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## Binary biofilm behaviour: sum of the synergistic and antagonistic interactions

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### Abstract

Biofilms can be defined as multicellular communities, embedded in a polymeric matrix, attached to a surface, where bacteria may show different phenotypic features that favour survival in hostile environments. Biofilms are colonizers of every surface being the leading cause of hospital infections and source of pathogenic bacteria. Several species can coexist in biofilms being its behaviour the sum of synergistic and antagonistic interactions, as they can produce metabolites that can interfere negatively or positively with growth and biofilm formation. In this work, the interactions between *S.aureus*, *S.sciuri*, *P.aeruginosa*, *P.fluorescens*, and *E.coli*, in single and binary biofilms, were examined. Biofilms were characterized by total mass, through crystal violet, metabolic activity, through XTT method, and number of cells. Single biofilms formed by *E. coli* and *S. sciuri* had the highest values of biomass. However, *S.sciuri* biofilms are less active holding the smallest number of cells. These results emphasised that each bacteria has an intrinsic sessile formation behaviour that leads to biofilms with different phenotypic features that might be on the basis of distinct answers to antimicrobials in a disinfection scenario. The biofilms resulting from the combination of *P. aeruginosa* with, respectively, *E.coli* and *S.aureus*, leads to binary biofilms with clearly more mass but less activity than the singles ones, being however the number of the binary biofilm-grown cells higher. The same behaviour was also observed for the binary biofilm formed by *P. fluorescens* and *S. sciuri*. It can be said that the growth of these species in a mixed biofilm clearly favours the sessile growth of each species, which may be disadvantageous in terms of sanitation. The number of cells does not seem to be proportional with metabolic activity, being the formation of binary biofilms favourable for some bacterial species and adverse for other. The reduced activity showed by binary biofilms can be explained by the fact that bacteria growing alone do not face cell-to-cell signalling stress. These data seem to indicate that the presence of *Pseudomonas* favoured the sessile growth of the other species, which may impair its eradication. In fact, the resulting binary biofilms seems to have cells in a latent state embedded in a matrix with more quantity of exopolymers that will shield bacteria from stress factors and make the access of antimicrobials more difficult.