells were obtained by centrifugation (8,000 g for 10 min at 4°C) and then washed with sterilized isotonic saline (0.85%) three times. The final concentration of intact cells was adjusted to 4.0×10^8 CFU/ml.

***In vitro* determination of antioxidant activity of LAB strains. Primary screening of LAB with antioxidant capacity.** The scavenging capacity against DPPH· of 481 LAB strains was evaluated based on the method described by Wang et al. (2017) with some modification. Briefly, 1.0 ml of LAB suspension was added to 2.0 ml of DPPH· solution (0.2 mM in ethanolic) and shaken well before incubation for 30 min in the dark at room temperature. In the control group, LAB suspension was replaced by sterile saline, and the DPPH free radical solution was replaced with ethanol solution in the blank group. After centrifugation at 8,000 g for 10 min, the absorbance of the supernatants was measured at 517 nm. The specific method for measuring DPPH· free radical scavenging capacity was as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_{sample} - A_{blank})/A_{control}] \times 100$$

Hydroxyl radical scavenging. The method for the measurement of hydroxyl radical scavenging ability of the preliminarily screened LAB strains was based on Lin's test method (Lin et al. 2018a) with some modifications. Briefly, 1 ml of the LAB suspension was added to 2.5 ml mixture containing 0.5 ml of O-phenanthroline (2.5 mM), 1.0 ml of PBS (10 mM, pH = 7.4), 0.5 ml of $FeSO_4$ (2.5 mM), and 0.5 ml of H_2O_2 (20 mM). The mixture was shaken well and incubated in a water bath at 37°C for 1.5 h. The control group used sterile saline instead of LAB suspension. After centrifugation at 8,000 g for 10 minutes, the absorbance of the supernatant was measured at 510 nm. The specific details of the measurement of hydroxyl radical scavenging ability of LAB cells were as follows:

$$\text{Scavenging activity (\%)} = [(A_{sample} - A_{control}) / (A_{blank} - A_{control})] \times 100$$

Lipid peroxidation inhibition rate. The lipid peroxidation inhibition rate of the primary screening LAB strains was determined based on a Kullisaar's test method (Kullisaar et al. 2003) with some modifications. Briefly, 1.0 ml of the LAB suspension was added to a solution containing 0.5 ml of deionized water, 0.2 ml of $FeSO_4$ (0.01%, w/v), 0.02 ml of ascorbic acid (0.01%, w/v), and 1.0 ml of linoleic acid emulsion (20 ml linoleic acid emulsion includes 0.2 ml of tween 20, 0.1 ml of linoleic acid, and 19.7 ml of sterile saline). The mixture was incubated at 37°C for 12 h. Then, 0.2 ml of 4% TCA, 2 ml of thiobarbituric acid (TBA, 0.8%), and 0.2 ml of butylated hydroxytoluene (BHT, 0.4%) were added to the mixture and shaken well. After reaction in a heated water bath at 100°C for 30 min, the solution was rapidly cooled by using an ice bath and extracted by 2 ml butyl alcohol. The supernatant was obtained by centrifugation at 8,000 g for 10 min, and the absorbance at 532 nm was determined as A_{sample}. The absorbance of the mixture without the LAB cells was determined as $A_{control}$. The specific calculation method of the lipid peroxidation inhibition rate was as follows:

$$\text{Lipid peroxidation inhibition capacity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Tolerance of the selected strains to simulated bile. The tolerance of the six LAB strains to simulated bile

was evaluated based on the test method described by Argyri et al. (2013). LAB cells (4×10^8 CFU/ml) were collected and resuspended into 1 ml simulated bile (a sterile saline solution containing 1 mg/ml pancreatic enzymes, and 0.5% (w/v) of bile salt. The LAB cells were incubated at 37°C for 1 h, and then the samples were diluted and plated on MRS solid plates. After 48 h of incubation at 37°C, the survival rate (SR) of cells was determined by a plate count method. The survival rate was determined as follows:

$$SR\,(\%) = S_2 / S_1 \times 100$$

where, S_1 is the initial number of cells, S_2 is the final number of cells.

Tolerance of the selected strains to simulated gastric and intestinal fluids. Tolerance of the selected six strains to simulated gastric and intestinal fluids was tested based on the method described by Huang and Adams (2004). Briefly, LAB cells (4×10^8 CFU/ml) were collected and resuspended into 1 ml of the simulated gastric fluid or intestinal fluid. The simulated gastric and intestinal fluids were made in the same way as in the previous study (Huang and Adams 2004). LAB strains in the simulated gastric fluid were incubated at 37°C for 3 h, and LAB strains in the simulated intestinal fluid were incubated at 37°C for 24 h. Then, LAB strains were diluted and spread on MRS plates. After 48 h of incubation at 37°C respectively, the survival rate was determined as shown above for the SR(%) equation.

Assay antioxidant activity of *L. fermentum* JX306 *in vivo* using the D-galactose-induced aging mice model. Animal experiment designs. Sixty male KM mice (20 ± 2 g), purchased from Jinan Pengyue Animal Experimental Center (Jinan, China), were randomly divided into six groups after one-week adaptation: the normal control group (NC), model control group (MC), positive control group (PC), low-dose group (LD), middle-dose group (MD), and high-dose group (HD). The treatments of each group were shown in Table I. After feeding for eight weeks, mice were sacrificed. The specific methods and details of animal experiments have been approved by the Ethics Committee of Shandong

Agriculture University and comply with relevant guidelines in the European Union (Directive 2010/63/EU).

