The ionic liquid 1-alkyl-3-methylimidazolium demonstrates comparable antimicrobial and antibiofilm behavior to a cationic surfactant

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Biofilms are problematic in health and industry because they are resistant to various antimicrobial treatments. Ionic liquids are a novel class of low temperature liquid salts consisting of discrete anions and cations, and have attracted considerable interest as safer alternatives to organic solvents. Ionic liquids have interesting antimicrobial properties and some could find use in the development of novel antiseptics, biocides and antifouling agents. The antimicrobial and antibiofilm activity of 1-dodecyl-3-methylimidazolium iodide ([C12MIM]I) was studied using the clinically important bacterial pathogens, *Staphylococcus aureus* SAV329 and *Pseudomonas aeruginosa* PAO1. The ionic liquid increased cell membrane permeability in both *S. aureus* and *P. aeruginosa* cells and impaired their growth, attachment and biofilm development. The ionic liquid exhibited superior antimicrobial and antibiofilm activity against the Gram-positive *S. aureus* compared to the Gram-negative *P. aeruginosa* cells. BacLight™ staining and confocal microscope imaging confirmed that the ionic liquid treatment increased the cell membrane permeability of both the Gram-positive and Gram-negative bacteria. In addition, the antimicrobial and antibiofilm properties of [C12MIM]I were similar or superior to those of cetyltrimethylammonium bromide (CTAB), a well-known cationic surfactant. It is concluded that the ionic liquid induced damage to bacterial cells by disrupting cell membrane, leading to inhibition of growth and biofilm formation. Overall, the results indicate that the ionic liquid 1-dodecyl-3-methylimidazolium iodide was effective in preventing *S. aureus* and *P. aeruginosa* biofilms and could have applications in the control of bacterial biofilms.

**Keywords:** 1-alkyl-3-methylimidazolium; antibiofilm; BacLight™ bacterial staining; biofilm control; CTAB; ionic liquid

**Introduction**

Most bacteria form biofilms, when they grow on surfaces in industrial and medical settings. In industry, biofilm growth can impede heat transfer and flux across membranes, and cause food spoilage and equipment contamination. In health, biofilms are problematic because they tend to grow on teeth and on implanted medical devices and cause persistent infections. Biofilm bacteria exhibit increased resistance to antimicrobials (Mah and O'Toole 2001), thus making biofilm-mediated infections difficult to control. Therefore, development of new antimicrobials and methods to eradicate biofilms in industrial and in clinical settings is of considerable interest.

Ionic liquids (ILs) are novel class compounds, existing as liquid salts at ~100°C, consisting entirely of ions (Rogers 2003). Ionic liquids have no detectable vapour pressure and do not contribute volatile organic compounds to the atmosphere. Thus, ILs have attracted great interest as safer alternatives to organic solvents in the chemical industry (Pham et al. 2010). The properties of ILs have led to their use in synthesis, solvent extraction, and catalysis (Sheldon et al. 2002), in biomass dissolution (Singh et al. 2009; Nancharaiah and Francis 2011), and as active pharmaceutical ingredients (APIs) (Hough et al. 2007) and antimicrobials (Pernak et al. 2003; Pham et al. 2010). Typically, ILs are composed of a combination of an organic cation with an organic or inorganic anion. The tunability by way of independent modification of the constituent ions (cations and anions) provides an unprecedented flexibility in the design of ionic liquids with unique properties. There is a considerable interest in the development of ionic liquids with novel physical, chemical and biological properties. Though generally considered green, the toxicity of water-miscible ionic liquids is widely debated. Research shows that certain ionic liquids exhibit potent antimicrobial activities (Pernak et al. 2003; Demberelnyamba et al. 2004). Since the toxicity itself is a tunable property, it would be possible to design ionic liquids for use as antiseptics,
disinfectants and antifouling agents (Pernak et al. 2004).

