USE OF CV AND XTT TO SCREEN BIOFILM SUSCEPTIBILITY TO NEW QAC´S COMPOUNDS

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ABSTRACT
This study evaluates the biocide ability of new quaternary ammonium compounds, against *Pseudomonas fluorescens* (a Gram- bacterium) and *Staphylococcus sciuri* (a Gram+ bacterium) biofilms. For comparison purposes the results were compared with the data obtained with a commercial QAC, cetyltrimethylammonium bromide (CTAB), often used in the sanitation of medical and industrial surfaces. The effect of the QAC´s preconditioning of the adhesion surfaces was also assessed. The new series of fluorinated ammonium compounds were achieved by the introduction of new molecular parameters in the chemical structure of the traditional surfactant, and catalogued as MF 6.8, AB 6.6 and A 6.8 according their chemical nature. The modified microtiter plate technique, using crystal violet (CV), together with the colorimetric assay using 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium (XTT) salt, was used to assess, respectively, the total attached biomass and respiratory activity of the biofilms. The results showed that the application of the new compounds, as well as CTAB, did not cause biomass removal, regardless the biofilm-formed bacterium. In fact, the contact of the biofilms with all the QAC´s results, in general, in an increase of the amount of the biofilm mass adhered to the wells of the microtiter plate, being the increase more evident for *S. sciuri* biofilms. Concerning respiratory activity, biofilms formed by the *S. sciuri* bacteria presents a different response to the QAC´s treatment when compared with the *P.fluorescens* biofilms. Conversely to *P.fluorescens* biofilms, the activity of the *S. sciuri* biofilms was significantly reduced after the application of the fluorinated ammonium compounds, even if total inactivation was not achieved. The treatment with CTAB resulted in the preservation or increase of the respiratory activity of both biofilms.
The conditioning of the wells of the microtiter plate with the QAC´s, for 30 min, changed biofilms response to the antimicrobial products, specially noticed in terms of respiratory activity. The application of the QAC´s to the biofilms formed on the conditioning wells stills to increase the amount of the adhered biomass, independently of the kind of biofilm-forming bacteria, but it reduces drastically the respiratory activity of the *S. sciuri* biofilms. In the latter, AB 6.6 caused total inactivation (MBC = 0.3 mM). Concerning *P. fluorescens* biofilms, the respiratory reduction is only observed for higher QAC´s concentrations and clearly in a less extent that the one observed with the *S. sciuri* biofilms. Regarding CTAB, the conditioning of the wells did not cause any significant alteration of biofilms susceptibility. The overall results showed that the new QAC´s compounds seems to have a promising better sanitation action than the traditional one, even though none of them presents both marked biofilm removal and biofilm inactivation at once. The results also highlighted that biofilms formed by a Gram- bacterium are more resistant than the ones formed by a Gram+ microrganism.