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Research Article

Study of Antibacterial Properties of Cinnamaldehyde against *Aeromonas hydrophila*

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Antibacterial properties of cinnamaldehyde against *Aeromonas hydrophila* were assayed in this study. To investigate the action mechanisms of cinnamaldehyde against *A. hydrophila*, we examined the antibacterial activity, bacterial membrane permeability, and ultrastructure of *A. hydrophila* cells treated with cinnamaldehyde. The results showed that the minimum inhibitory concentration (MIC) value of cinnamaldehyde against *A. hydrophila* NJ-35 was found to be 0.039 mg/mL. The trends of the growth curve of *A. hydrophila* treated with different concentrations of cinnamaldehyde (from 1/4 MIC to 2 MIC) were different, and there was a significant difference in the growth curve of different groups of treatment. There were significant differences in the K^+ concentration among all treatment groups from 1 h to 5 h after incubation compared with that of the control. The highest K^+ concentration was observed in the 1 MIC group of cinnamaldehyde. The ultrastructure of *A. hydrophila* cells treated with cinnamaldehyde was destroyed, and the morphology changed. These results indicated that cinnamaldehyde could inhibit the growth of *A. hydrophila*, increase bacterial membrane permeability, and damage cell membrane integrity, resulting in leakage of the *A. hydrophila* cell contents.

1. Introduction

China is one of the countries with the longest history of aquaculture in the world. Over the past decade, China has accounted for more than 60% of global aquaculture, which is still in development. However, there are so many kinds of fish diseases caused by the pathogen [1], such as bacteria [2], including motile aeromonad septicemia in carp, tilapia, perch, catfish, and salmon; red sore disease in bass and carp; ulcerative infections such as epizootic ulcerative syndrome in catfish, cod, carp, and goby [3], which have been caused a huge economic loss in aquaculture [4]. Among many invasive diseases, bacterial diseases are more serious to aquatic animals [5], around 6 million dollars are lost in aquaculture annually due to infectious diseases, bacterial infections account for half of the deaths of aquatic animals [6], and one of the main pathogens causing the occurrence of diseases is *Aeromonas* [7].

Aeromonas hydrophila is a gram-negative short bacillus, a thermophilic and motional *Aeromonas* [8], which is widely distributed in various water bodies, soil, body surface of aquatic animals, and digestive tract in nature, causing great harm [9]. *A. hydrophila* is an important bacterial pathogen and is associated with several fish diseases, such as hemorrhagic septicemia, fin and tail rot, and epizootic ulcerative syndrome [10, 11]. These diseases have caused high mortality in freshwater fish resulting in extensive losses around the world [12]. Antibiotic and chemotherapeutics are used to

control these diseases and result in the development of drug-resistant bacteria, environmental pollution, and residues in fish. With increasing the demand for organic aquaculture, there has been growing interest in using plant extract [13, 14] in aquaculture to prevent diseases of their lesser side effects than antibiotics.

Cinnamaldehyde is the main active ingredient of cinnamon [15]. Its molecular formula is $C_6H_5CH=CHCHO$, and it is an aromatic compound with an aldehyde group. Both natural and synthetic cinnamaldehyde have trans-structures, as shown in Figure 1 [16]. Cinnamaldehyde is a yellow oily liquid with a cinnamon odor and sweet taste [17], which is difficult to dissolve in water, glycerin, and easily soluble in ethanol and ether. Cinnamaldehyde has the advantages of a fast metabolism, no residue, no environmental pollution, and less susceptible to drug resistance. It also has analgesic [18], antibacterial [19, 20], and antioxidant [21, 22] effects and is considered a green and environmentally friendly feed additive. For example, cinnamaldehyde has a strong antagonistic effect against *Escherichia coli* [23], *Candida Albicans* and *Candida Glabrata* [24], *Mycobacterium tuberculosis* [25], and *Streptococcus mutans* [26]. An earlier study by Nogueira et al. [27] indicated that after cinnamaldehyde treatment, the structural changes of *Staphylococcus aureus* and *Escherichia coli* cells were observed, which induced the disruption of cell membrane integrity. Faleye et al. [28] reported that cinnamaldehyde and some of its derivatives eliminated *V. parahaemolyticus* and exhibited similar antimicrobial and antibiofilm activities against *Vibrio harveyi*.

According to the Food and Drug Administration (FDA), cinnamaldehyde is a safe food additive and can be used as a preservative to inhibit food-borne pathogens [29]. Under the circumstance of the prohibition of adding antibiotics, the research and development of environment-friendly functional aquatic compound feed additives are gradually becoming a research hotspot [30]. Therefore, it is very necessary to study the antifungal agents with strong antibacterial effects and small side effects [31]. The purpose of this study was to determine the antibacterial mechanism of cinnamaldehyde against *A. hydrophila* and to provide a theoretical basis for the application of cinnamaldehyde in aquaculture.

2. Materials and Methods

2.1. Microorganisms and Reagents. *A. hydrophila* NJ-35 was obtained from Nanjing Agricultural University. The cinnamaldehyde product was obtained from Yangzhidao Feed Co., Ltd. (Jiangsu, China). The composition and content of cinnamaldehyde were cinnamaldehyde ≥40%; thymol ≥5%; carvacrol ≥15% (the main component is cinnamaldehyde). The LB medium was formulated according to the formula of 5 g/L yeast extract, 10 g/L peptone, and 5 g/L sodium chloride.

