Similarities between Plasmids of the P-incompatibility Group Derived from Different Bacterial Genera

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(Received 8 August 1975; revised 8 January 1976)

SUMMARY

The properties of four P-group plasmids, R26, R527, R751 and R906, which differ in resistance phenotype or in the bacterial species in which they were first detected, have been compared with the prototype of this group, RP1. Two of the plasmids, R26 and R527, are new isolates which have been assigned to the P group because of their incompatibility with R751. The properties studied include response to aeruginocin and to male and female sex-specific phages, interaction with prophage B3 and fertility inhibition by plasmid R38. Strains harbouring these plasmids behaved similarly in all tests except those involving aeruginocin. This suggests that the locus for aeruginocin-insensitivity is one of the R-determinants whereas the genes controlling the remaining characteristics are closely linked to the transfer factor. These plasmids may therefore have a common ancestor and their differences in resistance phenotype may simply reflect recombination events which they have undergone subsequently. Their similarity is also seen in transduction experiments, since determinants from two of these plasmids can be 'rescued' by the P-group plasmid R18 if this is already present in the recipient cell and the host recombination system is functional.

INTRODUCTION

The R plasmid, RP4, which was first detected in *Pseudomonas aeruginosa*, is readily transferable to a wide variety of bacterial species and is the prototype of the P-incompatibility group (Datta *et al.*, 1971). This plasmid is probably identical to RP1 (Grinsted *et al.*, 1972; Holloway & Richmond, 1973) and R18 (Chandler & Krishnapillai, 1974b; Stanisich, Bennett & Ortiz, 1976), since all were derived from *P. aeruginosa* 1822, have the same molecular properties, and confer the same resistance phenotype [carbenicillin (Cb), neomycin/kanamycin (Nm/Km), tetracycline (Tc)]. Recently, P-group plasmids with different resistance phenotypes have been reported and their geographical distribution seems widespread (Davey & Pittard, 1974; Grant & Pittard, 1974; Hedges, Jacob & Smith, 1974; Jobanputra & Datta, 1974; Hedges, Rodriguez-Lemoine & Datta, 1975). The object of the present study was to assess the uniformity of properties among P-group plasmids which, in terms of resistance phenotype or initial carrier strain, differed from those previously described. We have used the plasmids R751 (Jobanputra & Datta, 1974) and R906 (Hedges *et al.*, 1974) which confer resistance to trimethoprim (Tp) and to ampicillin (Ap), streptomycin (Sm), sulphonamides (Su) and mercuric salts (Hg) respectively, together with two new plasmids, R26 and R527, derived from *Serratia* and *Pseudomonas* species isolated in Spain. R26 and R527 are assigned to the P group by their incompatibility with R751; and the properties of the four plasmids, R26, R527, R751 and R906, are then compared with those determined by RP1.

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Table 1. *Bacterial strains*

Strain	Genotype	Reference
<i>Escherichia coli</i> K12		
UB1301 = W3110	<i>rif</i>	Grinsted <i>et al.</i> (1972)
JC3272	<i>his, lys, trp, strA</i>	Achtman <i>et al.</i> (1971)
185 <i>nal</i>	prototroph, <i>nal</i>	S. Falkow (personal communication)
<i>Pseudomonas</i>		
<i>aeruginosa</i> PAO		
PAO8	<i>met28, ilv202, strI</i>	Isaac & Holloway (1968)
PAO2001	<i>arg32, str39, chl2</i>	Chandler & Krishnapillai (1974a)
PAO2002	<i>arg32, str39, chl2, rec1</i>	Chandler & Krishnapillai (1974a)
PAO2635	<i>trp54, rif3, fon101</i>	This paper
PAO2637	prototroph, <i>chl2, fon100</i>	This paper
PAO9501	<i>ade136, leu8, chl3, rif1</i>	This paper
PAO9502	<i>ade136, leu8, chl3, rif1, (B3)</i>	This paper

Resistance genes: *chl*, chloramphenicol; *fon*, phage F116L; *nal*, nalidixic acid; *rif*, rifampicin; *str*, streptomycin. Nutritional requirements: *ade*, adenine; *arg*, arginine; *ilv*, isoleucine plus valine; *his*, histidine; *leu*, leucine; *lys*, lysine; *met*, methionine; *trp*, tryptophan.

