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# Phenotypic properties of R factors of *Pseudomonas aeruginosa*: R factors transferable only in *Pseudomonas aeruginosa*

BY P. M. CHANDLER AND V. KRISHNAPILLAI
*Department of Genetics, Monash University, Clayton, Victoria 3168, Australia*

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SUMMARY

A study was made of the R factors from two multiply drug resistant wild type isolates of *Pseudomonas aeruginosa* from a Birmingham hospital (Lowbury *et al.* 1969) from which, in contrast to other strains from the same source (Chandler & Krishnapillai, 1974*a*), drug resistance was not transferable to *Escherichia coli* K12 or *Salmonella typhimurium*. Transfer of drug resistance occurred at a low frequency to *Shigella flexneri*, although drug resistance in this species was subsequently non-transferable.

In *P. aeruginosa* there are several features of these two R factors which distinguish them from the group 1 and 2 R factors described previously (Chandler & Krishnapillai, 1974*a*). Although coding for resistance to neomycin and tetracycline, they did not express this resistance in two strains of *P. aeruginosa* examined, in contrast to the wild type strains they were isolated in.

The control of transfer of the two R factors is different to the group 1 and 2 R factors in that derepression of transfer could be demonstrated following physiological treatments or mutagenesis. The R factors of this third group were compatible with the group 2 R factors, but did not repress their pilus synthesis on the basis of R factor specific phage plating.

## 1. INTRODUCTION

In a previous paper (Chandler & Krishnapillai, 1974*a*) we described the properties of two types of R factor isolated from *P. aeruginosa* strains from a Birmingham hospital (Lowbury *et al.* 1969).

This paper describes the properties of a third type of R factor from the Birmingham strains. This group apparently codes for essentially the same functions as one of the previous groups, but the expression of those functions is controlled quite differently.

## 2. MATERIALS AND METHODS

(*a*) *Bacterial strains*. The strains and their characteristics are described by Chandler & Krishnapillai (1974*a*), except for *E. coli* C (W2438), a prototrophic strain obtained from E. Lederberg.

(*b*) *Derivation of R factor strains*. The two multiply resistant wild type *P. aeruginosa* strains 1954 and 9169 were isolated in the MRC Industrial Injuries and

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Burns Unit, Birmingham Accident Hospital, England (Holloway & Richmond, 1973). They show resistance to the antibiotics carbenicillin, neomycin/kanamycin and tetracycline.

(*c*) *Media, transfer of R factors, determination of minimal inhibitory concentrations (M.I.C.) of antibiotics and R factor stability.* These have been described (Chandler & Krishnapillai, 1974*a*).

(*d*) *Determination of derepressibility of R factor transfer.* The extent of repression of R factor transfer was determined according to the method of Meynell & Meynell (1970). For both *Pseudomonas* strains PAO and PAT, which are obligate aerobes overnight incubation was performed in nutrient broth supplemented with sodium nitrate (0·1 % w/v) (Loutit *et al.* 1968; Hamilton & Shelley, 1971).

(*e*) *Ethyl methane sulfonate (EMS) mutagenesis.* The method used was that of Meynell & Meynell (1970).

(*f*) *Ultraviolet irradiation.* Exponential phase bacterial cells suspended in buffer were exposed to 360 ergs/mm$^2$ from a Philips germicidal lamp. One millilitre of the irradiated suspension (approximately $3 \times 10^7$ viable cells) was inoculated into 10 ml of nutrient broth and grown with aeration at 37 °C for 90 min. This culture provided the donor cells used in R factor matings.

## 3. RESULTS

### (i) *R factor transfer from donor strains*

The R factors from *P. aeruginosa* wild type strains 1954 and 9169 were transferable by plate matings into the recipient species *P. aeruginosa* and *Sh. flexneri*. No transfer to either *E. coli* K12 or *S. typhimurium* was observed (Table 1).

