In vitro Antimicrobial Activities and Mechanism of 1-Octen-3-ol against Food-related Bacteria and Pathogenic Fungi

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*

Abstract: 1-Octen-3-ol, known as mushroom alcohol, is a natural product extracted from fungi and plants. Its antimicrobial activities against five common food-related bacteria and two pathogenic fungi were evaluated in this paper, including Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Fusarium tricinctum and Fusarium oxysporum. The results showed that 1-octen-3-ol had a strong antibacterial activity against the tested bacteria, especially against Gram-positive bacteria, and it can also inhibit fungal growth and spore germination. The minimum inhibitory concentrations (MICs) for Gram-positive bacteria and Gram-negative bacteria were 1.0 and 2.0 mg/mL, respectively. The minimum bactericidal concentrations (MBCs) for Gram-positive bacteria and Gram-negative bacteria were 4.0 and 8.0 mg/mL, respectively. The completely inhibitory concentrations for fungal growth and spore germination were 8.0 and 2.0 mg/mL, respectively. Cell constituents’ leakage and scanning electron microscope assays indicated that 1-octen-3-ol changed the permeability of the cell membrane. Discrepant antimicrobial activity between 1-octen-3-ol and 1-octen-3-one indicated that hydroxyl may play a decisive role in antimicrobial activity. It is suggested that 1-octen-3-ol, with attractive mushroom aroma and antimicrobial activity, has potential applications in control of pathogens.

Key words: 1-octen-3-ol, antimicrobial activity, mechanism, scanning electron microscope

1 Introduction

1-Octen-3-ol is a natural product derived from linoleic acid during oxidative breakdown, which was first isolated from mushroom and thereafter from other fungi and plants. It, known as mushroom alcohol, was considered to be the major contributor to mushroom flavor in most species of edible mushrooms, together with a series of aliphatic compounds containing eight carbons, such as 1-octen-3-one, 1-octene, (E)-2-octenal, octanal and phenylacetaldehyde.

1-Octen-3-ol can trap biting insects such as mosquitoes, in combination with carbon dioxide, to kill them with certain electronic devices. It is approved by US Food and Drug Administration (ASP 1154, Regnum 172.515) as a food additive and also considered a wine fault. In an animal study, octenol has been found to disrupt dopamine homeostasis and may be an environmental agent involved in parkinsonism.

Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food, which can lead to spoilage or cause infection and illness. With the increasing demand of consumers for healthy, food safety was asked to focus on multifunctional food additives. Therefore, the development of food additive with antibacterial activity for the prevention of food contamination is becoming increasingly attractive. Previous studies frequently reported that 1-octen-3-ol was one of the main volatile components of many plants, such as Origanum vulgare, L. Lopez-palacii, Phyllanthus emblica and Scutellaria barbata, and these essential oils were reported to...
have antimicrobial activity\textsuperscript{[16–19]}. 1-Octen-3-ol, with attractive mushrooms aroma, was used in a variety of foods as a famous food additive. It is still unknown whether 1-octen-3-ol has antimicrobial activities to inhibit food-related bacteria.

In this study, the antimicrobial activities of 1-octen-3-ol against five common food-related bacteria and two pathogenic fungi were evaluated by measuring growth inhibition zone diameters and fungal spore germination. The potential antibacterial mechanisms were determined by permeability, integrity of cell wall and membrane and scanning electron microscopy (SEM) observation.

2 EXPERIMENTAL PROCEDURES

2.1 Chemicals and microbial strains

1-Octen-3-ol (CAS # 3391-86-4, racemate, purity ≥ 99\%) and 1-octen-3-one (CAS # 4312-99-6, purity ≥ 99\%) were purchased from Sigma–Aldrich (Shanghai, China). Penicillin G, Hygromycin B and all other chemicals of analytical grade were purchased from Ast Creative (Chengdu, China). Five common food-related bacterial strains (three Gram positive and two Gram negative) and two pathogenic fungi were selected for the study. The Gram positive bacteria were Staphylococcus aureus 1.8721, Bacillus subtilis 1.4225 and Staphylococcus epidermidis 1.4260, while the Gram negative bacteria were Escherichia coli 1.8732 and Pseudomonas aeruginosa 1.10712. Two pathogenic fungi were Fusarium tricinctum 3.4731 and Fusarium oxysporum 3.6864. All strains were obtained from the China General Microbiological Culture Collection Center, CGMCC (Beijing, China).