Table 2. *Origins and phenotypes of P-group plasmids*

Plasmid	Resistance phenotypes	Carrier strain	Geographical origin	Reference
RP1	Cb Nm/Km Tc	<i>Pseudomonas aeruginosa</i> 1822	U.K. (Birmingham)	Grinsted <i>et al.</i> (1972)
R26	Cb Nm/Km Tc Sm Su Gm Hg	<i>Serratia marcescens</i>	Spain	This paper
R527	Cb Nm/Km Tc Sm Su Gm Hg	<i>Pseudomonas aeruginosa</i>	Spain	This paper
R751	Tp	<i>Klebsiella aerogenes</i>	U.K.	Jobanputra & Datta (1974)
RP1	Ap Sm Hg Su	<i>Bordetella bronchiseptica</i>	Japan	Hedges <i>et al.</i> (1974)

METHODS

Media. The nutrient agar (NA), nutrient overlay agar for phage propagation, and nutrient broth (NB), were described by Beard, Howe & Richmond (1972). The compositions of minimal medium (MM) for *P. aeruginosa* and for *Escherichia coli* were given by Stanisich & Holloway (1972) and Grinsted *et al.* (1972) respectively.

Bacteria. The derivatives of *E. coli* K12 and *P. aeruginosa* PAO used in this work are shown in Table 1. Plasmid-carrying sub-lines were constructed for some of these strains (see Tables 3 to 7). The aeruginocinogenic strain *P. aeruginosa* 41 (Holloway *et al.*, 1974), provided by B. W. Holloway, Monash University, Australia, was also used.

Plasmids. The origins and resistance phenotypes of the plasmids are shown in Table 2. *Escherichia coli* 353 and 353-2 carrying R751 and R906 respectively, were provided by N. Datta, Royal Postgraduate Medical School, Hammersmith Hospital, London. Clinical isolates of *Serratia marcescens* and *P. aeruginosa* carrying R26 and R527 respectively, were provided by D. Damaso, Clinica Puerta de Hierro, Madrid, Spain. These plasmids were transferred to JC3272 (provided by A. J. Clark, University of California, Berkeley, U.S.A.) directly or, in the case of R26 and R527, via *E. coli* 185. RP1 was transferred to UB1301 from *P. aeruginosa* PAO8.RP1 (Stanisich, 1974b). R91, its derepressed derivative

Table 3. Resistance phenotype of *R*⁻ and *R*⁺ lines of *E. coli* K12 and *P. aeruginosa* PAO

Strain	Minimum inhibitory concentration ($\mu\text{g ml}^{-1}$)							
	Cb	Nm	Km	Tc	Sm	Gm	Hg	Su
<i>E. coli</i> UB1301								
R ⁻	< 3	0.3	< 3	< 3	0.6	< 0.1	< 2	< 7
R906	30	0.3	< 3	< 3	7	< 0.1	< 2	500
RP1	> 2000	30	125	125	0.6	< 0.1	< 2	< 7
R26, R527	> 2000	15	125	125	7	10	40	250
<i>P. aeruginosa</i> PAO2635								
R ⁻	30	7	30	7	7	0.6	< 2	15
R906	125	7	30	7	500	0.6	40	2000
RP1	> 2000	500	> 1000	500	7	0.6	< 2	15
R26, R527	1000	500	> 1000	500	250	> 40	40	< 2000

R91-21 and R38 have been described previously (Stanisich, 1974*b*). R38-5 is a derivative of R38 which does not inhibit the fertility of RP1. R18-18 is a carbenicillin-sensitive derivative of R18 (Chandler & Krishnapillai, 1974*b*) provided by P. M. Chandler, Monash University, Australia. R18-91 is a transfer-defective derivative of R18-18 selected by its hosts' resistance to phage PR4.

Phages. These were the male-specific phages PRR1 and PRD1 (Olsen & Thomas, 1973; Olsen, Siak & Gray, 1974) provided by R. H. Olsen, Michigan University, U.S.A., and PR3, PR4, and Pf3 (Stanisich, 1974*b*), the temperate phages F116L, G101, B3, B33 and B39, and the virulent phage E79 (Krishnapillai, 1974; Holloway & Krishnapillai, 1975; Morgan & Stanisich, 1976). The mutant phage G101-1 was provided by P. M. Chandler.

General methods. Mating experiments, propagation of phages, transduction with F116L and spot assays to determine phage titre or efficiency of plating (e.o.p.) were all carried out as previously described (Stanisich, 1974*a, b*). The preparation of aeruginocin by mitomycin C induction and the determination of its titre was as described by Holloway *et al.* (1974).

