

Sporicidal activity mechanism of lactic acid and peracetic acid on bovine pathogenic *Bacillus cereus* spores

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Abstract

In recent years, the pathogenicity of *Bacillus cereus* (*B. cereus*) has increased significantly, its host adaptability has continued to expand, and the infection spectrum has also expanded. At the same time, food contamination by *B. cereus* is also common. Therefore, it is of practical significance to select green, safe and effective inhibitors. Lactic acid (LA) and peroxyacetic acid (PAA) possess good antibacterial properties, making them environmentally friendly, safe, and non-toxic. The purpose of this experiment was to preliminarily explore the inhibition mechanism of LA and PAA on *B. cereus* spores. The effects of LA and PAA on *B. cereus* spores were evaluated by measuring the MIC and MBC of LA and PAA inhibiting *B. cereus* spores, the effect of spore germination, the turbidity of spore suspension (OD₆₀₀), the release of macromolecules in spores, the content of ROS in spores, the release of DPA in spores and the hydrophobicity of spore surface. The results showed that the MIC and MBC of LA against spores were 0.315% and 40.375%, respectively, and the MIC and MBC of PAA against spores were 0.030% and 0.119%, respectively. Different concentrations of LA and PAA could decrease the spore germination rate, spore suspension turbidity, and spore hydrophobicity of *B. cereus*, and also increase the release of macromolecules in *B. cereus* spores, the content of spore reactive oxygen species (ROS), and the release of spore dipicolinic acid (2,6-pyridinedicarboxylic acid, DPA). The above results showed that LA and PAA could inactivate *B. cereus* spores by damaging the integrity of the cell membrane and cell wall of spores.

Keywords: *Bacillus cereus*, lactic acid, peracetic acid, spores

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Introduction

Bacillus cereus belongs to the genus *Bacillus* and is a Gram-positive rod-shaped bacterium. Because it can form spores, it shows strong adaptability and tolerance in the environment (Kim *et al.*, 2025). When the spores are exposed to nutrients, they will withdraw from the dormant state, germinate rapidly and resume growth (Wang *et al.*, 2019; Setlow and Christie, 2023; Gao *et al.*, 2023). The spore has a multi-layered structure. The inner layer of the spore is called the inner core. The inner core is surrounded by a low-permeability inner membrane, a germ cell wall, an extra peptidoglycan layer called the cortex, several layers of protein shells, and in some cases the outermost layer called the exospore (Delbrück *et al.*, 2021) (Fig. 1). Spores can increase the resistance of strains to various adverse external environments to reduce external damage to themselves. Therefore, it is of practical significance to effectively control and eliminate strain spores in food.

The antibacterial activity of organic acids is mainly derived from monocarboxylic acids (such as butyric acid, propionic acid, acetic acid, and formic acid) or hydroxyl-containing carboxylic acids (such as tartaric acid, citric acid, malic acid, and lactic acid) (Khan *et al.*, 2022). Its molecular weight, pKa value, properties (undissociated or dissociated), and specific antibacterial effects all affect the antibacterial effect of organic acids (Aljumaah *et al.*, 2020). The antibacterial activity of the acid also depends on its concentration and pH value (Chaveerach *et al.*, 2004). In addition to pathogens, organic acids can activate the host immune system, promote the production of bactericidal substances such as antimicrobial peptides, and increase the permeability of cell membranes by depolymerization of lipopolysaccharide, thereby inhibiting the growth of pathogens. Lactic acid has been certified by the U.S. Food and Drug Administration (FDA) as a safe and effective disinfection ingredient. At the same time, based on scientific evaluation, the European EFSA has also granted it the Qualified Presumption of Safety (QPS) qualification and recognized its safety (Tian *et al.*, 2022; Feng *et al.*, 2022; Rodríguez *et al.*, 2017; Koutsoumanis *et al.*, 2020). Qiao *et al.* (2008) pointed out that the antibacterial and bactericidal effects of lactic acid are mainly achieved by prolonging the metabolism of pathogenic bacteria and destroying the cell membrane potential. Other studies have shown that 1.5% milk combined with modified atmosphere packaging can effectively reduce the number of *B. cereus* bacteria in fresh-cut vegetables (Ahmad *et al.*, 2019). Combined treatment with LA and ultraviolet C (UV-C) inhibits *Salmonella* Enteritidis biofilms on food contact surfaces and chicken skin (Byun *et al.*, 2022). Lactic acid has a significant inhibitory effect on a variety of microorganisms. PAA is a strong oxidant formed by acetic acid and H₂O₂ under acidic conditions and can be used for disinfection, Sporicidal activity, bleaching and chemical synthesis. It is also a widely used organic peroxide. PAA was introduced into Urban Wastewater Treatment Plants (UWTP) and Drinking Water Treatment Plants (DWTP) as a promising disinfectant in the early 1980s (Sciscenko *et al.*, 2024). It has been