Determination of serum, liver, and kidney antioxidative parameters. Blood samples of each group were obtained by moving mice eyeballs after 12 h of the final administration. Subsequently, the samples were centrifuged (8,000 g, 10 min at 4°C) to obtain the serum samples. The serum samples were stored at −80°C for further analysis. The liver and kidney samples of each mouse (0.1 g) were added to 0.9 ml of sterile saline and fully homogenized. After centrifugation at 8,000 g for 10 min at 4°C, the supernatants of each group were gathered for further analysis. Four oxidative stress products, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) were determined in the serum, liver, and kidney samples according to the details in the kit (Nanjing Jiancheng Bioengineering Institute, China).

Changes of the antioxidant-related genes' transcription in the liver. The total RNA of liver tissue in each group was extracted according to the specific details in the RNAiso Plus (Takara, China). The purity and quality of total RNA were determined by OD_{260}/OD_{280} and agar gel electrophoresis. Then, the reversed transcription to synthesize cDNA was performed according to the details in the PrimeScriptII First Strand cDNA Synthesis Kit (Takara, China), and considering the needs of the next experiment, the cDNA was placed at −20°C. The 7500 Fast Real-Time PCR System (ABI, USA) and SYBR Green PCR Kit (TransGen, China) were used for operating the RT-PCR. The PCR procedure contained a denaturation step at 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 5 s, annealing, and extension at 60°C for 30 s.

The primers (Table SII) for the amplification of the genes encoding for peroxiredoxin-1 (Prdx1), glutathione peroxidase (Gsr), glutathione peroxidase (Gpx), and thioredoxin reductase (TR3), used for quantitative-PCR, were as described previously (Zhao et al. 2018). The gene encoding for β-actin was considered as a reference gene. The transcriptional levels of

Table I
Groups of experimental animals.

Group	Number	Experimental treatments	
		Oral administration	Intraperitoneal injection
NC	10	Normal saline (0.85%, 20 ml/kg BW)	Normal saline (0.85%, 20 ml/kg BW)
MC	10	Normal saline (0.85%, 20 ml/kg BW)	D-galactose (30 g/l, 200 mg/kg BW)
PC	10	Vitamin C (30 g/l, 200 mg/kg BW)	D-galactose (30 g/l, 200 mg/kg BW)
LD	10	*L. fermentum* JX306 (10^8 CFU/day)	D-galactose (30 g/l, 200 mg/kg BW)
MD	10	*L. fermentum* JX306 (10^9 CFU/day)	D-galactose (30 g/l, 200 mg/kg BW)
HD	10	*L. fermentum* JX306 (10^{10} CFU/day)	D-galactose (30 g/l, 200 mg/kg BW)

four genes encoding antioxidant enzymes Prdx1, Gsr, Gpx, and TR3 in the liver were evaluated according to the standard curve of quantitative analysis. The specific result of the relative expression of the gene is expressed by the formula $2^{-\Delta\Delta CT}$.

Histological analysis. Liver and kidney tissues were fixed with 10% formalin for 24 h and then embedded in conventional paraffin. For each paraffin block, thin sections (4−5 μm thickness) were gained, and then HE stained, and professional pathologists were invited to interpret the section results. Subsequently, these sections were used for light microscopic evaluation.

Statistical analysis. The software SPSS 20 was used to perform the statistical evaluation. Mean ± standard deviations were used to interpret the data. One-way ANOVA was operated to analyze the data. Significant differences between experimental groups were determined using LSD's and Tukey's tests. The data are significantly different with $p < 0.05$.

Results

Screening of antioxidant LAB strains. In this study, 481 strains isolated from Chinese traditional fermented vegetable samples were firstly screened for their DPPH free radical scavenging ability. As shown in Table II, six LAB strains were selected based on their high DPPH radical scavenging capacity. The strongest scavenging effects on the DPPH radical scavenging capacity were found for *L. fermentum* 306 (37.29%).

The antioxidant activity of the strains selected was further evaluated by HO· scavenging ability, and the lipid peroxidation inhibition rate. As shown in Table II, among the strains selected, *L. fermentum* JX306 exhibited the highest HO· scavenging capability, and the strongest lipid peroxidation inhibition activity with the HO· scavenging rate of 37.29%, and lipid peroxidation inhibition rate of 37.9%.

Tolerance to bile salts and the simulated gastric and intestinal fluids. Prerequisites for the application of LAB strains for commercial use include resistance to bile salt-mediated growth inhibition (Jamalifar et al. 2010), and their survival in an acidic, alkaline gastrointestinal environment. Oral lactic acid bacteria must overcome these adverse conditions to live to the intestines and, therefore, play a beneficial health effect. Thus, the tolerance of the strains selected for simulated bile, gastric fluid, and intestinal fluid was determined, as shown in Table III, *L. fermentum* JX306 showed the highest survival rates after incubation in these three simulated solutions. The survival rates were 78.28% for simulated bile, 53.05% for simulated gastric fluid, and 42.07% for simulated intestinal fluid, respectively.

Table II
The selected LAB strains with high antioxidant activity.