2.2. Antibacterial Activity. According to the reference of Naghmouchi et al. [32], the antimicrobial activities of cinnamaldehyde were determined by using a twofold microdilution broth method. *A. hydrophila* NJ-35 was grown to midlog phase in LB medium at 28°C for 16 h. Sterile PBS solution was used, and the bacterial suspension was corrected to a concentration of about 1×10^6 CFU/mL. In a sterile 96-well cell culture plate, cinnamaldehyde was gradient diluted with LB medium, and the final concentrations were 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.782, 0.391, 0 μg/mL, respectively. 5 μL of bacterial suspension was added to each well, and the culture medium with bacterial suspension was used as a positive control. After incubation at 37°C for 24 h, it was taken out for observation, and the minimum concentration of cinnamaldehyde was MIC value. The experiment was three replications.

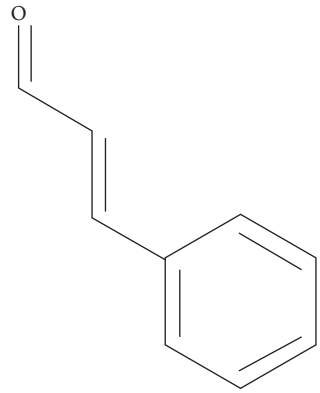

Figure 1: Structure of cinnamaldehyde.

2.3. Bacteriostatic Zone. According to the reference of Kassaw et al. [33], *A. hydrophila* NJ-35 was cultured to the log phase and diluted to 1×10^6 CFU/mL with a sterile PBS solution. Under aseptic conditions, 100 μL of *A. hydrophila* solution was absorbed on LB nutritional agar plate, then evenly coated with a cotter (alcohol burning) on the plate, and then drilled with a hole punch.

The cinnamaldehyde was treated with three temperature gradients: room temperature (T), 80°C (T1), and 110°C (T2). The heating time of 80°C and 110°C was set as 20 min, 40 min, 80 min, and 120 min, respectively. Then, 100 μL cinnamaldehyde was added to the test well, and the concentrations were 4, 2, 1, and 0.5 mg/mL, respectively. 100 μL PBS solution was added to the well for the negative control. The plate was put into a constant temperature incubator and cultured at 28°C for 24 h. Then, the diameter of the inhibition zone was measured at each concentration and photographed. The experiment was repeated three times, and the data were averaged.

2.4. Growth Curve. According to the reference of Zhang et al. [34], A. h*ydrophila* NJ-35 was grown to the log phase at 28°C for 16 h. The bacterial suspension was made to a final concentration of 1×10^6 CFU/mL, and the bacteria were inoculated into 100 mL LB liquid medium with 1/4 MIC, 1/2 MIC, 1 MIC, and 2 MIC cinnamaldehyde at a 1% ratio. LB liquid medium without drugs was used as the control group, and the medium was incubated at 28°C and 180 r/min on

a shaking table. Samples were taken every 1h, the sample volume was 1 mL, and the OD value at 600 nm was determined under a 745 UV/VIS spectrometer. The experiment was repeated three times.

2.5. Bacterial Membrane Permeability. According to the reference of Lu et al. [35], *A. hydrophila* NJ-35 was incubated at 28°C for the log phase, and then, the bacterial cells were collected and finally resuspended with 1 mL sterile deionized water. Cinnamaldehyde was added into the bacterial suspension (with the final concentration of 1/2 MIC and 1 MIC cinnamaldehyde) and cultured in a shaking table at 28°C and 180 /min for different times, and ultrapure water was in the control group. Samples were taken every 1h and centrifuged at 3000 r/min for 5 min, and the supernatant was absorbed by an atomic absorption spectrometer (Spectr AA 220; VARIAN, USA) and detected the concentration of K^+. The experiment was repeated three times.

2.6. DNA Extravasation. According to the reference of Zhan et al. [36], *A. hydrophila* NJ-35 was cultured at 28°C for 16 h and centrifuged at 3000 r/min for 3 minutes, and the bacteria were collected and resuspended in a sterile PBS solution so that the final concentration was about 1×10^6 CFU/mL. Cinnamaldehyde with a final concentration of 1/2 MIC and 1MIC was added, and the control group was treated with the same volume of tylene-20. Samples were taken after 5 h of culture, the supernatant was collected by centrifugation (4500 r/min, 4°C, 10 min), and the DNA content in the supernatant was determined by NanoDrop One, a microspectrophotometer.

2.7. Crystal Violet Biofilm Adhesion Test. According to the reference of Li et al. [37], *A. hydrophila* NJ-35 was cultured at 28°C to the log phase. The *A. hydrophila* was diluted to a concentration of 1×10^6 CFU/mL, and 200 μL was added to each well in the 96-well plate and cultured at 28°C for 48 h. After incubation, the supernatant was sucked up and cleaned gently with sterile PBS buffer, then 1/2 MIC and 1 MIC cinnamaldehyde were added to the culture medium, and the culture medium was in the control group at 28°C for 24 h. The supernatant was removed, washed 3 times with PBS buffer, 1% crystal violet was added, stained at 37°C for 15 min, and then, the floating color was washed with sterile PBS solution, placed in a ventilated place, and photographed after the membrane dried, adding 200 μL 33% glacial acetic acid to each well to dissolve. Using a microplate reader, the OD value was detected and recorded at 590 nm [38], with repeated determination three times.