reported that the addition of PAA to strawberries or washing water can effectively reduce the artificially inoculated *L. innocua* and further reduce the cross-contamination in the cleaning step (Nicolau-Lapeña *et al.*, 2019). In addition, PAA can also effectively inhibit the number of *Listeria monocytogenes* on red mustard leaves and kale leaves (Lee *et al.*, 2020). Due to its strong oxidizing and acidic properties, PAA is often used as a broad-spectrum antibacterial agent for microbial control and can be used in many industries, such as medical and food (Li *et al.*, 2022; Wang *et al.*, 2023). The purpose of this study is to provide a theoretical basis for further study on the inhibition mechanism of LA and PAA on *B. cereus* spores and comprehensive prevention and control strategies, so as to fundamentally reduce the harm of the disease to animals and humans.

Materials and Methods

Strain origin: *B. cereus* was from the Veterinary Pathology Laboratory of Inner Mongolia Minzu University for Nationalities, and the GeneBank serial number was CP129005.1.

Main reagents: Manganese sulfate monohydrate (M9490), malachite green solution (G1630), 96-well plates (YZ-3599), DPA (PQ0050), LA (L8630), and TSB liquid medium (LA0110) were purchased from Solarbio. PAA disinfectant was purchased from Hangzhou Metso Biotechnology Co., Ltd.; alkaline phosphatase kit (item number: A059-2-2) was purchased from Nanjing Jiancheng Bioengineering Institute, reactive oxygen species detection kit (item number: S0033S) was purchased from Beyotime, n-hexadecane (item number: YZ-BW0110251) was purchased from Hongmeng.

Instrument and equipment: The thermostatic incubator was purchased from LAB-LINE; the microplate reader was purchased from Thermo Fisher; the upgraded laser scanning confocal system was purchased from Yongxing Optics; the high-speed centrifuge was purchased from Eppendorf; the high-pressure steam Sporicidal activity pot was purchased from BIOBASE; the microbalance was purchased from OHAUS; the three-hole electrothermal thermostatic water tank was purchased from Shanghai Yiheng Scientific Instrument Co., Ltd.; the ultra-clean bench was purchased from Suzhou Purification Equipment Co., Ltd.

Preparation of *B. cereus* spore suspension: 100 µL of *B. cereus* solution (OD₅₉₀ = 0.095) was added to the nutrient agar medium containing 0.05g / L manganese sulfate monohydrate and coated and then cultured at 37 °C for 7 d to induce the formation of spores. After the culture, the spores on the surface of the plate were scraped into a centrifuge tube containing sterile water using a sterile glass slide. Centrifuge the tubes at 4°C and 6000 rpm for 10min. After centrifugation, discard the supernatant, resuspend the cells in sterile water, and wash them three times until the OD₅₉₀ < 0.01 to thoroughly remove residual nutrient cell debris. Place the resulting sample in a water bath at 80°C for 15min

to kill the trophozoites, then centrifuge and wash twice more before resuspending in sterile water. After 15min, place a drop of the liquid on a microscope slide, stain with a 5% malachite green solution for 5min, wash with water, and counterstain with 0.5% eosin for 30s. If the stained area appears blue green under a microscope, the spore suspension has been successfully prepared. Simultaneously, 100μL of the suspension is spread onto a nutrient agar plate and incubated at 37°C for 24h to confirm the absence of colony growth, indicating that no live vegetative cells remain.

Determination of minimum spore inhibition concentration of LA and PAA on *B. cereus* spores: The minimum inhibitory concentration (Wu *et al.*, 2018) of LA and PAA against *B. cereus* spores was determined using a twofold dilution method. Add 10 μL of spore suspension and 190μL of TSB medium containing LA or PAA to a 96-well plate, adjust the final concentration of the suspension to OD₆₀₀ = 0.1, and ensure that the initial LA concentration in the wells is 8.5% (v/v) and the PAA concentration is 0.25% (v/v). Then perform serial twofold dilutions. The experiment was repeated three times. The sterile TSB was set as a blank control. Incubate at 37 °C for 20 h, and observe the growth of spores in TSB medium after incubation. In the clear and transparent wells of the TSB medium, the well with the lowest organic acid concentration represents the minimum inhibitory concentration (MIC) of LA or PAA against *B. cereus* spores.

