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Phenotypic properties of R factors of *Pseudomonas aeruginosa*: R factors readily transferable between *Pseudomonas* and the Enterobacteriaceae

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

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SUMMARY

R factors have been demonstrated in multiply drug resistant strains of enterobacteria and *Pseudomonas aeruginosa* in a Birmingham hospital (Lowbury *et al.* 1969; Ingram, Richmond & Sykes, 1973). A comparative genetic analysis of these R factors has been initiated on the basis of a variety of phenotypic characteristics. This paper describes the properties of R factors derived from strains which could transfer multiple drug resistance to the recipient species *P. aeruginosa*, *Escherichia coli*, *Shigella flexneri* and *Salmonella typhimurium*. Two types of R factor could be recognized phenotypically. The single group 1 R factor, R18-1 which is probably the same as RP1-1 (Ingram *et al.* 1972) was different to the group 2 R factors in many respects, including host range, R factor-specific phage plating, cellular location, drug resistance pattern, and stability. The group 2 R factors were found to be very similar to RP1 (Grinsted *et al.* 1972) and R1822 (Olsen & Shipley, 1973) with respect to their wide host range, plating of a sex specific phage, extrachromosomal location, and drug resistance pattern. Compatibility was shown between the group 1 R factor and a group 2 R factor, providing additional evidence for significant genetic differences.

1. INTRODUCTION

Multiple drug resistance factors have been extensively studied in enteric bacteria (for reviews see Novick, 1969; Watanabe, 1971; Clowes, 1972; Meynell, 1972) but their study in *Pseudomonas aeruginosa* has only commenced relatively recently following their isolation in strains from a Birmingham Hospital (Lowbury *et al.* 1969).

A distinctive feature of some of the R factors from this source is their wide host range (Datta *et al.* 1971; Mattes *et al.* 1973; Olsen & Shipley, 1973) which makes them interesting both from a genetical and medical viewpoint. The studies on these R factors to date include investigations of their transferability to enterobacteria (Sykes & Richmond, 1970; Fullbrook, Elson & Slocombe, 1970; Roe, Jones & Lowbury, 1971), a study of the chromosome transfer they mediate in a strain of *P. aeruginosa* (Stanisich & Holloway, 1971), and their molecular characterization (Grinsted *et al.* 1972; Ingram *et al.* 1972, 1973).

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R factors have also been recently isolated in *P. aeruginosa* in other geographical areas (Black & Girdwood, 1969; Bryan, Van Den Elzen & Jui Teng Tseng, 1972; Kawakami *et al.* 1972; Witchitz & Chabbert, 1972). On the basis of drug resistance pattern most of these R factors are different from those isolated initially.

The present study uses solely R factors from Birmingham (Lowbury *et al.* 1969), and its aim is to examine the phenotypic and genotypic characteristics of R factors isolated from a single source. In this paper we describe the properties of those R factors which were readily transferable to members of the Enterobacteriaceae.

2. MATERIAL AND METHODS

Bacterial strains. A list of these and their characteristics is given in Table 1.

Media. These have been described previously (Krishnapillai, 1971).

