Indole derivatives inhibited the formation of bacterial biofilm and modulated Ca$^{2+}$ efflux in diatom

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**Abstract**

Marine biofouling is a serious environmental problem worldwide. As an effort to find environmental friendly antifoulants, indole derivatives were determined for their activities to inhibit the growth of bacteria and diatom. The minimum inhibitory concentrations (MICs) of indole derivatives against bacteria were very low, especially for 6-chloroindole. It was proved that 6-chloroindole obviously inhibited the growth of bacteria, interfered with the formation of bacterial biofilm, destroyed bacterial cell morphology and also inhibited the growth of diatom *Cylindrotheca* sp. as well. By using noninvasive microtest technique (NMT), 6-chloroindole triggered algal cellular Ca$^{2+}$ efflux. The highest value was 72.03 pmol cm$^{-3}$ s$^{-1}$, 10.6 times of the control group. The present studies indicated that indole derivatives might have the potential to be new antifouling agents because of their excellent antibacterial and anti-algal activities. At the same time, Ca$^{2+}$ efflux might be one of the mechanisms that indole derivatives inhibited the growth of diatom.

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1. Introduction

Marine biofouling, the undesirable accumulation of microorganisms, algae and animals on all natural and man-made surfaces that are immersed in marine environment, causes tremendous technical and economic problems worldwide (Hellio et al., 2004). In order to control marine biofouling, antifouling coatings have been widely applied for many years. Those antifoulants, such as tributyltin (TBT)-based or copper-based compounds effectively prevent biofouling. However, the fact that their highly toxic to a wide of non-target organisms makes it very urgent to find effective and environmental friendly alternatives against fouling organisms (Yebra et al., 2004; Qi et al., 2009).

Many marine organisms protect the surface of their bodies with antifouling substances without causing serious fouling problems. Therefore, these substances may be expected to be utilized as new environmental friendly antifouling agents, especially those having high activities against fouling organisms, without biocide properties on other organisms (Omøe, 2006). Indeed, many natural products from a variety of organisms (microorganisms, aquatic plants and invertebrates) with strong antifouling activities have been isolated and reported (Qian et al., 2010; Cho, 2013). For example, 9-Z-oleic acid and 1-hydroxymyristic acid extracted from marine bacterium *Shewanella oneidensis*, eight terpenes from the red algae *Sphaerococcus coronopifolius* all showed anti-settlement activity against larvae of *Amphibalanus* (*Balanus*) *Amphitrite* (Bhattarai et al., 2005; Piazza et al., 2010). These reports indicate that the secondary metabolites from organisms may be one of the most promising matters as new and environmental friendly antifoulants.

The natural products of indole derivatives as promising antifoulants are classified into two kinds of compounds, indole-3-carbaldehyde and gramines, which are isolated from ascidian *Stomozoa murrayi* and bryozoan *Zoobotryon pellucidum* respectively. Especially, the halogenated indole derivatives of 6-bromoindole-3-carbaldehyde and 2, 5, 6-tribromo-1-methylgramine show excellent antifouling activities against the barnacle *Balanus amphitrite* and blue mussel *Mytilus edulis* (Olguin-Uribe et al., 1997). The antifouling activity of 2, 5, 6-tribromo-1-methylgramine is higher than that of CuSO$_4$ and 6 times as strong as that of tributyltin oxide, while its toxicity to the cyprid larvae is only one-tenth of that of tributyltin oxide (Omøe, 2006). However, natural products often present at very low concentrations in organisms, which makes it almost impossible to be widely applied as antifouling agents. Chemical synthesis is an effective method to solve this problem. It was reported that about one hundred kinds of indole compounds were synthesized and some showed excellent antifouling activities...
(Konya et al., 1994; Kawamata et al., 2006). However, the antifouling activities and mechanisms of indole derivatives are worthy to be further studied before they are applied as worldwide environmental friendly antifouling agents.