Table 1. *R factor transfer frequencies from multiply resistant strains into a variety of recipient strains**

| | | Frequency of CB-r transfer to | | | | |
|---|---|---|---|---|---|---|
| | | *P. aeruginosa* | | *E. coli* K12. | *Sh. flexneri 4a.* | *S. typhimurium* LT2. |
| Donor | R factor | PAO8 | PAT900 | AB1450 | 25SM | PV18SM |
| 1954 | R19 | $1 \times 10^{-6}$ | $1 \times 10^{-3}$ | $< 3 \times 10^{-8}$ | $6 \times 10^{-6}$ | $< 3 \times 10^{-8}$ |
| 9169 | R91 | $2 \times 10^{-7}$ | $1 \times 10^{-4}$ | $< 3 \times 10^{-8}$ | $4 \times 10^{-6}$ | $< 3 \times 10^{-8}$ |

* Transfers were accomplished by plate matings, and frequencies are expressed as CB-resistant recipients per donor cell.

### (ii) *Variation in the antibiotic resistance pattern of exconjugants*

There was a variable coinheritance of NM and TC resistance among the CB-resistant exconjugants from crosses involving both donors (Table 2). With both PAO and PAT recipients there was no co-inheritance of NM- and TC-resistance following selection for CB resistance. On the other hand, the majority of CB-resistant exconjugants formed in *Sh. flexneri* were also resistant to NM and TC.

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The nature of the recipient obviously exerts a marked influence on the coinheritance or expression of R factor genes.

The absence of NM and TC resistance in the R19 and R91 isolates of PAO8 and PAT900 (Table 3) could conceivably be due either to non-expression of the resistance genes or to their physical loss at some stage during or after R factor transfer.

Table 2. *Co-inheritance of NM and TC resistance following transfer of R19 and R91*

| Donor | Recipient | Drug resistance pattern of recipient |
|---|---|---|
| 1954 ($CB^rNM^rTC^r$) | *P. aeruginosa* PAO8 | 81/81 $CB^rNM^sTC^s$ |
| | *P. aeruginosa* PAT900 | 150/150 $CB^rNM^sTC^s$ |
| | *Sh. flexneri* 4a:25SM | 2/20 $CB^rNM^sTC^s$ |
| | | 18/20 $CB^rNM^rTC^r$ |
| 9169 ($CB^rNM^rTC^r$) | *P. aeruginosa* PAO8 | 142/142 $CB^rNM^sTC^s$ |
| | *P. aeruginosa* PAT900 | 122/122 $CB^rNM^sTC^s$ |
| | *Sh. flexneri* 4a:25SM | 94/94 $CB^rNM^rTC^r$ |

Table 3. *Antibiotic resistance of R factor strains*

| | M.I.C. ($\mu$g/ml) | | |
|---|---|---|---|
| | CB | NM | TC |
| *P. aeruginosa* | | | |
| 1954, 9169 | > 4000 | 2000– > 4000 | 500– > 1000 |
| PAO8 | 8–16 | 16–32 | 16–32 |
| PAO8 (R19), PAO8 (R91) | 1000 | 16–32 | 16–32 |
| PAT900 | 32–64 | 16 | 32–64 |
| PAT900 (R19), PAT900 (R91) | 4000 | 16 | 32–64 |
| *Sh. flexneri* | | | |
| 4a:25SM | < 4 | 8–16 | 2–4 |
| 4a:25SM (R19), 4a:25SM (R91) | 4000 | 128 | 64–128 |
| 4a:25SM (R19-1) | > 4000 | 8–16 | 2–4 |
| *E. coli* C | | | |
| W2438 | 1–5 | 5 | 5 |
| W2438 (R91) | > 4000 | 128–256 | 64–128 |

To test the first possibility attempts were made to retransfer CB resistance from the R19 and R91 derivatives of PAO and PAT into *Sh. flexneri* since in this species NM and TC resistance are known to be expressed (Table 3). In contrast to the original wild type strains however, no transfer ($< 10^{-9}$ per donor) of CB resistance from the PAO and PAT R factor derivatives to *Sh. flexneri* could be detected. Transfer of R91 at an extremely low frequency was detected in one case to *E. coli* C. These CB-resistant exconjugants were also resistant to NM and TC, implying that the genetic determinants for NM and TC resistance carried by R91 are present in both PAO and PAT, but are not expressed.



