Food Science of Animal Products

Research Article

Reduction of uric acid levels by lactic acid bacteria: *in vitro* mechanisms and antioxidant activities

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Abstract: To explore the *in vitro* mechanism of action of lactic acid bacteria (LAB) in reducing uric acid, this study focused on 16 LAB strains isolated from traditional fermented milk in Hulunbuir, Inner Mongolia, China. Through comprehensive evaluation of purine metabolite (xanthine, hypoxanthine, adenine, and guanine) degradation and time-dependent xanthine oxidase (XO) inhibition by viable/dead bacterial suspensions (intracellular/extracellular components), six strains with superior XO-inhibitory were screened. Suspensions of both live and dead cells, intracellular components, and extracellular secretions from these six strains all exhibited strong inhibitory effects on XO. Among these strains (whether live or dead), the extracellular secretions of *Lactiplantibacillus plantarum* HN2-3, *Lactobacillus helveticus* M1-1, *Lactobacillus rhamnosus* N1-4, and *L. plantarum* N2-4 exhibited significantly stronger inhibitory effects on XO compared to their intracellular extracts ($P < 0.05$). Meanwhile, in *Streptococcus thermophilus* ST0, there was no significant difference in inhibition activity between intracellular and extracellular components of both live and dead cells ($P > 0.05$). Finally, the extracellular secretions from live *L. helveticus* M1-2 cells and their intracellular components exhibited comparable levels of XO inhibition ($P > 0.05$). These results indicated some variability in the inhibition of XO. Furthermore, regardless of the viability of all the six strains, their suspensions, extracellular secretions, and intracellular components exhibited varying degrees of scavenging activity against hydroxyl radical, superoxide anion radical, and 1,1-diphenyl-2-picrylhydrazyl radical. In particular, the XO inhibition activity of live strains showed a positive correlation with the antioxidant activity of these bacteria. These findings provide important scientific evidence for the development of novel functional foods and medicines based on LAB.

Keywords: lactic acid bacteria; xanthine oxidase; antioxidant properties; uric acid-lowering

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1 Introduction

Uric acid (UA) is the end product of purine oxidation and is derived from adenosine triphosphate[1]. Unlike most mammals, humans exhibit relatively high serum concentrations of UA. However, the excessive production and inadequate excretion of UA can lead to crystal formation and deposition in the human body[2–3]. Due to the lack of uricase, which converts urate salts into the water-soluble compound allantoin, hyperuricemia may occur, potentially leading to gout. The main causes of hyperuricemia include the overproduction and decreased excretion of UA, and these processes are influenced by factors such as genetics, medication, and chronic kidney disease. Several studies indicate that probiotics, specifically lactic acid bacteria (LAB), can exert positive effects on host health. While the literature on the role of LAB in reducing UA levels is limited, they may play a role in preventing hyperuricemia.

In the LAB's purine metabolism pathway, adenosine monophosphate (AMP) is converted to inosine through two distinct mechanisms. First, AMP undergoes deamination via adenosine deaminase to form inosine monophosphate (IMP), which is then dephosphorylated by nucleotidase to generate inosine. Alternatively, AMP can be dephosphorylated to adenosine, which is subsequently deaminated by adenosine deaminase to yield inosine. Concurrently, guanosine monophosphate (GMP) is hydrolyzed by nucleotidase to form guanosine, which is then metabolized by purine nucleoside phosphorylase (PNP) into hypoxanthine or guanine. Hypoxanthine is oxidized to xanthine by xanthine oxidase (XO), whereas guanine is deaminated by guanine deaminase to produce xanthine[4]. Finally, xanthine is subsequently oxidized by XO to the final product UA. LAB degrades UA by reducing its absorption in the intestines or its production, potentially via the inhibition of XO. Research suggests that LAB can inhibit XO activity through various pathways. Ni et al.[5] proposed that probiotics regulate the gut microbiota to promote the production of short-chain fatty acids (SCFAs), directly inhibiting XO. Meanwhile, SCFAs can also indirectly inhibit XO by maintaining gut barrier function and preventing toxic compounds, such as lipopolysaccharides, from entering the liver and bloodstream. Interestingly, Wu et al.[6] found that the fermentation products of the *Lactobacillus* strain JL-3 also inhibit XO activity, although the specific substances responsible for this inhibition remain to be identified[7].

During UA formation catalyzed by XO, free radicals are produced, and free radicals in the blood can lead to various diseases, including gout. In this context, substances that inhibit XO and exhibit antioxidant activity are crucial for reducing production and clearing free radicals. The combined action of antioxidants and XO inhibitors presents a promising approach for the treatment of hyperuricemia. While multiple studies demonstrate the beneficial effects of LAB on hyperuricemia, further research is needed to determine if LAB strains universally degrade UA and inhibit XO across metabolic disorders. Additionally, more studies are needed to investigate the mechanisms through which LAB strains exert UA-lowering effects, and to identify LAB with XO inhibitory effects through *in vitro* experiments to aid the development of functional foods for preventing and treating hyperuricemia.

The LAB screened in this study can serve as a potential natural source for lowering UA. Furthermore, by comparing their inhibitory effects across multiple dimensions—including live and dead cells, intracellular components, and extracellular secretions—the differences in active components among different strains were elucidated. Meanwhile, the research integrated antioxidant activity evaluation to explore its correlation with XO inhibitory function, thereby revealing the potential mechanisms underlying the UA-reducing effects of LAB. This provides new insights for developing novel functional foods aimed at preventing and treating hyperuricemia.

2 Materials and methods

2.1 Materials and reagents

The LAB used in this study were all isolated from traditional fermented milk in Hulunbuir, Inner Mongolia, and were provided by the Key Laboratory of Dairy Biotechnology and Engineering of the Ministry of Education at the College of Food Science and Engineering, Inner Mongolia Agricultural University. Specific source information was shown in Table 1.

M17 medium was purchased from Qingdao Haibo Biotechnology Co., Ltd. (Qingdao, China). Guanine, adenine, xanthine, hypoxanthine, UA, *O*-diazepine, lysoenzyme, and Tris-HCl were obtained from Beijing Solarbio Reagent Co., Ltd. (Beijing, China). XO, potassium phosphate, and triphenol were supplied by Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ferrous sulfate were purchased from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (Tianjin, China).

2.2 Strain activation

The strains were activated, and a 2% inoculum was cultured. *S. thermophilus* was inoculated in M17 liquid medium (42.3 g of M17 broth medium was dissolved in 1 L of distilled water, sterilized at 121 °C for 15 min) and cultured at 42 °C for 18 h to the third generation. The remaining strains were inoculated into MRS liquid medium (10.0 g soy peptone, 10.0 g yeast extract, 10.0 g beef extract, 20.0 g glucose, 1.0 g Tween-80, 5.0 g anhydrous sodium acetate, 2.0 g sodium citrate, 2.0 g K_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, and 0.05 g $MnSO_4{\cdot}5H_2O$ were dissolved to 1 L of distilled water, sterilized at 121 °C for 15 min) and cultured at 37 °C for 18 h to the third generation.

2.3 Screening of LAB for purine degradation

A mixed standard solution of xanthine-hypoxanthine-adenine-guanine was prepared by dissolving the purines in 10 mmol/L K_3PO_4 buffer (adjusted to pH 7.0 with H_3PO_4). After filtering through a 0.22-μm membrane, 0.9 mL of the solution was mixed with 0.1 mL of stop solution (0.1 mol/L perchloric acid). High performance liquid chromatography (HPLC) was performed using an Agilent Poroshell120 EC-C_{18} reverse-phase column (4.6 mm × 150 mm, 4.0 μm) with 50 mmol/L KH_2PO_4 buffer (pH 3.7):methanol (96:4, *V*/*V*) as mobile phase at 0.4 mL/min flow rate, 30 °C column temperature, and 254 nm detection wavelength, 5 μL injection volume for 15 min. The retention times of purines were identified by comparison with reference standards, while a quantitative standard curve was constructed using the external standard method.

For bacterial treatment, the LAB suspension was centrifuged at 4 000 r/min for 10 min, washed twice, and adjusted to 10^8 CFU/mL. Then, 2 mL of bacterial suspension was centrifuged (4 °C, 4 000 r/min, 10 min), resuspended with 0.75 mL purine mixture, and incubated at 37 °C for 60 min. After centrifugation (4 °C, 4 000 r/min, 10 min), the supernatant was collected, mixed with

Table 1 LAB in experiment and their sources.

