

DISPERSION OF BOFILM MASS AND ACTIVITY VALUES - SIGN OF HYPERMUTATION IN *P. AERUGINOSA* BIOFILMS?

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Background: Biofilms are thought to be the main mode of growth for bacteria in Nature. This mode of growth confers many advantages to bacteria when facing stresses, such as increased antimicrobial resistance and physical protection due to the EPS matrix. Nowadays, it has been recognized another important advantage - the rapid development of genetic diversity among members of the biofilm community.

Aim: To investigate the role of chemical stress on biofilm microbial diversity in order to explain the biofilm unsorted behavior.

Methods: *P. aeruginosa* non-adapted and adapted to Benzalkonium Chloride (BZK) were used as biofilm producers. Biofilms were formed for 24 h, in BZK conditioned and non-conditioned surfaces, being afterward attacked with BZK and Ciprofloxacin. Biofilms were characterized in terms of biomass, through crystal violet (CV), and respiratory activity, using XTT.

Results: Data of 7 independent experiments revealed that biofilms formed by adapted bacteria in the conditioned surfaces or after antimicrobial attack presented dispersed characteristics: biomass ranging from 9.0 to 17.0 and respiratory activity from 0.8 to 1.55. This dispersion may be a sign of hypermutable behavior since biofilm experiments did not result in exact copies albeit the experimental conditions were kept constant.

Conclusion: CV and XTT data are often represented as column graphs where mean and SD are plotted. This representation option can lead to misunderstanding in interpreting overall results and “mask” the hypermutable behavior. Biofilms formed with diluted inocula and submitted to several chemical stresses, may spontaneously generate mutants that can form biofilms with unsorted behavior.