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Quaternary Ammonium Compounds (QACs) induced inactivation of *Pseudomonas* spp.: Effect of material surface

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ABSTRACT

In this study, we investigated the adaptation of selected *Pseudomonas* spp. to QACs and monitored the changes in susceptibility to these compounds after serial exposure to QACs. Additional study was performed on the QACs efficacy on cells on different types of surfaces and the data were correlated to surface properties. Results showed that wild type *Pseudomonas fluorescens* and *Pseudomonas fragi* exhibited more susceptibility to QACs in comparison with *Pseudomonas putida*. In addition, the investigations carried out on the adhesion of both wild and adapted strains to different types of surface materials using thermodynamic approaches showed that hydrophilic *Pseudomonas* strains exhibited highest adhesion to the tile surface, while least adhesion was observed on the stainless steel surface material. A diverse relationship was detected between the thermodynamic affinity of cells to the surfaces (higher water contact angle and energy of adhesion) and cell inactivation. The results can be useful to the hygiene manufacture of food processing facilities or to choose appropriate sanitation strategies for the existing plants.

1. Introduction

Quaternary Ammonium Compounds are widely used disinfectants in food processing environments (Duong, 2005; Kim et al., 2007; To et al., 2002). These agents are reported to be effective against a wide range of microorganisms including yeast, mold and gram positive bacteria. However, some gram negative bacteria such as *Pseudomonas* spp. can easily adapt to sanitizers containing QACs as an active ingredient (Duong, 2005; Langsrud et al., 2003; Lucchesi et al., 2010; To et al., 2002). Therefore, unintentional residue deposition of QACs on surfaces after cleaning procedures could promote the adaptation of *Pseudomonas* spp. In addition, QACs residue deposition on surfaces could modify surface characteristics influencing the attachment of bacteria, which could eventually affect the safety and quality of the final food product (Machado et al., 2011; Oliveira et al., 2006; Pereira et al., 2006; Wang et al., 2008).

Several studies demonstrated that bacterial exposure to a sub-lethal level of QACs could result in the acquired resistance to these compounds (Langsrud et al., 2003; Lucchesi et al., 2010; Mc Cay et al., 2010). This increase in resistance to QACs can be demonstrated by the significant increase in the MIC value (minimum inhibitory concentration) in adapted cells (Haddadin et al., 2010; To et al., 2002). Degradation of these compounds and, changes in fatty acid composition and expression of efflux pump genes are some adaptive

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mechanisms to QACs showed by *Pseudomonas* spp. (Langsrud et al., 2003; Lucchesi et al., 2010; Mc Cay et al., 2010; To et al., 2002).

The alteration in cell surface composition due to exposure to QACs could induce changes in physico-chemical properties of cells, inhibiting or improving cell adhesion. According to Machado et al. (2011), significant physiological changes in lab adapted *Pseudomonas aeruginosa* after QACs exposure were reported. These alterations were more obvious in the free energy of interaction between polystyrene and the cell surface.

Apart from the cell physicochemical properties, surface material characteristics such as surface roughness, surface tension can influence initial cell adhesion (Van Houdt and Michiels, 2010). In addition, the susceptibility of bacteria to antimicrobial agents could also be affected by the type of contact materials (Bower et al., 1996; Krysinski et al., 1992; Pan et al., 2006; Sinde and Carballo, 2000). Based on Krysinski et al. (1992) findings, the greatest susceptibility to antimicrobial agents of *Lysteria monocytogenes* occurred on polyethylene/polyurethane surfaces followed by polyethylene and stainless steel surfaces (Krysinski et al., 1992). However, in another study (Aarnisalo et al., 2007), a similar decrease in cell population was obserevd on both surfaces. Simões et al. (2008) showed that treatment of glass surface with QACs increased the attachment of *Pseudomonas fluorescens*, but could also efficiently deactivate the bacteria remaining on the surface. However, a layer of such dead cells may provide a surface to which other microorganisms can attach, and therefore, facilitate the process of biofilm formation.

The objective of this study was to investigate the adaptation of selected *Pseudomonas* spp. to QACs and monitor the changes in susceptibility to these compounds after serial exposure to QACs. In addition, the adhesion of both wild and adapted strains to different types of surface materials commonly used in the food industry was predicted using thermodynamic approaches. The QACs efficacy on dried cells on different types of surfaces was also evaluated in order to be able to propose recommendations for cleaning and sanitation programs in different processing zones especially where wet cleaning is not frequently applied.