Species	Code	Source
Streptococcus thermophilus	ST0	Fermented cow's milk
Lactobacillus helveticus	LH3	Fermented camel milk
L. helveticus	M2-1	Fermented mare's milk
L. helveticus	M1-2	Fermented cow's milk
L. helveticus	M1-1	Fermented cow's milk
L. helveticus	T1(1)-1	Fermented camel milk
Lactiplantibacillus plantarum	HN2-7	Fermented cow's milk
L. plantarum	HN2-3	Fermented cow's milk
L. plantarum	HN2-5	Fermented cow's milk
L. plantarum	N2-4	Fermented cow's milk
Lacticaseibacillus rhamnosus	N1-2	Fermented cow's milk
L. rhamnosus	N1-4	Fermented cow's milk
L. rhamnosus	N1-5	Fermented cow's milk
Lacticaseibacillus paracasei	M2-4	Fermented mare's milk
L. paracasei	LP4	Fermented mare's milk
Lactococcus lactis	HN2-4	Fermented cow's milk

perchloric acid stop solution, filtered (0.22 μm), and analyzed by HPLC. The purine degradation rate was calculated according to formula (1):

$$\text{Degradation rate}\ (\%) = \frac{c_0 - c_1}{c_0} \times 100 \quad (1)$$

Where c_0 is the standard solution concentration (mmol/L), and c_1 is the sample concentration (mmol/L).

2.4 Screening of LAB with XO inhibitory activity

2.4.1 Preparation of bacterial suspensions

Third-generation LAB cultures were centrifuged (4 000 r/min, 5 min), and the pellets were washed thrice with sterile phosphate-buffered saline (PBS) before resuspension in PBS. Bacterial suspensions were adjusted to concentrations of 0.6×10^8, 0.8×10^8, 1.0×10^8, 1.2×10^8, and 1.4×10^8 CFU/mL for live-cell assays. Parallel samples were heat-inactivated (121 °C, 20 min) to prepare inactivated whole-cell suspensions.

2.4.2 Preparation of intracellular components and extracellular supernatant

For intracellular components, bacterial suspensions were ultrasonicated (200 W, 5 s-on/5 s-off, 6 min total) and centrifuged (12 000 r/min, 10 min)[8]. For extracellular supernatant, suspensions were incubated at 37 °C for 12 h, centrifuged (12 000 r/min, 10 min), and filtered (0.22 μm)[9]. Inactivated LAB derivatives (intracellular components and extracellular supernatant) were prepared identically.

2.4.3 XO activity inhibition assay

The XO inhibitory activity was evaluated using a modified protocol based on Umamaheswari et al.[10]. In a 96-well plate, phosphate buffer, test samples, and XO solution (0.2 U/mL) were sequentially added and pre-incubated at 37 °C for 4 min. The reaction was initiated by adding 100 μL xanthine substrate (1 mmol/L). Absorbance at 295 nm was monitored every 2 min for 12 min using a microplate reader. To account for background interference from xanthine absorbance (295 nm), control measurements without samples and without enzyme were subtracted. Each sample is tested in triplicate[11]. The inhibition rate was calculated according to formula (2):

$$\text{Inhibition rate}\ (\%) = 1 - \frac{A_3 - A_4}{A_1 - A_2} \times 100 \quad (2)$$

Where A_1 represents the absorbance of complete reaction system containing 50 μL phosphate buffer, 50 μL XO, and 100 μL xanthine substrate; A_2 denotes the absorbance of background control with only 100 μL buffer and 100 μL xanthine (accounting for inherent substrate absorbance); A_3 indicates absorbance of 50 μL test sample, 50 μL XO, and 100 μL xanthine; and A_4 corresponds to the absorbance of sample blank comprising 50 μL buffer, 50 μL sample, and 100 μL xanthine without XO activity.

2.5 Determination of the antioxidant activity of LAB with XO inhibition activity

2.5.1 Determination of hydroxyl radical scavenging activity

The hydroxyl radical scavenging capacity was evaluated by monitoring the decrease in absorbance of 2,2′-dipyridyl at 536 nm. The reaction mixture contained 0.5 mL 2,2′-dipyridyl (6 mmol/L), 0.5 mL $FeSO_4$ (6 mmol/L), 1 mL PBS, 0.5 mL test sample, and 0.5 mL H_2O_2 (0.1%, *V*/*V*). After incubation at 37 °C for 1 h and centrifugation (4 000 r/min, 30 min), 200 μL supernatant was analyzed in a 96-well plate using a microplate reader (SpectraMAX M2, Molecular Devices, San Jose, CA, USA). The hydroxyl radical scavenging rate was calculated according to formula (3):

$$\text{Hydroxyl radical scavenging rate}\ (\%) = \frac{A_1 - A}{A_0 - A} \times 100 \quad (3)$$

Where A_1 is the absorbance of the sample group; A is the absorbance of the control group without the sample; and A_0 is the absorbance of the control group without the sample and H_2O_2.

2.5.2 Determination of superoxide anion radical scavenging activity

A 0.6 mL sample was mixed with 2 mL Tris-HCl buffer (pH 8.2, 150 mmol/L) and incubated at 25 °C for 10 min. After adding 0.4 mL quercetin solution (1.2 mmol/L), the mixture was further incubated for 20 min. After centrifugation (4 000 r/min, 30 min), 200 μL of the supernatant was used to measure the absorbance at 325 nm using a microplate reader (SpectraMAX M2, Molecular Devices, San Jose, CA, USA). The superoxide anion radical scavenging rate was calculated according to formula (4):

$$\text{Superoxide anion radical scavenging rate}\ (\%) = \frac{A_0 - A}{A_0} \times 100 \quad (4)$$

Where A_0 is the absorbance of the control group (sample solutions without Tris-HCl); and A is the absorbance of the sample group.

2.5.3 Determination of DPPH radical scavenging activity

A 1:1 mixture of sample and DPPH-ethanol solution was incubated in the dark (30 min, room temperature). After centrifugation (4 000 r/min, 30 min), the absorbance at 517 nm was measured. The DPPH radical scavenging rate was calculated according to formula (5):

$$\text{DPPH radical scavenging rate}\ (\%) = 1 - \frac{A_1 - A_2}{A_0} \times 100 \quad (5)$$

Where A_1 is the absorbance of the sample group; A_2 is the absorbance of the mixture of absolute ethanol and the sample; and A_0 is the absorbance of the mixture of DPPH-ethanol solution and absolute ethanol.

2.6 Statistical analysis

Data are expressed as the mean ± standard deviation of three independent experiments. Statistical analysis was conducted using SPSS 25 software and metaX, ANOVA was used to establish the statistical significance of intergroup differences, with a *P*-value less than 0.05 considered statistically significant.

3 Results and discussion

3.1 Screening of purine-lowering LAB

3.1.1 Standard curve generation

An HPLC-based analytical method was developed to quantify four key purines degraded by LAB[12]. The retention times of guanine, hypoxanthine, adenine, and xanthine were measured to be 7.085, 7.619, 8.137, and 8.624 min, respectively. The corresponding

standard curves, demonstrating linear relationships between concentration and peak area, were shown in Figure 1.

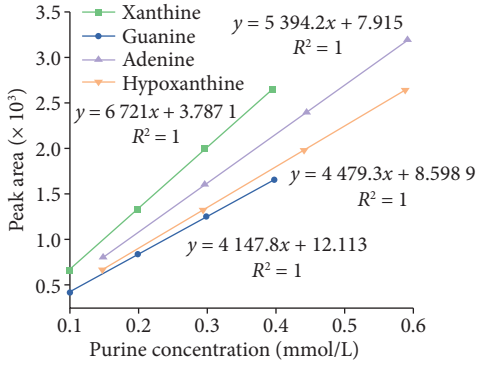

Figure 1 Standard curves of purine metabolites.

3.1.2 *Identification of purine-lowering LAB*

Based on the chromatographic profiles of the 16 tested bacterial strains, the degradation rates of different purines were calculated. *Ex vivo* purine degradation by LAB was depicted in Figure 2. The results showed that the 16 strains of LAB exerted certain degradative effects on xanthine, hypoxanthine, adenine, and guanine, but the degradation rates did not exceed 20%. This suggested that the purine degradation abilities of the LAB selected in this study were relatively weak. Therefore, the inhibitory effects of these 16 strains of LAB against XO were tested in subsequent experiments.

3.2 XO inhibition activity of LAB

3.2.1 *XO inhibition activity of bacterial suspensions at different concentrations*

LAB with XO inhibitory activity were identified in this study by examining the ability of various bacterial suspensions at different concentrations to inhibit this enzyme. Allopurinol, a common XO inhibitor widely used for treating hyperuricemia and gout, was used as a positive control for these experiments[13]. As shown in Figure 3, the inhibition rates induced by the 16 strains of bacteria increased with increasing concentrations but started to plateau or decrease beyond a bacterial concentration of 1.0 × 10⁸ CFU/mL. Therefore, a bacterial concentration of 1.0 × 10⁸ CFU/mL was selected for the next step of the study.