Recently, the anti-biofilm activities of certain ionic liquids (ie 1-alkyl-3-methylimidazolium chlorides, 1-alkylquinolinium bromides) have been evaluated against bacteria (Carson et al. 2009; Busetti et al. 2010). However, thorough investigations on the effect of these novel compounds on bacterial adhesion and biofilm formation are limited. Many common ionic liquids share structural similarity with API's and cationic-surfactants, which kill cells by disrupting cell membrane (Simões et al. 2005). There are a few studies on the similarities between the surface activity of ionic liquids and cationic surfactants (Pernak et al. 2003; Łuczak et al. 2010; McLaughlin et al. 2011). The antimicrobial activity was related to the alkyl group chain length of ionic liquids (Pernak et al. 2003). Because of the structural similarity with cationic surfactants, it was indicated that ionic liquids interact with the negatively charged bacterial cell membrane and may lead to cytoplasmic membrane disruption. However, there is no evidence for such an effect of alkyl-methylimidazolium ionic liquids on bacterial cells.

Due to the structural similarity with cationic surfactants, the authors hypothesized that ionic liquids may exhibit antimicrobial and/or anti-biofilm activity through cytoplasmic membrane damage. In the present study, the antimicrobial and anti-biofilm activity of a selected ionic liquid, 1-dodecyl-3-methylimidazolium iodide ([C12MIM]I) was studied using a Gram-positive bacterium and a Gram-negative bacterium. [C12MIM]I was chosen for the study because 1-alkyl-3-methylimidazolium halides are easy to synthesize and are relatively inexpensive (Carson et al. 2009). The antimicrobial and anti-biofilm potency of [C12MIM]I was compared with that of a well-known cationic surfactant, cetyl-trimethylammonium bromide (CTAB). The LIVE/DEAD® BacLight™ bacterial viability stain and confocal laser scanning microscopy (CLSM) were used to elucidate the mode of action of the ionic liquid on bacterial cell membranes. In addition, the fluorescence intensity of SYTO 9 and propidium iodide (PI) in bacterial cells exposed to different concentrations of ionic liquid was determined. Further experimental evidence for cytoplasmic membrane damage was obtained by monitoring the leakage of UV-absorbing material and ATP from bacterial cells exposed to ionic liquid.

**Materials and methods**

**Ionic liquids**

The chemicals, [C12MIM]I and CTAB were obtained from Sigma-Aldrich and used as received. The chemical structures of the ionic liquid and CTAB are given in Table 1. Stock solutions of [C12MIM]I and CTAB were prepared in sterile ultrapure water and stored at room temperature.

**Bacterial strains and growth conditions**

The Gram-positive bacterium, *Staphylococcus aureus* V329 and the Gram-negative bacterium, *Pseudomonas aeruginosa* PAO1 were used in this study. *S. aureus* V329 is a strong biofilm forming bacterium isolated from bovine mastitis (Cucrella et al. 2001). *P. aeruginosa* PAO1 is an environmental bacterium and opportunistic human pathogen and one of the best studied model organisms for biofilm formation (Klausen et al. 2003). The bacteria were routinely maintained on tryptic soy agar (TSA) and cultured in half-strength tryptic soy broth (TSB) supplemented with 0.25% (w/v) glucose. After inoculation, TSB was incubated at

| Compound name                  | Abbreviation | Structure |
|-------------------------------|--------------|-----------|
| 1-dodecyl-3-methylimidazolium iodide | [C12MIM]I   | ![Structure](image) |
| Cetyl-trimethylammonium bromide | CTAB         | ![Structure](image) |
30°C in an orbital shaker at 100 rpm. Overnight grown cultures were used as the inoculum in growth, adhesion and biofilm experiments.

**Antimicrobial activity**

The broth micro-dilution method was used for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). [C$_{12}$MIM]I and CTAB were diluted in half-strength TSB ranging from 0 to 250 µM for *P. aeruginosa* and 0 to 250 µM for *S. aureus* by 2-fold serial dilutions. Inoculum (OD$_{600}$ = 0.2) was added and 200 µl aliquots were transferred to each well of a 96-well microtiter plate. The final cell density in each well was ~2 x 10^5 CFU ml$^{-1}$. The plates were then incubated at 30°C in an orbital shaker at 100 rpm for 24 h. Planktonic growth of bacteria was monitored by measuring the optical density (OD) at 600 nm. The concentration which caused complete inhibition of planktonic growth of the bacteria was described as MIC. At the end of 24 h, the planktonic culture was spread plated onto TSA plates. The concentration at which no colonies appeared on agar plates was described as MBC. In an experiment, six wells were recorded for each tested concentration. Experiments were carried out in triplicates.