2.2 Antifungal activity

The antifungal in vitro activity test against two tested fungi was carried out using agar dilution method with some modification\textsuperscript{20}. Serial two fold dilutions of 1-octen-3-ol were prepared in Potato Dextrose Agar (PDA) (Baisi, Hangzhou) at concentrations of 4.0, 2.0, 1.0 and 0.5 mg/mL. The fungus cake (8.0 mm diameter) with Fusarium tricinctum or Fusarium oxysporum hyphae were taken up with a perforator and placed on PDA with different concentrations of 1-octen-3-ol. PDA without 1-octen-3-ol was set as control and with 0.5-1.0 mg/mL Hygromycin B was set as positive control. 0.8\% Tween-40 (w/w) was used to promote the dissolution of volatiles in PDA. Tween-40 and volatiles were added to the PDA by filtration sterilization. The growth rate of fungi in PDA with 4.0 mg/mL 1-octen-3-one was also tested. All plates were incubated at 28°C, the growth diameter of hypha was measured every day. All experiments were conducted in triplicate. The inhibition rate \( r \) was expressed by the following equation:

\[
r = \frac{(D_b - D_o + 8.0)}{D_o} \times 100\% 
\]

\( D_b \) (mm) represented the growth diameter of pathogenic fungi on control plate and \( D_o \) (mm) represented the growth diameter of pathogenic fungi on the plates with different concentrations of 1-octen-3-ol.

2.3 Spore germination assay

The effect of 1-octen-3-ol on spore germination rate of two tested fungi was evaluated according to Rana and others\textsuperscript{21} with some modifications. Pathogenic fungal spores were collected and diluted with sterile water at the concentrations of 30~100 spores each view of microscope (× 100). And then they were dealt with different concentrations of 4.0, 2.0, 1.0, and 0.5 mg/ml 1-octen-3-ol with 0.8\% Tween-40 (w/w) as chaotropic agent. Sterile water without 1-octen-3-ol served as control. All samples were incubated at 28°C for 12 h. The number of germinated spores was calculated through microscope counting. The length of germ tube greater than the spore short radius was regarded as germinating successful. All experiments were conducted in triplicate.

2.4 Paper disk diffusion assay

The antibacterial activity of 1-octen-3-ol was determined by the paper disc diffusion method described by Özer and others\textsuperscript{22} and Zhao and others\textsuperscript{23} with some modification. Five tested bacteria in logarithmic growth period were evenly daubed on the Luria-Bertani (LB) (Baisi, Hangzhou) plates by SS-Spreader. Then small pieces of sterile filter paper (13 mm diameter) were placed on the plate using the sterilized tweezers. 20 μL different concentrations of 8.0, 4.0, 2.0, 1.0, and 0.5 mg/ml 1-octen-3-ol were added on the paper using pipett. 1-Octen-3-one at the concentrations of 8.0 mg/mL was also conducted in the same way. The same volume of sterile water served as control. 0.8\% Tween-40 (w/w) was used to promote the dissolution of volatiles. All plates were incubated at 37°C for 12 h. The inhibition zone diameter was measured. All experiments were performed in triplicate.

2.5 Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

Five tested bacteria were selected to determine the MIC and MBC using broth macrodilution assay\textsuperscript{24}. Serial two fold dilutions of 1-octen-3-ol were prepared in Luria-Bertani (LB) medium at concentrations of 8.0, 4.0, 2.0, 1.0, and 0.5 mg/mL. One tube with the same volume of LB was set as control. The same volume of LB with 0.3 \( \times 10^6 \) CFU/mL Penicillin G served as positive control. 0.8\% Tween-40 (w/w) was used to promote the dissolution of volatiles. Inocula were added into all the tubes to achieve an initial inoculum of approximate 6 \( \times 10^6 \) CFU/mL. All tubes were incubated at 37°C, 24 h later, a 1 mL portion was extracted from each
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tube for colony counting by decimal dilution in 0.85% (w/v) sodium chloride solution, and plated onto LB Medium. All experiments were conducted in triplicate. MIC was defined based on the logarithmic difference in population \((\log \text{DP})\). The log DP was expressed by the following equation\(^\text{21}\):

\[
\log \text{DP} = \log (N/N_0) = (\log N) - (\log N_0)
\]

N is the population after incubation for 24 h and \(N_0\) is the initial population. MIC is defined as the lowest concentration resulting in maintenance or reduction of inoculum viability \((\log \text{DP} \leq 0)\). The minimum bactericidal concentration \((\text{MBC})\) is defined as the concentration where 99.9% or more of the initial inoculum are killed \((\log \text{DP} \leq 3)\).