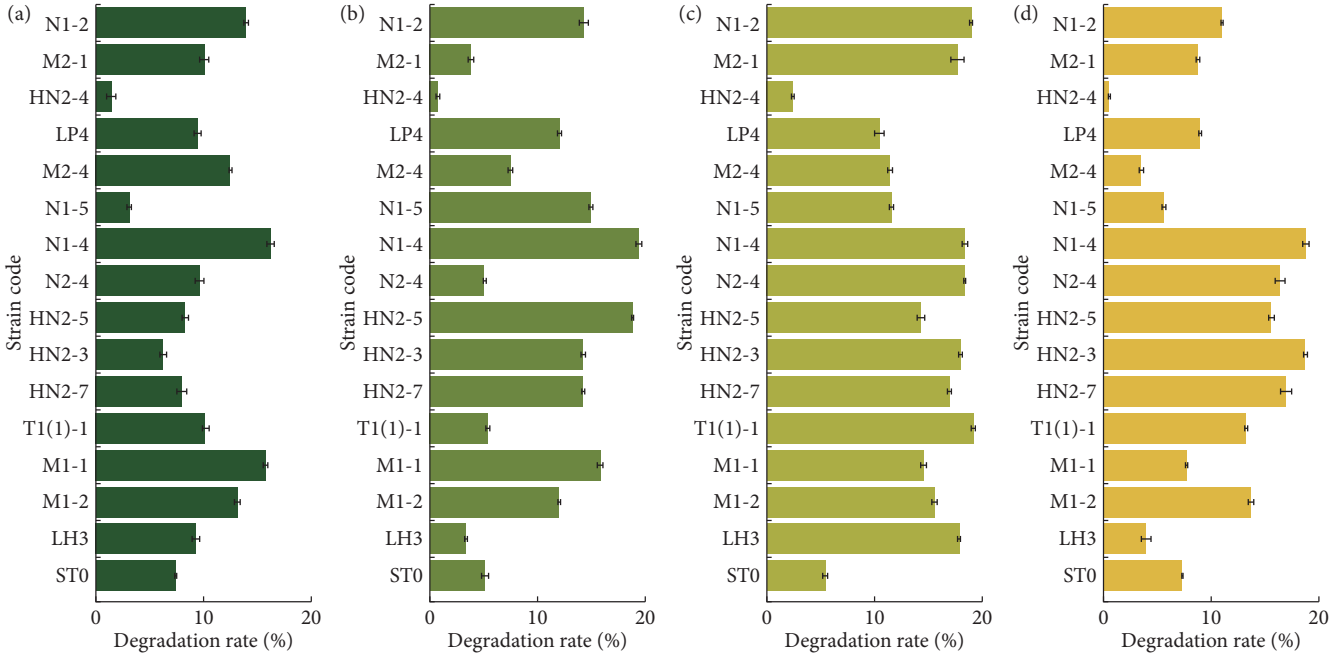

Figure 2 Ability of LAB to degrade purine metabolites (a) xanthine, (b) hypoxanthine, (c) adenine, and (d) guanine.

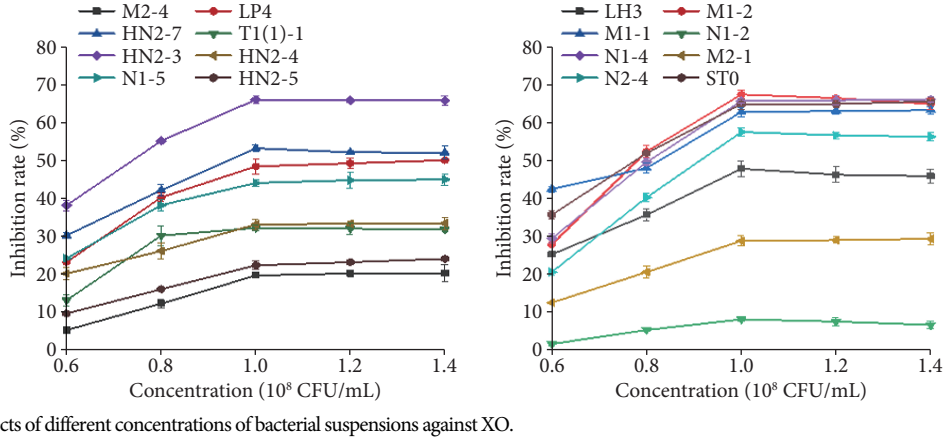

Figure 3 Inhibitory effects of different concentrations of bacterial suspensions against XO.

3.2.2 XO inhibition activity of different bacterial suspensions at different time points

Among the 16 strains of LAB, the live cell suspension of *L. helveticus* M1-2 exhibited the strongest inhibitory effect against XO, achieving an inhibition rate of 67.56% at 8 min. Meanwhile, live cell suspensions of HN2-3, N1-4, ST0, M1-1, and N2-4 exhibited XO inhibition rates of 66.26%, 64.99%, 65.84%, 62.88%, and 57.63%, respectively, at 10 min (Figure 4).

3.3 Preliminary investigation of the mechanism underlying the inhibition of XO by LAB

3.3.1 Effect of strain activity on XO inhibition

To preliminarily investigate the inhibitory mechanisms of LAB strains against XO, the inhibitory effects of dead bacteria on XO were determined. Even after inactivation, all 16 strains of LAB retained their XO inhibition capacity. Among them, the dead cell suspension of M1-2 exhibited the highest inhibition rate against XO, which peaked at 69.46% at 8 min. Meanwhile, the highest inhibition rates achieved by the dead cell suspensions of HN2-3, M1-1, and N2-4 were 65.83%, 66.34%, and 65.66% at 8 min, whereas N1-4 and ST0 achieved their highest inhibition rates of 58.26% and 62.81% at 10 min, respectively (Figure 5a). Collectively, the findings showed that LAB strains can inhibit XO even after they are rendered inactive. For some strains, there was no significant difference in inhibition rates between dead and live bacteria ($P > 0.05$). In fact, for most strains, the inhibitory effects of deactivated bacteria were significantly stronger than those of live bacteria ($P < 0.05$) (Figure 5b), indicating that the activity of the tested LAB strains had a minimal influence on their XO inhibitory effects.

3.3.2 XO inhibition by intracellular components at different time points

Given the effects of dead bacteria on XO activity, the inhibitory effects of the intracellular components and extracellular secretions of live and dead LAB against XO were investigated. First, the effects of intracellular components on XO were determined at different time intervals. The results showed that the intracellular components of strain HN2-3 enabled a stronger inhibition of XO than those of other strains, achieving a maximum inhibition rate of 52.00% at 8 min (Figure 6a). Similarly, the inhibition of XO by the intracellular components of inactivated N2-4 also peaked at 55.84% after 8 min (Figure 6b). The other strains in which the intracellular components derived from live cells showed high XO inhibition activity included ST0 (50.65%), N2-4 (48.65%), N1-4 (48.56%), M1-2 (46.47%), and M1-1 (43.85%), with all achieving their maximum inhibition rates at 8 min (Figure 6a). Meanwhile, the strains for which the intracellular components derived from dead cells showed high XO inhibition activity included ST0 (48.73%), HN2-3 (48.70%), M1-2 (48.18%), M1-1 (47.39%), and N1-4 (43.09%). Of these, HN2-3 and N1-4 reached their maximum XO inhibition rates at 8 min, while ST0, M1-2, and M1-1 reached this

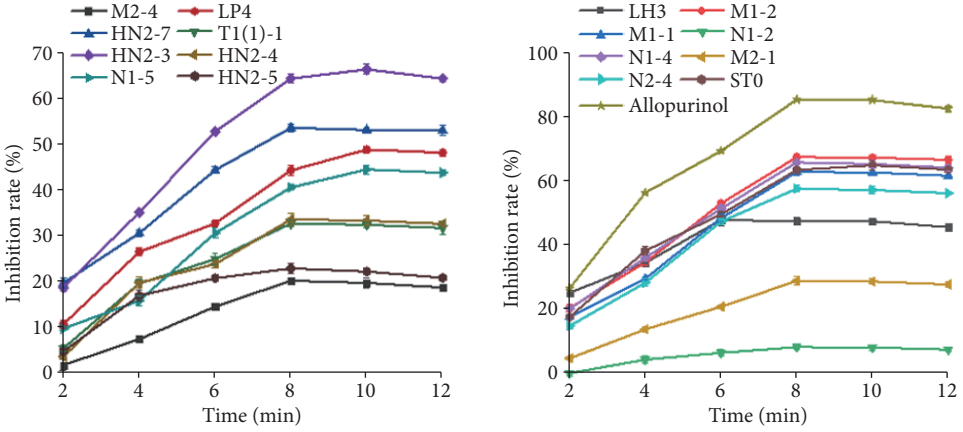

Figure 4 XO inhibition activity of viable bacterial cell suspensions at different time points.

