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Chromosome transfer in *Pseudomonas aeruginosa* mediated by R factors

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SUMMARY

R factors present in *Pseudomonas aeruginosa* strains of clinical origin can be transferred to other strains of *P. aeruginosa* and may act to promote host chromosome transfer. In general, their properties are similar to those R factors in Enterobacteria. The different R factors studied vary with respect to transferability, transfer of specific resistance properties, repressibility, and ability to promote chromosome transfer.

1. INTRODUCTION

Strains of *Pseudomonas aeruginosa* have recently been reported to have become resistant to carbenicillin as a result of acquisition of genetic structures similar to the R factors of Enterobacteria (Lowbury *et al.* 1969). Sykes & Richmond (1970) and Fullbrook, Elson & Slocombe (1970) have demonstrated that certain strains could transfer carbenicillin resistance to *Escherichia coli* K12 and that the β-lactamase from these strains was indistinguishable from that found in enteric bacteria showing R-factor-mediated resistance to penicillin. We have examined the R factors of some of these strains in terms of their transmissibility to other strains of *P. aeruginosa* and their ability to promote chromosome transfer.

2. RESULTS AND DISCUSSION

The R factor-carrying strains of *P. aeruginosa*, R9169, R6886, R1822 and R3425 kindly provided by Dr E. J. L. Lowbury (Lowbury 1969) all display multiple resistance to the antibiotics carbenicillin (CB), neomycin/kanamycin (NM/KM) and tetracycline (TC). To determine whether the factors conferring CB resistance can be transferred to other strains of *P. aeruginosa*, genetically marked derivatives of two strains, strain 1 (PAO) and strain 2 (PAT) were used as recipients (Holloway, 1969). CB resistance was transferred from R9169, R1822 and R6886 to the recipient *P. aeruginosa* strain PAT900 (histidine-requiring, streptomycin-resistant, FP^-) by mixed incubation for 4 h at 37 °C of log phase cultures of a CB-resistant streptomycin-sensitive strain and PAT900 at an inoculum ratio of 100:1 and a final cell density of *c.* 5×10^8/ml. Subsequent selection on Nutrient Agar supplemented with both carbenicillin and streptomycin at 1 mg/ml revealed frequencies of transfer of CB resistance ranging between 10^{-2}/recipient cell for R9169, 10^{-5}/recipient cell for R1822, and 10^{-6}/recipient cell for R6886. No transfer of CB resistance to PAT900 was found when R3425 was used as donor. By a similar procedure it was possible to transfer CB resistance to strain PAO, using in this case the PAT900 R^+ derivatives as donors. These three R factors are stable in both strains PAO

and PAT, with the exception of the R determinant from *P. aeruginosa 6886*, which shows some degree of instability in strain PAT.

Fig. 1. shows the results of an experiment to compare R transfer from PAT900 R^+ donors to a CB-sensitive strain PAT967 (methionine-requiring, FP^-). The extent of R-factor transfer at 5 min indicates that the ratios of active to inactive donors in the populations of R1822, R9169 and R6886 during this time interval are 1/10, 1/700, and 1/900 respectively. These differences in transmissibility may reflect various degrees of repression of the various R factors (Meynell, Meynell & Datta, 1968). The transfer of CB resistance in $R^+FP^- \times FP^-$ or $R^+FP^+ \times FP^+$ crosses indicates that these R factors are able to mediate cell contact and their own transfer independently of the presence or absence of other known sex-determining plasmids in the cell. We also found that the rates of R factor transfer were not markedly affected by the presence of the FP2 sex factor in the recipient strain.