2.6 Time-kill analysis

The time-kill test was performed according to D’Arrigo and others\(^\text{27}\) to determine the killing kinetics of 1-octen-3-ol against four tested bacteria. The bacteria with a density of \(6 \times 10^5\) CFU/mL were exposed to 1-octen-3-ol broth dilutions with the final concentrations ranging from 1 \(\times\) MIC to 4 \(\times\) MIC. LB broth was used as the control. 0.8 \(\times\) MIC to 4 \(\times\) MIC LB broth dilutions with the final concentrations ranging from 1 \(\times\) MIC to 4 \(\times\) MIC. LB broth was used as the control. 0.8 \(\times\) MIC to 4 \(\times\) MIC was used to promote the dissolution of volatiles. The electrophoresis was performed for 30 min under 110 V at room temperature. After incubation, all the solutions were inoculated at 37°C and 110 rpm/min in constant temperature oscillator. After 4, 8, 12, 16, 20, and 24 h of incubation, aliquots of 100 \(\mu\)L were taken out, serially diluted, and inoculated on LB plates for 24 h at 37°C. Then the number of survivors \((\text{CFU/mL})\) was determined by counting the colonies. Time-kill curves were constructed by plotting the \(\log \text{CFU/mL}\) versus time. The experiments were conducted in triplicate.

2.7 Cell constituents’ release

The cell integrity was examined by determining the release of cell constituents into supernatant according to the method described by Lv and others\(^\text{27}\) and Diao and others\(^\text{30}\) with some modifications. Cells were collected from the working culture of tested bacteria by centrifuging for 30 min at 5000 \(g\), followed by being washed three times with 0.1 M phosphate buffer solution \(\text{PBS, pH 7.2}\), and resuspended in the same buffer. Twenty-five milliliters of cell suspension were incubated at 37°C under agitation for 6 h in the presence of 1-octen-3-ol at the MIC concentrations. Then, 2 \(\mu\)L of each sample was harvested by centrifugation at 10,000 \(g\) for 5 min. The bacterial supernatant without 1-octen-3-ol treatments were tested similarly, which was set as the Control. 0.8 \(\times\) Tween-40 \((\text{v/v})\) was used to promote the dissolution of volatiles. The release of DNA and RNA from the cytoplasm in supernatant were estimated by the detection of absorbance at 260 nm. The concentrations of proteins in supernatants were determined using Modified Bradford Protein Assay Kit (Sangon Biotech, Shanghai) following the instructions. Reducing sugars in supernatants were determined by 3,5-dinitrosali-}

cyclic acid (DNS) assay\(^\text{29}\) with some modifications. A mixture of 1 mL of diluted supernatant and 1 mL of DNS reagent was heated in boiling water for 10 min. After cooling to room temperature under running tap water, 10 mL of distilled water was added. The absorbance of the reaction mixtures at 540 nm was recorded on a Spectra Max plus microplate reader (Molecular Devices, USA) and compared with a glucose calibration curve to quantify the concentration of reducing sugars.

2.8 Scanning electron microscopy (SEM) analysis

To determine the efficacy of the 1-octen-3-ol and the morphological changes of bacteria strains, SEM studies were carried out as previously reported with some modifications\(^\text{30}\). Two tested bacteria in logarithmic growth phase \((\text{OD}_{600} = 1.0)\) were treated with 1-octen-3-ol at MIC value. 0.8% Tween-40 \((\text{v/v})\) was used to promote the dissolution of volatiles. All samples and the control \((\text{without 1-octen-3-ol})\) were incubated at 37°C for 6 h. After incubation, cells were harvested by centrifugation \((10,000 \, g, 5 \, \text{min})\) and washed twice with 0.1 M phosphate buffer solution \(\text{PBS, pH 7.2}\). Then the cells were fixed with 2.5% \((\text{v/v})\) glutaraldehyde in PBS for 4 h. After centrifugation, the cells were further dehydrated using a graded series of ethanol \((30\%, 50\%, 70\%, 80\%, 90\% \text{ and } 100\%)\). Finally, the samples were fixed on SEM support, and sputter-coated with gold under vacuum, followed by microscopic examinations using a scanning electron microscope (Tescan s.r.o., Brno, Czech Republic).