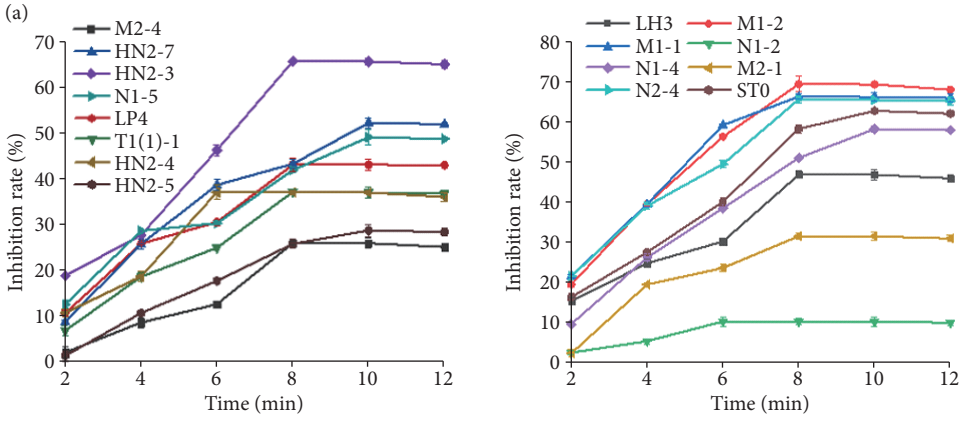

Figure 5 (a) XO inhibition by suspensions of dead bacteria at different time points and (b) comparison of the XO activities of live and dead bacterial suspensions. Bars labeled with different lowercase letters indicate statistically significant differences among different components of the same strain ($P < 0.05$).

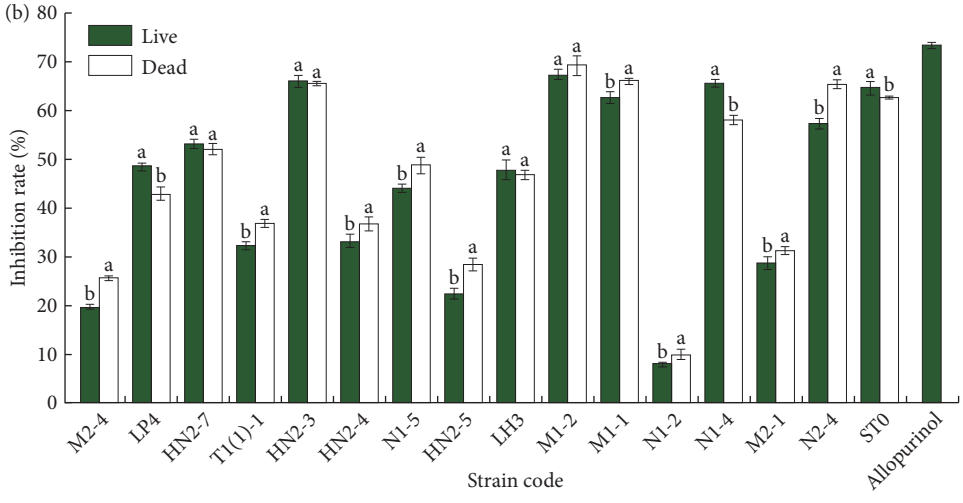

Figure 5 (*Continued*)

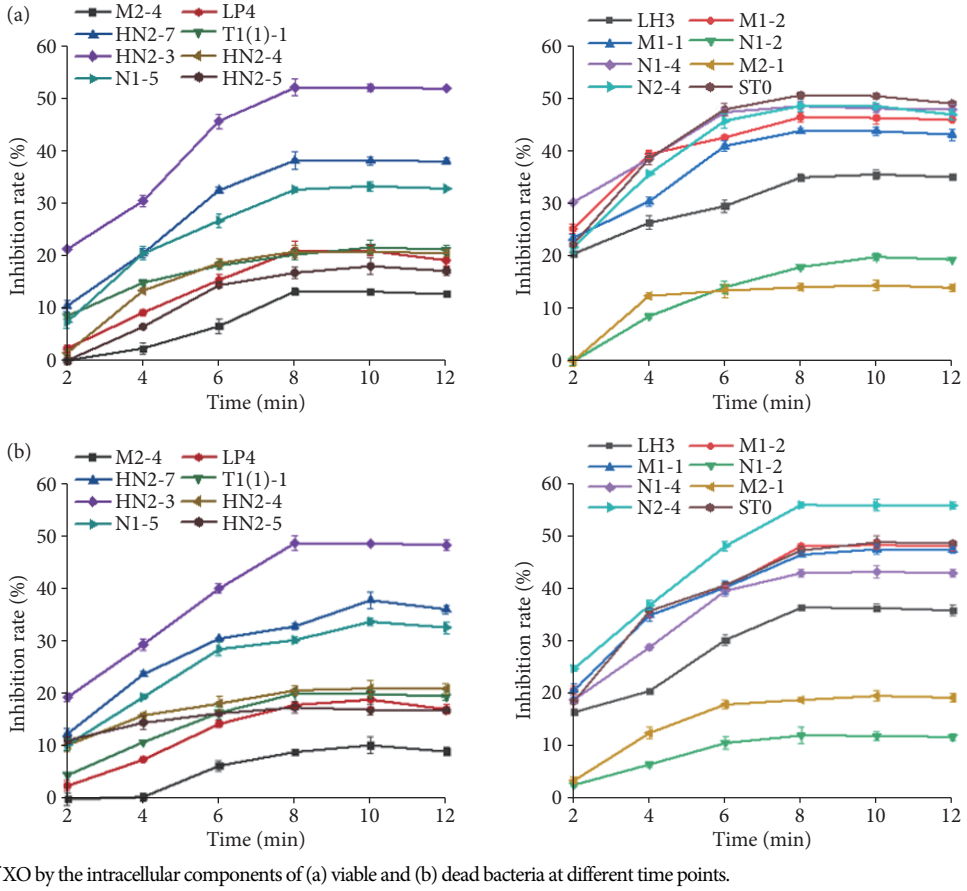

Figure 6 Inhibition of XO by the intracellular components of (a) viable and (b) dead bacteria at different time points.

milestone at 10 min (Figure 6b). This indicated that the intracellular components of both live and dead bacterial cells show inhibitory effects against XO. Moreover, the inhibition rates and durations required to achieve peak inhibition vary among different LAB strains.

3.3.3 XO inhibition by extracellular secretions at different time points

As shown in Figure 7, regardless of LAB activity, the extracellular secretions of the bacteria continued to exhibit inhibitory effects on XO. Extracellular secretions from both the dead and live cells of strain HN2-3 induced higher XO inhibition rates than those of other strains. Extracellular secretions from live cells achieved the highest XO inhibition rate of 58.79% at 8 min (Figure 7a), while those from dead cells achieved the highest inhibition rate of 59.10% at 6 min (Figure 7b). Other LAB for which the extracellular secretions of live cells exhibited high XO inhibition rates included N2-4 (54.89%), N1-4 (53.85%), ST0 (50.26%), M1-1 (47.65%), and M1-2 (46.14%), with all except N2-4 reaching their maximum inhibitory potential at 8 min (Figure 7a). Meanwhile, high XO inhibition rates were also observed for the extracellular secretions of

