Influence of ionic strength in *P. aeruginosa* early-biofilm development

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Many factors may affect bacterial attachment to surfaces and subsequent biofilm development. The strain type, the culturing method and concentration of the organism, contact time, temperature, presence and concentration of ionic species in the adhesion media, are some points to consider. Initial adhesion is one of the most important steps in biofilm formation. Bacteria adhere to surfaces by the formation of mechanical attachment organelles and by several physical mechanisms involving electrostatic and hydrophobic interactions. The aim of this work was to evaluate the influence of several salts with different ionic strengths in bacterial adhesion, and to compare the results with the ones observed with the addition of glucose.

Pseudomonas aeruginosa in a concentration of 10^7 CFU/ml was used as a biofilm producer strain. The various media used where: ultrapure water, and ultrapure water supplemented with several salts and a carbon source, with a final concentration of 1g/L (Glucose, Glucose+$NaPO_4$, NaCl, $MgSO_4$, NaCl+$MgSO_4$, $NaPO_4$). The biofilm experiments were undertaken for 24 h, at 10 and 37ºC, at static conditions. Biofilm was characterized by enumeration of colony forming units as CFU/cm^2, and by total biomass through Cristal Violet (CV) staining.

At 10ºC, the presence of NaCl and $MgSO_4$ clearly favored adhesion, while the medium with glucose, when compared to the ultrapure water medium, did not seem to affect the number of culturable cells after 24 h. On the other hand, the presence of glucose when biofilms were developed at 37ºC undoubtedly favored the final number of cells. Adhesion in the presence of salts was not affected by temperature.

In what concerns the total biomass of biofilms formed in the several media tested, at 10ºC, the CV method did not seem to be sensitive enough to provide significant values. At 37ºC, biofilm biomass was detectable, being the results dependent on the medium composition, however the differences were not as evident as the ones pointed out by CFU/cm^2.

At low temperatures, changes in the cell wall physico-chemical properties may promote different levels of surface adhesion. In this work, ionic strength provided by the presence of different salts in water played a significant role in cell wall properties and may even influence the conditioning of the substratum. Because at 10°C glucose failed to promote biofilm formation, the adhesion observed seems to be nonspecific and entirely determined by general physicochemical properties of the cell wall and the substrata. At higher temperatures, *P. aeruginosa* is able to consume glucose as a carbon source, and as such adhesion in the

glucose medium is likely to be more cell-mediated. Adhesion may probably imply specialized structures of the cell surface and cell metabolism that might be related to secreted proteins responsible for the adhesion process.

Acknowledgments: IBB-CEB and FCT, for the financial support (Project PTDC/SAU-ESA/64609/2006; PhD Grant SFRH/BD/31065/2006).