2.9 DNA agarose gel electrophoresis

Agarose gel electrophoresis assay was performed to detect the effect of 1-octen-3-ol on DNA according to the procedures described by He and others\(^\text{31}\). The pBR322 plasmid DNA \((0.2 \, \mu\text{g})\) in Tris–HCl/EDTA buffer \(\text{pH 7.2}\) was treated with different concentrations of 1-octen-3-ol, followed by dilution with the Tris–HCl buffer to a total volume of 20 \(\mu\)L. Then, the reaction mixtures were incubated at 37°C for 1 h before being loaded onto a 1% agarose gel. 0.8% Tween-40 \((\text{v/v})\) was used to promote the dissolution of volatiles. The electrophoresis was performed for 30 min under 110 V at room temperature.

2.10 Statistical analysis

Data were expressed as means \(\pm\) SD. Statistical analysis was performed using the One-way ANOVA and Duncan test at the 95% significance level to express the difference between two groups by SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). \(p < 0.05\) was considered statistically significant.
3 Results and discussion

3.1 Antifungal Activity

Two pathogenic fungi, Fusarium tricinctum and Fusarium oxysporum were selected to test the antifungal activity of 1-octen-3-ol. The growth diameter of the two pathogenic fungi on PDA medium containing different concentrations of 4, 2, 1, and 0.5 mg/mL 1-octen-3-ol were measured every day. As shown in Fig. 1, the diameter of the colonies were reduced by 1-octen-3-ol as compared to that of the control. The average growth diameter of F. tricinctum (0.25 ± 0.01 cm/d) on PDA medium containing 2.0 mg/mL 1-octen-3-ol was significantly smaller than Control (1.28 ± 0.01 cm/d). Inhibition rate (r) of 2.0 mg/ml 1-octen-3-ol against F. tricinctum was as high as 82.4%. F. tricinctum on medium with 4.0 mg/mL 1-octen-3-ol could never grow. Similarly, the inhibition rate of 4.0 mg/mL 1-octen-3-ol against F. oxysporum was 100%. Overall, 1-octen-3-ol can completely inhibit the growth of tested pathogenic fungi at the concentration of 4.0 mg/mL. However, 1-octen-3-one had no effect on the growth rate of tested fungi at the same concentration. It was suggested that hydroxyl in 1-octen-3-ol may play an important role in its antifungal activity.

1-Octen-3-ol can also inhibit fungal spore germination (Fig. 2). Two tested fungal spore germination rate at different concentration of 1-octen-3-ol reduced significantly. Compared with Control, the spore germination rate slowed down to 50% and 10% of the Control by 0.5 mg/mL and 1.0 mg/mL 1-octen-3-ol addition respectively. And the fungal spores cannot germinate at the concentration of 2.0 mg/mL 1-octen-3-ol. Compared to the positive control, the antifungal activity of 1-octene -3-ol was a little weaker, but it did not show significant differences in the range of tested concentration.

In our previous study, 1-octen-3-ol was found to be the most abundant volatile component in the prized edible mushroom (Tricholoma matsutake)\(^9\), which can protect T. matsutake fruiting bodies from mycophagous Proisotoma minuta\(^32\). Many studies have indicated a decrease of fungal communities in the T. matsutake fairy ring zone depending on the growth of T. matsutake\(^33\). However, the reason for it has not been fully understood. In this study, 1-octen-3-ol has shown its efficient antifungal activity. As the most abundant volatile in T. matsutake, 1-octen-3-ol may contribute to the decrease of fungal communities in the T. matsutake fairy ring zone. Its ecologic function also deserved further research.