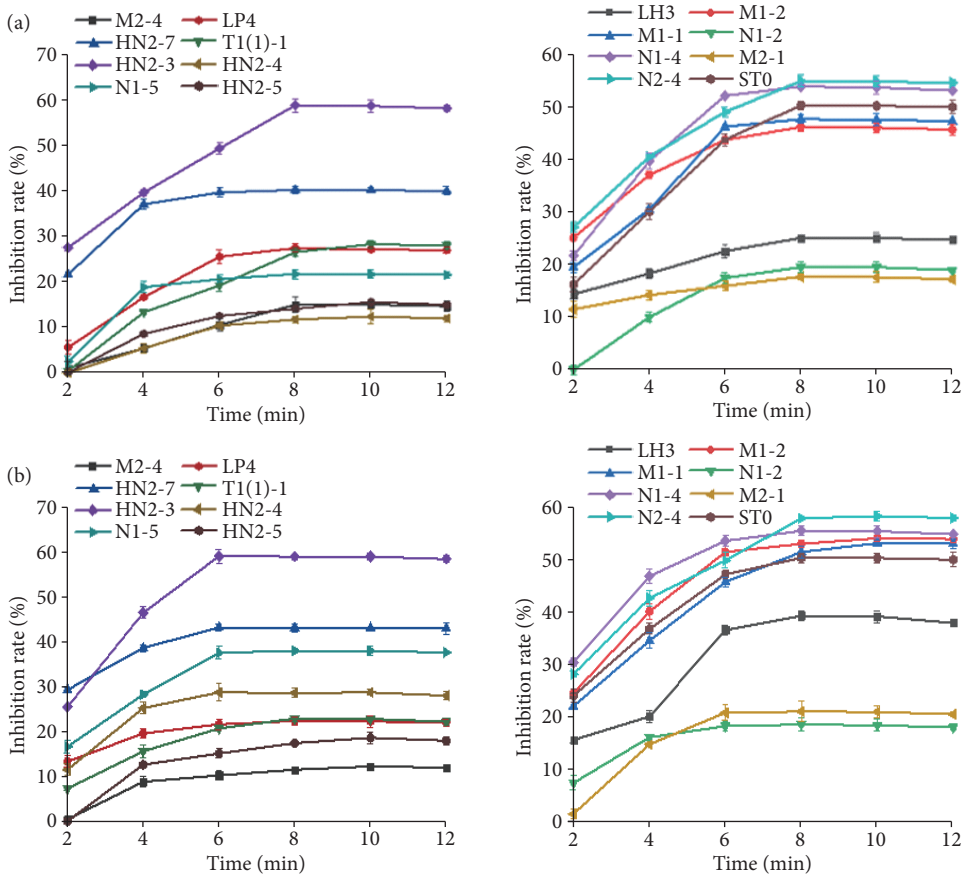

Figure 7 Inhibition of XO by the extracellular secretions of (a) viable and (b) dead bacteria at different time points.

dead cells belonging to the N2-4 (58.37%), N1-4 (55.57%), and M1-2 (54.15%). However, all these strains achieved peak inhibition rates at different time points (Figure 7b).

These experimental results indicated that extracellular secretions from both dead and live bacterial cells, in addition to the intracellular components of these cells, produce certain inhibitory effects against XO. However, further research is warranted to identify the specific components responsible for these effects. Based on the aforementioned experimental results, the strains HN2-3, M1-1, M1-2, N1-4, N2-4, and ST0—which exhibit higher rates of XO inhibition—were selected for further analysis.

3.3.4 XO inhibition by intracellular substances and extracellular secretions

Figure 8 shows that regardless of strain viability, extracellular secretions from HN2-3, M1-1, N1-4, and N2-4 exhibited significantly higher rates of inhibition against XO than the intracellular components ($P < 0.05$). This reflected the predominant role of the extracellular secretions from these four strains in XO inhibition. In contrast, there was no significant difference ($P > 0.05$) observed between the intracellular and extracellular secretions derived from live or dead ST0 cells, indicating that both the intracellular and extracellular components of this strain mediate XO inhibition. Additionally, the extracellular secretions and intracellular components of live strain M1-2 cultures showed comparable levels ($P > 0.05$) of XO inhibition, while the extracellular secretions of dead M1-2 cultures showed significantly higher XO inhibition activity than their intracellular components ($P < 0.05$), reflecting the primary inhibitory role of the extracellular secretions. These findings highlighted the differential effects of different LAB components on XO inhibition[14].

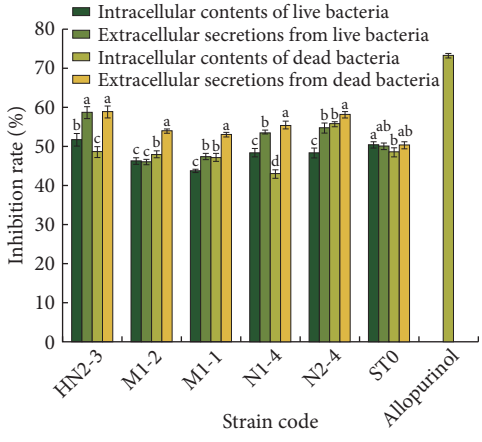

Figure 8 Inhibitory effects of extracellular secretions and intracellular substances from the six screened bacterial strains against XO. Bars labeled with different lowercase letters indicate statistically significant differences among different components of the same strain ($P < 0.05$).

3.4 Antioxidant activity of LAB with inhibitory effects against XO

Previous studies have demonstrated that in plant-derived compounds, the strength of XO inhibition is positively correlated with antioxidant activity. Therefore, in the subsequent analysis, we evaluated the antioxidant properties of live and dead LAB cultures, as well as their intracellular and extracellular secretions.

3.4.1 Antioxidant activity of different concentrations of dead and live bacterial suspensions

This study tested the concentration-dependent antioxidant activity of six screened bacterial strains. As shown in Figure 9, with the increase in bacterial suspension concentration, the scavenging rates of hydroxyl radical, superoxide anion radical, and DPPH radical all increased, exhibiting a positive correlation, which is consistent with the findings of Lu et al.[15]. This indicates that, regardless of whether the bacteria are alive or dead, the antioxidant activity of the six LAB strains is enhanced as the bacterial suspension concentration increases. In the next phase, this study will use vitamin C (VC) as a control group to investigate the differences in antioxidant activity among the six LAB strains.

3.4.2 Antioxidant capacity of bacterial suspensions, intracellular components, and extracellular secretions

Figure 10a shows the hydroxyl radical scavenging abilities of the strains, with VC as the positive control. The results indicated that regardless of the viability of all six screened strains, the cell suspensions, extracellular supernatant, and intracellular components could scavenge hydroxyl radical. In all strains, the hydroxyl radical scavenging rates of the cell suspensions were higher than those of the extracellular supernatant and intracellular components. Moreover, there were some differences in the effectiveness of the extracellular supernatant and intracellular components among the strains. Although there were some differences in the compositions and components of the suspensions before and after inactivation for some strains, the hydroxyl radical scavenging rates of most live cell suspensions, intracellular components, and extracellular secretions were higher than those of inactivated suspensions.

As depicted in Figure 10b, both live and inactivated LAB suspensions, extracellular secretions, and intracellular components exhibited considerable scavenging activities against superoxide anion radical, although they were less effective than VC. Among the different components tested for each strain, the suspensions of HN2-3 and M1-2 (whether live or dead cells) showed the highest