Determination of the minimum spore-killing concentration of LA and PAA against *B. cereus* spores: The initial concentration of LA in the well was 85% (v/v), and the concentration of PAA was 0.5% (v/v). The minimum spore-killing concentration test was carried out, and all the clear medium in the 96-well plate of the minimum spore-killing concentration test was coated. After 20h of culture, the colony growth was observed. If there was no colony growth on the plate, the minimum concentration of organic acid contained in the culture medium was called the minimum spore killing concentration, and the experiment was repeated three times.

Effects of LA and PAA on the germination of *B. cereus* spores: Adjust the concentration of the spore suspension to OD₆₀₀ = 0.1. Add LA and PAA to the spore suspension to achieve final concentrations of 1/2 MIC, MIC, and 2 MIC. The control group receives no addition of LA or PAA. Incubate at 37°C for 20h. The number of spores after LA and PAA treatment was determined using the colony count method to calculate the relative spore survival rate. As shown in the formula:

$$\text{Relative survival rate (\%)} = \frac{\text{Number of spores in the experimental group}}{\text{Spore count in the control group}} \times 100 \%$$

In the formula, the spore count for the experimental group refers to the number of colonies (CFU) after 20 h of incubation following treatment with LA and PAA, while the spore count for the control group refers to the

total number of colonies (CFU) after 20 h of incubation in the control group without the addition of LA and PAA.

Determination of turbidity (OD₆₀₀) of *B. cereus* spore suspension: Add 190 μL of medium containing different concentrations of LA and PAA (MBC, MIC, 1/2 MIC) and 10 μL of spore suspension with OD₆₀₀ = 0.1 to a 96-well plate, mix thoroughly, Measure the OD₆₀₀ values (three replicates per group), and use the spore suspension without acid as the positive control and sterile water as the blank control.

Effects of LA and PAA on the release of macromolecules from *B. cereus* spores: In a *B. cereus* spore suspension (OD₆₀₀ = 0.1), LA and PAA were added at initial concentrations of 85% (v/v) and 0.5% (v/v), respectively. Different concentrations of LA and PAA were adjusted to achieve final concentrations corresponding to the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), with a control group containing no LA or PAA. Samples were collected at different time points (1 h, 2 h, 4 h, 6 h, 8 h), centrifuged, and the supernatant was measured for absorbance at 260 nm and 280 nm (three replicates per group).

Effects of LA and PAA on ROS content of *B. cereus* spores: 1mL of spore suspension (OD₆₀₀ = 0.1) was added to 19mL of LA, PAA (MIC, MBC) at different concentrations, and the control group was added to the same volume of medium, and cultured at 37°C, 180 rpm for 20h. Subsequently, the samples were treated according to the steps of the active oxygen detection kit, and the fluorescence intensity of the filtrate in each group was detected by laser confocal microscopy.

Effects of LA and PAA on the release of DPA from *B. cereus* spores: The prepared DPA standard solutions with different concentrations (10, 20, 40, 80, 120, 160, 200 μg/mL) were mixed with DPA colormetric reaction solution (100mL 0.50mol/L sodium acetate, 1 g ascorbic acid, 1 g ammonium ferrous sulfate, pH = 5.2) in a ratio of 4: 1 and stood for 1.5h. After 1.5h, the value of OD₄₄₀ was detected by a microplate reader, and the standard curve was drawn for DPA detection of subsequent samples (standard curve: $y = 570.21x - 24.825$, $R^2 = 0.9993$). The spore suspension treated under different conditions was centrifuged at 4°C, 9000 r/min for 15min. After centrifugation, the supernatant was mixed with the DPA chromogenic reaction solution in a ratio of 4:1, and the OD₄₄₀ was detected according to the same test steps above. The detected values were brought into the standard curve to further calculate the DPA release. At the same time, the DPA release amount calculated from the spore suspension treated at 121°C for 30min was set as a positive control, and the DPA leakage rate was calculated according to the following formula:

$$F = \frac{F_1 - F_2}{F_0} \times 100\%$$

In the formula, F₂ represents the concentration of DPA in the supernatant of different treatment groups,

$\mu\text{g/mL}$; f_1 represents the concentration of DPA in the supernatant of the untreated group, $\mu\text{g/mL}$; f_0 represents the concentration of DPA in the supernatant after 30min treatment at 121°C , $\mu\text{g/mL}$.

Effects of LA and PAA on the surface hydrophobicity of *B. cereus* spores: Mix 3mL of untreated (control group) and LA- and PAA-treated spore suspensions with 0.6mL of n-hexadecane, vortex for 20 seconds, and let it sit for 10min. The OD_{600} of the spore suspension in the control group was A_0 . The aqueous phase was measured again, and the OD_{600} was A_1 . Finally, the final results were calculated using the following formula.

$$\text{RHS}(\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

Data processing: Data analysis and graphing were performed using GraphPad Prism 5.

