Detection of resistant mutants within *Pseudomonas aeruginosa* colony morphology variants in lung cystic fibrosis environment

Sousa, A. M., Pereira, M. O.

IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710–057 Braga, Portugal

Bacterial infections caused mainly by *P. aeruginosa* are typical of cystic fibrosis (CF) lung disease. Despite the long and aggressive antibiotic therapy, CF patients still died because of these chronic infections. The deprived bacterial eradication is mainly due to several strategies adopted by bacteria to achieve CF airways adaptation and tolerance to antibiotics. Biofilm formation and phenotypic switching are among the most relevant adaptive biological processes. Triggering those processes bacteria have the potential to better survive to CF conditions and antibiotics action. Phenotypic switching provides a source of microbial diversity through switch between two phenotypic states, analogue to a mechanism ON/OFF, without the fitness costs of irreversible mutations. This interchange of states, visible by differential colony morphology, can have serious impact on bacterial virulence, antimicrobial resistance and persistence.

The present work aims to investigate the specific colony variants-forming bacteria responsible by typical CF chronic infections. Through isolation and deep characterization of those colony variants, including discriminatory antibiotic susceptibility profiles and virulence characterization, it is intended to determine the mechanisms underlying the inefficiency of antimicrobial therapies of airway CF.

P. aeruginosa strains (collection and clinical isolated) and LB medium were used to simulate airway CF infections. Clonal diversification of *P. aeruginosa* was checked after 24, 48, 72 and 120 hours after media inoculation (or initial infection) by colony morphology observation. The different colonies were further deep characterized in terms of resistance to in-use antibiotics including piperacillin (PRL), aztreonam (ATM), cefepime (FEP) and ceftazidime (CAZ) in Mueller-Hinton agar plates. Biofilm formation was also tested since it is one of the most relevant virulence factors in airway CF context.

Data obtained revealed that new colony variants arisen according infection development stage. These new variants were generally more resistant than the normal *P. aeruginosa* colony morphology. Interestingly, the population were generally composed by colony variants with distinct resistance profiles. Some colony variants exhibited high specific resistance to CAZ, other variants to PRL or FEP. Some colony variants multi-resistant were as well observed. Such heterogeneous behaviours were also observed concerning biofilm formation ability. These results seemed to corroborate the “insurance hypothesis” that posits biodiversity as a mechanism to ensure population survival. In addition, data also highlight novel evidences since it was noted that single and well-differentiated colonies seemed to encompass bacteria with distinct phenotypes. Such evidences emerged when a single colony was used to test its susceptibility and it was observed resistant mutants within the inhibition zones. The inclusion of resistant mutants was not exclusive of small colony variants, well known for its high and diverse antibiotic resistance. Several other colony morphologies showed to encompass resistant mutants in their populations. This evidence might revolutionize the actual colony morphology knowledge. Colony variants have been so far studied and considered as an amount of identical bacteria. So, it is not just need the simple profiling of colony variants but the deep knowledge about bacteria that composed them. The ignorance or under valorisation of these mutants may be the reason of recalcitrance of CF infections. CF antimicrobial therapies must consider those mutants.

Keywords: cystic fibrosis, antibiotic resistance, colony variants, P. aeruginosa, resistant mutants

Acknowledgments: The financial support from IBB-CEB and FCT and European Community fund FEDER, through Program COMPETE (FCT PTDC/SAU-SAP/113196/2009/ FCOMP-01-0124-FEDER-016012) and Ana Margarida Sousa PhD Grant (SFRH/BD/72551/2010) are gratefully acknowledged.