MA07. Proteomic characterization of Benzalkonium Chloride- and Ciprofloxacin-adapted *Pseudomonas aeruginosa* biofilms

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Abstract

Bacteria are able to adapt to several environmental stresses such as the presence of antimicrobial molecules and, as consequence, bacterial resistance may increase with increasing exposure to antimicrobials. The most impressive mechanism of the bacterial mode of life is their grow as part of a sessile community referred to as biofilm [1]. Biofilm formation is an important aspect of many bacterial diseases, especially those related with medical devices [2]. When biofilms are identified as the cause of infection, treatment becomes very difficult since bacteria within biofilms demonstrate peculiar features, that confer them increased resistance to biocides. The adaptive response to antimicrobial stresses of sessile bacteria is more effective than the corresponding planktonic populations. Adaptive resistance to antimicrobials has been widely reported in planktonic state and characterized in terms of phenotypic traits and proteomic analysis [3,4]. Concerning biofilm adaptation, the response of the biofilm-entrapped cells to chemical stress conditions is not yet well studied. This work aimed to examine whether exposure of *Pseudomonas aeruginosa* biofilms to benzalkonium chloride (BC) and ciprofloxacin (CIP) during a laboratory adaptation process could induce any proteomic alterations in the outer membrane (OM) of the biofilm cells. Biofilms were formed in 6-well plates for 24 h being after submitted to the presence of 324 mg/L of BC and 6.0 mg/L of CIP, during 13 days. The obtained biofilm-cells were separated from the biofilm matrix and the OM proteins extracted. Protein patterns were analyzed by 2-DE and gels by Progenesis SameSpot software. Protein spots from the bacterial populations were considered to display significant quantitative differences if they fulfilled the following criteria: p values ≤ 0.05 (t-test); detection threshold, average volume ≥ 20 (n = 3); differential tolerance, fold change ≥ 2. Excised spots from three different gels of each adapted bacteria were identified by LC-MS/MS. Biofilm proteome analysis showed that *P. aeruginosa* adaptation to BC and CIP changed the expression of six proteins. The biofilm exposure to both antimicrobials generated common down-regulation of three proteins: GroEL, major capsid protein and putative tail sheath protein, revealing a possible similar stress response. The type 4 fimbrial biogenesis outer membrane protein PilQ precursor was over-expressed only in biofilms submitted to BC, while the probable bacteriophage protein and the hypothetical protein PA0537 were overexpressed in CIP exposed biofilms. When bacteria are within biofilms and exposed to chemical stress, the regulation of OM proteins expression can contribute to increase the biofilm resistance. The proteins involved in adhesion, oxidative stress response, as well as in synthesis of lipopolysaccharide, were up- or down-regulated in adapted *P. aruginosa* biofilms. These acquired proteomic profiles may be associated with antimicrobial tolerance.

References

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