Minimum inhibitory concentration (m.i.c.). The concentration of antibacterial agents inhibitory to bacterial growth was determined by plating single-colony-forming units on to NA containing the agent at appropriate concentrations. For sulphathiazole, lysed horse blood NA was used. The blood was lysed with saponin (2 %, v/v) then added to molten NA at about 48 °C to a final concentration of 5 % (v/v). Sulphathiazole was included as required and the plates stored at 4 °C for 24 h prior to use.

RESULTS

Resistance phenotype

The m.i.c. of various antibacterial agents to *R*⁻ and *R*⁺ lines of *E. coli* K12 and *P. aeruginosa* PAO are shown in Table 3. R26 and R527 both share with RP1 the resistance pattern Cb Nm/Km Tc, but in addition confer resistance to mercuric ions, streptomycin, sulphathiazole (Su) and gentamicin (Gm). The responses of R751⁺ lines were similar to their *R*⁻ parents (data not shown) except with trimethoprim. The m.i.c. of this compound to *R*⁻ *E. coli* and *P. aeruginosa* was 0.5 and 60 $\mu\text{g ml}^{-1}$ respectively, but to R751⁺ bacteria it exceeded 1 mg ml^{-1} .

Exclusion and incompatibility

The plasmids R26 and R527 were assigned to the P-incompatibility group by their exclusion and incompatibility responses with the previously described P-group plasmid, R751 (Jobanputra & Datta, 1974). R26 and R527 were used as the superinfecting or resident

Table 4. *Exclusion and incompatibility responses of P plasmids in E. coli K12*

For broth matings (45 min at 37 °C), equal volumes of donor and recipient bacteria at 5×10^8 bacteria/ml were used. Donors were R⁺ lines of UB1301 and recipients were JC3272 and its R⁺ derivatives. Selection was on supplemented MM using Cb (250 µg ml⁻¹) and Tp (60 µg ml⁻¹) as appropriate. Streptomycin (250 µg ml⁻¹) was used for counter selection.

Donor	Recipient	Selected marker	Frequency of resistant transcipts/donor cell	Phenotype of transcipts
R751	R ⁻	Tp	8.1×10^{-4}	—
R751	RP1	Tp	5.1×10^{-6}	40/40 Tp-r Cb-s
R751	R26	Tp	5.2×10^{-6}	40/40 Tp-r Cb-s
R751	R527	Tp	6.9×10^{-6}	35/35 Tp-r Cb-s
RP1	R ⁻	Cb	2.6×10^{-3}	—
RP1	R751	Cb	1.4×10^{-4}	20/20 Cb-r Tp-s
R26	R ⁻	Cb	3.6×10^{-5}	—
R26	R751	Cb	1.5×10^{-6}	32/40 Cb-r Tp-s
R527	R ⁻	Cb	2.4×10^{-3}	—
R527	R751	Cb	0.8×10^{-4}	20/20 Cb-r Tp-s

plasmids in a series of reciprocal matings with bacteria harbouring R751. Similar experiments using RP1⁺ and R751⁺ bacteria were included as controls (Table 4). Exclusion was evident in each of these pairwise matings since the inheritance of R751, as indicated by the recovery of Tp-r transcipts, was reduced 100-fold by bacteria harbouring RP1, R26 or R527 and, conversely, the inheritance of these three plasmids was reduced 20-fold by bacteria harbouring R751.

The compatibility properties of the superinfecting and resident plasmids were determined from the resistance phenotypes of the Cb-r and Tp-r transcipts recovered. In almost every case the resident plasmid had been displaced by the superinfecting one, indicating that R751 was incompatible with RP1, R26 and R527 and hence that all four plasmids were members of the P group. Although the resistance phenotypes of R26 and R527 precluded the formal demonstration of incompatibility between these plasmids or between them and RP1, exclusion by bacteria harbouring the latter could be demonstrated (data not shown). It seems likely, therefore, that matings involving all combinations of these plasmids would give similar results to those obtained with R751.

Properties of P-group plasmids

To determine the similarity between the various P-group plasmids, the properties they confer were compared with those determined by RP1. Table 5 summarizes the results.