2. Material and methods

2.1. Strains and cultures

Pseudomonas putida ATCC 49128, *Pseudomonas fragi* DSM 3456 and *P. fluorescens* DSM 50090 were supplied by Ashtown Food Research Centre, Ireland. Stock cultures were maintained in Tryptic Soy Broth (TSB, Sigma, Ireland) and 20% glycerol at −70 °C. Prior to each experiment, bacterial cells were grown on Tryptic Soy Agar (TSA, Sigma, Ireland) plates for 24 h, at 37 °C. To evaluate the growth of the selected *Pseudomonas* strains, bacterial cells were firstly grown on TSA plates for 24 h, at 37 °C. Thereafter, a single colony of each *Pseudomonas* strains (wild-type and the adapted strain) was collected from the TSA plates and used to inoculate 5 mL of Tryptic Soy Broth (TSB, Sigma, Ireland). The inoculated medium was kept an overnight at 37 °C, in a horizontal shaker (200 rpm). For growth evaluation, samples were taken every 5 h and 8 h for wild and adapted strains, respectively, and the relevant optical densities (OD = 600 nm) were recorded using a Double beam Unicam, UV-3 spectrophotometer (UK). The OD was obtained by reading the absorbance of the samples against the blank sample containing only TSB. The absorbance at 600 nm was plotted versus the related microbial population to produce a calibration curve. Bacterial population was also determined by standard plate count method (SPC) and reported as CFU (Colony Forming Units) mL^{-1}.

2.2. Antimicrobial agents

Tetradecyl Dimethyl Benzyl Ammonium Chloride (one of the mostly used QACs in biocide formulation for disinfection purposes) was purchased from Sigma–Aldrich, Ireland (CAS number: 122-18-9). QACs solutions were prepared to the required concentration of 500 mg Tetradecyl Dimethyl Benzyl Ammonium Chloride in 1 L of distilled water (recommended concentration of QACs in the sanitation process of food plants) (Stanfield, 2003) and were filter sterilized using cellulose acetate syringe filters (0.45 μm, Cat. No. 28145-481, VWR, Ireland).

2.3. Development of adapted strains resistant to QACs

QACs adaptive resistance was laboratory induced for the *Pseudomonas* wild-type strain to further study the effect of QACs exposure on the physicochemical properties of the cell surface and the attachment to different surface materials. The adaptation process was based on the previously developed method by Machado et al. (2013). Adaptive resistance was induced by sub-culturing *Pseudomonas* strains in TSB in the presence of increasing concentrations of QACs. For adaptation to grow in the presence of QACs, the first used concentration was 5 mg L^{-1}. Bacterial growth was monitored by optical density measurement at 600 nm. Bacteria showing growth after 24–72 h in the presence of this concentration were used to inoculate the next culture series (increasing by 5 mg L^{-1} for each step). Adapted strains were consecutively preserved in TSA supplemented with final QACs concentration exhibiting maximum tolerance.

2.4. MIC (minimum inhibitory concentration) determination

The MIC of both wild and adapted *Pseudomonas* strains was determined using the microbroth dilution method in a 96-well microplate (8 rows and 12 columns). The test strains were grown overnight in TSB at 37 °C. Overnight cultures of both wild and adapted *Pseudomonas* strains were diluted in TSB to achieve a final cell number of approximately 10^5 CFU mL^{-1}. In order to test the susceptibility of the wild and adapted strains to QACs, QACs stock solution (500 μg mL^{-1}) was firstly made up and filter sterilized. Then, 200 μL of the stock solution was added to column 1 of the 96-well microplate and 100 μL of sterile water was added to the all other columns. Thereafter, ½ serial dilution of QACs was performed across the plate leaving out the last column.100 μL of inoculum was added to each well. Finally, the plate was incubated for 18 h (minimum) at 37 °C and the wells were examined for growth (turbidity). The MIC was reported as the concentration in which no increase in turbidity was detected.

2.5. Surfaces

Stainless steel 304 (2B finish, 0.9 mm thickness, 0.75, 5.04, 0.96, 9.73 and −4.6 μm for Ra, Rp, Rq, Rt and Rv respectively, AMARI Ireland), PVC cladding (3 cm thickness 0.11, 1.71, 0.15, 3.16