Strains	DPPH scavenging rate (%)	Hydrogen radicals scavenging rate (%)	Inhibition rate of lipid peroxidation (%)
L. fermentum JX306	37.29 ± 1.75[a]	37.90 ± 0.29[a]	28.14 ± 2.97[a]
L. fermentum GZ394	34.92 ± 3.57[a]	35.02 ± 1.70[b]	23.89 ± 1.60[b]
L. plantarum SC34	23.09 ± 4.00[c]	34.50 ± 1.44[b]	12.69 ± 0.23[d]
L. plantarum GZ328	27.83 ± 2.25[b]	29.21 ± 1.60[c]	20.85 ± 2.07[bc]
Pediococcus pentosaceus GZ430	16.22 ± 0.89[d]	23.32 ± 1.62[d]	18.01 ± 2.09[c]
Leuconostoc mesenteroides YN295	15.26 ± 0.67[d]	34.31 ± 1.18[b]	14.43 ± 1.29[d]

[a-d] An average value within a list with different superscript alphabets differ ($p < 0.05$)
Data are shown as means ± SD from triplicate results

Table III
The survival rate (%) of LAB in simulated bile, gastric fluid, and intestinal fluid.

Strains	Simulated bile	Simulated gastric fluid	Simulated intestinal fluid
L. fermentum JX306	78.28 ± 0.18[a]	53.05 ± 1.75[a]	42.07 ± 6.52[a]
L. fermentum GZ394	74.61 ± 4.67[a]	30.57 ± 3.68[b]	27.26 ± 4.95[b]
L. plantarum SC34	65.09 ± 6.33[b]	5.34 ± 1.92[c]	12.23 ± 1.85[e]
L. splantarum GZ328	74.48 ± 1.79[a]	1.51 ± 0.22[e]	9.75 ± 0.45[f]
Pediococcus pentosaceus GZ430	22.99 ± 3.12[d]	3.62 ± 1.11[d]	15.26 ± 2.34[d]
Leuconostoc mesenteroides YN295	33.89 ± 0.96[c]	5.34 ± 0.79[c]	17.28 ± 0.56[c]

[a-f] Average value within a list with different superscript alphabets differ ($p < 0.05$)
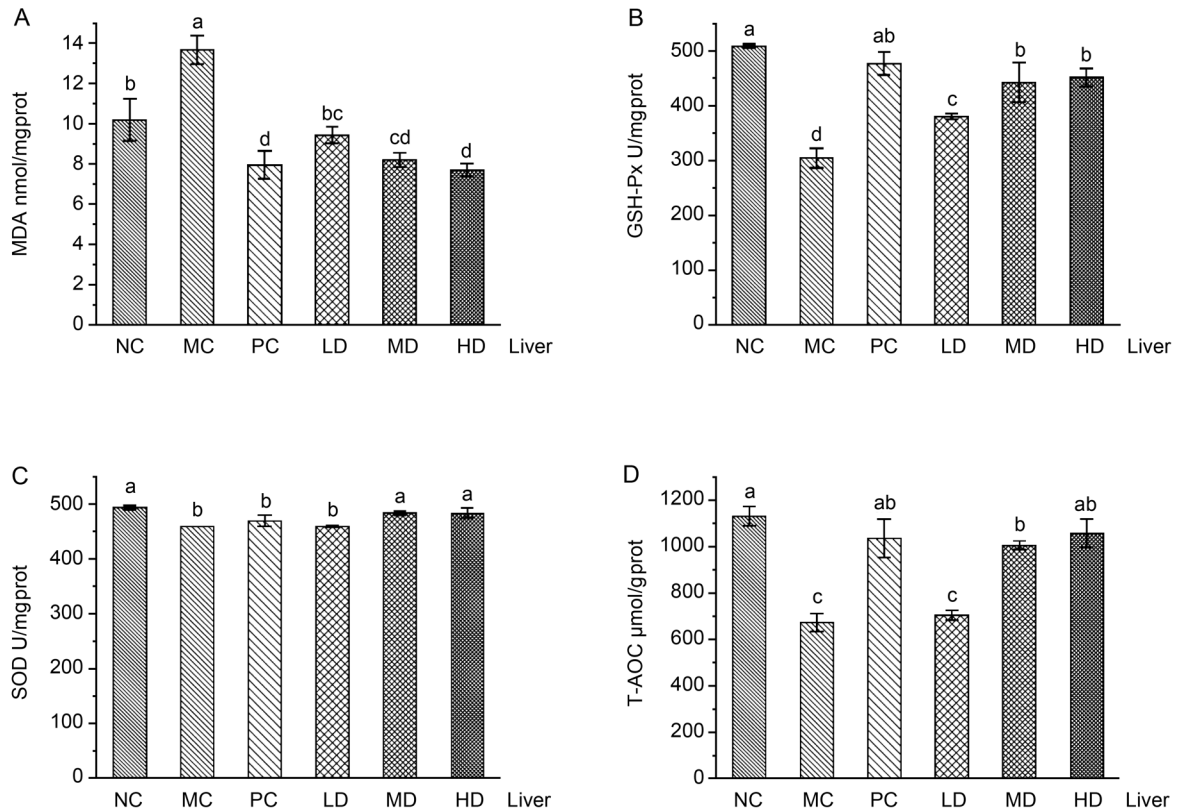Data are shown as means ± SD from triplicate results

Fig. 1. Effect of *L. fermentum* JX306 on GSH-Px, SOD, T-AOC activities, and MDA concentration in the liver of mice with oxidative stress induced by D-galactose. (A) MDA; (B) GSH-Px; (C) SOD; (D) T-AOC. All data are presented as mean ± SD (n = 3). Bars with different letters were significantly different ($p < 0.05$).