In marine environment, biofilm mainly consists of numerous species of bacteria and diatom incorporated into a matrix of extracellular polymers which is composed of high molecular weight polysaccharide (Dobretsov, 2008). Many literatures reported that marine biofilm might secrete chemical cues to attract invertebrate larvae and macro-algal spores and enhance the settlement of larval and spores (Joint et al., 2002; Dobretsov et al., 2009; Tait and Havenhand, 2013). So it is very important to inhibit the growth of bacteria and diatom for controlling marine biofouling. In our experiment, six kinds of indole derivatives of simple halogenated structures were screened for their antifouling activities on the growth of bacteria and diatom, and the formation of bacterial biofilm. The aim was to reveal the potential activities of the halogenated indole derivatives on inhibiting the formation of fouling biofilm. Meanwhile, a new technique (NMT) was used to determine the Ca2+ velocity in algal cells for preliminary exploring the physiological mechanism of the inhibitory effect of indole derivatives on the diatom. The results showed that indole derivatives with only a halogen substituent extremely inhibited the formation of bacterial biofilm and the growth of diatom, which might be developed as new antifouling agents in the industry for their high activities.

2. Materials and methods

2.1. Reagents and organisms culture

Indole, 5-chloroindole, 5-bromoindole, 6-chloroindole, 6-chloroindole, and gramine were purchased from Alfa Aesar Company, Tianjing. The structures of indole derivatives were shown in Table 1. All other chemicals were analytical or higher grades. Bacterial strains Escherichia coli, Bacillus subtilis-F and Staphylococcus aureus were stored in Key Laboratory of Coastal Biology and Biological Research Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, kindly provided by Dr. Jianming Ren. Bacillus subtilis-S and Pseudomonas aeruginosa were purchased from Marine Culture Collection of China (MCCC). All bacterial strains were cultured with LB medium and distilled water at 30 °C in darkness, in addition to B. subtilis-S and P. aeruginosa with seawater filtered by 0.45 μm membrane. The diatom Cylindrotheca sp. was also stored in the Key Laboratory of Coastal Biology and Biological Research Utilization, cultured with f/2 medium in light incubator, at light intensity of 48 μmol photons m−2 s−1 and 20 °C with a 12:12 h light:dark cycle. All bacterial strains and diatom were cultured to the exponential phase before inoculation in the following experiments. The commercial biocidal agent CuSO4 was tested as a reference.

2.2. Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of these compounds were determined by a dilution method (Qi et al., 2009). The bacterial strains were grown in LB medium to the exponential phase at a concentration of 106 CFU mL−1. Bacterial culture was diluted with LB to give a final concentration of 105 CFU mL−1. The concentrations of tested compounds were orderly diluted to 80, 40, 20, 10, 5, 2.5, 1.25 mg L−1. Each concentration culture of 100 μL was added into wells of 96-well plates containing 100 μL diluted bacterial suspension. The wells containing 100 μL bacterial suspension and 100 μL LB medium were determined as the control. The 96-well plates were incubated at 30 °C for 24 h, the bacterial growth was determined by the optical absorbance at 600 nm. All experiments were repeated six times and the values of MIC were calculated by 10% inhibition of bacterial growth according to logistic fitting curve based on equation.

2.3. Effect of indole derivatives on the formation of bacterial biofilm by microplate assay

The effects of these compounds on P. aeruginosa biofilm were carried out in 96-well plate using a method developed by Kawarai et al. (2009). With some modifications, bacteria were cultured to exponential phase and diluted to the concentration of 106 CFU mL−1. All these compound solutions were added into 96-well plates and incubated at 30 °C for 24 h, the bacterial biofilms were formed, then washed twice with PBS (0.1 M, pH 7.0) to remove any non-adherent bacteria. The bacteria attached to the walls were stained with 250 μL 1% crystal violet for 15 min, washed three times with PBS, then the crystal violet dye was solubilized with 250 μL 95% ethanol per well. The optical density of each well was measured at 590 nm to represent biofilm formation.

2.4. Effect of indole derivatives on the formation of bacterial biofilm by slide assay

P. aeruginosa was grown to the exponential phase and diluted into the concentration of 105 CFU mL−1 for the following experiment. LB medium of 3 mL were added into 12-well sterile polyethylene plate, which contained 0.1 mL bacterial inoculum and 1 × 1 cm2 slides. In order to evaluate the inhibitory activities of these compounds on biofilm formation, compounds of 3 mM were added into the wells of plate, which were incubated at 30 °C for 24 h, then the slides were taken out to analyze biofilm formation by detecting cell density and biofilm morphology.

