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OMP ALTERATIONS IN *PSEUDOMONAS AERUGINOSA* AFTER BENZALKONIUM CHLORIDE AND CIPROFLOXACIN BIOFILM ADAPTATION

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Outer membrane proteins, Biofilms, chemical bacterial adaptation, stress responses

INTRODUCTION

Bacteria in natural, industrial and clinical settings predominantly live in surface-associated communities denominated biofilms. Biofilm-associated bacteria present a different phenotype that can be responsible for the increased resistance to biocides, disinfectants, and antibiotics. Moreover, biofilms may be a niche in which pathogens can survive and persist to regular cleaning and sanitation procedures and to immune system. Therefore, the occurrence of a biofilm normally leads to a variety of serious problems in clinical area, to persistent inanimate surfaces contamination and to chronic or lethal infections in human and animals. According to US National Institutes of Health, biofilms account for over 80 percent of microbial infections in the body.

Antimicrobial chemical agents are widely employed to reduce microbial levels on inanimate objects. Some of them may occasionally act as chemosterilants, and many are employed as high-level disinfectants. During sanitation procedures, water and antimicrobial agents can be left unintentionally in an unknown concentration as a thin layer on the surfaces. The presence of antimicrobials on the surfaces, may lead to some unexpected serious consequences. Residual antimicrobial concentrations on cleaned surfaces are expected to be low, but a marked fluctuation in such levels is conceivable as a consequence of the frequent use or reuse of disinfectants. There have been some reports referring that the widespread use of biocides and disinfectants in hospitals, and to a lesser extent at home, even where there is a clear benefit could act as selective pressure for antibiotic-resistant bacteria. Some authors refer to hospital sinks, where disinfectants and detergents are disposed, as one of the main environmental source of several *Pseudomonas aeruginosa* outbreaks in clinical settings. Bacterial isolates collected from industrial or clinical sources often show decreased susceptibility to biocides or disinfectants and antibiotics compared with culture collection strains. So, one of the factors that can contribute to the understanding of the parameters involved in sanitation failure is to establish whether pre-contact of bacteria with a chemical, can contribute to their reduced susceptibility to that product, or even to resistance to antibiotics.

Several studies have suggested that the failure of the antimicrobial action of Quaternary ammonium compounds (QAC) can be due to the low level of permeability of the outer-membrane and/or changes of the efflux pumps levels. It is now believed that there is not a unique phenotypic change that gives rise to the increase in the acquired resistance to QAC, being more a “collection” of changes in the outer membrane of the cell. In this context outer membrane proteins (OMPs) of Gram-negative bacteria play a key role in the associated molecular processes, including cellular adhesion, signal transduction and pathogenesis.

Adaptive resistance to antimicrobials has been widely reported in planktonic studied trough phenotypic characterization and proteomic analysis.

Concerning biofilm adaptation, the response of biofilm-entrapped cells to chemical stress conditions is not yet well studied. There is evidence that proteins involved in oxidative stress response, cell envelope synthesis, as well as in synthesis of EPS become up- or down-regulated in biofilms, indicating that these altered phenotypes might contribute to antimicrobial tolerance.

GOALS

This work aimed to examine whether exposure of *Pseudomonas aeruginosa* biofilms to a surfactant, benzalkonium chloride (BC), and to an antibiotic, ciprofloxacin (CIP), could induce any change in the proteomic maps of OMPs (membranome) of the biofilm-entrapped bacteria as a stress adaptive response.

MATERIAL AND METHODS

Biofilms were formed in 6-well plates for 24 h being after submitted to the presence of 0.9 mM BC and 6.0 ug/ml CIP, during 13 days. The obtained biofilm-cells were separated and the OMP extracted by the spheroplast procedure. OMP patterns were analyzed by two-dimensional gel electrophoresis (2-DE) and spot quantification was achieved by computing scanning densitometry (ProXPRESS 2D, PerkinElmer Sciex) and gels were analyzed using SameSpot software.

RESULTS

Two-dimensional gel electrophoresis (2DE) revealed distinct and reproducible phenotypic differences between adapted and non-adapted biofilms. OMP expression pattern showed that 33 OMP (≈ 47 %) where down-regulated after biofilm adaptation to BC but not after adaptation to CIP, 10 OMP (≈ 14 %) where down-regulated after biofilm adaptation to both BC and CIP.

Gel analysis also revealed up-regulated proteins in CIP adapted biofilms (7 %), BC adapted biofilms (18 %) For both adapted biofilms (4 %) OMP expression was changed in common by both antimicrobials, revealing a possible similar stress response. Protein identification and spot picking are in progress

Membrane proteins play key roles in cell life cycles and are central in resistance mechanisms. The outer membrane is an important compartment where metabolites and ions are transported and constitutes a protective barrier but also more than half of all known drug targets.

Data demonstrated that stepwise exposure to a disinfectant can induce a mechanism of resistance able to confer an increased resistance to antibiotics. The outer membrane (OM) may be involved. The present study shows that, the presence of BC and CIP during biofilm development can be one driving force that can encourage material colonization by adapted or even resistant strains.

CONCLUSIONS

Biofilms matured in the presence of surfactants and/or antibiotics can embrace OMP pattern that can increase bacteria virulence and be favourable to encourage resistance. There might be some common OMP regulation when bacteria within biofilms face chemical stress from antimicrobials and antibiotics.

This conclusion can support the fact that bacteria may have similar responses to both types of products and that biofilm-entrapped bacteria may gain tolerance to Ciprofloxacin, as a result of exposure to a similarly acting substance like Benzalkonium Chloride. This particular response to the environment can be one of the causes of the well-known biofilm resistance phenotype.

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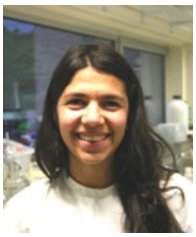

IDALINA MACHADO was born in Santo Tirso, Portugal and went to the University of Minho, where she studied Biological Engineering and obtained his degree in 2002. She worked for a couple of years for “SPO3” before moving in 2004 to the University of Minho where she did some studies for the Biofilm research group in the field of Biofilm control. Her main interests are the proteomic characterization of microorganisms in Biofilms and the relation between these characteristics with resistance phenomena She is know finishing her Phd entitled “Physiological characterization of bacterial biofilms challenged by emergent antimicrobial stress agents –characterization of the bacterial resistance mechanisms”.

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