3.2 Antibacterial activity

The antibacterial activity of 1-octen-3-ol against five tested food-related bacteria was conducted using the paper disk diffusion assay, which was determined by the diameter of inhibition zones. As shown in Table 1, different concentration of 1-octen-3-ol displayed a variable degree of antibacterial activity against different tested strains. Its anti-
bacterial activity became stronger with the concentration of 1-octen-3-ol increasing. The diameter of inhibition zone of Gram-positive bacteria was larger than that of Gram-negative bacteria by 1-octen-3-ol addition, which indicated stronger antibacterial effects against Gram-positive bacteria. Likewise, 1-octen-3-one was inactive against bacteria. It suggested that hydroxyl played a determinant role in exhibiting antibacterial activity of 1-octen-3-ol.

3.3 MICs and MBCs of 1-octen-3-ol
The MICs and MBCs of 1-octen-3-ol against the tested bacteria are shown in Table 1. The MICs and MBCs for Gram-positive bacteria were 1 and 4.0 mg/mL, respectively. The MICs and MBCs for Gram-negative bacteria were 2.0 and 8.0 mg/mL, which were 2-folds higher than that for Gram-positive bacteria. The results indicated that Gram-negative bacteria were more resistant to 1-octen-3-ol than Gram-positive bacteria, which may be attribute to their outer membrane, restricting diffusion of 1-octen-3-ol through their lipopolysaccharide covering. The results were in accordance with other literature. The different inhibitory activity of 1-octen-3-ol against gram positive or gram negative bacteria was not as great as the inhibitory activity of penicillin G against gram positive or gram negative bacteria, since gram negative bacteria are penicillin-resistant, indicating the stable antibacterial activity of 1-octen-3-ol.

3.4 Time-kill analysis
To further investigate the antibacterial activities of 1-octen-3-ol, time-kill assays were carried out to study the concentration and time-dependent killing effect. The time-kill curves were summarized in Fig. 3. For all four selected bacteria, higher concentration of 1-octen-3-ol led to a more rapid decrease in bacterial number. The growth of bacteria was suppressed by 1-octen-3-ol at 1 × MIC, indicating a bacteriostatic effect. The numbers of viable cells of five bacteria were significantly lower than the initial value after treatment with 2 × MIC 1-octen-3-ol for 24 h. When 1-octen-3-ol concentration attained to 4 × MIC, there were 3.7, 3.3, 2.0 and 2.5 log CFU/mL drops in colony counts for S. aureus, B. subtilis, P. aeruginosa and E. coli after treatment for 24 h, respectively (Fig. 3a–d).

3.5 Integrity of cell membrane
To explore the antibacterial mechanism of 1-octen-3-ol, the integrity of the cell membrane was examined by measuring the release of cell constituents including protein, reducing sugar in the supernatant of tested bacteria. As shown in Table 2, the release of cell constituents increased significantly after treatment of 1-octen-3-ol for 6 h. The absorbance of supernatant from tested bacteria treated with 1-octen-3-ol at 260 nm was 1.5 times as high as that of the control. Moreover, the concentration of total protein and reducing sugar in the supernatant of tested bacteria increased by 1.5 times and 2.5 times as high as that of the control. The results indicated that 1-octen-3-ol may induce the cytoplasmic membranes destruction, which led to an efflux of the intercellular content and the death of bacteria.

3.6 Scanning electron microscopy (SEM) analysis
The morphological changes of B. subtilis and S. epidermidis were observed by SEM analysis. As shown in Fig. 4, untreated cells showed regular and typical morphology, with a plump and smooth surface, and were uniform in size and distribution (Fig. 4a and 4c). In contrast, B. subtilis and S. epidermidis cells treated with 1-octen-3-ol at MIC appeared a severe destruction on the cell morphology, showing an irregularly wrinkled and coarse outer surface (Fig. 4b and 4d). This indicated that 1-octen-3-ol treatment may result in damage to the bacterial cell wall and cytoplasmic membrane.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Inhibition zone diameter (mm)</th>
<th>MIC (μg/mL)</th>
<th>MBC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 mg/mL</td>
<td>0.5 mg/mL</td>
<td>1.0 mg/mL</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>13.0 ± 0.0</td>
<td>13.7 ± 0.3</td>
<td>14.0 ± 0.3</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>13.0 ± 0.0</td>
<td>13.6 ± 0.2</td>
<td>14.2 ± 0.5</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>13.0 ± 0.0</td>
<td>13.2 ± 0.6</td>
<td>14.4 ± 0.3</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13.0 ± 0.0</td>
<td>13.5 ± 0.4</td>
<td>13.6 ± 0.4</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>13.0 ± 0.0</td>
<td>13.4 ± 0.7</td>
<td>13.5 ± 0.2</td>
</tr>
</tbody>
</table>