**Microtiter plate biofilm assay**

Half-strength TSB with different concentrations of [C$_{12}$MIM]I or CTAB was inoculated with *P. aeruginosa* or *S. aureus* and 200 µl aliquots were transferred to wells of a polystyrene microtiter plate. The plates were covered and incubated at 30°C and 100 rpm in an orbital shaker for 24 h. The spent medium was discarded. The wells were washed thoroughly with sterile water and stained by addition of 200 µl of 0.2% crystal violet (CV) to each well for 5 min. The unbound stain was removed by thorough washing with water and the bound CV was eluted into 95% ethanol. CV absorbance was measured at 570 nm and used to represent biofilm biomass.

The broad-spectrum antibiofilm activity of the ionic liquid was tested using freshwater (the cooling water of a fast breeder test reactor, Kalpakkam, India) as the inoculum. The freshwater was supplemented with 0.2% (w/v) glucose to stimulate biofilm formation. Various concentrations of [C$_{12}$MIM]I were prepared in freshwater and dispensed into 96-well microtiter plate as 200 µl aliquots. Freshwater without the ionic liquid served as the control. Sterilized freshwater was used as the blank. Microtiter plates were incubated for 48 h as described above. Biofilm was stained with crystal violet, eluted with ethanol and quantified by measuring absorbance at 570 nm. Planktonic growth was determined by counting the colony forming units (CFU) on nutrient agar. The CFU were counted after incubation for 5 days.

**Glass slide biofilm assay**

Twenty ml of half-strength TSB containing varying concentrations of [C$_{12}$MIM]I or CTAB was dispensed into 50 ml falcon tubes and inoculated with 50 µl of overnight grown culture (OD 600 nm = 0.2). Clean glass slides were sterilized by immersing in alcohol followed by flaming. Each sterile glass slide was transferred to a falcon tube and was incubated at 30°C in an orbital shaker at 100 rpm. At different time intervals, the glass slides were retrieved, washed and stained with acridine orange. Selected slides were also stained using LIVE/DEAD® BacLight™ bacterial viability kit (L-7012, Molecular Probes, USA). The slides were washed and covered with a glass coverslip using a sealant. Stained biofilms were imaged using confocal microscopy.

**Adhesion assay**

Cultures were grown overnight, and then adjusted with sterile medium to an OD$_{600}$ of 0.2. Cell suspensions with varying concentrations of [C$_{12}$MIM]I or CTAB were dispensed as 200 µl aliquots into 96-well microtiter plate and incubated at 30°C and 100 rpm on an orbital shaker for 2 h. After incubation, the medium was discarded and the wells were rinsed thoroughly with water before staining with 0.2% CV for 5 min. Adherent cells were quantified after eluting the bound CV into 95% ethanol, whose absorbance was measured at 570 nm.

**Effect of ionic liquid on cell membrane permeability**

Ionic liquid and CTAB were diluted 2-fold in cell suspension (OD$_{600}$ = 0.2) in 15 ml falcon tubes. These cell suspensions were incubated at 30°C and 100 rpm in an orbital shaker. A 0.5 ml sample was collected after 1 h contact time, centrifuged, washed and stained with the LIVE/DEAD® BacLight™ bacterial viability staining kit.

**Leakage of UV-absorbing substances**

In order to discern damage to cytoplasmic membranes, leakage of intracellular substances from bacterial cells upon exposure to [C$_{12}$MIM]I was determined according to a previously reported protocol (Virto et al. 2005). Overnight grown cells were harvested, suspended in distilled water by adjusting OD$_{600}$ to 0.5. These cells were treated with different concentrations of ionic liquid for 1 h. Treated and untreated cells were centrifuged at 6000 × g for 10 min. The absorbance of
the supernatant was measured at 260 and 280 nm using a spectrophotometer (Shimadzu, Japan).