Results

Determination of MIC and MBC of LA and PAA against *B. cereus* spores: The minimum inhibitory

concentration (MIC) of LA and PAA against *B. cereus* spores was determined using a two-fold dilution method. As shown in Table 1, the MIC values of LA and PAA against *B. cereus* spores were 0.315% and 0.030%, respectively; the minimum bactericidal concentration (MBC) values of LA and PAA against *B. cereus* spores were 40.375% and 0.119%, respectively.

Effects of LA and PAA on spore germination of *B. cereus*: Under the inhibition of different concentrations of LA and PAA, the germination results of *B. cereus* spores were also different. As shown in Figure. 2, under 1/2 MIC treatment, there was no significant difference in spore germination rate between the LA and PAA treatment groups. However, under MIC and 2 MIC treatments, the spore germination rate after LA treatment was significantly higher than that of the PAA treatment group. With the increase in concentration, the germination rate of spores decreased continuously. The above results showed that organic acids (LA, PAA) at MIC and 2 MIC could significantly inhibit spore germination.

Table 1 MIC and MBC of LA and PAA on *B. cereus* bacterial cells.

Parameters	1/2 MIC	MIC	MBC
LA	0.630%	0.315%	40.375%
PAA	0.060%	0.030%	0.119%

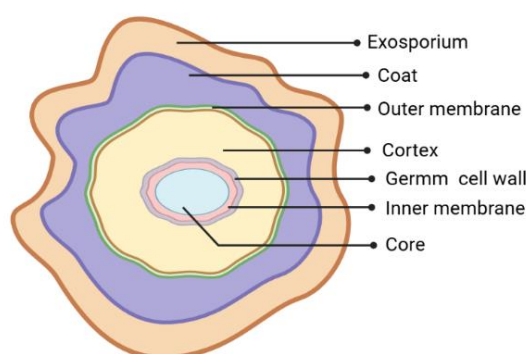


Figure 1 Schematic diagram of the structure of *Bacillus* spores.

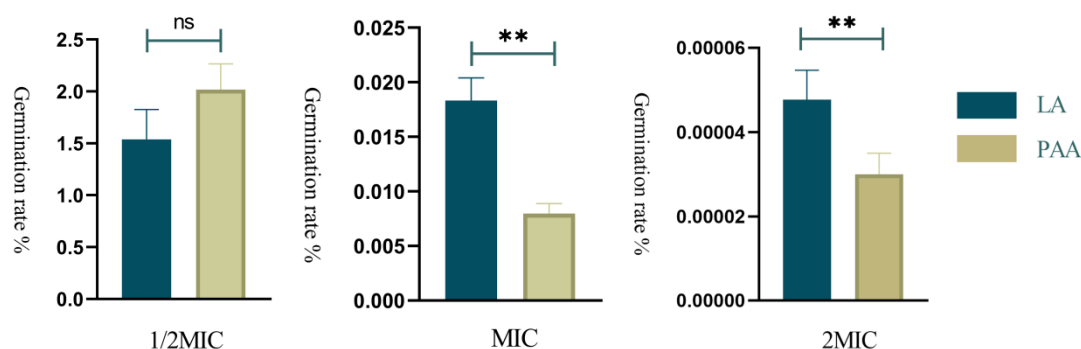


Figure 2 Effect of LA and PAA on the germination rate of *B. cereus* spores. Note: $P > 0.05$ is denoted as ns, $P < 0.05$ is denoted as *, $P < 0.01$ is denoted as **, $P < 0.001$ is denoted as ***, $P < 0.0001$ is denoted as ****.

Determination of turbidity (OD_{600}) of spore suspension of *B. cereus*: The degree of hydration of the spore core was negatively correlated with its refractive index and stress resistance (Li et al., 2024). The effect of the above treatments on the degree of hydration of *B. cereus* spore nuclei was detected by measuring the OD_{600} values of *B. cereus* spore suspensions before and after treatment with LA and PAA at different concentrations (1/2 MIC, MIC, MBC). It can be seen from Figure. 3 that the OD_{600} value of the untreated sample was 0.59. The OD_{600} value decreased significantly after LA and PAA treatment, and the increase in concentration was linear with the OD_{600} value. The higher the concentration, the smaller the OD_{600} value. In addition, the OD_{600} value of the 1/2 MIC PAA treatment group was higher than that of the 1/2 MIC LA treatment group; however, the OD_{600} value of the PAA group was lower than that of the LA group at the MIC concentration. In the MBC group, there was no significant difference between the two organic acid groups. This indicates that LA and PAA can inhibit the growth of *B. cereus* and even kill spores by increasing the degree of hydration of the spore core.