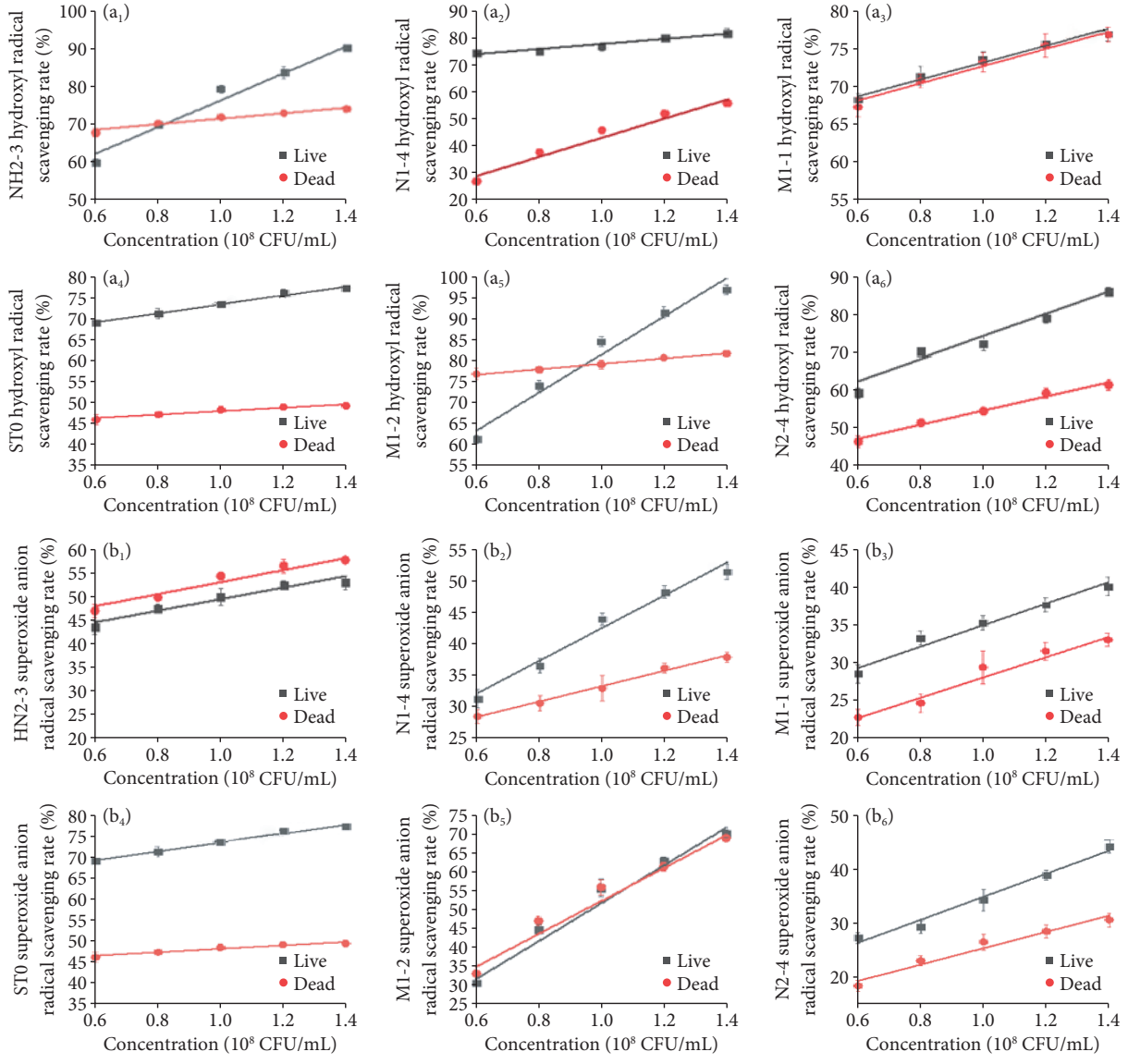

Figure 9 (a) Hydroxyl radical, (b) superoxide anion radical, and (c) DPPH radical scavenging rates of bacterial suspensions with different concentrations. Subscripts 1–6 represent NH2-3, N1-4, M1-1, ST0, M1-2, and N2-4 respectively.

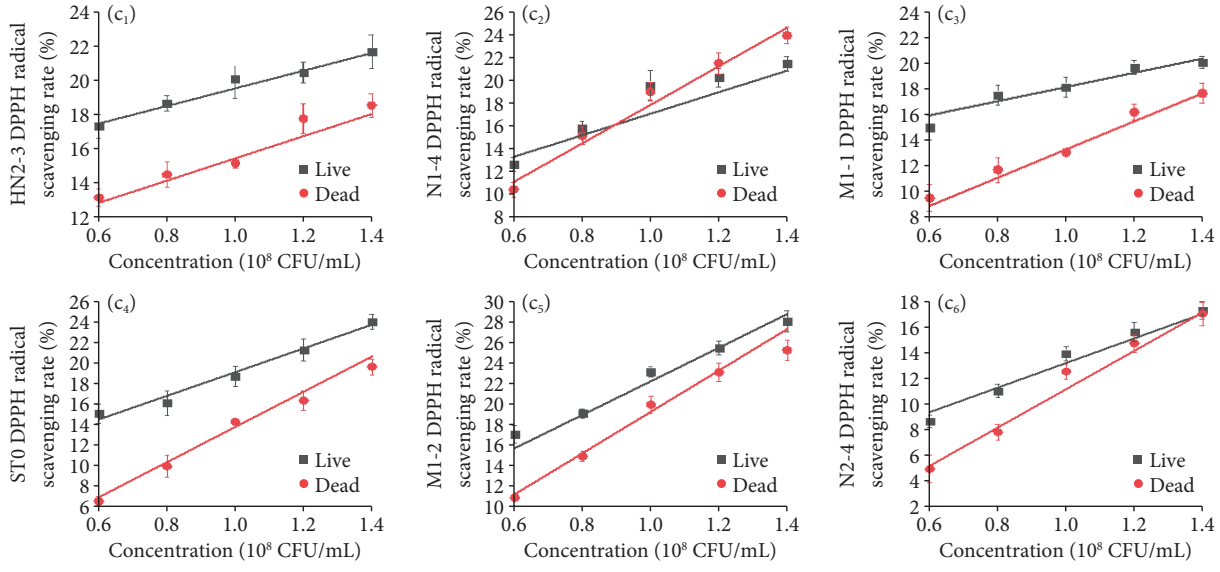

Figure 9 (*Continued*)

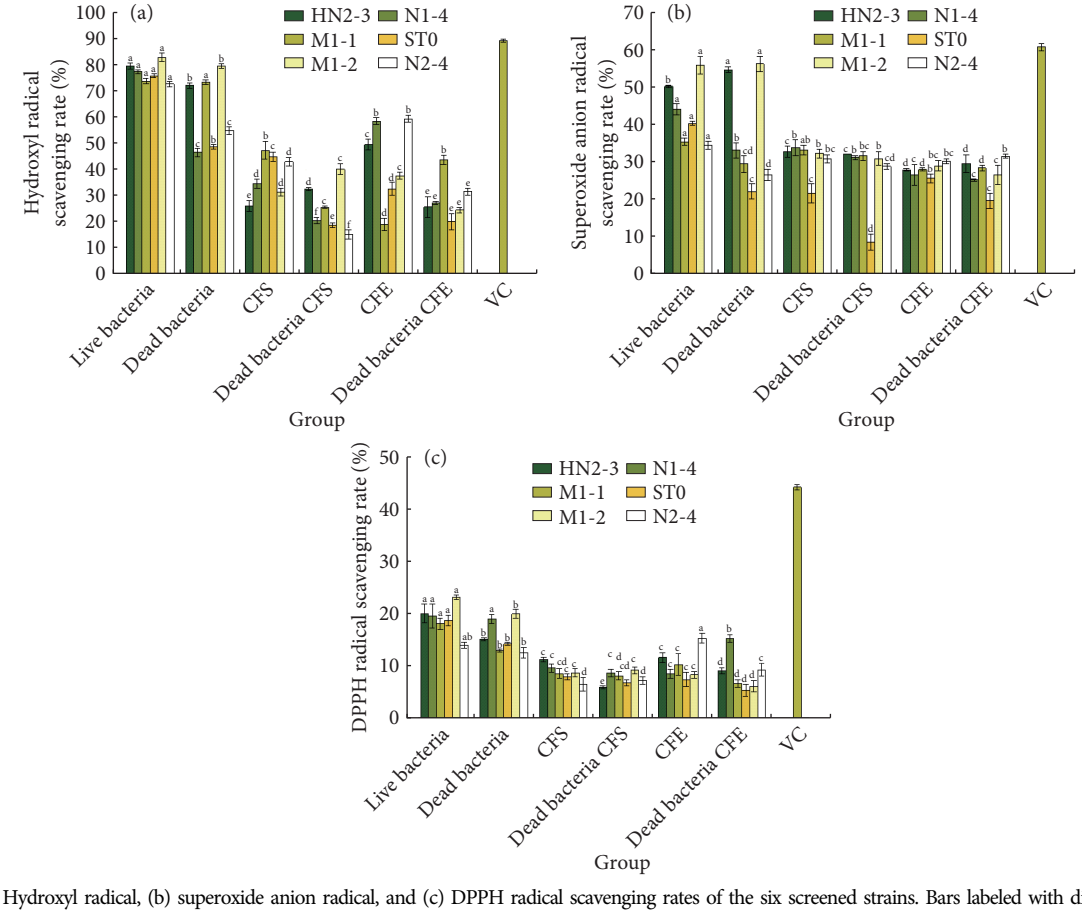

Figure 10 (a) Hydroxyl radical, (b) superoxide anion radical, and (c) DPPH radical scavenging rates of the six screened strains. Bars labeled with different lowercase letters indicate statistically significant differences among different components of the same strain ($P < 0.05$). CFE (cell-free intracellular extracts) and CFS (cell-free excretory supernatants) correspond to intracellular components and extracellular supernatant, respectively.

superoxide anion radical scavenging rates. The superoxide anion radical scavenging rates of HN2-3, N1-4, ST0, and M1-2 suspensions (dead cells) were higher than those of their corresponding intracellular components and extracellular secretions. Meanwhile, the superoxide anion radical scavenging ability of the dead M1-1 cell suspension was comparable to those of the intracellular components and extracellular supernatants ($P > 0.05$). Furthermore, N2-4 dead cell suspensions had lower scavenging rates than the corresponding extracellular supernatant and intracellular components. Additionally, the intracellular components of ST0 and N2-4 dead cells showed significantly stronger superoxide anion radical scavenging capacity than the

extracellular secretions of these cells ($P < 0.05$). However, in the other strains, extracellular secretions showed higher scavenging rates against superoxide anion radical than intracellular extracts, regardless of cell viability. Except for ST0 cells, in which the intracellular components and extracellular secretions of live cells showed stronger superoxide anion radical scavenging than the corresponding components of dead cells ($P < 0.05$), there was no significant difference in scavenging rates between intracellular extracts and extracellular secretions before and after inactivation ($P > 0.05$).