Host range of male-specific phages. RP1⁺ bacteria are hosts for three types of male-specific phages: the RNA phage PRR1 (Olsen & Thomas, 1973), the double-stranded DNA phages PRD1, PR3 and PR4 (Olsen *et al.*, 1974; Bradley, 1974; Stanisich, 1974*b*; Bradley & Rutherford, 1975), and the filamentous phage Pf3 (Bradley, 1974; Stanisich, 1974*b*). The double-stranded DNA phages can infect bacteria harbouring plasmids of incompatibility groups other than P (Olsen *et al.*, 1974; Kontomichalou & Papachristou, 1975; Bradley & Rutherford, 1975), but so far this broad host range has not been found for PRR1 or Pf3. Table 5 shows the plating efficiencies of these phages on various R⁺ lines of *E. coli* K12 compared with those obtained on *P. aeruginosa* PAO harbouring RP1.

Of the two bacterial species, *P. aeruginosa* was the more suitable as high plating

Table 5. Phenotypic properties of *P* plasmids in *P. aeruginosa* PAO and *E. coli* K12

Plasmid	Efficiency of plating*							Plasmid transfer to B3 lysogen§	Response of P ⁺ bacteria to aeruginocin AP41	Fertility inhibition of P plasmid by ¶	
	Male-specific phage†					Female-specific phage‡				R38	R38-5
	PRR1	PR3	PR4	PRD1	Pf3	G101	G101-I				
RP1	10 ⁻³	(10 ⁻⁵)	10 ⁻¹	10 ⁻¹	(10 ⁻⁶)	10 ⁻⁴	I	10 ⁻⁴	R	+	—
R26	10 ⁻³	(10 ⁻⁵)	10 ⁻¹	10 ⁻¹	(10 ⁻⁶)	10 ⁻⁴	I	10 ⁻⁴	R	+	—
R527	10 ⁻³	(10 ⁻⁵)	10 ⁻¹	10 ⁻¹	(10 ⁻⁶)	10 ⁻⁴	I	10 ⁻⁴	R	+	—
R906	10 ⁻³	< 10 ⁻⁶	10 ⁻¹	10 ⁻¹	(10 ⁻⁶)	10 ⁻⁴	I	10 ⁻³	S	+	—
R751	(10 ⁻⁷)	< 10 ⁻⁶	(10 ⁻⁶)	(10 ⁻⁶)	(10 ⁻⁷)	10 ⁻⁴	I	10 ⁻³	S	+	—

* Efficiencies of plating were determined by spot assays. Parentheses indicate that complete lysis of the bacterial lawn occurred at the e.o.p. shown but that single plaques were not formed at lower dilutions.

† Efficiencies of plating are relative to those on *P. aeruginosa* RP1⁺. The data shown are responses using P⁺ sub-lines of *E. coli* 1C3272. Efficiencies of plating using P⁺ lines of *P. aeruginosa* PAO2635 were all 1.0.

‡ Efficiencies of plating were determined using P⁺ lines of *P. aeruginosa* PAO8, and are relative to those obtained on PAO8.

§ Transfer frequencies to *P. aeruginosa* PAO9502 are relative to those obtained with PAO9501. Donors were derivatives of PAO2635. Matings were for 1 h using equal volumes of parental bacteria (about 5 × 10⁸ bacteria/ml) in NB. Selection was on NA supplemented with Cb (250 µg ml⁻¹) or Hg (18 µg ml⁻¹), or on MM supplemented with Tp (1 mg ml⁻¹). Rifampicin (150 µg ml⁻¹) was used for counterselection.

|| Sensitivity (S) or insensitivity (R) to AP41 was determined using P⁺ sub-lines of *P. aeruginosa* PAO9502 and aeruginocin of titre 8-16.

¶ Bacteria harbouring the P-group plasmids together with R38 or R38-5 were constructed in *P. aeruginosa* PAO2635. Fertility inhibition of the P plasmid (+) was detected by resistance of the strain to phages PRR1 and Pf3, and by decreased transferability of the P plasmid in matings with PAO2637.

efficiencies were obtained for all the phages on the R⁺ sub-lines used. In contrast, the plating efficiencies on *E. coli* hosts varied widely, and although lysis could generally be detected using high titres of phage, plaque formation was often absent. The possibility that a correlation existed between the frequency of plasmid transfer and the efficiency of phage plating was examined, but none was found. Thus, transfer frequencies using *P. aeruginosa* donors ranged from 1.0 × 10⁻² to 2.1 × 10⁻³/donor during 30 min mating (selecting Cb-r, Tp-r or Hg-r transcipts as appropriate) whereas with *E. coli* they were about 10 times lower (1.5 × 10⁻³ to 6.3 × 10⁻⁵). However, despite the wide differences in plating efficiencies, the host range of both PRR1 and Pf3 extended to P⁺ hosts other than those harbouring RP1, and could be detected in both *P. aeruginosa* and *E. coli*.