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### (iii) *Stability of R factors*

The CB resistance of R19 and R91 was stable in both *P. aeruginosa* recipient strains (< 0·1 % segregation per generation). Drug resistance in *Sh. flexneri* was similarly stable in the case of the triply resistant (R19, R91) isolates, but unstable if the isolate was resistant only to CB (1–2 % segregation per generation). There was no observed loss of transfer ability (< 0·1 %) in both *P. aeruginosa* recipients.

### (iv) *Intrastrain R factor transfer*

No detectable transfer (< $10^{-9}$) of drug resistance was found from either the triply resistant or singly resistant isolates of *Sh. flexneri*. Transfer from both PAO and PAT occurred at a frequency of approximately $1\text{–}5 \times 10^{-4}$ per donor cell in 15 min broth matings. Partial derepression occurred following transfer to an intermediate host and complete derepression following u.v.-irradiation (Table 4).

Table 4. *Derepression of R factor transfer in* P. aeruginosa *strain PAO*

| Host | R factors | Prior treatment of R factor donor | Frequency of CB$^r$ transfer* |
|---|---|---|---|
| PAO8 | R19, R91 | None | $1\text{–}5 \times 10^{-4}$ |
| | R19, R91 | Conjugal derepression† | $1\text{–}5 \times 10^{-2}$ |
| | R19, R91 | u.v.-irradiation† | 1–2 |
| | R19-4, R91-5 | Genetic derepression† | 1·0 |

* Transfers were accomplished by 15 min broth matings, and frequencies are expressed as CB$^r$ recipients per CB$^r$ donor.

† These treatments are described in more detail in the text.

### (v) *Isolation and characteristics of R factor mutants derepressed for transfer*

Stably derepressed mutants of R19 and R91 (R19-4 and R91-5 respectively) were isolated following EMS mutagenesis by the successive mating technique of Edwards & Meynell (1968). Transfer of both of these mutant R factors occurred at a frequency of 100 % in 15 min broth matings (Table 4). Derepression of these R factors was associated with the appearance of sensitivity to the R factor specific RNA phage PRR1 described by Olsen & Shipley (1973), which is believed to adsorb to R factor-specified pili (R. Olsen, personal communication). The phage plated with an efficiency of < $10^{-10}$ on the repressed R factor strains relative to the group 2 R factors (Chandler & Krishnapillai, 1974*a*), but with an efficiency of 1 on the derepressed mutants. PRR1 also plated with an efficiency of 1 on strains carrying both a group 2 R factor (R18) and either R19 or R91, indicating that the mechanism inhibiting transfer or pilus synthesis by R19 and R91 does not affect pilus synthesis by R18.

### (vi) *Transfer of R19 and R91 to a recombination-deficient mutant of* P. aeruginosa

The results obtained when R19 and R91 were transferred to a recombination-deficient derivative of PAO and to the *rec*$^+$ parent are given in Table 5. The recom-

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bination-deficient strain is analogous to the *recA* mutants of *E. coli* K12 in many respects (Chandler & Krishnapillai, 1974*b*), and the observation that there was no significant difference in the frequency of transfer to a $rec^-$ as opposed to a $rec^+$ host suggests that both R19 and R91 are extrachromosomal in strain PAO. It is unlikely that these R factors integrate stably into the chromosome *via* an R factor specified recombination enzyme, since chromosome transfer mediated by them occurs at extremely low frequencies (P. M. Chandler, unpublished observations).