Figure 10c demonstrates that both the live and inactivated cell suspensions, extracellular secretions, and intracellular components of all six strains of LAB exhibited DPPH radical scavenging abilities. Specifically, HN2-3, N1-4, M1-1, ST0, and M1-2 showed significantly higher DPPH radical scavenging rates as suspensions, before and after inactivation, than their intracellular components and extracellular secretions ($P < 0.05$). The live suspension of N2-4 showed no significant difference in DPPH radical scavenging rate when compared to its intracellular components ($P > 0.05$), but it showed significantly higher DPPH radical scavenging rates than the corresponding extracellular supernatant ($P < 0.05$). Meanwhile, the dead cell suspensions of N2-4 showed significantly higher DPPH radical scavenging rates than both the intracellular components and extracellular supernatant ($P < 0.05$). Notably, there was no significant difference in DPPH radical scavenging rates between the intracellular components and extracellular secretions of live HN2-3, N1-4, M1-1, ST0, and M1-2 cells ($P > 0.05$). The N2-4 intracellular components showed significantly stronger DPPH radical scavenging effects than its extracellular supernatant ($P < 0.05$), while for dead M1-1 and ST0, there was no significant difference between the scavenging activity of the intracellular components and extracellular supernatant ($P > 0.05$). However, the DPPH radical scavenging rate of the extracellular supernatant from dead M1-2 cells was significantly higher than that of the intracellular components ($P < 0.05$). Overall, there were minimal differences in the scavenging capabilities of various components before and after inactivation.

These results highlighted the antioxidant capacities of LAB in terms of scavenging hydroxyl radical, superoxide anion radical, and DPPH radical. Moreover, they delineated the differences in antioxidant effects among different cellular fractions and between live and dead cells.

3.5 Correlation analysis between XO inhibition activity and antioxidant activity

The abovementioned findings collectively indicated that both dead and live suspensions of the six LAB strains possessed varying degrees of hydroxyl radical, superoxide anion radical, and DPPH radical scavenging capabilities. Sui et al.[14] demonstrated that the inhibitory strength of plant-derived XO inhibitors is positively correlated with their antioxidant activity. Therefore, the LAB strain N1-4, which exhibited excellent antioxidant capabilities across all measures, was selected for a correlation analysis between XO inhibition and antioxidant properties. As shown in Table 2, the XO inhibition activity of live N1-4 suspensions was significantly correlated with their hydroxyl radical, superoxide anion radical, and DPPH radical scavenging rates ($P < 0.05$ or $P < 0.01$). In contrast, in dead N1-4 suspensions, the XO inhibition activity was only significantly correlated with the hydroxyl radical scavenging rate ($P < 0.05$), and there was no significant correlation with superoxide anion radical and DPPH radical scavenging rates ($P > 0.05$). Furthermore, the antioxidant properties of the strains were independent of their viability.

4 Discussions

This study first measured the XO inhibition capacity of bacterial suspensions at different concentrations. The findings revealed that as the concentration of the bacterial strains increased, the increase in the XO inhibition rate slowed down, and some strains showed lower inhibitory action[16]. This could be due to the saturation of binding sites between the strains and the enzyme/substrate, leading to a limited improvement in XO inhibitory activity beyond a certain bacterial concentration.

LAB are highly prone to strain inactivation. For a long time, cell viability was considered critical for the health benefits of probiotics. However, this study revealed that even after the strains were inactivated, their inhibitory effects on XO were retained, with some strains even showing higher inhibition rates after inactivation[17]. Accumulating evidence indicates that health benefits can still be achieved in the absence of strain viability if sufficient quantities of non-living microorganisms or their components are administered to hosts. This has led to the emergence of a concept called "postbiotics", in which the non-living intact bodies of LAB and their metabolites and cell lysates are used to provide health benefits[18]. The use of microbe-derived metabolites or fragments (i.e., "postbiotics") is an appealing strategy for disease treatment and prevention. Therefore, this study further analyzed the inhibitory effects of the extracellular and intracellular components of LAB on XO to explore the specific components responsible for XO inhibition.

Extracellular secretions and metabolites contain substances such as extracellular polysaccharides, acids, and enzymes[19]. Most current studies suggest that the inhibitory mechanism of XO involves peptides. Thus, we speculate that the inhibition of XO by the extracellular secretions in this study may be due to the extracellular peptides or other substances. Additionally, other studies indicate that the inhibition of XO by LAB is primarily mediated by intracellular substances. In this study, in two strains of LAB, the inhibitory activities of extracellular and intracellular substances

Table 2 Relationship between the XO inhibitory activity and antioxidant activity of the N1-4.

Item	Live bacterial suspension			Dead bacterial suspension		
	Hydroxyl radical scavenging rate	Superoxide anion radical scavenging rate	DPPH radical scavenging rate	Hydroxyl radical scavenging rate	Superoxide anion radical scavenging rate	DPPH radical scavenging rate
XO inhibition rate of live bacteria suspension	0.874*	0.855*	0.958**			
XO inhibition rate of dead bacteria suspension				0.841*	0.467	−0.041

Note: *$P < 0.05$, **$P < 0.01$.

were comparable. Thus, in these strains, the intracellular substances played a similar role as the extracellular secretions in inhibiting XO, although the specific active components remain to be identified. Moreover, it is possible that the inhibition of XO by inactivated bacterial suspensions, intracellular substances, and extracellular substances was mediated by non-enzymatic substances[20–21].

Further investigation revealed that regardless of viability, the six LAB strains exhibited strong XO inhibition capabilities, and their cell suspensions, intracellular components, and extracellular secretions all possessed antioxidative activities. Previous research has indicated that LAB primarily exerts antioxidative effects through certain antioxidant enzymes such as reduced form of nicotinamide-adenine dinucleotide (NADH) oxidase, NADH peroxidase, and catalase[22]. In this study, even after the strains were inactivated, they still retained their antioxidative properties. Some researchers suggest that the antioxidative properties exhibited by bacteria after inactivation may be attributed to non-enzymatic antioxidative substances[23]. Hence, our findings broaden the potential applications of these strains[24]. This study did not conduct an in-depth investigation into the potential mechanisms of XO inhibition and antioxidant activity. These experiments will be included in future research.

Current evidence points to a correlation between XO inhibition and antioxidative effects[25]. Substances with antioxidative properties, such as VC, flavonoids, and *Perilla* leaf extracts[26], have been discovered to exert strong inhibitory effects on XO. Interestingly, previous research suggests that the excellent XO inhibition activity of oligomeric proanthocyanidins, caffeic acid, and chlorogenic acid may be attributed to the superior antioxidative activity of these molecules. Masuoka et al.[27] demonstrated that flavonoids primarily inhibit the generation of superoxide anion radical by inhibiting XO, thereby suppressing UA synthesis. The correlation analysis in the present study revealed a positive correlation between the XO inhibition activity of the live suspensions of the N1-4 strain and its antioxidative properties. However, the correlation between the XO inhibition activity and antioxidative properties was weaker in the suspensions of inactivated LAB strains, suggesting that other complex XO inhibitory mechanisms, such as competition with substrates for XO binding sites, may contribute to the XO inhibition mediated by inactivated strains.

5 Conclusion

This study evaluated the performance of 16 LAB strains in terms of XO inhibition and antioxidant activity. The results showed that the XO inhibition rates of live and dead cell suspensions of M1-2, HN2-3, N1-4, ST0, M1-1, and N2-4 strains were all around 65%, demonstrating strong inhibitory effects. Among them, intracellular materials of HN2-3, ST0, N2-4, N1-4, M1-2, and M1-1 showed significant inhibition effects on XO, while both the extracellular and intracellular materials of ST0 played a role in this process. Furthermore, both the extracellular and intracellular materials of live M1-2 bacteria also exhibited inhibitory effects, while dead bacteria mainly relied on extracellular materials for inhibition. The study also found that the cell suspensions, intracellular materials, and extracellular secretions of all six strains exhibited certain scavenging activities against hydroxyl radical, superoxide anion radical, and DPPH radical. Overall, the XO inhibitory activity of the LAB strains was positively correlated with their antioxidant properties, supporting their potential application in the treatment of hyperuricemia and gout. By comprehensively considering the effects of live and dead bacteria, and their various secretions, this study not only reveals the inhibition mechanisms of different strains but also provides a more refined screening basis for future functional applications of LAB.