Effects of LA and PAA on the release of macromolecules from *B. cereus* spores: The effect of LA and PAA on the spore macromolecules of *B. cereus* was similar to that on the vegetative cells of the bacteria. It can be seen from Figure. 4 that the OD_{260} and OD_{280} values of the control group were low, but the OD_{260} and OD_{280} values of the spore suspension treated with LA and PAA increased significantly, and this increase was proportional to the increase in the action time and concentration. The above results indicate that LA and PAA have a significant detrimental effect on the permeability and integrity of each layer of spores, resulting in a substantial leakage of nucleic acids and proteins, which in turn affects the normal growth of spores.

Effects of LA and PAA on ROS content in *B. cereus* spores: This experiment uses the DCFH-DA fluorescent probe, which is inherently non-fluorescent and can freely cross the cell membrane, to detect ROS levels. Once DCFH-DA enters the cell, it is hydrolyzed by intracellular esterases into DCFH. Since DCFH cannot cross the cell membrane, the probe is readily transported into the cell. Intracellular reactive oxygen species (ROS) oxidize the non-fluorescent DCFH to produce fluorescent DCF. The level of intracellular ROS is determined by measuring the fluorescence of DCF. The effects of LA and PAA on the reactive oxygen species activity of *B. cereus* endospores were consistent with their effects on the vegetative cells of *B. cereus*. As

shown in Figure. 5, the control group exhibited minimal green fluorescence; the MIC LA and MIC PAA groups showed a significant increase in green fluorescence; and the MBC LA and MBC PAA groups exhibited the highest levels of green fluorescence.

Effects of LA and PAA on DPA release from *B. cereus* spores: DPA is a unique indicator biomarker of bacterial spores, which is the main factor affecting the hygrothermal resistance of spores, and its own release will reduce the resistance of spores (Baloh and Sorg, 2022). Therefore, the resistance of *B. cereus* spores to LA and PAA was evaluated by measuring the release of DPA. As shown in Figure. 6, the DPA concentration in the spore supernatant of the control group was 0.006 $\mu\text{g/mL}$, with a leakage rate of 13.85%; after treatment with MIC LA and MIC PAA, the DPA concentrations in the spore supernatants were 0.018 $\mu\text{g/mL}$ and 0.024 $\mu\text{g/mL}$, respectively, with leakage rates of 40.72% and 52.96%, respectively; After treatment with MBC LA and MBC PAA, the DPA concentrations in the spore supernatants were 0.028 $\mu\text{g/mL}$ and 0.028 $\mu\text{g/mL}$, respectively, with leakage rates of 62.21% and 62.71%, respectively; the absolute DPA content in the positive control was 0.0447 $\mu\text{g/mL}$. As shown by the data, in the MIC treatment group, PAA had a greater effect on the release of DPA from *B. cereus* spores than LA; however, in the MBC group, there was no significant difference in the effects of the two acids on spore DPA. Additionally, under the same acid conditions, the higher the acid concentration, the greater the effect on spore release. This indicates that LA and PAA can effectively reduce the resistance of *B. cereus* spores, leading to spore damage and inactivation.

Effects of LA and PAA on the surface hydrophobicity of *B. cereus* spores: It can be seen from Figure. 7 that the surface hydrophobicity of *B. cereus* in the control group was 49.36%. The surface hydrophobicity of spores treated with MIC LA and MIC PAA decreased significantly ($P < 0.05$), and the surface of spores changed from hydrophobic to hydrophilic. The value of the MIC LA group was higher than that of the MIC PAA group, which was 27.00% and 20.64%, respectively. In the MBC group, there was no difference in the RHS (%) values of the two organic acids (LA was 13.10%, PAA was 12.50%). The results showed that the effect of PAA on the hydrophobicity of spores at the MIC concentration was greater than that of LA. At the same time, with the increase in acid concentration, the effect on the hydrophobicity of the spore surface was also enhanced.