Therefore, based on the antioxidant activities and the survival responses of the strains selected to the simulated conditions in the human gastrointestinal tract, the strain of *L. fermentum* JX306 was finally selected for *in vivo* assay to examine their antioxidant profiles in the D-galactose-induced aging mice model.

Antioxidant activities of *L. fermentum* JX306 in the D-galactose-induced aging mice model. High levels of ROS lead to oxidative damage. D-galactose has low toxicity and slow action and could stimulate the body to produce a large number of free radicals. It has been used as a mature model to simulate the body's oxidative stress process. In this study, the antioxidant activities of *L. fermentum* JX306 were verified *in vivo* in the D-galactose-caused oxidative damaged mice. After intraperitoneal injection of D-galactose for eight weeks, compared with the NC group, the MC group exhibited obvious signs of aging. The skin color of the mice was dull, the skin elasticity was poor, and worse emotions such as irritability and lethargy appeared. It is worth noting that no mouse death occurred during the entire test. The weight of the mice was analyzed in detail without statistical differences in all groups ($p > 0.05$) (data not shown).

The level of antioxidant products in the D-galactose-induced aging mice liver. Compared with the NC

group, the levels of GSH-Px and T-AOC antioxidant products in the MC group showed a significant downward trend, while the activity of MDA showed an upward trend. ($p < 0.05$, Fig. 1). Oral treatment with *L. fermentum* JX306 strain could change significantly adverse effects, which were caused by the three oxidative stress products (Fig. 1). The MDA levels decreased with the increase of LAB dose, and it reached 9.43 nmol/mg protein, 8.19 nmol/mg protein, and 7.70 nmol/mg protein in the LD, MD, and HD group, respectively, which were 31.01%, 40.03%, and 43.65% lower than the results in MC group. It should be noted that the MDA levels in a high-dose group were significantly decreased compared to the normal control group, and comparable to the PC group. In the LAB groups, the GSH-Px and T-AOC levels increased with the increase of LAB dose, and it reached 451.80 U/mgprot, and 1057.13 μmol/gprot in a HD group respectively, which were 48.40% and 57.13% higher than the results in MC group. As shown in Fig. 1, the hepatic SOD activity was not significantly influenced by D-gal, vitamin C or *L. fermentum* JX306 strain application.

The level of antioxidant products in the D-galactose-induced aging mice kidney. When compared with the NC group, the injection of D-galactose to mice from the MC group generated a lot of free radicals. The
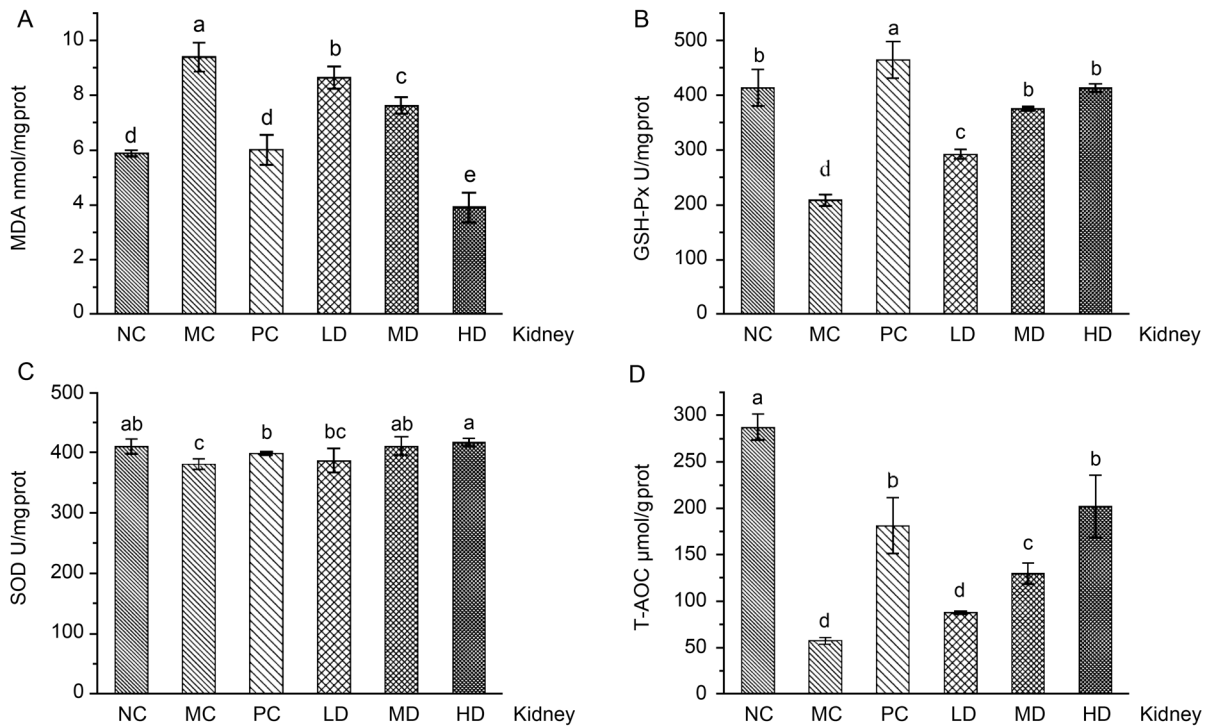
Fig. 2. Effect of *L. fermentum* JX306 on GSH-Px, SOD, T-AOC activities, and MDA concentration in the kidney of mice with oxidative stress induced by D-galactose. (A) MDA; (B) GSH-Px; (C) SOD; (D) T-AOC. All data are presented as mean ± SD (n = 3). Bars with different letters were significantly different ($p < 0.05$).