2.8. Dyeing Test

2.8.1. DAPI Staining. According to the reference of Chazotte [39], *A. hydrophila* NJ-35 was cultured at 28°C to the late log phase, centrifuged and collected, and suspended with sterile PBS solution, and 1/2 MIC and 1 MIC concentrations of cinnamaldehyde were added, respectively. Staining solution with three times the volume of the sample was stained, mixed, and incubated at room temperature for 5–10 minutes, then wash 2–3 times with sterile PBS solution for 3–5 minutes each time, then the DAPI dye was removed, washed with sterile PBS solution for 2–3 times, 3–5 minutes each time. After washing out the uncombined DAPI, the bacteria showed blue fluorescence. The fluorescence microscopy with excitation wavelength of 360 nm and emission wavelength of 460 nm was used.

2.8.2. PI Staining. According to the reference of Watts [40], *A. hydrophila* NJ-35 was cultured to the late log phase, and 1/2 MIC and 1 MIC concentrations of cinnamaldehyde were added, respectively, and cultured at 28°C for 4 h. 10 mL samples were taken from each group, centrifuged at 5000 r/min for 3 min, the supernatant was removed, and the cells were fully cleaned with 1 × Assay Buffer for 2-3 times. The cell suspension was prepared with 1 × Assay Buffer, and its density was 1×10^5–1×10^6 cells/mL. 100 μL staining solution was added into 200 μL cell suspension, mixed well, and incubated at 37°C for 15 min. Then, 5 μL drops were absorbed on the slide and observed by fluorescence microscope. Dead bacteria were stained by PI and showed red fluorescence.

2.9. Ultrastructure Analysis. According to the reference of [41], *A. hydrophila* NJ-35 was grown to log phase in LB broth, 1/2 MIC and 1 MIC concentrations of cinnamaldehyde were added to the bacterial, and the control group was not added. At 28°C, 180 r/min, the solution was cultured for 4 h in a shaking table. 10 mL samples were taken from each group and centrifuged at 3000 r/min for 3 minutes, and the thallus was collected, washed twice with sterile PBS solution, then fixed with 2.5% glutaraldehyde buffer, sealed and preserved with sealing membrane, and sent to Max Biotechnology Co., LTD. (Wuhan, China) for detection, and the ultrastructure of bacterial cells was observed by transmission electron microscopy.

2.10. The Radical Scavenging Ability. According to the reference of Singh et al. [42], cinnamaldehyde was treated with three temperature gradients at room temperature (T), 80°C (T1), and 110°C (T2), and each temperature gradient was configured with four concentrations of 100%, 50%, 20%, and 10%, respectively. Solarbio DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability or hydroxyl radical scavenging ability was detected by the commercial test kit (BC4755) or commercial test kit (A018-1-1) from Nanjing Jiancheng Bioengineering Institute P.R. China (Nanjing, China). The experiment was repeated three times.

2.11. Data Statistics and Analysis. All data are presented as means ± SEM (standard error of the mean). SPSS Statistics 26.0 was used to analyze the experimental data. Among them, Figure2(c) the inhibition zone diameter and Figure 3 the free radical scavenging ability were analyzed by two-way ANOVA, and Figure 4 the growth curve, Figure 2(b) the

inhibition zone diameter, Figure 5 concentration of K^+ and DNA extravasation, and Figure 6 biofilm were analyzed by one-way ANOVA. Duncan's test was used for multiple comparisons, and the level of significant difference was set at $P < 0.05$.

3. Results

3.1. Antibacterial Activity of Cinnamaldehyde. The MIC value of cinnamaldehyde against *A. hydrophila* was 0.039 mg/mL. Figure 4 shows that the bacteria in the control and 1/4 MIC groups showed logarithmic growth in the early stage of incubation and reached a stable period of bacterial growth at 16 h. The 1/2 MIC group reached a logarithmic growth period by 16 h and stabilized at 24 h. The 1 MIC and 2 MIC groups both made the OD value trend horizontal, and the bacteria could not grow, and there were significant differences ($P < 0.05$) between different groups at 16 h, indicating that the growth of *A. hydrophila* was inhibited by the addition of cinnamaldehyde in different concentrations. Cinnamaldehyde with the concentration of 1 MIC and 2 MIC could completely kill *A. hydrophila*, and cinnamaldehyde with the concentration of 1/2 MIC had a certain antibacterial effect, and cinnamaldehyde with the concentration of 1/4 MIC basically did not inhibit the growth of *A. hydrophila*, but the overall growth level of *A. hydrophila* was lower than that of the control group.

3.2. Diameter of Bacteriostatic Zone. Figure 2(a) indicated that cinnamaldehyde could produce an obvious bacteriostatic zone against *A. hydrophila*. As shown in Figure 2(b), the diameter of the inhibition zone is positively correlated with the concentration of cinnamaldehyde, and *A. hydrophila* is highly sensitive to cinnamaldehyde concentrations of 2 mg/mL and 4 mg/mL.

As shown in Figure 2(c), there was no significant difference in the diameter of the antibacterial zone at different temperatures and heating times ($P > 0.05$), which indicated that high temperature would not affect the antibacterial effect of cinnamaldehyde.