Table 1. *Bacterial strains used*

Species	Strain	Characteristics	Reference
P. aeruginosa PAO	PAO1	Prototroph, *chl*-2, FP2⁻	Isaac & Holloway (1968)
	PAO8	*met*-28, *ilv*-202, *str*-1, FP2⁻	Isaac & Holloway (1968)
	PAO2001	*arg*-32, *str*-39, *chl*-2, FP2⁻	Chandler & Krishnapillai (1974)
	PAO2003	*arg*-32, *str*-39, *chl*-2, *rec*-2, FP⁻2	Chandler & Krishnapillai (1974)
PAT	PAT900	*his*-404, *str*–1100, FP2⁻	Stanisich & Holloway (1971)
	PAT964	Prototroph, FP2⁻	Stanisich & Holloway (1971)
E. coli K-12	AB1450	*ilv*-16, *arg*-1, *met*-1, *thi*-2, *his*-1, *xyl*-4, *mal*-1, *tsx*	Pittard *et al.* (1963)
	C600NAL†	*thr, leu, thi, lac, ton,* (λ)⁻, *nal*	Appleyard (1954)
B	WP2	*trp*	Bridges *et al.* (1972)
	WP2 recA	*trp recA*	Bridges *et al.* (1972)
S. typhimurium LT2	PV18SM†	*hisC*527, *trp*-48, *H2enx*, *vh*2⁻, *str*	B. A. D. Stocker (personal communication)
	D3052NAL†	*his*D3052, *nal*	Ames *et al.* (1972)
Sh. flexneri Serotype 4a	25SM†	*nic, str*	R. B. Davey (personal communication)
	25NAL†	*nic, nal*	R. B. Davey (personal communication)

† The nalidixic-acid resistant mutants were selected by the method of Davey & Pittard (1971) using a 6 h incubation prior to plating. Streptomycin-resistant mutants were selected on NA supplemented with 1 mg/ml streptomycin. *Sh. flexneri* 25 SM was a spontaneous mutant, whereas *S. typhimurium* PV18SM was induced by ethyl methane sulfonate. The genotype symbols designate the following: *arg*, arginine; *his*, histidine; *ilv*, isoleucine and valine; *leu*, leucine; *met*, methionine; *thr*, threonine; *trp*, tryptophan; *lac*, lactose; *mal*, maltose, *xyl*, xylose; *nic*, nicotinic acid; *thi*, thiamine; *chl*, chloramphenicol resistance; *nal*, nalidixic acid resistance; *rec*, recombination deficiency; *str*, streptomycin resistance; *ton*, phage T1 resistance; *tsx*, phage T6 resistance; FP2⁻, lack of *P. aeruginosa* sex factor FP2; *vh*2, control of rate of phase variation; *H2enx*, phase two flagellar antigen.

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Derivation of R factor strains. All the multiply resistant wild type strains were isolated in the M.R.C. Industrial Injuries and Burns Unit, Birmingham Accident Hospital, England, and are described in Table 2 (Holloway & Richmond, 1973). All showed resistance to the antibiotics carbenicillin, neomycin/kanamycin, and tetracycline.

Table 2. *Multiply resistant strains of* P. aeruginosa *and* E. coli

Strain	R factor(s)	Origin of R factor
P. aeruginosa		
1822	R18, R18-1†	Wild type isolate
P14 (R1822)	R18-3	From 1822, via *E. coli* K12
3098	R30	Wild type isolate
6886	R68	Wild type isolate
P14 (R74)	R74	From *E. coli* (R7475)
6888	R88	Wild type isolate
E. coli		
E. coli (R7475)‡	R74-3	From *K. aerogenes* 7475

† Strain 1822 yields two R factors when mated with a variety of recipients (see Results).
‡ R7475 is an R factor derived from a strain of *Klebsiella aerogenes* which was present in a burn also colonized with *P. aeruginosa* showing the same drug resistance phenotype. The host *E. coli* strain was not specified on acquisition of the strains.

Transfer of R factors. R factors were transferred from the strains in Table 2 to genetically characterized recipient strains, except that R74-3 was first transferred to the streptomycin-sensitive strain *E. coli* K12 C600 which was used as the donor for R74-3. Plate-matings were accomplished by the mixed plating of stationary phase recipients and logarithmic phase donors (suitably diluted) on nutrient agar (NA) plates supplemented with streptomycin (SM) and carbenicillin (CB) both at 500 μg/ml. In broth matings logarithmic phase donors and stationary phase recipients were mixed in the ratio of 1:20 at a final cell density of about 7×10^8/ml in nutrient broth at 37 °C. After 15 min, matings were interrupted by agitation on a vortex mixer. Dilutions of the mating mixture were plated, utilizing CB selection for R factor-carrying bacteria and streptomycin or a nutritional marker contraselection. In some cases all R^+ derivatives of the *Pseudomonas* recipients PAO and PAT following plate-mating with the wild type strains were found to be lysogenized with phages from the donor strains. Therefore, non-lysogenic strains were constructed by first transferring the R factor into PAO1 by a 5 min broth mating, and after verifying that the PAO1 (R) ex-conjugants were non-lysogenic, transferring the R factor into PAO8 and PAT900. This procedure was used to construct the following strains: PAO8 (R68), PAO8 (R88), PAT900 (R18), PAT900 (R18-1), PAT900 (R68) and PAT900 (R88).