**Leakage of adenosine triphosphate (ATP)**

Leakage of ATP from bacterial cells upon exposure to ionic liquid was determined using the luciferase assay. Overnight grown cells were harvested, washed and suspended in distilled water by adjusting the OD$_{600}$ to 0.2. These cells were exposed to different concentrations of [C$_{12}$MIM]I for 1 h. Subsequently, the supernatant was collected by centrifugation at 6000 × g for 5 min for monitoring ATP using the Firefly Lantern Extract (Sigma) kit. A 100 µl of reagent was added to 100 µl of supernatant in 96-well microtiter plate. Light emission was monitored in a Synergy HT Multi-Mode microplate reader (BioTek, USA) and expressed as relative luminescence units (RLU).

**Fluorimetric detection of cell membrane permeability**

Overnight grown cells were harvested, washed and suspended in distilled water by adjusting the OD$_{600}$ to 0.2. Untreated and treated cells were centrifuged, and the cell pellets were washed, and re-suspended in BacLight viability stain. After staining for 15 min in the dark, the fluorescence was measured using a Synergy HT Multi-Mode microplate reader (BioTek, USA). The fluorescence of cells excited at a wavelength of 485 nm was collected at 530 nm (SYTO 9: green) and 630 nm (PI: red). Results are presented as the ratio of the green and red fluorescence intensities.

**Staining and confocal laser scanning microscopy (CLSM)**

Stained planktonic cells or biofilms were imaged using a TCS SP2 AOB5 confocal laser scanning microscope (Leica Microsystems, Germany). A 63×1.2 NA water immersion objective was used for imaging. A 488 nm laser was used for excitation. Emission was collected between 500 and 520 nm for SYTO 9 and between 600 and 620 nm for PI. Images were collected using 1.2 airy pinhole and 2× line averaging. Images are presented using Leica confocal software 2.0.

**Results and discussion**

Alkyl-methylimidazolium salts were found to be efficient in inhibiting the growth of both Gram-positive and Gram-negative bacteria (Pernak et al. 2003; Demberelnyamba et al. 2004). Ionic liquids containing 1-alkyl-3-methylimidazolium cation were evaluated to be effective in controlling bacterial growth and biofilm formation (Carson et al. 2009). These studies show promising applications of 1-alkyl-methylimidazolium ionic liquids in the development of effective antimicrobials. However, the mechanism of action of these novel compounds on bacteria and on biofilm formation remains to be elucidated.

Growth of *S. aureus* and biofilm formation by this bacterium in the presence of different concentrations of ionic liquid and CTAB are shown in Figure 1. Complete inhibition of growth of *S. aureus* was observed at a [C$_{12}$MIM]I concentration of 0.31 µM. Typically, ionic liquids are salts, composed of heterocyclic organic cations and organic or inorganic anions. The growth and biofilm formation by *S. aureus* and *P. aeruginosa* were unaffected by the anion group, iodide and 1-methylimidazole (Figures S1 to S4) [Supplementary material is available via a multimedia link on the online article webpage]. Thus, the antimicrobial activity of the [C$_{12}$MIM]I could be attributed solely to the whole cation, 1-dodecyl-3-methylimidazolium. The antimicrobial activity appears to be determined mainly by the cationic component at least in the case of

