

## Effect of subinhibitory concentrations of benzalkonium chloride on the competitiveness of *Pseudomonas aeruginosa* grown in continuous culture

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This study investigates the link between adaptation to biocides and antibiotics in *Pseudomonas aeruginosa*. An enrichment continuous culture of *P. aeruginosa* NCIMB 10421 (MIC 25 mg BKC l<sup>-1</sup>) was operated ( $D=0.04\text{ h}^{-1}$ , 792 h) with added benzalkonium chloride (BKC). A derivative, PA-29 (696 h), demonstrated a >12-fold decrease in sensitivity to the biocide (MIC >350 mg BKC l<sup>-1</sup>). The variant demonstrated a 256-fold increase in resistance to ciprofloxacin, with a mutation in the *gyrA* gene (Thr-83→Ile). Similarly, culturing of the original strain in a continuous-culture system with ciprofloxacin selection pressure led to the evolution of BKC-adapted populations (MIC 100 mg BKC l<sup>-1</sup>). Efflux pump activity predominantly contributed to the developed phenotype of PA-29. An amino acid substitution (Val-51→Ala) in *nfxB*, the Mex efflux system regulator gene, was observed for PA-29. Overexpression of both MexAB-OprM and MexCD-OprJ was recorded for PA-29. Similarly, *mexR*, a repressor of the Mex system, was downregulated. Competition studies were carried out in continuous culture between PA-29 and the original strain (in the presence of subinhibitory concentrations of BKC). The outcome of competition was influenced by the concentration of biocide used and the nature of limiting nutrient. The inclusion of 1 mg BKC l<sup>-1</sup> in the medium feed was sufficient to select ( $S=0.011$ ) for the BKC-adapted strain in magnesium-limited culture. Conversely, the presence of 10 mg BKC l<sup>-1</sup> in the medium supply was insufficient to select for the same organism ( $S=-0.017$ ) in the glucose-limited culture. These results indicate the importance of environmental conditions on selection and maintenance of biocide adaptation.