excessive radicals hurt all biological macromolecules and cause oxidative damage to tissues and cells; thus, they led to the decrease of GSH-Px and T-AOC activities and the increase of MDA levels ($p < 0.05$, Fig. 2). The administration of *L. fermentum* JX306 could effectively reverse the adverse changes, which were caused by D-galactose ($p < 0.05$). The T-AOC levels boosted with the increase of the LAB dose and reached 87.80 µmol/g protein, 129.80 µmol/g protein, and 202.27 µmol/g protein in the LD, MD, and HD group respectively. These levels were 53.12%, 126.34%, and 252.76% higher than the results demonstrated for the MC group. It is worth mentioning that the activity of GSH-Px with high-dose of *L. fermentum* JX306 was higher, and there was no statistically significant difference when compared to the NC group. This result showed that the high-dose of *L. fermentum* JX306 had the most significant effect in inhibiting oxidative damage, which was caused by excessive free radicals. The MDA levels decreased with the increase of the LAB dose, and reached 8.64 nmol/mg protein, 7.62 nmol/mg protein, and 3.91 nmol/mg protein in the LD, MD, and HD group, respectively. These levels were 7.92%, 18.79%, and 58.32% lower when compared to the MC group. It should be noted that the MDA levels in the high-dose group were significantly decreased in comparison to the normal control group and PC group. The administration of *L. fermentum* JX306 did not significantly affect SOD activity in the kidney when compared to that of the MC group.

Effect of antioxidant products in the serum from the D-galactose-induced aging mice. As shown in Fig. 3, a significant decrease of T-AOC activities and an increase of MDA levels were observed in the serum of the D-galactose induced aging mice's control group when compared to those of the normal control group. *L. fermentum* JX306 administration reversed the changes in T-AOC and MDA levels. The T-AOC levels enlarged with the increase of the LAB dose and reached 641.25 µmol/g protein, 772.53 µmol/g protein, and 793.53 µmol/g protein in the LD, MD, and HD group, respectively. These values were 4.45%, 25.83%, and 29.25% higher than the results in the MC group. The MDA levels decreased with the increase of LAB dose, and reached 1.92 nmol/mg protein in the HD group, and were 35.75%, 27.64%, and 20.44% lower than the results in the MC group, NC, and PC group, respectively. The GSH-Px levers in sera of *L. fermentum* JX306 administration mice showed only a slight increase. The serum SOD activities were not significantly influenced by D-galactose, vitamin C or *L. fermentum* JX306.