![Figure 1. Growth (a) and biofilm formation (b) by *S. aureus* in TSB supplemented with various concentrations of ionic liquid or CTAB. Growth was significantly inhibited at concentrations of the ionic liquid as low as 0.31 µM. Cells were cultured in 96-well plates and stained with crystal violet to quantify biofilm formation. The ionic liquid and CTAB impaired biofilm formation in *S. aureus* at concentration as low as 0.31 µM.](image-url)
1-alkyl-3-methylimidazolium halides (Pernak et al. 2003; Carson et al. 2009). This is because the cationic components can interact with the bacterial cell envelope by displacing cations. Subsequent interactions with the cytoplasmic membrane components can result in membrane disruption and leakage of cytoplasmic material (Moore et al. 2008). Biofilm formation in *S. aureus* was completely impaired by a concentration of 0.31 μM and higher of [C12MIM][I]. The cationic surfactant, CTAB inhibited growth and biofilm formation in *S. aureus* at 0.31 μM. The MIC and MBC values (Table 2) of the ionic liquid obtained in the present study were smaller compared to those reported in earlier studies (Carson et al. 2009). These differences in the absolute MIC values could be due to basic differences in bacterial strains, medium composition, inoculum cell density and culture conditions.

Unlike the case in *S. aureus*, growth and biofilm formation in *P. aeruginosa* was inhibited at higher concentrations of the ionic liquid (Figure 2). Growth was significantly reduced at 100 μM and complete inhibition of biofilm formation was observed at a [C12MIM][I] concentration of 125 μM. Interestingly, CTAB exhibited antimicrobial activity against *P.

### Table 2. MIC and MBC values (μM) of [C12MIM][I] and CTAB.

| Compound     | *S. aureus* MIC | *S. aureus* MBC | *P. aeruginosa* PAO1 MIC | *P. aeruginosa* PAO1 MBC |
|--------------|-----------------|-----------------|--------------------------|--------------------------|
| [C12MIM][I] (μM) | 0.31            | 5               | 125                      | 250                      |
| CTAB (μM)    | 0.31            | 5               | 125                      | 250                      |

Note: *Determined by measuring the OD after incubation for 24 h.

Figure 2. (a) Growth of *P. aeruginosa* PAO1 in the presence of different concentrations of ionic liquid and CTAB. Growth was inhibited completely at a concentration of 125 μM. Cells of PAO1 were cultured in 96-well plates for 24 h in the presence of different concentrations of ionic liquid and CTAB. (b) Biofilm was quantified by crystal violet (CV) staining. Biofilm formation was significantly impaired by ionic liquid at a concentration of 125 μM.

Figure 3. Adhesion of *S. aureus* and *P. aeruginosa* cells in 96-well plates in the presence of different concentrations of ionic liquid and CTAB. Overnight grown cells were harvested, the OD was adjusted to 0.2 in TSB medium and the cells were allowed to adhere to polystyrene wells for 2 h. Attached cells were quantified by crystal violet (CV) staining and elution into 95% ethanol. Absorbance of CV was measured at 570 nm. Adhesion of *S. aureus* was impaired by ionic liquid at a concentration of 0.8 μM and adhesion of PAO1 was impaired by ionic liquid at a concentration of 250 μM. IL2 = [C12MIM][I].
in a dose-dependent manner similar to that of \([\text{C}_{12}\text{MIM}]\). In order to determine the anti-adhesion ability, bacterial adhesion was determined after incubation for 2 h in the presence of different concentrations of ionic liquid and CTAB. Adhesion of both \(S.\ \text{aureus}\) and \(P.\ \text{aeruginosa}\) cells was impaired in the presence of \([\text{C}_{12}\text{MIM}]\) (Figure 3). In \(S.\ \text{aureus}\), adhesion was impaired by a \([\text{C}_{12}\text{MIM}]\) concentration of 0.8 \(\mu\text{M}\) and higher. Adhesion of \(P.\ \text{aeruginosa}\) was unaffected by the presence of lower concentrations of \([\text{C}_{12}\text{MIM}]\) but adhesion of this bacterium was impaired by 250 \(\mu\text{M}\) of \([\text{C}_{12}\text{MIM}]\). The cationic surfactant, CTAB impaired adhesion both in \(S.\ \text{aureus}\) and \(P.\ \text{aeruginosa}\) cells. However, the impairment of adhesion in \(P.\ \text{aeruginosa}\) required higher concentrations of CTAB (Figure 3). Confocal imaging confirmed the anti-biofilm activity of ionic liquid on \(S.\ \text{aureus}\) and \(P.\ \text{aeruginosa}\) (Figure 4). It is evident from the data that \([\text{C}_{12}\text{MIM}]\) exhibited potent antimicrobial, anti-adhesion and anti-biofilm activities.