Table 5. *Transfer of R19 and R91 to a recombination deficient mutant of strain PAO*

| Donor | Frequency of $CB^r$ transfer to* | |
|---|---|---|
| | PAO2001 ($Rec^+$) | PAO2003 ($Rec^-$) |
| PAO1 (R19) | $2{\cdot}5\times10^{-4}$ | $2{\cdot}4\times10^{-4}$ |
| PAO1 (R91) | $1{\cdot}3\times10^{-3}$ | $1{\cdot}2\times10^{-3}$ |

* Transfers were accomplished by plate matings, using streptomycin contraselection against the donor. Frequencies are expressed as CB-resistant recipients per CB-resistant donor cell.

## 4. DISCUSSION

The two R factors described in this paper show significant differences to the group 1 and 2 R factors described previously (Chandler & Krishnapillai, 1974*a*) and constitute a third phenotypic group of R factors from the Birmingham outbreak (Lowbury *et al.* 1969). The control of expression of group 3 R factor genes is different to that of group 2 R factor genes although their known somatic functions appear to be very similar. The pili of both group 2 and 3 R factors are probably very similar since both types plate the pilus adsorbing phage PRR1 (Olsen & Shipley, 1973; R. Olsen, personal communication). It was surprising then to find that the group 3R factors were normally repressed for pilus synthesis, in contrast to the group 2 R factors. Furthermore the 'repressor' of the group 3 R factors did not reduce pilus synthesis by the group 2 R factors when both were present in the same cell on the basis of pilus specific phage plating.

Neomycin and tetracycline resistance coded for by the group 3 R factors was not expressed in either *P. aeruginosa* strain tested, although it was in the original wild type isolates. The presence of the resistance genes was inferred from their expression in a subsequent recipient species. The mechanism preventing the expression of of NM and TC resistance in strains PAO and PAT did not alter the expression of the corresponding resistance genes carried by the group 2 R factors (P. M. Chandler, unpublished observations) providing a second example of a difference in control of apparently similar functions carried by these R factors.

Ingram, Richmond & Sykes (1973) studied in some detail the molecular characteristics of an R factor from strain 9169. Their results show an expected high degree (80–90 %) of sequence homology between this and RP1, which is representative of the group 2 R factors. There are unaccountable differences however between



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the behaviour of drug resistance from their strain 9169 and ours, in particular their transfer of R9169 to *E. coli* K12, and the frequency of transfer relative to RP1 in *Pseudomonas* × *Pseudomonas* crosses (cf. Tables 3 and 9 in Chandler & Krishnapillai, 1974*a*).

Table 6. *Phenotypic classification of R factors of* P. aeruginosa *derived from a Birmingham hospital*

| Group | R factors | Transferable to | Transferable in | Transfer | Drug resistance genes | Stability in *P. aeruginosa* strain PAT |
|---|---|---|---|---|---|---|
| 1 | R18-1 | Pseudomonas<br>Escherichia<br>Shigella | Pseudomonas* | Not depressible | CB | Stable |
| 2 | R18, R18-3, R30, R68, R74, R74-3, R88 | Pseudomonas<br>Escherichia<br>Salmonella<br>Shigella | Pseudomonas<br>Escherichia<br>Salmonella<br>Shigella | Not derepressible | CB, NM, TC | Unstable |
| 3 | R19, R91 | Pseudomonas<br>Shigella | Pseudomonas | Derepressible | CB (NM, TC)† | Stable |

* The chromosomally integrated form of R18-1 in *E. coli* (see text) is transferable at very low frequencies. Significant transfer occurs only in *P. aeruginosa*.

† The bracketed NM and TC refers to the observation with R91 that these resistance markers were not expressed in the *P. aeruginosa* recipients used, but their presence could be deduced (see text). R19 is probably similar.

It is interesting that the group 2 and 3 R factors share very similar gene functions, but apparently control their expression quite differently (Table 6). We hope that further genetic analysis and molecular characterization will aid the evolutionary interpretation of these differences.

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