Generally, at least one hundred clones of the CB-resistant *P. aeruginosa* and *E. coli* K-12 recipients from each plate-mating with the wild-type donor were screened for co-inheritance of neomycin (NM) and tetracycline (TC) resistance by spotting to selective media after purification. For the *Salmonella* and *Shigella* recipients smaller numbers were screened.

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Determination of minimal inhibitory concentrations (M.I.C.) of antibiotics. Logarithmic phase cultures grown in nutrient broth containing CB at 250 μg/ml were diluted by a factor of 2×10^5 and 0·01 ml aliquots spotted on to NA containing various concentrations of the antibiotics. The M.I.C. was scored after incubation at 37 °C for 18 h (and again at 42 h) and was taken to be the lowest concentration of antibiotic producing a significant inhibition of single clone growth.

R factor stability. Strains grown overnight in nutrient broth containing 250 μg/ml CB were diluted and approximately 100 cells sub-cultured into CB-free broth. These were grown for 8–9 h at 37 °C with aeration until cell density of 10^6–10^7/ml was reached. Dilutions of both the initial and final populations were plated on NA and incubated overnight. One hundred clones from each were spotted to NA, and the proportion of R factor-carrying cells determined by replica-plating to antibiotic-containing media. For R factor derivatives of *P. aeruginosa* strains PAO and PAT, the proportion of CB-resistant cells which retained transfer ability was determined by replica-plating to minimal medium containing CB (500 μg/ml) which had previously been spread with a prototrophic, CB-sensitive strain (PAO1 or PAT964).

3. RESULTS

R factor transfer from donor strains into a variety of recipient strains. The R factors from the *P. aeruginosa* strains and an *E. coli* strain were transferred by plate-matings to the following recipient species: *P. aeruginosa, E. coli, Sh. flexneri,* and *S. typhimurium*. The transfer frequencies are shown in Table 3.

Table 3. *R factor transfer frequencies from multiply resistant strains into a variety of recipient strains*†

		Frequency of CB-r transfer to:				
		P. aeruginosa			*Sh. flexneri* 4a	*S. typhimurium* LT2
Donor	R factor	PAO8	PAT900	*E. coli K*12 AB1450	25SM	PV18SM
1822	R18, R18-1	1×10^{-5}	2×10^{-4}	4×10^{-3}	4×10^{-2}	2×10^{-7}
P14(R18-3)	R18-3	5×10^{-7}	3×10^{-5}	9×10^{-3}	2×10^{-1}	3×10^{-8}
3098	R30	1×10^{-6}	9×10^{-3}	6×10^{-3}	3×10^{-2}	3×10^{-7}
6886	R68	3×10^{-8}	1×10^{-5}	2×10^{-5}	3×10^{-2}	3×10^{-8}
P14(R74)	R74	2×10^{-6}	7×10^{-6}	6×10^{-3}	1×10^{-1}	3×10^{-7}
C600(R74-3)	R74-3	1×10^{-5}	7×10^{-3}	4×10^{-1}	3×10^{-1}	2×10^{-7}
6888	R88	1×10^{-7}	2×10^{-4}	2×10^{-4}	3×10^{-2}	1×10^{-7}

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

In most *Pseudomonas* × *Pseudomonas* crosses one parent was sensitive to either phage or aeruginocin produced by the other parent, and the transfer frequencies obtained are therefore likely to be underestimates. Consequently, a comparison between the transfer frequencies with *Pseudomonas* and enterobacterial recipients is not valid.