In order to understand the mode of action of ionic liquid on bacterial cells, viability staining based on a \(\text{SYTO} \ 9 – \text{PI}\) combination was used. According to this protocol, bacteria cells having normal cytoplasmic membranes are stained by \(\text{SYTO} \ 9\) but not by \(\text{PI}\). Being a large molecule, \(\text{PI}\) can enter cells having a cell membrane with higher permeability (compromised cell membranes). Once inside the cells, \(\text{PI}\) can replace and exclude \(\text{SYTO} \ 9\) from the cells because of its higher affinity towards nucleic acids (Virto et al. 2005; Saravanan et al. 2006; Kim et al. 2008). As evident from the confocal images, the cell membrane permeability of both \(S.\ \text{aureus}\) and \(P.\ \text{aeruginosa}\) was markedly increased by the interaction with the ionic liquid (Figure 5). It is clear that the ionic liquid increased the membrane permeability that led to the extensive staining of cells by the \(\text{PI}\). Moreover, the damage was dose dependent, because a higher proportion of cells were stained by the \(\text{PI}\) at higher concentrations of \([\text{C}_{12}\text{MIM}]\). At lower concentrations, some of the treated cells were either green or yellow, indicating no damage or partial damage (Figure 5). These cells could resume growth and biofilm formation during extended period of

![Figure 4. Biofilm formation by \(S.\ \text{aureus}\) and \(P.\ \text{aeruginosa}\) PAO1 in TSB medium (control) and in the presence of \([\text{C}_{12}\text{MIM}]\). Biofilms of \(S.\ \text{aureus}\) were stained with acridine orange and imaged using confocal microscopy. \(P.\ \text{aeruginosa}\) biofilms were stained with BacLight live/dead bacterial viability staining. Green = \(\text{SYTO} \ 9\); red = propidium iodide.](image-url)
incubation (data not shown). At higher concentrations, all the cells were damaged such that they were unable to resume growth and biofilm formation. CTAB also caused membrane damage in a large proportion of cells (Figure S5) [Supplementary information is available via a multimedia link on the online article webpage]. However, the effect of CTAB on membrane permeability was less compared to the ionic liquid. The ionic liquid used in the study appears to be mechanistically similar to CTAB in its interaction with the bacterial cells. However, the activity of the ionic liquid cannot be directly comparable to CTAB, due to the difference in the head group and alkyl chain. The alkyl chain length is much shorter in the chosen IL (C_{12}) as compared to the alkyl chain (C_{16}) of CTAB. From these results, it is argued that the ionic liquid increased the cell membrane permeability in the Gram-positive and the Gram-negative bacteria, thereby inhibiting growth, adhesion and biofilm formation. The broad-spectrum activity of [C_{12}MIM]I was clearly evident when it prevented biofilm formation on polystyrene surfaces exposed to freshwater (Figure 6). [C_{12}MIM]I exhibited a clear dose response effect on multispecies biofilm formation in freshwater. Biofilm formation was unaffected at [C_{12}MIM]I concentrations of 0.01–0.02 μM, while it was moderately affected at 0.05–0.1 μM. Inhibition of biofilm formation was >80% at [C_{12}MIM]I concentrations of 0.2–0.4 μM. Complete inhibition in biofilm formation was observed at [C_{12}MIM]I concentrations ≥0.8 μM. Planktonic growth was also affected in freshwater dosed with different concentrations of the ionic liquid (Table S1) [Supplementary material is available via a multimedia link on the online article webpage].