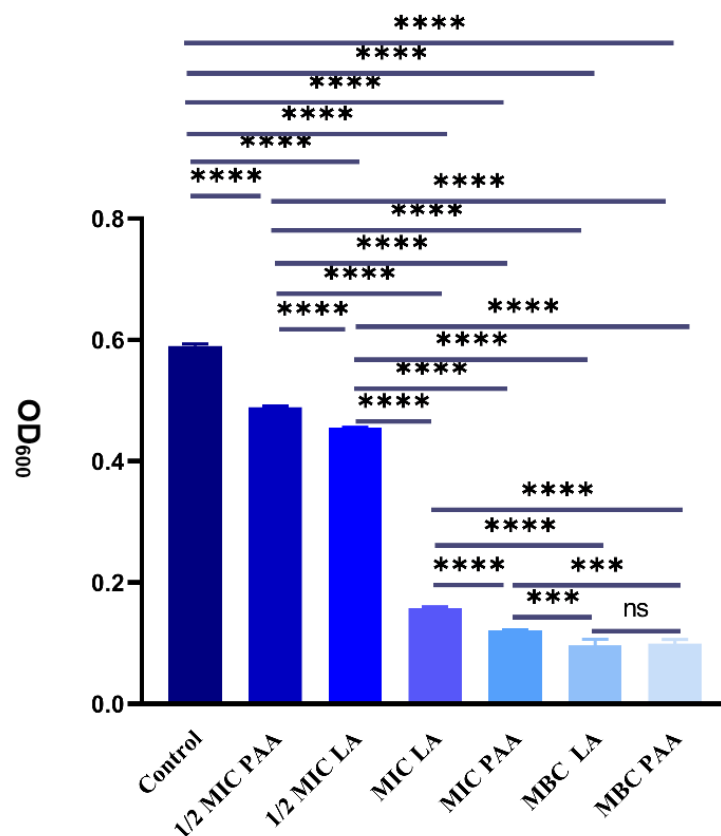


Figure 3 Changes in turbidity of *B. cereus* spore suspensions after LA and PAA treatment. Note: $P > 0.05$ is denoted as ns, $P < 0.05$ is denoted as *, $P < 0.01$ is denoted as **, $P < 0.001$ is denoted as ***, $P < 0.0001$ is denoted as ****.

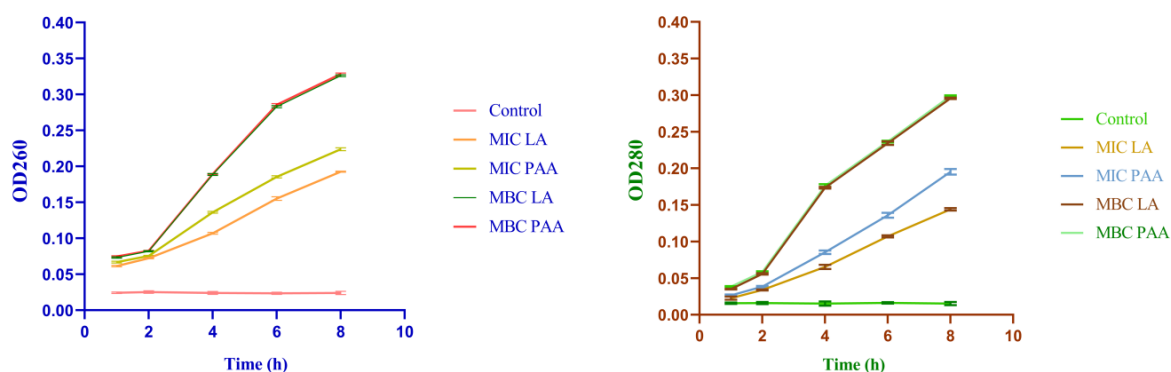


Figure 4 Nucleic acid and protein leakage from *B. cereus* spores after LA and PAA treatment.

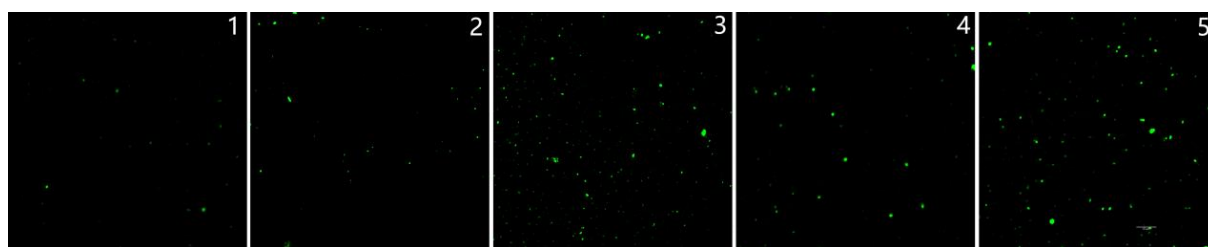


Figure 5 Effect of LA and PAA on ROS of *B. cereus* spores. Note: 1 is the control group, 2 is the MIC LA group, 3 is the MBC LA group, 4 is the MIC PAA group, and 5 is the MBC PAA group.