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Shigella flexneri was the most efficient enterobacterial recipient, followed by *E. coli* K12. Both of these species accepted the R factors far more readily than *S. typhimurium*.

Variation in the antibiotic resistance pattern of exconjugants. Since selection was imposed only for CB resistance during R factor transfer, it was possible that some R^+ exconjugants would not inherit or express the determinants for NM and TC resistance. In all cases except the donor strain 1822, however, it was found that selection for CB resistance resulted in 100% co-inheritance of NM and TC resistance. Table 4 shows that with both *P. aeruginosa* and *Sh. flexneri* recipients there was less than 100% co-inheritance of NM and TC resistance from 1822. This indicated that this strain either contained two R factors (one conferring CB resistance and one conferring CB, NM, TC resistance) or that it contained an R factor which undergoes dissociation. This point is considered further below.

Table 4. *Variation in the drug resistance patterns of recipients following inheritance of R factor from wild type* P. aeruginosa *strain 1822*

Recipient strain	Drug resistance pattern of recipients
P. aeruginosa PAO8	60/150(36%) $CB^rNM^rTC^r$
	90/150(64%) CB^r
PAT900	68/150(43%) $CB^rNM^rTC^r$
	82/150(57%) CB^r
E. coli K12 AB1450	150/150(100%) $CB^rNM^rTC^r$
S. typhimurium PV18SM	9/9 $CB^rNM^rTC^r$
Sh. flexneri 25SM	60/83 $CB^rNM^rTC^r$
	23/83 CB^r

Expression of antibiotic resistance in recipient strains. The wild type strains of *P. aeruginosa* used in these experiments were characterized by very high levels of resistance to CB, NM and TC (Table 5). When the R factors were transferred to other strains CB resistance was still expressed at very high levels, but NM and TC resistance did not occur at the same high levels in the enterobacteria (Table 5).

Stability of R factors. The stability of drug resistant phenotype was examined for all R factors in all recipient strains. In addition, the stability of transferability was examined in both *P. aeruginosa* recipient strains. Table 6 shows that the R factors which confer triple drug resistance were extremely stable in all recipients except for *P. aeruginosa* strain PAT.

The one R factor which confers only CB resistance, R18-1, was stable in both *P. aeruginosa* recipients, but when transferred to the enterobacteria, CB-resistance was unstable. However, stable forms could be generated in *E. coli* K12 by continuous purification in the presence of CB. These stable forms are probably analogous to the chromosomally integrated R factor described by Richmond & Sykes (1972), since their inheritance after transfer to *E. coli* is dependent on a functional *recA* gene (Table 8). The stable form of R18-1 in *E. coli* will be designated R18-1*.

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In both *P. aeruginosa* strains there was no observed instance (< 0·1 % per generation) of segregation between the genes for CB resistance and transferability.

Cell location of R factors. The chromosomal or extrachromosomal status of all the R factors in strain PAO was determined by crossing suitable R factor donors with a recombination-deficient mutant of this strain. This mutant appears to be analogous to the *recA* mutants of *E. coli* K12 (Clark, 1971) in that it does not act as a