Data (means ± SD, n = 3)
MIC, minimal inhibitory concentration.
MBC, minimum bactericidal concentration.
NT, not tested.
* 13.0 mm, the diameter of sterile filter paper used for paper disk diffusion assay.
Fig. 3  Time-kill curves of 1-octen-3-ol against four food-related bacteria. (a) *Staphylococcus aureus*; (b) *Bacillus subtilis*; (c) *Pseudomonas aeruginosa*; (d) *Escherichia coli*.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentrations</th>
<th>Protein (μg/mL)</th>
<th>Reducing sugar (μg/mL)</th>
<th>Cell constituents (OD260 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Control</td>
<td>3.52 ± 0.14</td>
<td>12.34 ± 1.96</td>
<td>2.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1 × MIC</td>
<td>5.06 ± 0.14*</td>
<td>41.89 ± 2.78*</td>
<td>3.53 ± 0.01*</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Control</td>
<td>2.95 ± 0.12</td>
<td>19.71 ± 3.05</td>
<td>2.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1 × MIC</td>
<td>4.59 ± 0.13*</td>
<td>48.56 ± 4.66*</td>
<td>3.61 ± 0.02*</td>
</tr>
<tr>
<td><em>Staphylococcus epidermis</em></td>
<td>Control</td>
<td>3.14 ± 0.11</td>
<td>16.93 ± 2.37</td>
<td>2.39 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1 × MIC</td>
<td>4.94 ± 0.08*</td>
<td>45.32 ± 4.02*</td>
<td>3.61 ± 0.02*</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Control</td>
<td>3.50 ± 0.15</td>
<td>21.51 ± 1.89</td>
<td>2.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1 × MIC</td>
<td>5.12 ± 0.14*</td>
<td>49.89 ± 3.21*</td>
<td>3.59 ± 0.02*</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Control</td>
<td>3.33 ± 0.09</td>
<td>23.67 ± 1.38</td>
<td>2.38 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>1 × MIC</td>
<td>4.92 ± 0.13*</td>
<td>51.66 ± 4.17*</td>
<td>3.54 ± 0.02*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD (n = 3). Asterisks indicate statistically significant difference from the control group (*p < 0.05*).
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3.7 DNA agarose gel electrophoresis

The interaction between 1-octen-3-ol and DNA was investigated, since it had a potential to reach the inner structure of cells through a damaged membrane. Agarose gel electrophoresis of control DNA and DNA with increasing concentration of 1-octen-3-ol was used to evaluate whether it had DNA destruction ability (Fig. 5). When circular plasmid DNA (pBR322) is subjected to electrophoresis study, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form will be generated which migrates in between. As shown in Fig. 5, the bands of plasmid pBR322 DNA treated with different concentrations of 1-octen-3-ol were similar to that of control (lane 0). The result indicated that 1-octen-3-ol had no ability to cause DNA destruction.

4 Conclusion

The antimicrobial activities of 1-octen-3-ol against five...
bacteria and two pathogenic fungi were evaluated in this study. As a food additive, it showed a strong antibacterial activity against the tested bacteria, especially against Gram-positive bacteria, and it can also inhibit fungal growth and spore germination. With the increase of concentration, the antibacterial activity of 1-octen-3-ol has been increasing. The release of cell constituents and SEM observation suggested that 1-octen-3-ol exerted its antibacterial effect through affecting the permeability of the cell membrane, leading to leakage of some cellular components such as proteins, reducing sugars and 260 nm absorbing materials. Moreover, 1-octen-3-ol had no direct effect on cellular DNA migration profiles. Comparative analysis found that hydroxyl in 1-octen-3-ol may play an important role in its antimicrobial activity. In conclusion, 1-octen-3-ol has a potential to be used in the control of food-related bacteria and pathogenic fungi, but the high concentrations of MICs and MBCs limited its application as an antimicrobial food additive. Further studies are required to fully understand the antimicrobial efficacy of 1-octen-3-ol, such as its effect against other pathogenic bacteria and fungi, and its antifungal mechanism, in order to promote its application in our life.

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