Plating efficiencies of female-specific phages. The presence of RP1 in *P. aeruginosa* PAO reduces the plating of the temperate phage G101 by about 10⁻⁴ (Krishnapillai, 1974) and a similar reduction was found with bacteria harbouring R26, R527, R751 and R906 (Table 5). In contrast, phages B33, B39, E79 and F116L which show interactions with other *Pseudomonas* R plasmids (Krishnapillai, 1974; Morgan & Stanisich, 1976) were not affected by the P-group plasmids (data not shown). The mutant phage G101-1 was used to distinguish the specificity of the inhibition mediated by the P-group plasmids from that which occurs with R91. This plasmid is compatible with RP1 and mutants of it which are derepressed for transfer function interfere with the plating of both G101 and G101-1 (e.o.p., 10⁻⁴; Chandler, 1975). However, G101-1 is not susceptible to inhibition by RP1, nor was this observed with any of the other P-group plasmids. Since the sensitivity of P⁺ bacteria to infection by the plasmid-specific phage PRR1 suggests that they are derepressed for transfer function, it was concluded that the specificity of the interaction between G101 and the plasmids R26, R527, R751 and R906 was similar to that mediated by RP1.

Interaction with prophage B3. The presence of prophage B3 in *P. aeruginosa* PAO reduces the bacteria's inheritance of the RP1-like plasmid R18-3 (Krishnapillai, 1975). A reduction of about 10^{-3} was found in the recovery of all the P-group plasmids when matings involved B3 lysogenic recipients rather than non-lysogens. This suggests that the interaction between B3 and plasmids of the P group is a general one.

Insensitivity to aeruginocin AP41. *P. aeruginosa* 41 produces an aeruginocin, AP41, which is lethal to *P. aeruginosa* PAO (Holloway *et al.*, 1974). However, sub-lines harbouring R18, a plasmid identical to RP1 (Stanisich *et al.*, 1976) can tolerate it (Chandler, 1975). Bacteria harbouring RP1, R26 and R527 were found to be insensitive to AP41 whereas those with R751 and R906 were as sensitive as the R⁻ strain (Table 5). Whether RP1, R26 and R527 conferred resistance or tolerance to the aeruginocin was not examined.

Fertility inhibition. The plasmid R38 is compatible with RP1 but inhibits its fertility when in the same cell (Stanisich, 1974*b*). R38⁺ RP1⁺ bacteria therefore show a reduction both in sensitivity to male-specific phages and in the conjugal transfer of RP1. R38-5 is a mutant of R38 which has no effect on the fertility of RP1 (Stanisich, unpublished data). Bacteria carrying R38 or R38-5 together with any of the plasmids R26, R527, R751 or R906 were found to have the same phenotypes as did those with RP1 (Table 5). R38 therefore seems to inhibit the fertility of each of these P-group plasmids by a similar mechanism.

With the exception of response to aeruginocin, the various P-group plasmids conferred similar or identical phenotypes for the range of characteristics studied, and it was concluded that these plasmids have several genes in common. The possible origin of these genes and their distribution on the plasmid molecule is discussed below.

Transduction and marker rescue

RP1 has a molecular weight of 38×10^6 daltons and is transducible by F116L which has a genome of 40×10^6 daltons (Stanisich & Richmond, 1975). Attempts to transduce R26 and R527 with F116L were unsuccessful indicating that these plasmids were larger than RP1. Since the preceding data suggested a similarity between RP1, R26 and R527, the transduction experiments were repeated using recipients carrying R18-18, a Cb-s derivative of R18. Cb-r and Hg-r transductants could then be recovered at frequencies that were 5 to 10 times lower than when selection was for the chromosomal allele *arg32*⁺, or a plasmid marker (Cb-r) from RP1 (Table 6, lines 1 and 2).

Table 7 shows the phenotypes of the transductants obtained in a similar experiment in which the recipient carried R18-91, a transfer-defective derivative of R18-18. Linkage of the resistance determinants Cb Sm Su Gm Hg is seen in their coinheritance by 80 to 90% of the Cb-r and Hg-r transductants. Furthermore, the determinants Sm Su Gm Hg are more closely linked to each other than to Cb since these may be inherited independently of Cb but not of each other. These five determinants are also coinherited with transfer function suggesting that both R26 and R527 are composite plasmids in which the sex factor is continuous with the resistance genes.

