Role of planktonic and sessile extracellular signals on interspecies relationships

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ABSTRACT

It is well established that in nature, bacteria are found primarily as residents of surface-associated communities called biofilms, which are likely to consist of consortia of species that influence each other in synergistic and antagonistic manners. Although few reports specifically address interactions within multispecies biofilms, recent studies have shown that certain bacterial species, as a microbial defence mechanism, secrete extracellular products that interfere with biofilm formation and cell-to-cell communication. This study aimed to evaluate whether synergistic or antagonistic associations occur during multispecies planktonic growth and, single and binary biofilm formation and activity.

P. aeruginosa (ATCC 10145) and *Escherichia coli* k12 were the strains used in this study. Previously, supernatants obtained under planktonic and biofilm cultures of each single species were recovered, filtered and stored for further experiments. The latter supernatants were supplemented with TSB and used as the growth media to planktonic and sessile growth of both single- and two-species cultures. Planktonic bacterial growth on 96-wells plates was examined through OD_{640} measurement. Biofilms were obtained after 24 h and evaluated in terms of biomass, through CV and respiratory activity, with XTT. For dual-species growth it was used a combination of 50% of suspended inocula of each species.

Results indicated that both biofilm supernatants had an inhibitory effect on the growth of all planktonic cultures, mainly in the exponential stage. Conversely, single- and two-species planktonic growth has been stimulated in the presence of the *P.aeruginosa* planktonic supernatant. Concerning biofilm studies, it was found that the supernatants resulting from bacterial biofilm cultures favoured biomass accumulation. This may be due to the release of signalling molecules secreted by bacteria within the biofilms, inducing neighbouring cells to shift from planktonic to sessile growth. None of the supernatants tested had effect in *P.aeruginosa* biofilms, although all stimulated *E. coli* biomass accumulation. Concerning *E. coli* biofilm supernatant, all the biofilms formed in this medium showed biomass increase, more pronounced for dual-species biofilms. Thus, it is possible that *E. coli* have produced signalling molecules for the bulk media that later may be on the basis of a cooperative biofilm formation by both strains. Regarding respiratory activity, it was observed that this parameter was not significantly altered as biomass values. However, it was noted a slight decrease when the biofilms grew in planktonic supernatants, probably due to the presence of a secondary metabolite released by bacteria in planktonic conditions that may disturb biofilm activity. Since few studies have reported the complex web of interactions within biofilm communities, these results help to understand the behaviour of bacteria when facing microbial defense mechanisms promoted by other species.