Effect of *L. fermentum* JX306 on the relative gene expression in the liver of D-galactose-induced aging mice. In order to further explore the related antioxidant mechanism of *L. fermentum* JX306 at the gene level, we have completed a quantitative analysis of the relative expression of the key antioxidant genes in the thioredox system (TRX) and the glutathione system (GSH). As shown in Fig. 4, when compared with the normal group,
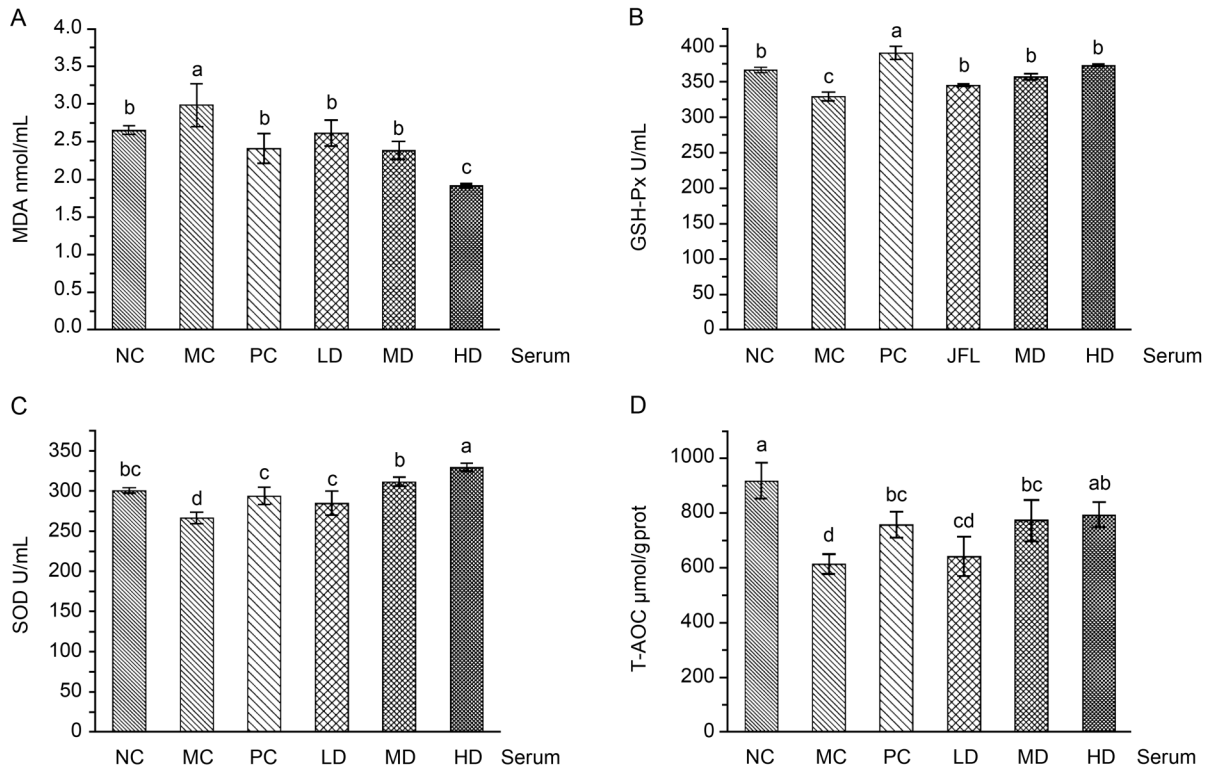
Fig. 3. Effect of *L. fermentum* JX306 on GSH-Px, SOD, T-AOC activities and MDA concentration in the serum of mice with oxidative stress induced by D-galactose. (A) MDA; (B) GSH-Px; (C) SOD; (D) T-AOC. All data are presented as mean ± SD (n = 3). Bars with different letters were significantly different ($p < 0.05$).
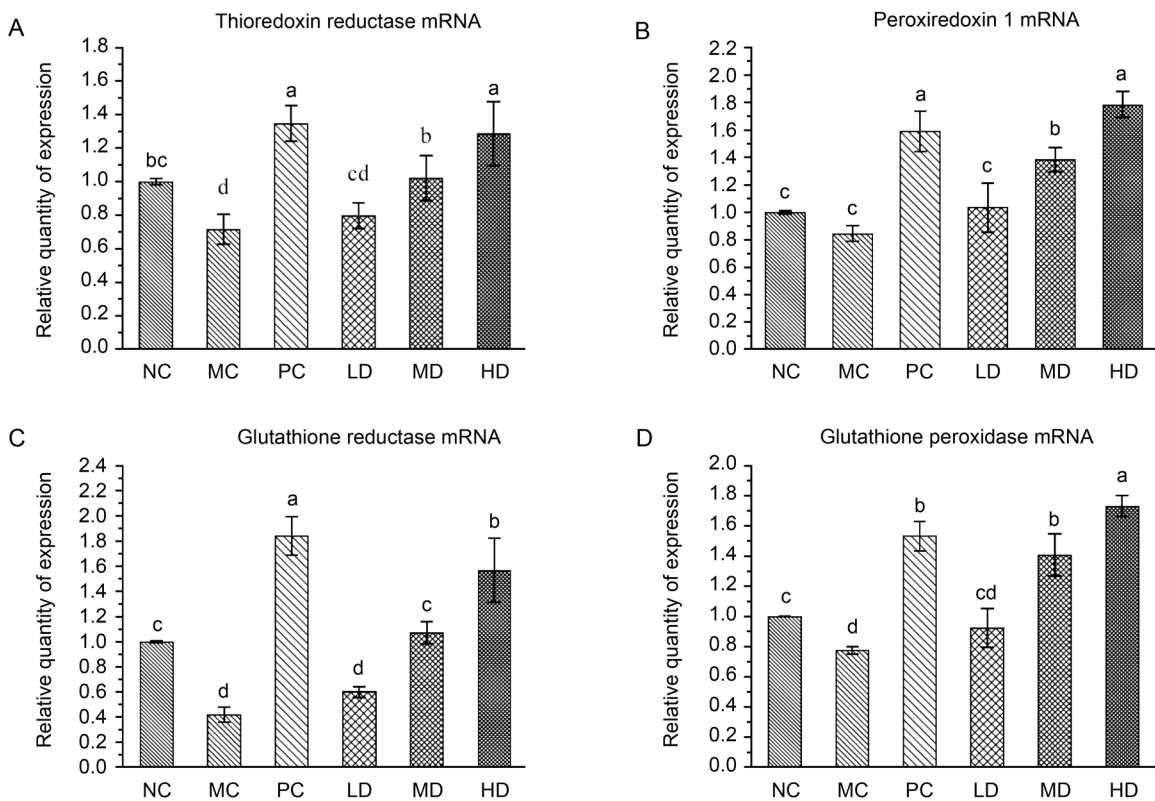