Cell density was analyzed by agar plate counting method. The biofilm-coated glass slides were taken out after 24 h incubation and washed twice with sterile PBS (0.1 M, pH 7.0) to remove non-adherent bacteria, the biofilm was scraped into PBS solution, mixed well and diluted gradiently for the total numbers of cells.

Biofilm morphology was observed with light microscope, fluorescence microscope and scanning electron microscope (SEM, Hitachi S-4800, Japan). The biofilm-coated glass slides were taken out after 24 h incubation and washed twice with sterile PBS (0.1 M, pH 7.0) to remove non-adherent bacteria for the following experiment. A portion of samples were directly observed with light microscope. Another portion of samples were stained with DAPI (4′, 6-diamidino-2-phenylindole dihydrochloride, 10 mg L−1 working solution), then incubated in the dark for 5 min and washed twice with PBS. DAPI-stained cells were visualized by fluo- rescence microscope (Olympus BX51, Japan). The last part of samples were fixed with 4% formaldehyde for 2 h, followed by dehydrating in a gradient series of aqueous solutions of 25%, 50%, 75% and 95% ethanol for 20 min. The slides were air dried and kept in a dessicator overnight for SEM examination (Fang et al., 2002; Villa et al., 2009).

2.5. Effect of indole derivatives on the growth of diatom Cylindrotheca sp.

The inhibitory effect of indole derivatives on the growth of Cylindrotheca sp. was determined in 96-well microplates, each well containing 200 μL of f/2 medium inoculated with algal cells at 2.5 × 104 cells mL−1. The cells were exposed to a range of final concentrations of indole derivatives (0, 0.5, 1, 2, 5, 10, 20 and 50 mg L−1). The optical density of 630 nm was measured by using
multi-well plate reader (Infinite M2000, Tecan) to represent algal growth. The EC50 values which represent the inhibitory effect of compounds on the growth of algal cells were calculated by logistic curve fitting based on equation (Origin 7.5 for Windows) (Leflaive and Ten-Hage, 2011; Yang et al., 2013).

2.6. Measurement of Ca2+ flux in Cylindrotheca sp.

To reflect the effect of indole derivatives on algal cells, the net Ca2+ flux were measured by using non-invasive micro-test technique (NMT, Younger USA). The Ca2+ ion-selective microelectrodes were purchased from the Xu-Yue Company (Beijing, China; http://www.xuyue.net) and this experiment was carried out in Public Service Technology Center of Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences. The microelectrodes were calibrated in 0.05, 0.1 and 1 mM Ca2+ prior to the Ca2+ flux measurement.

The cells of Cylindrotheca sp. were adhered to glass coverslips by being cultured in light incubator for 24 h and washed three times with measuring solution (0.1 mM KCl, 0.1 mM CaCl2, 0.1 mM MgCl2, 0.5 mM NaCl, 0.3 mM MES, 0.2 mM Na2SO4 and 0.1% sucrose, pH 7.8), then transferred to the measuring chamber containing 3 mL measuring solution in the presence or absence of 6-chloroindole. The Ca2+ flux data were recorded for about 10–20 min. The flux data were obtained according to the NMT velocity conversion table JCal V3.2 provided by Xu-Yue Company.

2.7. Statistics

All the data shown in the study were the means ± S.D. of at least three independent experiments and were evaluated by using one-way analysis of variance (ANOVA) followed by the least significant difference test (LSD), *p* < 0.01 and *p* < 0.05 (Origin 7.5 for Windows).

3. Results

3.1. Antibacterial activities of indole derivatives

For antibacterial activities of indole derivatives, microplate assays determined the values of MIC (Table 2) against five kinds of bacteria strains including three kinds of freshwater bacteria strains and two kinds of marine bacteria strains. The results of MIC values showed that the antibacterial activity of 6-chloroindole was better than that of other indole derivatives and CuSO4 except that the activity of 6-chloroindole against *S. aureus* was not determined in this experiment. Based on the calculated MIC values, the antibacterial activities of these compounds for marine bacteria (*B. subtilis*-S and *P. aeruginosa*) were orderly 6-chloroindole > 6-chlorooxindole > indole > 5-chloroindole > 5-bromoindole > CuSO4 > gramine. However, the antibacterial activities of these compounds for freshwater bacteria were dependent on the bacterial species. The antibacterial activities of indole derivatives were generally better than that of CuSO4 against two freshwater bacteria (*E. coli* and *B. subtilis*) except for *S. aureus*.