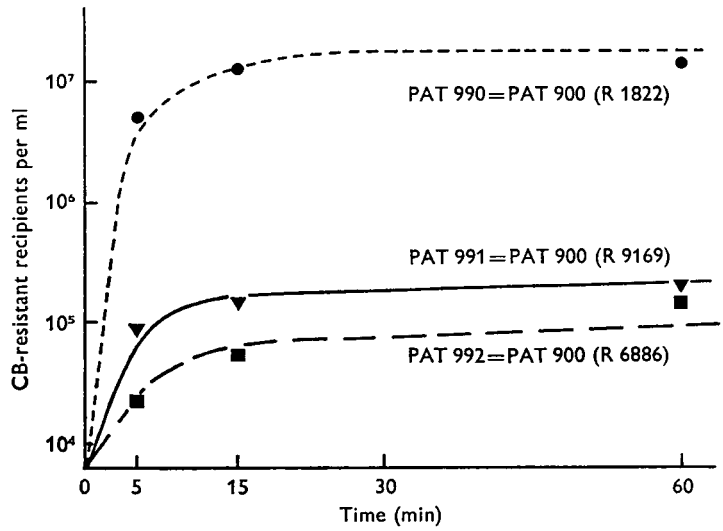

Fig. 1. Transfer of CB resistance from the R^+ (*his*) donors PAT990, PAT991 and PAT992 to the recipient PAT967 (*met*). Equal numbers of log phase cells of R^+ and R^- parents were mixed to a final density of *c.* 10^8 cells/ml. At time intervals samples were vortexed and plated on to minimal medium supplemented with methionine and CB (1000 μg/ml).

When PAT R^+ strains were tested for the inheritance of multiple drug resistance, only strains carrying the R factor from R6886 were found to possess resistance to all four antibiotics at levels similar to those of R6886. PAT R^+ and PAO R^+ strains carrying the R factor from either R1822 or R9169 were resistant to CB only, and showed no change in the levels of resistance to NM/KM and TC. The lack of inheritance of the NM/KM and TC resistance phenotypes from R1822 or R9169 indicates that these strains either fail to transfer that portion of the plasmid genome carrying the NM/KM and TC determinants or that these determinants exist on a separate plasmid. It is also possible that the NM/KM and TC determinants are lost or are not expressed following transfer to the recipient strains used.

The ability of three of the R factors to mediate chromosome transfer was assessed in plate mating experiments. Derivatives of the FP^- strain PAT900 containing the R factors were used as genetic donors and the ability of these strains to transfer chromosomal

markers to auxotrophic PAT FP$^-$ recipients was measured. A log phase donor culture consisting of a mixture of PAT900 and the isogenic R$^+$ derivative (inoculum ratio 10:1) was prepared. This procedure was followed to obtain cells freshly infected with R factors which presumably would be derepressed and show a higher frequency of transfer (Meynell *et al.* 1968). The donor ability of these cultures was compared with that obtained using log phase cultures of the R$^+$ derivatives alone (Table 1). It may be seen that R9169 and R6886 are capable of chromosome transfer at frequencies which may even be greater than those mediated by the sex factor FP2. The fact that higher levels of recombinants were obtained when a mixed inoculum was used for R9169, compared to that obtained with the R factor carrying strain used alone, supports the idea that this R factor is more subject to repression than the others tested. The range of recovery frequencies observed with the different markers when R9169 and R6886 promote chromosome transfer suggests that these R factors may promote transfer from different sites of origin.

Table 1. *Recombinants/10^8 R$^+$ or FP$^+$ donors*

Selected marker in recipient FP$^-$ strain	Donor parent: PAT990 (R1822) Alone	PAT990 (R1822) Mixed culture	PAT991 (R9169) Alone	PAT991 (R9169) Mixed culture	PAT992 (R6886) Alone	PAT992 (R6886) Mixed culture	PAT404 (FP$^+$)
met-1105	14	14	14	2370	409	936	740
ilv-1104	7	7	7	824	48	177	532
ilv-1105	14	13	7	1842	279	820	512
arg-1104	13	11	6	2237	640	1077	448
pur-1108	10	8	2	71	595	1486	117
met-1106	11	17	1	74	5380	7143	57

(Ability of R factors to mediate chromosome transfer was compared with that of the natural sex factor FP2 in isogenic strains of PAT. R$^+$ donor cultures were either grown alone or in mixed culture with an isogenic FP$^-$ strain prior to mating with a range of independently isolated auxotrophic mutants. 0·1 ml aliquots of saline suspensions of R$^+$ or FP$^+$ and FP$^-$ cultures at *c.* 10^9 cell/ml were plated to minimal medium. PAT990, PAT991 and PAT992 are R$^+$ derivatives of PAT900 (*his*, FP$^-$). PAT404 has the same *his* mutation and is FP$^+$.)

Such R factors should prove to be a valuable additional tool for genetic analysis in *P. aeruginosa* and may well provide a means of establishing a range of donor strains promoting transfer from different parts of the chromosome. This would yield a variety of male strains similar to the range of Hfr strains available in *E. coli*.

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