3.3. Bacterial Membrane Permeability. Figure 5(a) shows that a significant potassium efflux from bacteria cells was induced after incubation, and the K^+ efflux increased with increasing incubation time from 0 to 1 h, and when the incubation time was increased further, only slight changes were observed. There was a significant difference ($P < 0.05$) in the K^+ concentration of bacteria cells between 1MIC group and control group after 1–5 h incubation. In addition, the K^+ concentration of *A. hydrophila* cells treated with 1 MIC cinnamaldehyde was significantly ($P < 0.05$) higher than that of *A. hydrophila* cells treated with 1/2 MIC cinnamaldehyde after 1–5 h incubation. In a word, the membrane permeability of *A. hydrophila* cells treated with cinnamaldehyde was 1 MIC group >1/2 MIC group > control group.

As shown in Figure 5(b), at the same incubation time, the extracellular DNA content of cinnamaldehyde treatment groups was significantly higher than that of the control group ($P < 0.05$), and the amount of DNA exosmosis of *A. hydrophila* was 1 MIC group >1/2 MIC group > control group.

Figure 6(a) shows that the biofilm of the control group was relatively intact and attached to the bottom of the 96-well plate. After *A. hydrophila* was treated with different concentrations of cinnamaldehyde, it could be observed that the biofilm attached to the bottom gradually decreases until it disappeared completely with the increase of concentration. Absorbance at 590 nm was measured, as shown in Figure 6(b), the amount of biofilm decreased significantly compared with the control group ($P < 0.05$) at concentration of 1 MIC, and the clearance rate of biofilm reached about 66% ($P < 0.05$) at concentration of 4 MIC.

As shown in Figure 7(a), the blue fluorescence density in the control group was higher, while in the experimental group, the fluorescence density gradually decreased with the increase of cinnamaldehyde concentration, indicating a decrease in the number of living cells. Figure 7(b) shows that with the increase of cinnamaldehyde concentration, the red fluorescence density increased significantly, indicating an increase in the number of dead cells.

3.4. Transmission Electron Microscopy. As shown in Figure 8, untreated *A. hydrophila* cells in the control group remained intact and showed a smooth surface, and it was evenly short rod, and in addition, the structure of untreated control *A. hydrophila* cells was not damaged (Figure 8(a)). However, some cell contents of *A. hydrophila* were spilled after 1/2 MIC cinnamaldehyde treatment (Figure 8(b)), and the *A. hydrophila* cells showed important morphological changes such as irregular or malformed cell, uneven cell wall surface, fracture and breakage of cell wall, and membrane after treatment with 1 MIC cinnamaldehyde (Figures 8(c) and 8(d)).

3.5. Effect of High Temperature on Antioxidant Ability of Cinnamaldehyde. Figure 3(a) shows that with the increase of cinnamaldehyde concentration, the scavenging ability of cinnamaldehyde on hydroxyl radical was significantly increased at all temperatures ($P < 0.05$), and temperatures did not have a significant effect on it. As shown in Figure 3(b), the scavenging ability of cinnamaldehyde on DPPH radical increased significantly with the increase of cinnamaldehyde concentration ($P < 0.05$), and high temperature treatment did not affect the scavenging ability of cinnamaldehyde on radical.

4. Discussion

The antibacterial effect of cinnamaldehyde on *A. hydrophila* was studied in this study. The results showed that *A. hydrophila* was highly sensitive to the higher than 2 mg/mL cinnamaldehyde. The diameter of the inhibition zone with 4 mg/mL cinnamaldehyde to *A. hydrophila* was 28.3 mm, and the MIC value was 0.039 mg/mL. The analysis of the antibacterial activity of cinnamaldehyde indicated that