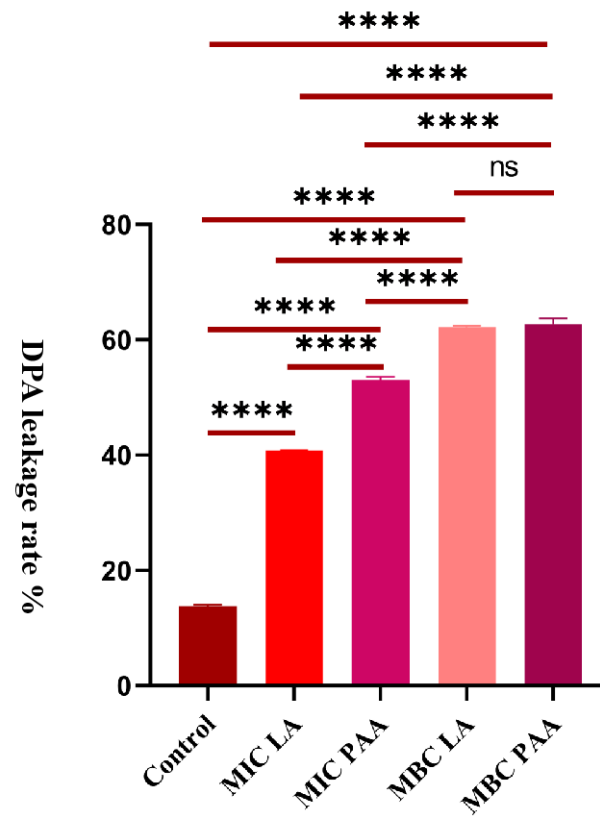


Figure 6 Changes in the leakage rate of budding DPA after LA and PAA treatments. Note: $P > 0.05$ is denoted as ns, $P < 0.05$ is denoted as *, $P < 0.01$ is denoted as **, $P < 0.001$ is denoted as ***, $P < 0.0001$ is denoted as ****.

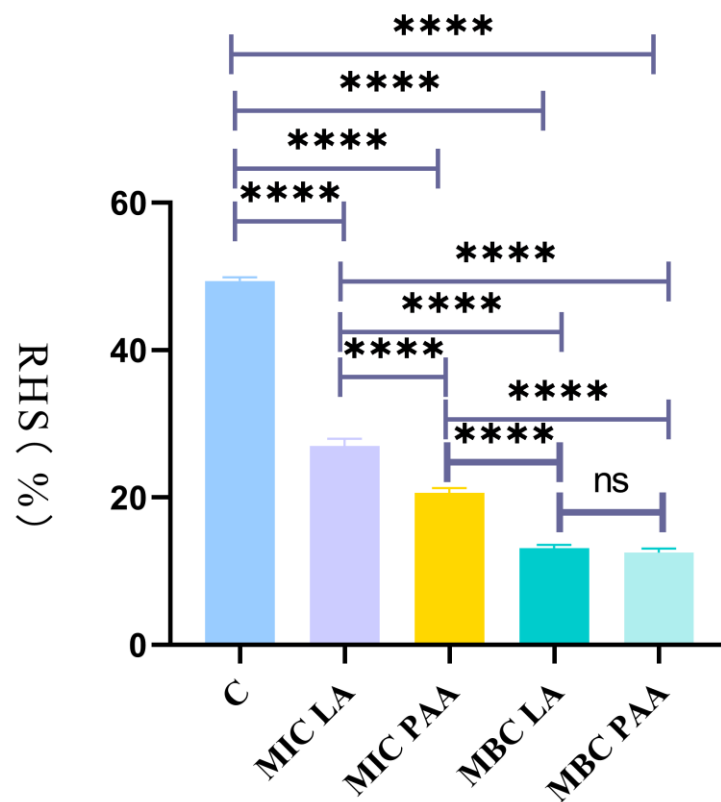


Figure 7 Effect of LA and PAA on the hydrophobicity of *B. cereus* spores. Note: $P > 0.05$ is denoted as ns, $P < 0.05$ is denoted as *, $P < 0.01$ is denoted as **, $P < 0.001$ is denoted as ***, $P < 0.0001$ is denoted as ****.

Discussion

Organic acids refer to a class of organic compounds containing carboxyl groups (-COOH) in the molecule. These carboxyl groups can release hydrogen ions (H⁺), making organic acids acidic (Wu *et al.*, 2025). The carboxyl group is the main functional group of organic acids, which determines the acidic properties of organic acids. In addition, organic acids can inhibit the growth of bacteria by reducing the pH value and exert antibacterial effects by penetrating the cell membrane and changing the concentration of protons and anions in the cytoplasm. This effect is affected by pH value, concentration, and specific organic acids (Bushell *et al.*, 2019; Qiu *et al.*, 2021; Zhang *et al.*, 2024). LA and PAA are organic acid members, which not only have a good bacteriostatic effect, but also have high safety. LA can be used to control the growth of microorganisms in the food industry, such as against *Salmonella* in beef (Man *et al.*, 2025). PAA, as an environmentally friendly strong oxidizing organic peroxide, can be rapidly degraded into harmless substances. Therefore, it is of practical significance to use these two safe and antibacterial organic acids (LA, PAA) to explore their antibacterial and bactericidal mechanisms against *B. cereus*.