Table 5. *Antibiotic resistance of R factor strains*

Species	Strain(s)	R factor(s)	M.I.C. (μg/ml) CB	NM	TC
P. aeruginosa	All wild type isolates	—	> 4000	2000– > 4000	500– > 1000
	PAO8	—	8–16	16–32	16–32
	PAO8	R18-1	2000–4000	16–32	16–32
	PAO8	R18, R18-3, R30, R68, R74, R74-3, R88	2000–4000	> 1000	250–500
	PAT900	—	32–64	16	32–64
	PAT900	R18-1	> 4000	16	32–64
	PAT900	R18, R18-3, R30, R68, R74, R74-3, R88	2000– > 4000	500– > 1000	128–500
E. coli	K-12:AB1450	—	< 4	2	2
	K-12:AB1450	R18-1*	> 4000	< 2	2
	K-12:AB1450	R18, R18-3, R30, R68, R74, R74-3, R88	> 4000	64	128–256
Sh. flexneri	4a:25SM	—	< 4	8–16	2–4
	4a:25SM	R18-1	> 4000	8–16	2–4
	4a:25SM	R18, R18-3, R30, R68, R74, R74-3, R88	> 4000	128	63–128
S. typhimurium	LT2:PV18SM	—	4	8	2–4
	LT2:PV18SM	R18, R18-3, R30, R68, R74, R74-3, R88	> 4000	128–256	128

recipient for chromosomal markers in either conjugation or transduction, is non-inducible for the synthesis of phage and aeruginocins, and is more sensitive to u.v.-irradiation, mitomycin C, and methyl methane-sulfonate than is the parent strain (Chandler & Krishnapillai, 1974). Table 7 demonstrates that the transfer of all the R factors in PAO is not significantly reduced by using this recombination deficient recipient. This, as well as the fact that plasmid transfer occurs at frequencies at least 10^4 times that of any chromosomal marker tested (Chandler, unpublished observations) suggests that in strain PAO all the R factors have an extrachromosomal location.

In *E. coli*, the establishment of stable CB resistance following the transfer of R18-1 is dependent on a functional *recA* gene. None of the other R factors show

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Table 6. *Stability of R factors in recipient strains*

Host strain	R factor(s)	Percentage of cells which are drug-resistant†		Number of generations of growth
		Initial population	Final population	
P. aeruginosa PAO8	R18, R18-1, R18-3, R30, R68, R74, R74-3, R88	100	100	15
PAT900	R18-1	100	100	15
	R18	75	18	9
	R18-3	99	97	13
	R30	99	90	10
	R68	70	57	8
	R74	92	45	9
	R74-3	50	19	10
	R88	88	63	8
E. coli K12 AB1450	R18, R18-1*, R18-3, R30, R74, R74-3, R88	100	100	15
	R68	100	99	15
S. typhimurium PV18SM	R18, R18-3, R30, R68, R74, R74–3, R88	100	100	15
Sh. flexneri 25SM	R18, R18-3, R30, R68, R74, R74-3, R88	100	100	15
	R18-1	60	1	15

† Resistance to CB was tested in the case of R18-1 and to CB, NM and TC for all other R factors.

Table 7. *R factor transfer to a recombination deficient strain of* P. aeruginosa

Donor	Frequency of transfer† of CB resistance to	
	PAO2001(Rec+)	PAO2003(Rec−)
PAO1 (R18)	$6\cdot9\times10^{-2}$	$2\cdot9\times10^{-2}$
PAO1 (R18-1)	$6\cdot6\times10^{-2}$	$6\cdot1\times10^{-2}$
PAO1 (R18-3)	$7\cdot0\times10^{-2}$	$3\cdot1\times10^{-2}$
PAO1 (R30)	$6\cdot8\times10^{-2}$	$4\cdot0\times10^{-2}$
PAO1 (R68)	$5\cdot5\times10^{-2}$	$1\cdot6\times10^{-2}$
PAO1 (R74)	$6\cdot6\times10^{-2}$	$2\cdot5\times10^{-2}$
PAO1 (R74-3)	$11\cdot5\times10^{-2}$	$3\cdot8\times10^{-2}$
PAO1 (R88)	$7\cdot5\times10^{-2}$	$4\cdot2\times10^{-2}$

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

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Table 8. *R factor transfer to a recombination-deficient strain of* E. coli

Donor	Frequency of transfer† of CB resistance to	
	WP2	*WP2 recA*
AB1450 R18, R18-3, R30, R74, R74-3, R88	$4–10 \times 10^{-6}$	$2–5 \times 10^{-6}$
R68	10^{-6}	7×10^{-7}
R18-1*	40/ml‡	< 1/ml‡

† Transfers were accomplished by plate matings, except for the R factor R18-1, where the very low transfer frequency necessitated the use of overnight broth matings. Nutritional contraselection was used against donors. Frequencies are expressed as CB-resistant recipient cells per donor, except for R18-1, where the frequency is expressed as CB-resistant recipient cells per ml of mating mixture.
‡ Values quoted are the means of six independent crosses.