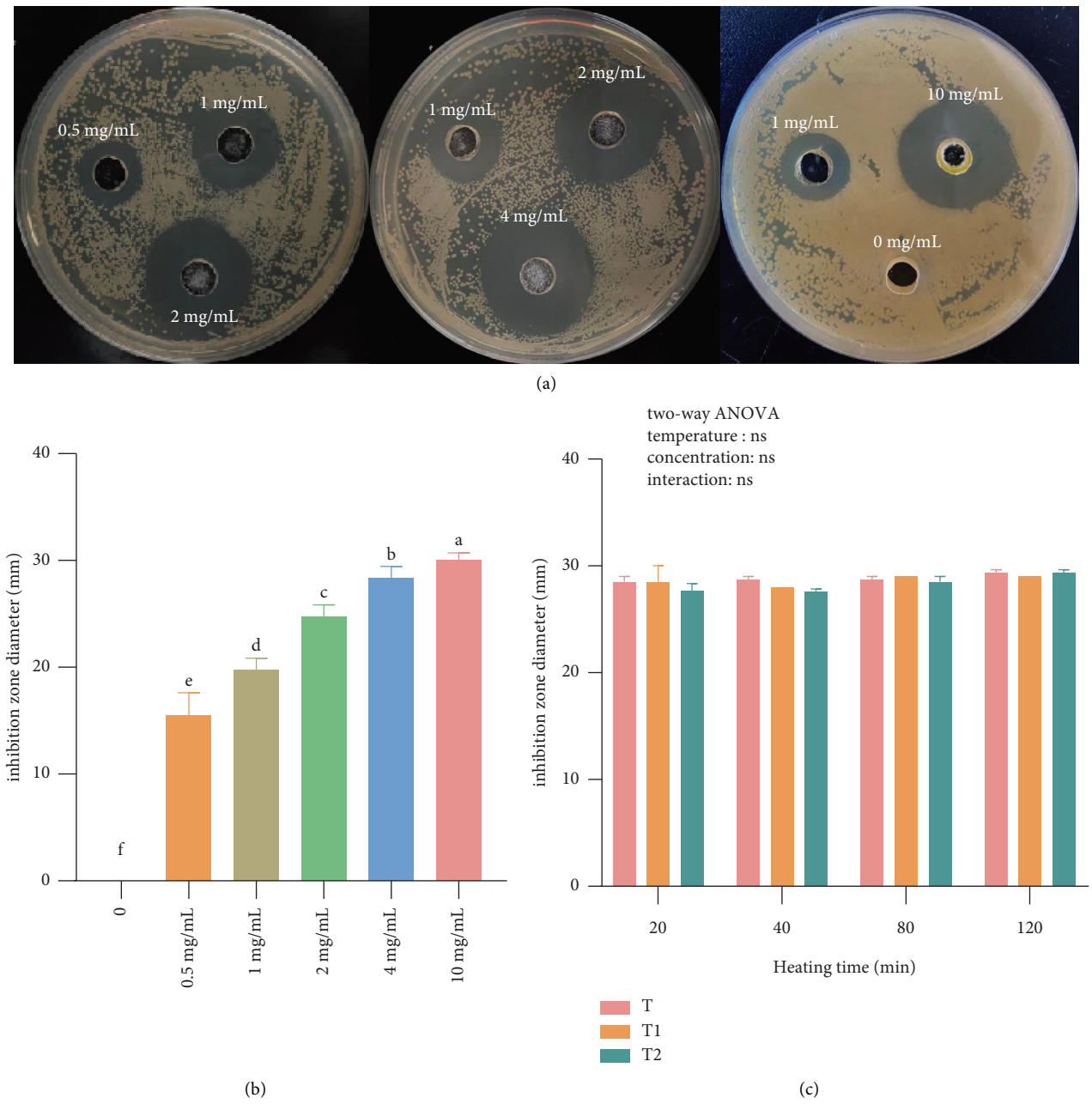

FIGURE 2: The effect of cinnamaldehyde on inhibition zone diameter. Note: Means with different superscripts are significantly different ($P < 0.05$); room temperature (T), 80°C (T1), and 110°C (T2). Note that, ns $P > 0.05$.

cinnamaldehyde had good antibacterial activity against *A. hydrophila*, the antibacterial activity of cinnamaldehyde was positively related to its concentration, and even low concentrations of cinnamaldehyde can inhibit the growth of *A. hydrophila*. In addition, cinnamaldehyde at the concentration of 1 MIC and 2 MIC could kill bacteria within 10 h after the incubation of *A. hydrophila* cells.

Bacterial membrane barrier provides a cytoplasmic environment for organelles of bacteria, which is composed of lipid compounds containing phosphatide protein and a minimal number of sugars and is responsible for intercellular transfers of chemicals [43]. Damage of the bacterial cell wall and cytoplasmic membrane might indicate loss of structural integrity and impact on the membrane's ability as a permeable barrier. When the bacterial membrane was damaged to a certain extent, small ions such as potassium and phosphate could be leached out, and some cytoplasmic constituents from the cells could be monitored. Therefore, the effect of cinnamaldehyde in the membrane permeability of *A. hydrophila* cells was investigated by measuring the amount of potassium ions released from drug-treated cells. The previous reports indicated that cinnamaldehyde damaged the cellular membrane of *Ps. aeruginosa*, leading to the collapse of membrane potential and

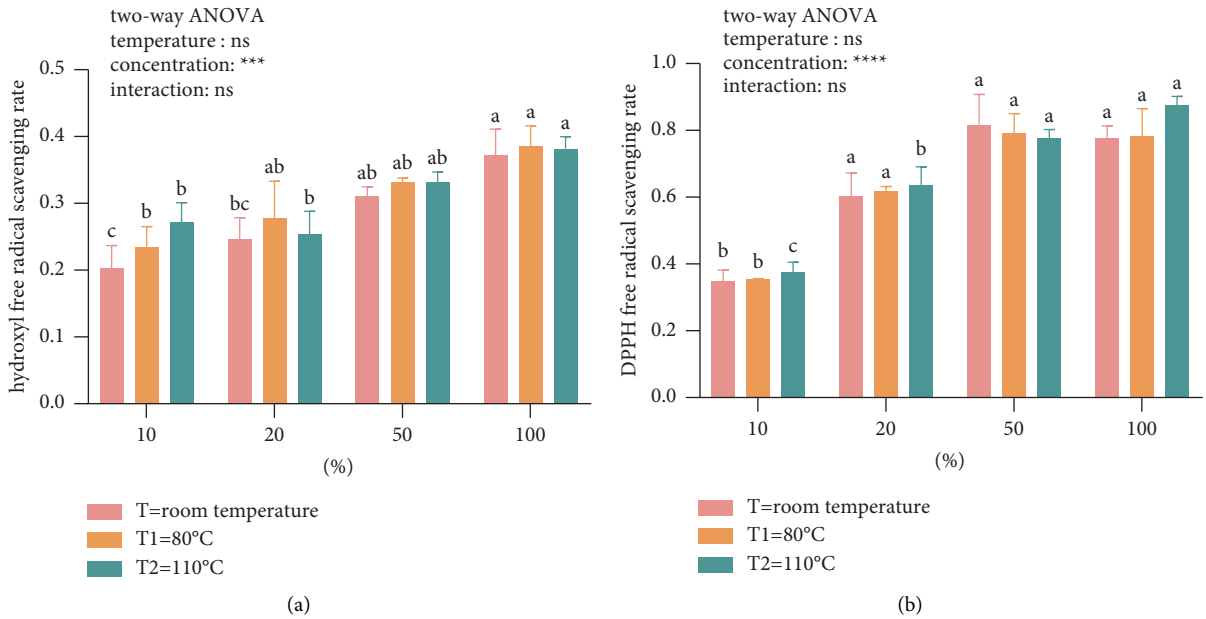

FIGURE 3: Radical scavenging rate of cinnamaldehyde at different concentrations and temperatures. Notes: Means in the same index with different superscripts are significantly different ($P < 0.05$). Note that, ns $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$.