Fig. 4. Effect of *L. fermentum* JX306 on the expression of the genes encoding for peroxiredoxin-1 (Prdx1), glutathione peroxidase (Gsr), glutathione peroxidase (Gpx), and thioredoxin reductase (TR3) in the liver of D-galactose induced aging mice.
(A) Thioredoxin reductase mRNA; (B) Peroxiredoxin1 mRNA; (C) Glutathione reductase mRNA; (D) Glutathione peroxidase mRNA.
All data are presented as mean ± SD (n = 3). Bars with different letters were significantly different ($p < 0.05$).
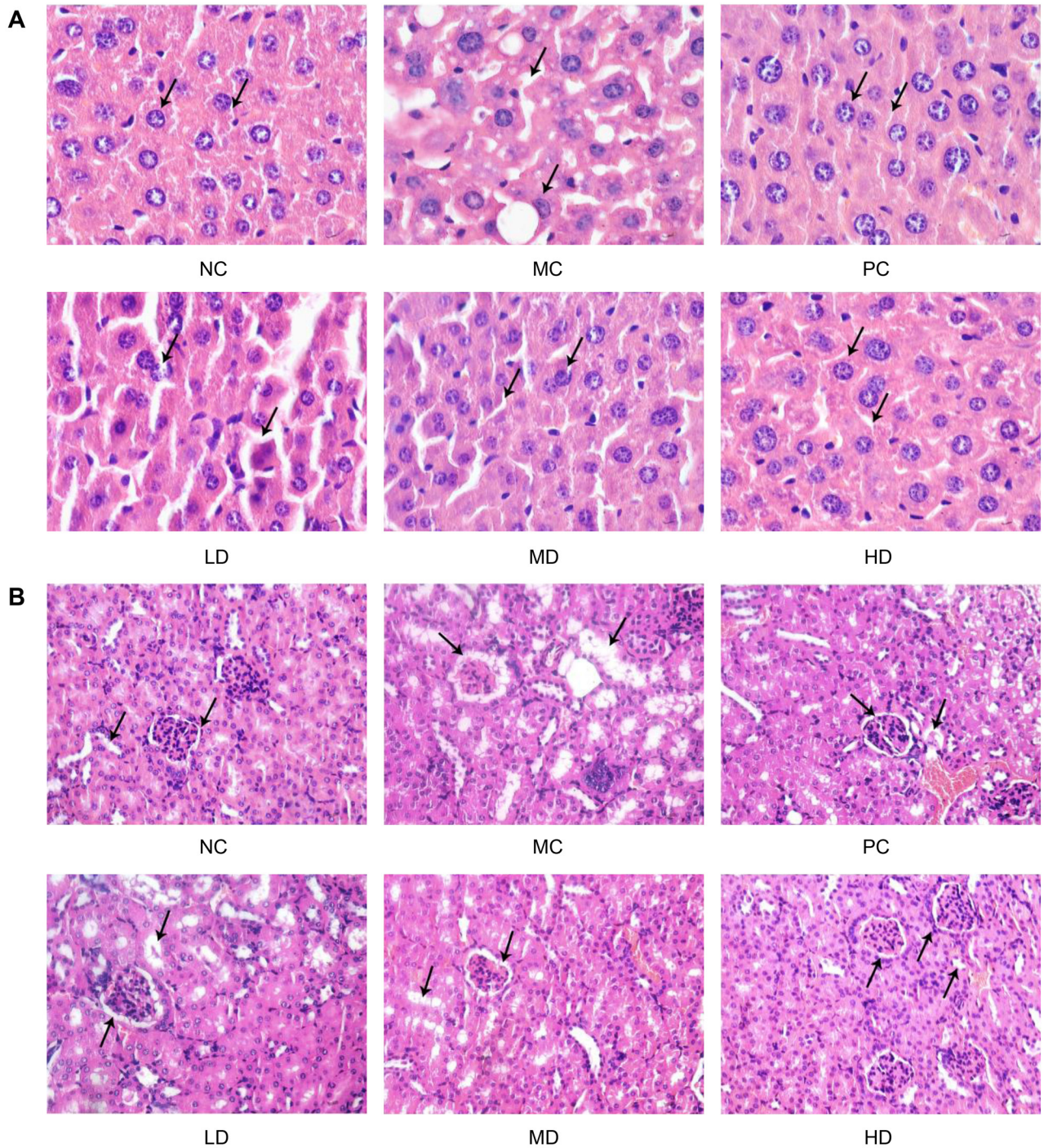
Fig. 5. Effects of *L. fermentum* JX306 treatment on organic damages in the liver (A), and the kidney (B) of D-galactose induced aging mice.

D-galactose treatment significantly reduced the relative gene expression levels of Prdx1, Gsr, Gpx, and TR3 ($p < 0.05$). However, it is worth noting that this phenomenon was significantly alleviated by *L. fermentum* JX306 intervention, and the relative gene expression levels of Prdx1, Gsr, Gpx, and TR3 were all significantly increased. Therefore, oral treatment with *L. fermentum* JX306 strain could significantly increase the transcription level of antioxidant genes in the liver and could play a key role in inhibiting oxidative damage.

Histopathological changes in mice livers and kidneys. As shown in Fig. 5, the HE sections showed that *L. fermentum* JX306 had an inhibitory effect on the oxidative damage caused by D-galactose in liver and kidney tissues. In the NC group, cells in the liver indicated large and round cell nucleus, nucleoli conspicuous, and entire cytoplasm (Fig. 5A). The renal tubules and glomerulus of the kidney showed an intact morphological structure (Fig. 5B). While for the MC group, the histological picture of the kidney indicated that glo-

meruli were severely damaged, as did tubulointerstitial lesions. A loss of brush borders and vacuolation of renal tubules were also observed. A histological picture of the liver showed necrotic spots, edema degeneration, and vacuoles degeneration. However, analysis of the three groups treated with *L. fermentum* JX306 indicated that D-galactose-induced pathologic changes could be alleviated with a dose-related effect. As especially observed in the HD group, the severe oxidative damages caused by D-galactose were strikingly improved to the level of the normal group.

Discussion

In recent years, due to long history of safe use and potential therapeutic benefits (Mokoena 2017; Garcia-Castillo et al. 2019; Zhao et al. 2019) for human health of probiotics, the antioxidative activity of LAB has attracted more and more attention (Amaretti et al. 2013; Tang et al. 2016; Zhao et al. 2018). Traditional Chinese fermented vegetables contain abundant LABs. In this study, for isolation of new LAB strains with high antioxidant activity, the variety of fermented vegetable samples from different areas of China were collected, and 481 LAB strains were isolated from these samples (Table SI). A DPPH free radical scavenging method can directly and rapidly reflect the antioxidant capacity of lactic acid bacteria and has been widely used to evaluate this activity (Antolovich et al. 2002; Ding et al. 2017; Lin et al. 2018b). After screening of the antioxidant activity of 481 LAB strains with a DPPH free radical scavenging method, six strains with a high DPPH radical scavenging rate were selected. It should be noted that these six strains were isolated from the fermented vegetable samples, which were collected from southwestern China. The *L. fermentum* JX306, isolated from Chinese sauerkraut in Jiangxi Province, showed the highest scavenging ability of DPPH radicals at a density of 10^8 CFU/ml (37.29%). Additionally, the strains of *L. plantarum* GZ328 and *L. fermentum* GZ394 (Table II), which showed high DPPH radical scavenging ability, were also isolated from Chinese sauerkraut. Therefore, the sauerkraut from southwestern China is a good source for the isolation of LAB with high antioxidant activity.