3.2. Effect of indole derivatives on bacterial growth and biofilm formation

In order to evaluate the biofilm of CuSO4 and indole derivatives-treated *P. aeruginosa*, the control and treated bacterial biofilms were stained by crystal violet in the microplate and the results were presented in Fig. 1. It was observed that indole, 5-bromoindole, 6-chloroindole and 6-chlorooxindole significantly inhibited the biofilm formation and the inhibitory rates were correspondingly 94.5%, 50.6%, 94.4% and 93.8% after cells exposed to the compounds. However, no obvious differences were found in the biofilm formation between the treatment groups of CuSO4, 5-chloroindole, gramine and the control group.

### Table 1

The nomenclature, chemical abstracts service (CAS) number and chemical structure of the indole derivatives used in this experiment.

<table>
<thead>
<tr>
<th>Chemical name and CAS number</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole (CAS:120-72-9)</td>
<td><img src="image" alt="Indole structure" /></td>
</tr>
<tr>
<td>5-Chloroindole (CAS:17422-32-1)</td>
<td><img src="image" alt="5-Chloroindole structure" /></td>
</tr>
<tr>
<td>5-Bromoindole (CAS: 10075-50-0)</td>
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<tr>
<td>6-Chloroindole (CAS: 17422-33-2)</td>
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</tr>
<tr>
<td>6-Chlorooxindole (CAS: 56341-37-8)</td>
<td><img src="image" alt="6-Chlorooxindole structure" /></td>
</tr>
<tr>
<td>Gramine (CAS: 87-52-5)</td>
<td><img src="image" alt="Gramine structure" /></td>
</tr>
</tbody>
</table>

### Table 2

The MIC values of indole derivatives on bacteria.

<table>
<thead>
<tr>
<th>Concentration (mg L⁻¹)</th>
<th>Bacterial strain</th>
<th>E. coli</th>
<th>B. subtilis-F</th>
<th>S. aureus</th>
<th>B. subtilis-S</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuSO4</td>
<td></td>
<td>51.97</td>
<td>6.26</td>
<td>&lt;2.5</td>
<td>53.68</td>
<td>118.86</td>
</tr>
<tr>
<td>Indole</td>
<td>n</td>
<td>160.08</td>
<td>26.87</td>
<td>3.32</td>
<td>3.32</td>
<td>n</td>
</tr>
<tr>
<td>5-Chloroindole</td>
<td>5.86</td>
<td>2.52</td>
<td>2.61</td>
<td>20.7</td>
<td>70.35</td>
<td></td>
</tr>
<tr>
<td>5-Bromoindole</td>
<td>5.86</td>
<td>7.55</td>
<td>2.73</td>
<td>41.82</td>
<td>92.02</td>
<td></td>
</tr>
<tr>
<td>6-Chloroindole</td>
<td>2.49</td>
<td>2.35</td>
<td>n</td>
<td>&lt;0.3125</td>
<td>3.35</td>
<td></td>
</tr>
<tr>
<td>6-Chlorooxindole</td>
<td>29.19</td>
<td>16.94</td>
<td>n</td>
<td>1.48</td>
<td>9.22</td>
<td></td>
</tr>
<tr>
<td>Gramine</td>
<td>16.92</td>
<td>&lt;2.5</td>
<td>6.26</td>
<td>253.44</td>
<td>338</td>
<td></td>
</tr>
</tbody>
</table>

n: Not determined.
For further assessing the effect of these compounds on bacterial growth and biofilm formation, bacterial cell numbers, cell morphology were determined by slides culture assay. The results of cells numbering showed that indole derivatives significantly inhibited bacterial adhesion on the surface of the slide. The numbers of attached cells on the slides when treated with indole, 5-chloroindole, 5-bromoindole, 6-chloroindole, 6-chlorooxindole and gramine were 4.9%, 13.6%, 10.4%, 3.5%, 7.7% and 78.1% of the control, respectively (Fig. 2). It was not significantly different for cell adhesion between the CuSO4-treated group and the control.