Table 9. *Interstrain R factor transferability*†

Host strain	R factor(s)	Frequency of CB^r transfer
E. coli K12 (AB1450)	R18, R18-3, R30, R74, R74-3, R88	$2–6 \times 10^{-6}$
	R18-1*, R68	$< 2 \times 10^{-6}$‡
S. typhimurium (PV18SM)	R18, R18-3, R30, R74, R74-3, R88	$3–7 \times 10^{-5}$
	R68	$< 2 \times 10^{-6}$‡
Sh. flexneri (25SM)	R18, R18-3, R30, R74, R74-3, R88	$< 2–8 \times 10^{-4}$
	R68	$< 2 \times 10^{-6}$‡
	R18-1	$< 2 \times 10^{-6}$§
P. aeruginosa (PAO8)	R18, R18-3, R30, R68, R74, R74-3, R88	$10^{-3} – 10^{-2}$
	R18-1	$10^{-3} – 10^{-2}$
P. aeruginosa (PAT900)	R18, R18-3, R30, R68, R74, R74-3, R88	$3 \times 10^{-3}–5 \times 10^{-2}$
	R18-1	0·5–0·75

† Determined by 15 min broth matings, and expressed as CB-resistant recipients per CB-resistant donor. Donor contraselection was nalidixic acid.
‡ The R factor was transferred at a low frequency in overnight matings.
§ The R factor was not detectably transferred in overnight matings.

any significant difference in transfer to a rec^- as opposed to a rec^+ recipient (Table 8).

R factor transferability in intrastrain crosses. The transfer abilities of R factors in the enterobacteria was measured and the results are given in Table 9. There are several interesting features:

(i) in all three species examined, the same six R factors were the most readily transferable (R18, R18-3, R30, R74, R74-3, and R88);

(ii) R68 was transferable at a very low rate in all species examined, and transfer could be shown only after overnight matings.

(iii) R18-1* was transferable in *E. coli* at a low frequency, and transfer of R18-1 in *Sh. flexneri* could not be demonstrated.

In intrastrain *Pseudomonas* crosses, the transfer frequencies obtained were higher than for enterobacterial crosses. An interesting feature was the behaviour

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of R18-1 which acted as a derepressed sex factor in PAT900 with 50–75 % of males transferring CB resistance in a 15 min mating.

R factors from wild type strain 1822. From Table 4 it is evident that when strain 1822 was mated with PAO8 or PAT900 about 40 % of the isolates were resistant to CB, NM and TC, and about 60 % resistant to only CB. Resistance from both of these isolates is transferable, the R factor conferring resistance to three drugs being referred to as R18, and that conferring only CB resistance as R18-1. A series

Cross

(1) 1822 (CB^{r}NM^{r}TC^r) × PAO8

PAO8 (CB^{r}NM^{r}TC^r) = 36% + PAO8 (CB^r) = 64%

(2) ×PAO1 ×PAO1

PAO1 (CB^r) = 86% + PAO1 (CB^{r}NM^{r}TC^r) = 14% PAO1 (CB^r) = 100%

(3) ×PAO8 ×PAO8 ×PAO8

PAO8 (CB^r) = 100%

(i) 11/14 donors tested each gave 100% triply-resistant PAO8 derivatives.

(ii) 3/14 donors tested each gave 70–90% triply-resistant and 10–30% singly-resistant PAO8 derivatives.