In order to confirm the influence of ionic liquid on permeabilization of bacterial cytoplasmic membranes, the leakage of intracellular material was determined. Extensive leakage of UV-absorbing substances was observed following ionic liquid treatment of cells (Figure 7a). The values for absorbance at 260 nm for supernatants of S. aureus and P. aeruginosa cells indicated permeabilization of cytoplasmic membranes. Exposure of cells to ionic liquid also caused leakage of ATP into the extracellular medium (Figure 7b). These observations further support the present findings based on live/dead staining which show that the ionic liquid increased cytoplasmic membrane permeability in S. aureus and P. aeruginosa cells. In addition, fluorimetric measurements also showed a decrease in the ratio of the green and red fluorescence intensities of bacterial cells upon exposure to ionic liquid (Figure S6) [Supplementary information is available via a multimedia link on the online article webpage], clearly indicating loss in viability.

Figure 5. Changes in cell membrane permeability of S. aureus and P. aeruginosa cells exposed to ionic liquid, [C_{12}MIM]I. Overnight grown cells were harvested and re-suspended in medium containing varying concentrations of ionic liquid. Green = SYTO 9 signal; red = propidium iodide signal. The cell membrane permeability of S. aureus was substantially increased by 1.6 μM [C_{12}MIM]I. In contrast, the cell membrane permeability of P. aeruginosa cells was substantially increased by 25 μM [C_{12}MIM]I.
The activity of the antimicrobial ionic liquid was comparable to that of the cationic surfactant in terms of (1) similar MIC values, (2) impaired adhesion and biofilm growth, and (3) superior antimicrobial activity against a Gram-positive bacterium compared with a Gram-negative bacterium. Moreover, the effect on bacterial cytoplasmic membranes was dose-dependent, indicating that both of these compounds cause damage to cytoplasmic membranes. It is apparent that the ionic liquid caused damage (increased cell membrane permeability, inhibition of growth, impairment in adhesion and biofilm formation) to bacterial cells in a manner similar to that of a well-known cationic surfactant such as CTAB. Typically, cationic surfactants are highly potent anti-biofilm compounds and their mode of action involves disruption of bacterial cell membranes (Simões et al. 2005). In general, they disrupt the lipid bilayer in bacterial cell membranes, causing leakage of cytoplasmic material and ultimately killing the bacterial cells (Gilbert and Moore 2005). Gram-negative bacteria are less susceptible to cationic surfactants because of the lipopolysaccharide layer of the outer cell membrane (Denyer and Maillard 2002; Gilbert and Moore 2005). Due to their lipophilic nature, the alkyl-chain of the cation of the ionic liquid may interact with the cell membrane constituents, thereby increasing membrane permeability. Here, for the first time, experimental evidence is presented which shows that antimicrobial ionic liquid [C12MIM]I disrupts bacterial cell membranes in a manner similar to a cationic surfactant, eventually leading to cell lysis and death. Recent studies show promising results on the design of more efficient antimicrobial ionic liquids (Alberto et al. 2011; Choi et al. 2011; Ismail Hossain et al. 2011; O’toole et al. 2012). Due to a broad spectrum and potent antimicrobial and anti-biofilm activities and tunability of their antimicrobial properties, ionic liquids could find applications in the control of microorganisms in industry and health.

**Conclusions**

The ionic liquid [C12MIM]I exhibited potent antimicrobial and anti-biofilm activity against *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative). The activity was found to be much higher against the Gram-positive bacterium compared to the Gram-negative bacterium. The antimicrobial and anti-biofilm activity of the ionic liquid was comparable to that of a cationic surfactant, CTAB. The ionic liquid increased the cell membrane permeability of both the Gram-
positive and the Gram-negative bacteria in a dose dependent manner. From the results, it is concluded that the ionic liquid disrupted the bacterial cytoplasmic membranes, thereby impairing growth, attachment and biofilm formation. In general, the ionic liquid 1-alkyl-3-methylimidazolium exhibited substantial antimicrobial and anti-biofilm activities and could find novel applications in the control of bacterial contamination and infections.

Supplementary information

Data on the effect of iodide and imidazole on growth and biofilm formation by *S. aureus* and *P. aeruginosa*, as well as fluorimetric measurements of the green/red intensities of the bacterial cells upon exposure to ionic liquid are included in the Supplementary information.

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