A D-galactose-induced oxidative stress model is a very mature model in many animal experiments (Ho et al. 2003). In this study, the antioxidant activities of *L. fermentum* JX306 were verified *in vivo* in a D-galactose-induced aging mice model. MDA is considered one of the by-products of the lipid peroxidation process, and its concentration is one of the most commonly used biomarkers to reflect the lipid peroxidation level (Nielsen et al. 1997). It was reported that *L. plantarum* CCFM10, *L. plantarum* AR501, and

L. delbrueckii subsp. bulgaricus F17 can decrease the level of MDA and inhibit the generation of an excess of free radicals (Ding et al. 2017; Lin et al. 2018b; Zhao et al. 2018). A comprehensive analysis of the literature and our results shows that lactic acid bacteria could indeed relieve the D-galactose-induced oxidative damage. After oral administration of *L. fermentum* JX306 for eight weeks, the MDA levels in serum, liver, and kidney were significantly lower when compared to the MD group. It is worth emphasizing that MDA levels in the high-dose group were also significantly lower than those in the NC group. Therefore, *L. fermentum* JX306 has high lipid antioxidant activity.

Generally, excess of free radicals is mainly eliminated by redox systems in the body, such as the sulfur oxygen reduction (TRX) system and the glutathione (GSH) system, which can reduce and control the occurrence of the oxidative stress-related diseases (Yu et al. 2015; Lin et al. 2018a). GSH-Px is the most important antioxidant factor in the glutathione (GSH) system, which could directly scavenge free radicals and prevent cell damage (Esposito et al. 2000; Ding et al. 2017). The oral treatment with *L. fermentum* JX306 strain could significantly increase the antioxidant enzymatic activity of GSH-Px in the liver and kidney of mice. SOD is the key enzyme for hastening the reaction of superoxide anions to H_2O_2 (Nordberg and Arnér 2001). However, almost no difference was observed in SOD levels among these groups. Our results were supported by previous research (Zhao et al. 2018), and the low toxicity and slow action of D-galactose may be the reason that no significant differences in SOD levels among these groups were observed (Zhao et al. 2018). Total antioxidant capacity (T-AOC) represents the ability of non-enzymatic antioxidant systems to scavenge an excess of free radicals (Zhang et al. 2013). After intragastric administration of *L. fermentum* JX306, T-AOC levels showed an upward trend in the serum, liver, and kidney. Similar to our findings, the previous researches reported that intragastric administration of *L. fermentum* JX306, *L. plantarum* AR501 (Lin et al. 2018a), *L. plantarum* CCFM10, and *L. plantarum* RS15-3 (Zhao et al. 2019) significantly increased T-AOC in the liver.

Normal dose of D-galactose can be metabolized into glucose through the liver enzymatic hydrolysis, and excessive concentration of D-galactose will induce the production of reactive oxygen radicals, causing oxidative damage to liver tissues. To further elucidate the antioxidant mechanism, the changes in the relative level of mRNA encoding Prdx1, Gsr, Gpx, and TR3 in the liver were determined. GSH serves as the most abundant cellular thiol resource and provides a buffer system to maintain the cellular redox status. Prdx1, Gsr, and Gpx are all part of a glutathione peroxidase/ glutathione/glutathione reductase antioxidant pathway

(Wu et al. 2019). TR3 is a component of the TRX system (Arnér and Holmgren 2000). TRX can directly remove intracellular ROS, such as hydrogen peroxide and oxygen free radicals, and regulate intracellular oxidation-reduction balance (Nordberg and Arnér 2000). This test proved that D-galactose harms all body's biological macromolecules, generates excessive free radicals, and causes oxidative damage. After oral administration of *L. fermentum* JX306, the expression of the genes encoding Prdx1, Gsr, Gpx, and TR3 was increased (Fig. 4), thus alleviating the D-galactose-induced liver injury in mice, and delaying the oxidative damage process. This was supported in this study by the histopathological analysis results. A high dose of *L. fermentum* JX306 was able to significantly improve the consequences of severe oxidative damage caused by D-galactose in the liver and kidney tissue.

In this study, *L. fermentum* JX306 with high antioxidant activities was isolated from traditional Chinese fermented vegetable samples. The strain not only showed excellent antioxidant activities *in vitro*, but also effectively restrained the oxidative damage in D-galactose-induced aging mice. While administered orally, the strain could significantly decrease the MDA levels and improve activities of SOD, GSH-Px. and TOC in the serum, liver, and kidney. It could also markedly up-regulate the transcription levels of the genes encoding for antioxidant-related enzymes in the livers of D-galactose-induced aging mice. Besides, the strain could improve the D-galactose-induced histological lesions, and a dose-effect relationship was observed in this case. Thus, *L. fermentum* JX306 could be regarded as a potential strain for further exploiting antioxidant functional products to treat the oxidative-stress-related restrictive disease.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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