Conflict of interest

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

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Authors contribution

Peixi Wang: Writing-original draft, methodology, investigation, conceptualization, methodology, data curation, conceptualization. Jingjing E: Methodology, investigation, conceptualization. Jing Li: Investigation, conceptualization. Qiaoling Zhang: Investigation, conceptualization, visualization, supervision, conceptualization. Yijian Zheng: Methodology, data curation, supervision, methodology. Wei Chen: Supervision, resources, formal analysis. Junguo Wang: Writing-review & editing, supervision, conceptualization.

Data availability

The data that has been used is confidential.

References

[1] Y. B. Cheng, Y. Li, et al., Hyperuricemia: does it matter for the progression from prehypertension to hypertension?, Hypertension 71(1) (2018) 66–67. https://doi.org/10.1161/hypertensionaha.117.10443.

[2] A. K. Mandal, D. B. Mount, The molecular physiology of uric acid homeostasis, Annu. Rev. Physiol. 77(1) (2015) 323–345. https://doi.org/10.1146/annurev-physiol-021113-170343.

[3] Y. Lee, P. Werlinger, J. W. Suh, et al., Potential probiotic *Lacticaseibacillus paracasei* MJM60396 prevents hyperuricemia in a multiple way by absorbing purine, suppressing xanthine oxidase and regulating urate excretion in mice, Microorganisms 10(5) (2022) 851. https://doi.org/10.3390/microorganisms10050851.

[4] J. Maiuolo, F. Oppedisano, S. Gratteri, et al., Regulation of uric acid metabolism and excretion, Int. J. Cardiol. 213 (2016) 8–14. https://doi.org/10.1016/j.ijcard.2015.08.109.

[5] C. X. Ni, X. Li, L. L. Wang, et al., Lactic acid bacteria strains relieve hyperuricaemia by suppressing xanthine oxidase activity via a short-chain fatty acid-dependent mechanism, Food Funct. 12(15) (2021) 7054–7067. https://doi.org/10.1039/D1FO00198A.

[6] Y. Wu, Z. Ye, P. Y. Feng, et al., *Limosilactobacillus fermentum* JL-3 isolated from "Jiangshui" ameliorates hyperuricemia by

degrading uric acid, Gut Microbes 13(1) (2021) 1897211. https://doi.org/10.1080/19490976.2021.1897211.

[7] H. Y. Zhao, Z. X. Lu, Y. J. Lu, et al., The potential of probiotics in the amelioration of hyperuricemia, Food Funct. 13(5) (2022) 2394–2414. https://doi.org/10.1039/D1FO03206B.

[8] Y. J. Li, J. Zhu, G. D. Lin, et al., Probiotic effects of *Lacticaseibacillus rhamnosus* 1155 and *Limosilactobacillus fermentum* 2644 on hyperuricemic rats, Front. Nutr. 9 (2022) 993951. https://doi.org/10.3389/fnut.2022.993951.

[9] Z. Zeng, J. Y. Luo, F. L. Zuo, et al., Screening for potential novel probiotic *Lactobacillus* strains based on high dipeptidyl peptidase IV and α-glucosidase inhibitory activity, J. Funct. Foods 20 (2016) 486–495. https://doi.org/10.1016/j.jff.2015.11.030.

[10] M. Umamaheswari, K. AsokKumar, A. Somasundaram, et al., Xanthine oxidase inhibitory activity of some Indian medical plants, J. Ethnopharmacol. 109(3) (2007) 547–551. https://doi.org/10.1016/j.jep.2006.08.020.

[11] X. Q. Wang, Z. Z. Cui, Y. Luo, et al., *In vitro* xanthine oxidase inhibitory and *in vivo* anti-hyperuricemic properties of sodium kaempferol-3′-sulfonate, Food Chem. Toxicol. 177 (2023) 113854. https://doi.org/10.1016/j.fct.2023.113854.

[12] M. Sekine, K. Okamoto, E. F. Pai, et al., Allopurinol and oxypurinol differ in their strength and mechanisms of inhibition of xanthine oxidoreductase, J. Biol. Chem. 299(9) (2023) 105189. https://doi.org/10.1016/j.jbc.2023.105189.

[13] V. Mohos, E. Fliszár-Nyúl, M. Poór, et al., Inhibition of xanthine oxidase-catalyzed xanthine and 6-mercaptopurine oxidation by flavonoid aglycones and some of their conjugates, Int. J. Mol. Sci. 21(9) (2020) 3256. https://doi.org/10.3390/ijms21093256.

[14] Y. Sui, J. B. Shi, S. Cai, et al., Metabolites of procyanidins from *Litchi chinensis* pericarp with xanthine oxidase inhibitory effect and antioxidant activity, Front. Nutr. 8 (2021) 676346. https://doi.org/10.3389/fnut.2021.676346.

[15] Q. H. Lu, B. Z. Liu, J. Lu, et al., Antioxidant and xanthine oxidase inhibitory theoretically evaluation of ferulic acid oligomers: tetramers as outstanding dual-functional candidates, Food Biosci. 74 (2025) 107784. https://doi.org/10.1016/j.fbio.2025.107784.

[16] Y. P. Meng, Y. S. Hu, M. Wei, et al., Amelioration of hyperuricemia by *Lactobacillus acidophilus* F02 with uric acid-lowering ability via modulation of NLRP3 inflammasome and gut microbiota homeostasis, J. Funct. Foods 111 (2023) 105903. https://doi.org/10.1016/j.jff.2023.105903.

[17] G. Vinderola, M. E. Sanders, S. Salminen, et al., The concept of postbiotics, Foods 11(8) (2022) 1077. https://doi.org/10.3390/foods11081077.

[18] B. Liang, D. M. Xing, The current and future perspectives of postbiotics, Probiotics Antimicrob. Proteins 15(6) (2023) 1626–1643. https://doi.org/10.1007/s12602-023-10045-x.

[19] J. Zhu, Y. J. Li, Z. G. Chen, et al., Screening of lactic acid bacteria strains with urate-lowering effect from fermented dairy products, J. Food Sci. 87(11) (2022) 5118–5127. https://doi.org/10.1111/1750-3841.16351.

[20] Q. Y. Li, C. C. Shi, M. Wang, et al., Tryptophan residue enhances *in vitro* walnut protein-derived peptides exerting xanthine oxidase inhibition and antioxidant activities, J. Funct. Foods 53 (2019) 276–285. https://doi.org/10.1016/j.jff.2018.11.024.

[21] W. W. He, G. W. Su, D. Sun-Waterhouse, et al., *In vivo* anti-hyperuricemic and xanthine oxidase inhibitory properties of tuna protein hydrolysates and its isolated fractions, Food Chem. 272 (2019) 453–461. https://doi.org/10.1016/j.foodchem.2018.08.057.

[22] A. Mehmood, A. Iftikhar, X. M. Chen, et al., Food-derived bioactive peptides with anti-hyperuricemic activity: a comprehensive review, Food Chem. 451 (2024) 139444. https://doi.org/10.1016/j.foodchem.2024.139444.

[23] W. Y. Li, L. E. Gao, W. K. Huang, et al., Antioxidant properties of lactic acid bacteria isolated from traditional fermented yak milk and their probiotic effects on the oxidative senescence of *Caenorhabditis elegans*, Food Funct. 13(6) (2022) 3690–3703. https://doi.org/10.1039/D1FO03538J.

[24] H. Y. Zhang, D. Wang, D. N. Li, et al., *Lactobacillus paracasei* N1115 alleviates hyperuricemia in mice: regulation of uric acid metabolism as well as its impact on gut microbiota and short-chain fatty acids, Front. Nutr. 12 (2025) 1651214. https://doi.org/10.3389/fnut.2025.1651214.

[25] M. D. Santi, M. Paulino Zunini, B. Vera, et al., Xanthine oxidase inhibitory activity of natural and hemisynthetic flavonoids from *Gardenia oudiepe* (Rubiaceae) *in vitro* and molecular docking studies, Eur. J. Med. Chem. 143 (2018) 577–582. https://doi.org/10.1016/j.ejmech.2017.11.071.

[26] T. Deguchi, Y. Hata, A. Tamai, et al., Inhibitory activity of Asana, heartwood of *Pterocarpus marsupium*, against xanthine oxidase, Nat. Prod. Commun. 14(12) (2019) 1934578X19887891. https://doi.org/10.1177/1934578X19887891.

[27] N. Masuoka, M. Matsuda, I. Kubo, et al., Characterisation of the antioxidant activity of flavonoids, Food Chem. 131(2) (2012) 541–545. https://doi.org/10.1016/j.foodchem.2011.09.020.