For the treatment groups of gramine, 6-chloroindole and the control, light microscope and DAPI staining fluorescence observations revealed that the adhesion cell numbers of the control group were greater than that of the treatment groups (Fig. 3). Especially, 6-chloroindole remarkably inhibited the adhesion of bacterial cells. As an example of its excellent inhibitory effect on bacterial cells, SEM images revealed that cell structures of *P. aeruginosa* were totally damaged (Fig. 4).

### 3.3. Effect of indole derivatives on the growth of *Cylindrotheca* sp.

The effect of indole derivatives on the growth of diatom *Cylindrotheca* sp. was conducted in this experiment. The growth rate of each given logarithm at defined treated concentrations was used to calculate dose–effect relationships and EC50 value was determined by a non-linear logistics equation. With increasing the concentration of indole derivatives and CuSO4, a decrease in the growth rate of algae was observed. The EC50 values calculated for CuSO4, indole, 5-chloroindole, 5-bromoindole, 6-chloroindole, 6-chlorooxindole and gramine were 11.24, 87.78, 12.9, 15.88, 3.32, 4.6 and 1.96 mg L⁻¹, respectively (Fig. 5). The inhibitory effects of 6-chloroindole, 6-chlorooxindole and gramine on the growth of diatom were much better than that of CuSO4 and other indole derivatives in this study.

### 3.4. Effect of 6-chloroindole on Ca²⁺ flux in *Cylindrotheca* sp.

The NMT was used to reveal the net Ca²⁺ flux of diatom *Cylindrotheca* sp. in the presence of 6-chloroindole, which was very effective to inhibit the growth and cell adhesion of bacteria and diatom according to the above report. In this experiment, the cells of *Cylindrotheca* sp. were treated with 10 mg L⁻¹ 6-chloroindole and then was immediately detected by NMT (Fig. 6). Fig. 6a showed that net Ca²⁺ efflux was triggered shortly after algal cells were treated with 6-chloroindole. With increasing the exposure time, a gradual decrease was observed in cellular net Ca²⁺ efflux, and finally the Ca²⁺ flux returned to the control level. The peak Ca²⁺ efflux of the treatment group was obviously higher than that of the control and the value was 72.03 pmol cm⁻² s⁻¹, which was 10.6 times of the control (Fig. 6b).

### 4. Discussion

As a part of continuing efforts to discover effective and environmental friendly alternatives instead of toxic antifoulants, many secondary metabolites from marine organisms have been reported (Xu et al., 2009; Shen et al., 2012; Zhao et al., 2013). Representative heterocyclic compounds, indole derivatives showed good antifouling activities against algae, bacteria, barnacle and mussel (Omae, 2006). Based on the simple structure and environmental friendly features, indole derivatives have been widely considered as potential antifoulant agents. Therefore, about one hundred kinds of indole compounds had been synthesized by chemical methods (Konya et al., 1994; Kawamata et al., 2006). Li et al. (2009) also
synthesized many kinds of indole derivatives and tested their anti-
agal activity. These reports prompted investigation of the properties of indole substances representing a diverse range of structural classes. However, there are little systematic antifouling activities and mechanisms evaluations of indole derivatives on bacteria and algae especially for simple indole substances. Here, for the first time, six kinds of indole substances were assessed for antibacterial and anti-algal activities which were represented by the growth and the formation of biofilm. CuSO$_4$ was used as a reference in order to evaluate the antifouling activity of indole substances compared with other reported bioactive substances.

In this study, indole derivatives showed good antibacterial activities by MIC evaluation. Overall, the antibacterial activities of 6-chloroindole and 6-chlorooxindole were better than that of 5-chloroindole and 5-bromoindole. Of these compounds, the activity of 6-chloroindole was the best and that of gramine was the worst. Several reports also focused on the antibacterial activity of some chemicals such as 3-phenyl-2-propenoic acid, Diketopiperazine (DKP) and zosteric acid (Newby et al., 2006; Qi et al., 2009). The MIC of 3-phenyl-2-propenoic acid against Loktanella hongkongensis was 80 mg L$^{-1}$ and the EC$_{50}$ of Zosteric acid against Pseudomonas putida was 166 mg L$^{-1}$. Compared with our results (Table 2), the MIC values of these chemicals against bacteria were generally higher than that of indole derivatives. Although the tested strains were different, the obtained results indicated that indole derivatives had good antimicrobial activities in this study.