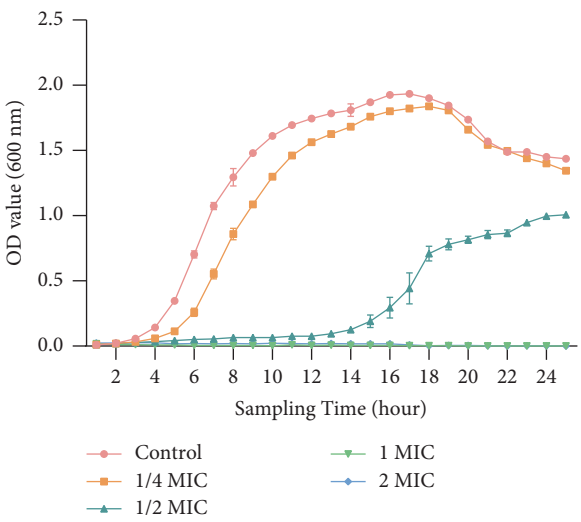

FIGURE 4: The effect of cinnamaldehyde on the growth curve of *A. hydrophila*.

loss of membrane-selective permeability, resulting in cell death [44]. The authors of [45] demonstrated that cinnamaldehyde could elongate bacterial cell morphology and cause its lysis. In agreement with these results, our results showed that the increase in the amount of K^+ released from *A. hydrophila* cells after treatment of cinnamaldehyde, which provided the evidence that cinnamaldehyde increased the plasma membrane permeability, caused potassium ion leakage from treated cells, and then led to *A. hydrophila* cells death [46].

Meanwhile, in order to investigate whether the antibacterial effect of cinnamaldehyde was induced by damage to the plasma membrane, the cells were stained by crystal violet (CV), DAPI, and PI [47–49], respectively. Crystal violet binds negatively charged molecules and thus stains both bacteria and the surrounding biofilm matrix [50]; in addition, CV is relatively stable and has little damage to bacterial structure, which can effectively reduce experimental error. DAPI can penetrate cell membranes and bind to double-stranded DNA in the nucleus for staining living cells, racking DNA in plants, microorganisms, multicellular animals, and bacterial cells. The fluorescence intensity of DAPI molecules bound to the double-stranded DNA increased by about 20 times, and stronger blue fluorescence could be observed using a fluorescence microscope [51, 52]. In this study, DNA extravasation amount detection and DAPI staining observation results showed that cinnamaldehyde could damage the cell wall and membrane of *A. hydrophila* and lead to DNA extravasation. PI cannot pass through the membrane of living cells but only through the disordered regions of dead cells to reach the nucleus, where it inserts into the DNA double helix to produce red fluorescence. The degree of cell membrane damage can be determined according to fluorescence intensity [53]. The above staining observation results were generally consistent, which confirmed that cinnamaldehyde had a significant and dose-dependent destructive effect on the biofilm of *A. hydrophila*.

Moreover, morphological changes and leakage of cytoplasmic contents were also demonstrated by electron micrographs of *A. hydrophila* cells treated with cinnamaldehyde, which elucidated that cinnamaldehyde increased membrane permeabilization and caused leakage of intracellular contents. Cell death might be the result of cell contents leakage [54, 55]. The previous reports indicated that cinnamaldehyde could bind and insert into the cell membrane, cause the damage of cytoplasmic membrane integrity [28, 56]. The present study was in agreement with these results.

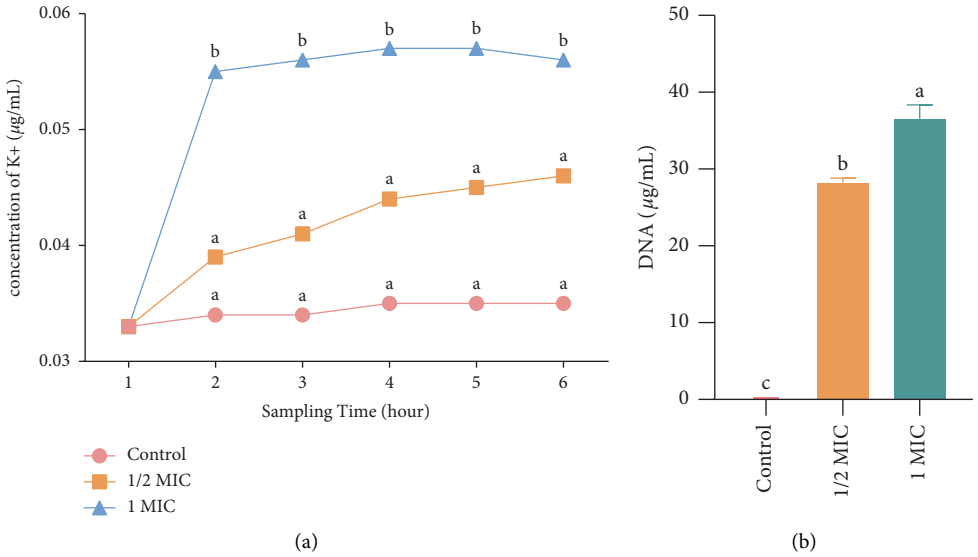

FIGURE 5: The effect of cinnamaldehyde on bacterial membrane permeability (a) and DNA extravasation (b) of *A. hydrophila*. Notes: Means in the same time points with different superscripts are significantly different ($P < 0.05$).