PAO8 (CB^r) = 100%

Fig. 1. R factors from 1822 in PAO

of experiments which imply that the PAO8 (R18) isolates obtained following plate-mating with 1822 also contain R18-1 is summarized in Fig. 1. Cross 1 describes the initial plate-mating from which the heterogeneous population of R$^+$ recipients was isolated. The singly-resistant derivatives (i.e. R18-1) were stable in subsequent matings. The presence of R18-1 in the triply-resistant PAO8 isolates from cross 1 cannot be directly determined since CB resistance is the only available marker on R18-1. Cross 2 describes a 10 min broth mating with PAO1 from which the majority of R$^+$ recipients were singly-resistant. If R18 dissociated to produce R18-1, which is a possible conclusion from the first two crosses, the triply-resistant isolates of PAO1 might be expected to show the same dissociation pattern in subsequent transfer. This was not observed (cross 3). An alternative explanation is that the triply-resistant PAO8 isolates obtained from cross 1 contained both R18 and R18-1, and the short mating time in cross 2 allowed most recipients to inherit only one of the R factors, although a small proportion (3/100) inherited both. Cross 3 reveals that the three donor types, predicted by the second explanation, were found. If this explanation is correct, the R18 and R18-1 are compatible in strain PAO. V. Stanisich (personal communication) has shown that two probably analogous R factors (RP1 and RP1-1) are compatible in strains PAO and Ps.18 of *P. aeruginosa*.

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Phage plating on R factor strain. Olsen & Shipley (1973) have described an RNA phage which plates specifically on cells carrying an R factor isolated from the wild type *P. aeruginosa* strain 1822. It was of interest to determine whether phage PRR1 plated on strains carrying any of the other R factors used in the present study. It was found that phage PRR1 plates with an efficiency of 1 on all R factor derivatives of PAO8 except for PAO8 (R18-1) where there was no detectable plating ($< 10^{-11}$).

4. DISCUSSION

The R factors studied here fall into two groups on the basis of several phenotypic differences: group 1 contains R18-1 only, while group 2 comprises R18, R18-3, R30, R68, R74, R74-3 and R88. Firstly, there is a significant difference in the stability of the two types of R factor in strain PAT of *P. aeruginosa*. The reason for the instability of the group 2 R factors in PAT is unknown, but its strain specificity strongly suggests that it depends upon factors in the host background rather than on maintenance functions carried by these R factors.

A second difference between the two R factor groups is their host range and frequency of interstrain transfer. The group 2 R factors were transferable to and within a wider variety of species than the group 1 R factor. The difference in pili between the two R factor groups, suggested by the difference in their plating of the pilus-adsorbing phage PRR1 (Olson & Shipley, 1973; R. Olsen, personal communication) may be partially responsible for the more restricted host range of R18-1, the group 1 R factor. However the instability of this R factor in *E. coli* and *Sh. flexneri* indicates that it also lacks some functions required for its stable maintenance in these species.

A third difference between R18-1 and the group 2 R factors lies in their somatic functions. The group 1 and 2 R factors both code for production of a type IIIa β-lactamase (Curtis, Richmond & Sykes, 1972) and the capacity to transfer this gene, but in addition the group 2 R factors code for resistance to NM and TC, interfere with the replication of phage G101 (Holloway, Krishnapillai & Stanisich, 1971; Krishnapillai, in preparation) and confer tolerance to an aeruginocin (Chandler, unpublished observations).

It would be interesting to determine the genetic basis of the phenotypic differences between the two R factor groups. It seems unlikely that R18-1 is a dissociation product of the group 2 R factor R18, and it may possess unique genes. Direct molecular comparisons between R18 and R18-1 have not yet been made, and attempts to compare two probably analogous R factors, RP1 and RP1-1 (Grinsted *et al.* 1972) have so far been unsuccessful owing to the fact that extrachromosomal DNA cannot be detected in strains harbouring RP1-1 (Ingram *et al.* 1972). However, there must be significant gene differences between them because they code for different pili, and belong to different compatibility groups.

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