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PRODUCTION OF SIDEROPHORES AND QUORUM SENSING ANTAGONISTS AND FREE ENERGY OF ADHESION REGULATES BACILLUS CEREUS AND PSEUDOMONAS FLUORESCENS INTERACTIONS

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Planktonic and biofilm interactions between Bacillus cereus, isolated from a disinfectant solution and a type strain Pseudomonas fluorescens were studied. Planktonic mixed growth assessment, in batch conditions, revealed that both bacteria co-existed equally during the exponential phase of growth. During the pre-stationary and the stationary phase of growth, P. fluorescens prevail due to production of microbial products which interfered with B. cereus, decreasing the number of culturable cells along time. The production of siderophores and quorum sensing antagonists was assessed according to Schwyn and Neilands (1987) and McLean et al. (2004), respectively, being found that only P. fluorescens produced such compounds. Mixed biofilms were formed in a bioreactor rotating system under a constant Reynolds number of agitation (N`ReA) of 2400 using ASI 316 stainless steel as adhesion surface (Simões et al., 2005). The biofilms were allowed to grow for seven days in order to reach the physiological steady-state, being, afterwards, stratified by the application of increased shear stress forces correspondent to N`ReA of 4000, 8100, 12100 and 16100. The number of B. cereus and P. fluorescens cells present in the layers of the biofilms were assessed by epifluorescence microscopy. The results demonstrated that biofilms were composed by log values of 13.9 and 13.6 of B. cereus and P. fluorescens, respectively, being about 99 % of the cells in a viable state. B. cereus existed in higher proportion in the outer layers of the biofilm, correspondent to the biofilm removed by the application of N`ReA of 4000 and 8100 (70-80 %) and P. fluorescens existed at higher extent in the layers removed by the application of N`ReA of 12100 and 16100 (70-80 %). This phenomenon is probably related with the production of quorum sensing antagonists and siderophores that were diluted in the outer layers of the biofilm as the system was continuously fed with fresh medium. The inner layer, i.e, the biofilm remaining on the surface was highly colonized by B. cereus (85 %), a fact that may be related with the favoured cell-substratum interaction. Studies of free energy of adhesion between the bacteria and stainless steel revealed that adhesion was thermodynamically favourable for B. cereus and unfavourable for P. fluorescens. Furthermore, both bacteria had similar motility. This study demonstrates that strength of cell-substratum interaction can play an important role in the colonization of biofilm inner layers. The action of siderophores and quorum-sensing antagonists was effective in closed

systems (as batch conditions), probably due to their residual concentration, being diluted in continuously operating systems. References McLean RJC, Pierson III LS, and Fuqua C (2004) A simple screening protocol for the identification of quorum signal antagonists. J. Microbiol. Methods 58:351-360. Schwyn B, and Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160:47-56. Simões M, Pereira MO, and Vieira MJ (2005) Effect of mechanical stress on biofilms challenged by different chemicals. Water Res. 39:5142-5152.