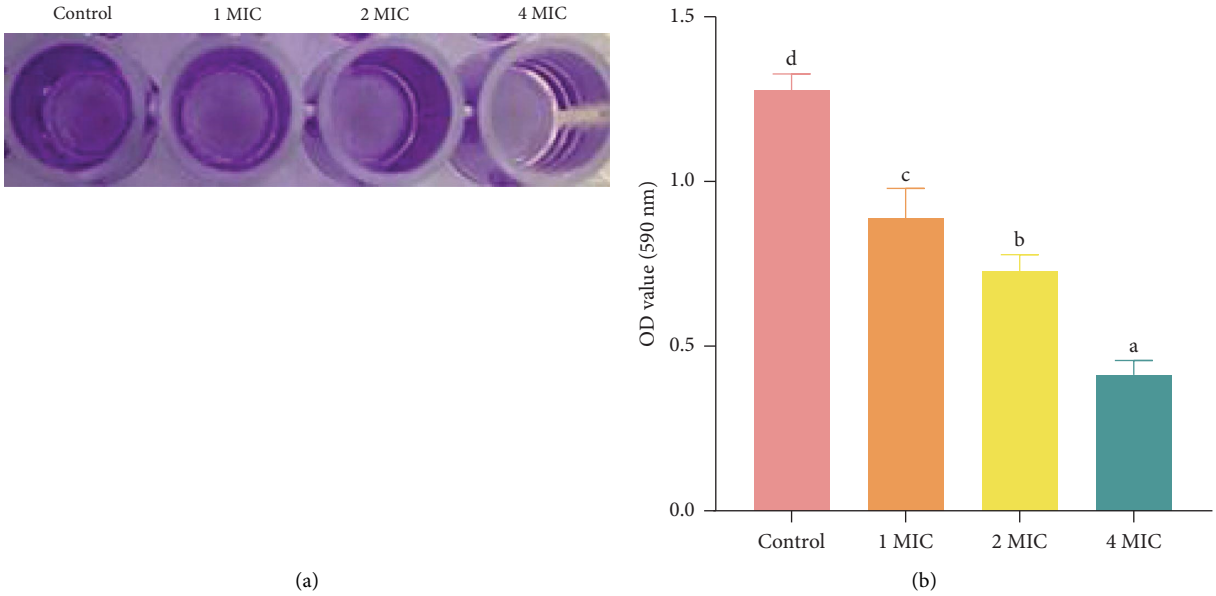

FIGURE 6: Effect of cinnamaldehyde on *A. hydrophila* biofilm formation. Notes: Means with different superscripts are significantly different ($P < 0.05$).

DPPH radical is widely used to evaluate the ability of compounds to operate as free-radical scavengers, and the DPPH test relies on the elimination of DPPH, a stabilized radical. DPPH is a dark-colored crystalline compound made up of stable free-radical particles. Once, the DPPH radical reduced and transformed into DPPH-H, and it turns colorless or light yellow [57]. In vitro, several extractions of plants have been shown to neutralize DPPH radical scavenging activity [58–61]. Hydroxyl radical is a kind of free radical produced in the process of metabolism, which is highly toxic and harmful to organisms. It can oxidize carbohydrates, amino acids, proteins, nucleic acids, and other substances in tissues, causing oxidative damage and destruction, leading to cell necrosis or mutation. Hydroxyl radical scavenging ability is one of the important indexes of antioxidant capacity, which has been widely used in the research of antioxidant health products and drugs [57]. Studies demonstrated that plant extracts could scavenge hydroxyl radicals [62–64]. The results showed that cinnamaldehyde had scavenging ability of DPPH radical and hydroxyl radical, which was positively correlated with the concentration, and high temperature had no effect on it. In addition, the antibacterial ability of cinnamaldehyde was not affected at the high temperature of 80°C and 110°C,