The slide culture is a very good method to observe the formation of bacterial biofilm (Kawarai et al., 2009). The quantitative analysis by counting bacterial numbers on the slide showed that indole substances effectively suppressed the growth and adhesion of bacteria on the glass slide, which were superior to that of CuSO$_4$. Patil and Jagadeesan (2011) also used microscopic observation severe. Several reports also focused on the antibacterial activity of some chemicals such as 3-phenyl-2-propenoic acid, Diketopiperazine (DKP) and zosteric acid (Newby et al., 2006; Qi et al., 2009). The MIC of 3-phenyl-2-propenoic acid against Loktanella hongkongensis was 80 mg L$^{-1}$ and the EC$_{50}$ of Zosteric acid against Pseudomonas putida was 166 mg L$^{-1}$. Compared with our results (Table 2), the MIC values of these chemicals against bacteria were generally higher than that of indole derivatives. Although the tested strains were different, the obtained results indicated that indole derivatives had good antimicrobial activities in this study.

Fig. 3. Micrographs of control, gramine and 6-chloroindole-treated biofilms of *P. aeruginosa*. a1 and c1. Light microscope; a2, b2 and c2. DAPI staining; a1 and a2. Control; b1 and b2. Gramine; c1 and c2. 6-Chloroindole.

Fig. 4. The scanning electron microscope (SEM) images of control and 6-chloroindole-treated biofilms of *P. aeruginosa* at different magnification. a1 and a2. Control; b1 and b2. 6-Chloroindole.
techniques to show that the development of marine biofilm was inhibited by chlorine as an effective antifouling technique. The only difference between them was that the report mainly focused on the biofilms dominated by diatom. The results exhibited that chlorine increased the numbers of dead cells in comparison with the control, reduced the photosynthesis and damaged cellular structure of *Amphora* and *Navicula*.

Diatom is the earliest eukaryotic colonizer of submerged surfaces and one of the most important organisms in biofouling. In our experiment, the diatom *Cylindrotheca* sp. isolated from fouling biofilm samples in Yantai Coastal Zone, was chosen as the test organism. The effect of indole derivatives on the growth of diatom was determined and expressed by EC$_{50}$. From the obtained results (Fig. 5), the anti-algal activity was orderly gramine > 6-chloroindole > 6-chlorooxindole > CuSO$_4$ > 5-chloroindole > 5-bromoindole > indole. Different from the antibacterial activities of these compounds, gramine exhibited strong inhibitory effect on the growth of diatom in this study. Similarly, 6-chloroindole and 6-chlorooxindole showed better anti-algal activities than that of 5-chloroindole and 5-bromoindole. Zhou et al. (2013) reported that nonivamide, a new antifouling agent, inhibited the growth of diatom *Phaeodactylum tricornutum* with the EC$_{50}$ value of 5.1 mg L$^{-1}$.

![Fig. 5. Effects of indole derivatives and CuSO$_4$ on the algal cells growth of Cylindrotheca sp. All error bars indicated S.D. of the six replicates. The EC$_{50}$ values were shown in the corresponding figures.](image-url)
Fig. 6. Effects of 6-chloroindole on net Ca$^{2+}$ fluxes (pmol cm$^{-2}$ s$^{-1}$) in Cylindrotheca sp. (a) Net Ca$^{2+}$ fluxes in algal cells were treated with 10 mg L$^{-1}$ 6-chloroindole. Arrow indicated that 6-chloroindole was added into the measuring solution including algal cells. (b) The maximum values of net Ca$^{2+}$ fluxes in algal cells. Error bars indicated the S.D. of the mean. **(p < 0.01) indicated significant differences compared to the control without 6-chloroindole.

However, the EC$_{50}$ values of 6-chloroindole and gramine against diatom Cylindrotheca sp. were only 3.32 and 1.96 mg L$^{-1}$. All the results indicated that indole derivatives had excellent anti-algal activities as potential antifouling agents.