In this experiment, LA and PAA could inhibit *B. cereus* spores, and the inhibition effect was different with the change of time and organic acid concentration. The results of this experiment showed that the germination rate of spores in spore suspensions treated with different concentrations of LA and PAA was significantly reduced, and the higher the concentration of organic acid, the lower the spore germination rate. Not only that, but other experiments also showed similar results, with the increase of concentration inhibition effect is more obvious. In the spore turbidity test and macromolecular substance release test, when the concentrations of LA and PAA increased, the spore turbidity decreased significantly, and the release of macromolecular substances increased. The reason for this result may be that LA and PAA damaged the inner membrane of the spores, disrupting their permeability, thereby inhibiting spore growth and even causing their death. Intracellular ROS levels increased significantly following LA and PAA treatment, suggesting that ROS may be involved in the bactericidal process. Lu *et al.* (2025) investigated the antibacterial effect of rosmarinic acid in sourdough bread dough containing *B. cereus*. The results showed that rosmarinic acid could increase the content of reactive oxygen species (ROS) within cells. This result was consistent with the findings of this study. DPA is a spore-specific substance. DPA leaks when the spore germinates or the inner core structure is damaged. Lin *et al.* (2023) used slightly acidic electrolyzed water (SAEW) to treat the spore suspension of *Clostridium perfringens* and tested the release rate of spore DPA. The test results showed that with the continuous increase of SAEW mass concentration, the spore core substance DPA was released in large quantities. The results are consistent with the results of this experiment. In this experiment, the DPA leakage rate increased significantly after *B. cereus* spores were treated with LA and PAA at

different concentrations. This indicates that LA and PAA can destroy the inner membrane of *B. cereus* spores, thereby increasing the permeability of the inner membrane and leading to a further increase in DPA release, thereby reducing the resistance of spores and inactivating them. Surface hydrophobicity is one of the key factors determining spore adhesion. As shown by the experimental results, the hydrophobicity of spores treated with LA and PAA significantly decreased, suggesting that LA and PAA may reduce their adhesion capacity by affecting the surface hydrophobicity of *B. cereus* spores.

In general, under the same acid, different concentrations of LA and PAA had significant inhibitory effects on *B. cereus* spores, and the degree of inhibition was positively correlated with the concentration of acid. The higher the concentration of acid, the better the inhibitory effect. MIC is the minimum inhibitory concentration, which only inhibits bacterial growth; MBC is the minimum bactericidal concentration that can kill bacteria. When treating severe infections such as mastitis, relying solely on MIC may underestimate the effectiveness of medication. Combining MBC can more scientifically assess the bactericidal ability of drugs, guide rational drug use, improve the success rate of infection control, and reduce the development of drug resistance. This not only provides a guarantee for the safety of the food industry, but also effectively prolongs the shelf life of food and replaces traditional disinfectants, acting on food packaging, fruit and vegetable surfaces, and other scenarios. *B. cereus* has a strong ability to form spores and resist the environment. It is an important pathogen causing environmental mastitis in dairy cows and can also contaminate raw milk and the infrastructure of dairy farms. By strictly implementing comprehensive control measures such as thorough disinfection of teats before and after milking, standardized on-site cleaning and disinfection procedures for milking equipment, maintaining dry and clean cowsheds, regularly changing bedding, and strengthening terminal disinfection of the environment, spore contamination can be effectively suppressed, significantly reducing the incidence of mastitis, thereby ensuring the health of dairy cows and the hygiene and safety of raw milk.

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Writing - original draft. **Haiying Zheng**: Formal analysis, Writing - original draft. **Lanlan Liu**: Formal analysis, Writing - original draft. **Wei Liu**: Formal analysis, Writing - original draft. **Mingshan Fu**: Conceptualization, Investigation, Writing - review & editing. **Weijuan Jia**: Conceptualization, Investigation, Writing - review & editing. **Zelin Jia**: Conceptualization, Investigation, Writing - review & editing. **Jiayu Cui**: Conceptualization, Investigation, Writing - review & editing. **Yuhang Zhang**: Conceptualization, Investigation, Writing - review & editing. **Huisheng Xiong**: Conceptualization, Investigation, Writing - review & editing. **Yujie Ma**: Conceptualization, Investigation, Writing - review & editing. **Xueli Wang**: Conceptualization, Writing - review & editing. **Haifeng Wang**: Conceptualization, Writing - review & editing.

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