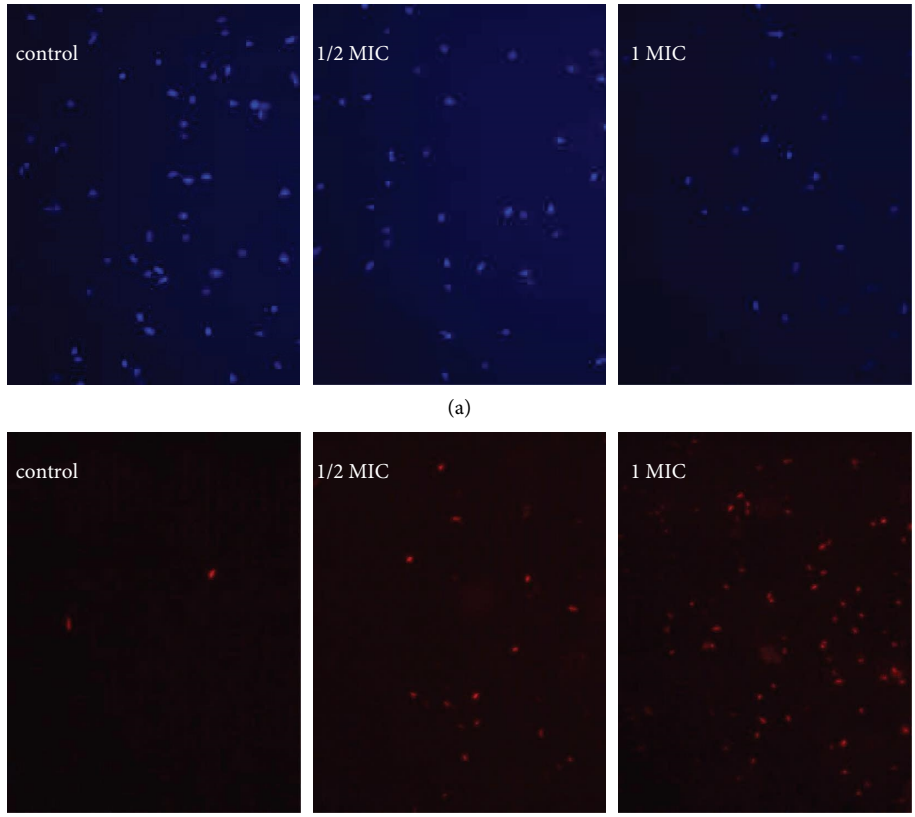

FIGURE 7: Effect of cinnamaldehyde on DAPI staining (a) and PI staining (b) of *A. hydrophila*. Notes: blue fluorescence indicates living cells after DAPI staining, and red fluorescence indicates apoptotic cells after PI staining.

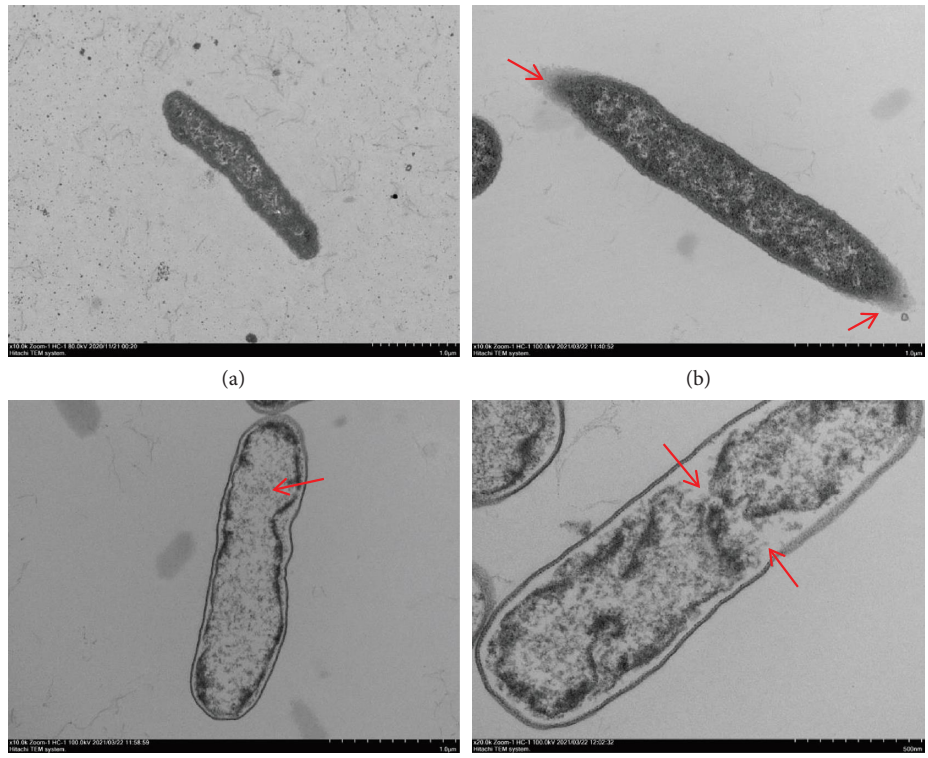

FIGURE 8: Scanning electron micrographs of the effects of cinnamaldehyde. Notes: The control group (a), 1/2 MIC cinnamaldehyde group (b), and 1 MIC cinnamaldehyde group (c, d). The arrow points to *A. hydrophila* cells damage.

indicating that cinnamaldehyde has less restriction on temperature.

In conclusion, all these results from the present investigation conclusively indicated that cinnamaldehyde could inhibit the growth of *A. hydrophila*, increase bacterial membrane permeability, damage bacterial cell membrane integrity, and scavenge DPPH radicals and hydroxyl radicals. The antibacterial ability and scavenging ability of DPPH radical and hydroxyl radical of cinnamaldehyde were not affected at the high temperature. It could play an important role in practical production.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jiaqiao Pei developed methodology and formal analysis, wrote the article, and prepared the original draft; Cunxin Sun wrote the article and reviewed and edited the article; Bo Liu gave ideas, prepared the formulation of overarching research goals and aims, and helped for project administration; Qunlan Zhou investigated the article; Xiaochuan Zheng developed visualization and supervised the article; Bo Liu investigated and validated the article; Chuanjiang Zhao & Chao Sun provided resources, production process and composition determination methodology.

Acknowledgments

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