The formation of biofilm is very important for the adhesion of subsequent fouling organisms. The studies have proved that bacterial biofilm of Pseudalteromonas spongiae was associated with its induction of larval settlement of the polychaete Hydroides elegans and the settlement was correlated with bacterial density (Lau et al., 2005; Huang et al., 2007), and the reports suggested that the extracellular polymers of benthic diatoms induced larval settlement in the polychaete Hydroides elegans (Lam et al., 2003, 2005). Similarly, bacterial biofilm promoted the settlement of Ulva spores and Balanus improvisus cyprid (Joint et al., 2002; Tait and Havenhand, 2013). Based on these reports, it is important to consider biofilm (bacteria and diatom) as antifouling models for screening bioactive substances. Bacteria and diatom as models have the advantages of more convenient cultivation, more rapid growth, lower price, more automated and faster counting method. Therefore, in the present study, bacteria and diatoms were used as models and it was found that indole derivatives had good antibacterial and anti-algal activities, in particular 6-chloroindole.

NMT is a useful tool for real-time measuring extracellular ions or molecular activities to noninvasively acquire information about their transportation in intact samples. It provides researchers a new approach to study the physiological mechanism in plants, animals and microorganisms. It had been reported that the technique was effective to detect the flux of ions, such as Ca$^{2+}$, H$^+$, Na$^+$, K$^+$, Cd$^{2+}$ and O$_2$ (Ma et al., 2010; Velard-Buendía et al., 2012; Zeng et al., 2012). In this experiment, NMT was used to determine the Ca$^{2+}$ flux in diatom Cylindrotheca sp. under 6-chloroindole treatment. The data indicated that the rate of Ca$^{2+}$ efflux in algal cells increased with the treatment of 6-chloroindole. Ca$^{2+}$ efflux was significantly induced by 6-chloroindole with a peak of 72.03 pmol cm$^{-2}$ s$^{-1}$, which was 10.6 times of the control. The results suggested that the induced Ca$^{2+}$ efflux in algal cells might be one of the reasons of 6-chloroindole resulting in obvious inhibitory effect on the growth of the diatom. The report about the cytotoxic mechanisms of gramine substances showed that indole derivatives 5,6-dibromo-1,2-dimethylgramine evoked Ca$^{2+}$ release from skeletal muscle Sarcoplasmic reticulum through ryanodine receptors and indicated that gramine derivatives were useful tools for the investigation of Ca$^{2+}$ release from Sarcoplasmic reticulum (Nakahata et al., 1999). Iwata et al. (2001) also reported that gramine derivatives 2,5,6-tribromo-1-methylgramine and 5,6-dibromo-1,2-dimethylgramine decreased cytosolic Ca$^{2+}$ level by inhibiting the transient increase in isolated rat aorta. Gramine derivatives reduced intracellular Ca$^{2+}$ abundance in animal cells, which might contribute to their inhibitory effects on the growth. Similarly, our results also showed that 6-chloroindole increased Ca$^{2+}$ efflux in algal cells. It was reported that calcium ion was closely related to algal many physiological activities and the adhesion of diatom also required the involvement of calcium (Cooksey and Cooksey, 1992; Vardi et al., 2006). Therefore, Ca$^{2+}$ efflux induced by 6-chloroindole might promote the inhibitory effect on the growth and adhesion of diatom. To our knowledge, this was the first time to report Ca$^{2+}$ flux in diatom and its dynamic changes induced by indole derivatives. The finding prompted us to hypothesize the mechanisms for anti-algal activities of indole derivatives were closely associated with cellular Ca$^{2+}$. However, the mechanisms of indole derivative against algae and bacteria were necessary to be further studied in the future work for their application in marine environment.

5. Conclusion

Bacteria and diatoms have the advantage as models for rapid screening the activities of substances. In our experiment, it was demonstrated that indole derivatives had good antibacterial and anti-algal activities as potential antifouling agents, especially 6-chloroindole. 6-chloroindole obviously inhibited the growth of bacteria, interfered with the formation of bacterial biofilm, destroyed bacterial cellular morphology and inhibited the growth of diatom. By using NMT analysis, 6-chloroindole triggered algal cellular Ca$^{2+}$ efflux, which might be associated with the inhibitory effect on the adhesion and growth of diatom. Indole derivatives have the potential to be new antifouling agents. Good understanding of the antifouling activities and mechanisms on fouling organisms is prerequisite for their commercial application in marine environment.

Acknowledgements

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References


