Impact of sessile growth state on *Pseudomonas aeruginosa* lipidome

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*Pseudomonas aeruginosa* is a common opportunistic and nosocomial pathogen and the leading cause of morbidity and mortality in cystic fibrosis patients. Many previous studies using genetic and proteomic approaches have revealed physiological differences between planktonic and sessile *P. aeruginosa*. One of these proteomic studies revealed that sessile *P. aeruginosa* cells accumulated some enzymes involved for fatty acid and phospholipids biosynthesis. These data question about the impact of the sessile growth state on the bacterial lipidome.

After inner membrane extraction of planktonic or sessile cells, lipid extraction was done according to Bligh and Dyer protocol. Lipids were analysed using Electrospray Ionization Mass Spectrometry. The impact of the biofilm growth mode on phospholipid organisation has been studied by reconstitution in monolayers and thus visualized by Brewster Angle Microscopy (BAM) and Atomic Force Microscopy (AFM).

The results obtained by mass spectrometry show a drastic decrease of the uneven-numbered chain phospholipids and a relative accumulation of long chain lipids in organisms grown in biofilms, suggesting better lipid stability in the bilayer and a decrease in membrane fluidity. The images taken by BAM and AFM showed that inner membrane lipids of *P. aeruginosa* could form domains (larger in biofilm bacteria) when the pressure is near to the physiological conditions. This observation is coherent with mass spectrometry analysis. This reflects a bacterial adaptation to the sessile mode of growth and might play a key role in the particular physiology of biofilm cells. This study is an innovative approach that could allow to a better understanding of the mechanism of biofilm formation and the switch between the two growth states.

**OMP Proteomic analysis of Benzalkonium Chloride and Ciprofloxacin adapted Biofilm cells**

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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. This work aimed to examine whether exposure of *Pseudomonas aeruginosa* biofilms to benzalkonium chloride (BC) and ciprofloxacin (CIP) could induce an adaptive response in bacteria. This was attained by inspection of proteome alterations of the outer membrane (OMP) in biofilm cells. 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. Protein patterns were analysed by 2-DE and gels by the SameSpot software. Biofilm-proteome showed that *P. aeruginosa* adaptation to BC promoted the down-regulation of 36 OMP and the up-regulation of only one. OMP 2DE of *P. aeruginosa* adapted to CIP revealed the down-regulation of 29 OMP. Six OMPs were changed in common by both antimicrobials, revealing a possible similar stress response. Proteins identification is in progress. This study highlighted that there might be an OMP regulation when bacteria within biofilms are submitted to chemical adaptation. This particular response to the environment can be one of the causes of the well-known biofilm resistance phenotype. Acknowledgments: IBB-CEB, FCT (PTDC/SAUESA/64609/2006; SFRH/BD/31065/2006)

**Impact of physical disturbance on the evolution of A-L interface biofilm structure**

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Bacterial growth results in assemblages ranging from micro-colonies to slimes and biofilms, with varying structure, rheology and ecological advantage. Growth at the meniscus of static liquid microcosms often produces air-liquid (A-L) interface biofilms in which oxygen and nutrient gradients are opposing, and maintenance at the surface involves meniscus attachment, hydrophobic structures and surface tension effects. A-L biofilm formation is a deep-rooted ability amongst environmental Pseudomonads, but as yet, the relationship between biofilm properties and ecological advantage is poorly understood. In order to investigate this, we have characterized three distinct A-L biofilms formed by the soil and plant-associated *Pseudomonas fluorescens* SBW25. Two of these, the Wrinkly Spreader (WS) and CBFS biofilms are the result of mutations, whilst the Viscous Mass (VM) biofilm is non-specifically induced by iron. These differ significantly in terms of biofilm strength, attachment and maintenance at the A-L interface, rheometry, cell hydrophobicity, stickiness, and surface recruitment. Each of the biofilms are resistant to low-level physical disturbance but not to extreme events, suggesting that there is a trade-off between physical resilience and the minimal structural requirements needed to colonise the A-L interface. Fitness assays confirm the advantage of biofilm formation in static liquid microcosms though...