THE PRODUCTION OF STABLE DEFINED CULTURES OF MUCOID *PSEUDOMONAS AERUGINOSA* IN CONTINUOUS CULTURE

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Laboratory studies of mucoid strains of *Pseudomonas aeruginosa* associated with cystic fibrosis are handicapped because the strains usually revert to non-mucoid strains *in vitro* (Govan, 1975). We have used the method of Govan (1976) to select mucoid strains and then tested their stability in batch and continuous culture.

Mucoid variants about 1 in 10^7 were selected by plating out log phase nutrient broth cultures of *P. aeruginosa* NCTC 6750 on nutrient agar containing 40 µg/ml carbenicillin. They were designated PA 6750 M1-M7. M2, M5 and M7 but not M1, M3, M4 or M6 produced mucoid slime in simple salts medium containing glucose or gluconate as the carbon source. Alcohol precipitated slime (Doggett & others, 1964) was white, fibrillar in glucose salts liquid medium and white, powdery in gluconate salts liquid medium. Differences also occurred between slime from glucose or gluconate salts agar.

Stability on batch subculture in nutrient broth or salts medium in a shaking water bath was tested with 0.5% sodium deoxycholate, 0.5% polysorbate 80 or 1% sodium lauryl sulphate. Samples were plated out and examined for revertants. All mucoid forms were rapidly replaced by revertants in nutrient broth and this was accelerated in the presence of deoxycholate and the other surfactants had no effect. In salts medium stability was increased. M1, M3, M4 and M6 were relatively unstable. M2 and M5 reverted after 4 subcultures. M7 did not revert even after 12 subcultures. This strain was also stable on nutrient agar but not in nutrient broth. In salts medium the surfactants decreased stability.

Separate chemostats were set up in which M7 was growth-limited by different nutrients in salts medium all at a dilution rate (D) of 0.05 h^{-1}. Stability was assessed by plating out and examining for revertants. During a 14 day experiment (about 24 generations) sulphate and magnesium limited cultures were fully stable. Iron limited cultures were relatively stable with 10% revertants after 14 days. The other 3 limited cultures were unstable from the start with revertants after 14 days being nitrogen 55%, phosphorus 75% and carbon 98%. The experiment was repeated and both the wild type-PA 6750 and M7 were separately grown in chemostats limited by sulphate, magnesium and iron, D 0.1 h^{-1}. After 2 days 10% mucoid was added to the wild type and 10% wild type added to the mucoid and stability followed for 10 days (about 34 generations). The stability of the original mucoid cultures was confirmed under these conditions. Only in the iron limited culture did the added mucoid tend to replace the wild type culture.

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