To determine the mechanism by which markers from R26 and R527 survived in R18-18 bacteria, transduction experiments were carried out using the recombination-deficient strain PAO2002 (Chandler & Krishnapillai, 1974*a*). Transduction of RP1 occurred to both PAO2002 and its R18-18 derivative, whereas transduction of R26 or R527 could not be detected to either strain (Table 6, lines 3 and 4). Thus while marker rescue in R18-18⁺ Rec⁺ hosts indicates that F116L can transduce portions of R26 and R527, the survival of such fragments in the new host is clearly dependent on the presence both of R18-18 and of a functional host recombination system. Despite the low frequency of transduction, it

Table 6. *Transduction of plasmids RP1 and R26 to Rec⁺ and Rec⁻ lines of P. aeruginosa PAO*

The recipients were PAO2001, its Rec⁻ derivative PAO2002, and their R18-18 sub-lines. Transducing preparations of phage F116L were from P⁺ sub-lines of PAO9501. Phage-bacterial mixtures (multiplicity of infection, m.o.i. 10 to 20) were maintained at 37 °C for 45 min: the cells were then concentrated about 10-fold and 0.1 ml samples plated on selective medium. Selection for Arg⁺ transductants was on MM; selection for Cb-r and Hg-r transductants was on NA containing Cb (250 µg ml⁻¹) and Hg (18 µg ml⁻¹).

Recipient	Transductants/10 ¹⁰ plaque-forming units					
	F116L.RP1			F116.R26*		
	Arg ⁺	Cb-r	Hg-r	Arg ⁺	Cb-r	Hg-r
Rec ⁺	101	54	0	130	0	0
Rec ⁺ . R18-18	103	43	0	131	11	27
Rec ⁻	0	20	0	0	0	0
Rec ⁻ . R18-18	0	22	0	0	0	0

* Similar results were obtained when phage was grown on R527 donors.

Table 7. *Analysis of R26 transductants from a recipient harbouring the plasmid R18-91*

PAO9501.R18-91 was the recipient for phage F116L propagated on PAO8.R26. The transductional procedure was as described in Table 6 except that the m.o.i. was 5.

Selected* marker	Unselected marker†				Percentage of transductants
	Cb	Hg	Sm, Su, Gm	Tra	
Cb-r		R	R	+	19
		R	R	—	60
		S	S	—	21
Hg-r	R		R	+	38
	R		R	—	47
	S		R	+	8
	S		R	—	7

* Numbers of transductants analysed were: Cb-r, 80; Hg-r, 120.

† R, Resistance; S, sensitivity to the antibiotics and mercuric ions. Transfer proficiency (+) or deficiency (—) was determined from the response to male-specific phage Pf3.

seems fair to conclude that marker rescue involves recombination of the transduced fragment with the resident plasmid, and that sufficient homology exists between these plasmids to allow these events to occur.

DISCUSSION

One of the most striking features of P-group plasmids is their ready transfer across generic boundaries, so the derivation of the plasmids used in this study from species of *Pseudomonas*, *Bordetella*, *Serratia* and *Klebsiella* is not in itself particularly surprising (see Table 2). By a comparative study of these plasmids based primarily on phenotypic criteria, we have shown that despite their differences in resistance phenotype they share several properties in common and therefore may be derived from a single element which is becoming more widespread or is already endemic in different areas. Their gross similarity at the molecular level probably accounts for the recombination that occurs between them when determinants from R26 and R527 are rescued by R18. Recombination is implicated in this process because

transductants are recovered only from Rec⁺ recipients (Table 6), but direct evidence has been obtained in a separate study (Stanisich *et al.*, 1976) in which the sizes of the plasmids carried by these transductants has been determined.

Most of the characteristics common to the P-group plasmids are those which might be expected to be controlled by genes on the transfer factor: for example, interaction with sex-specific phages and inhibition by R38. However, only those plasmids with the resistance pattern Cb Nm/Km Tc were insensitive to aeruginocin AP41, and it may be that this phenotype is controlled by a gene linked to the R-determinants. This possibility is supported by the phenotypes exhibited by recombinants between RP1 and the non-transmissible plasmid pVS1. One such recombinant class retains all the characteristics of pVS1 but has acquired, presumably in a single event, resistance to both carbenicillin and AP41 (Stanisich, 1974*a*; Stanisich, unpublished data). This implies close linkage between these two resistance determinants.

In addition to P-group plasmids, other drug-resistance plasmids occur in *P. aeruginosa* (Stanisich, 1976). These can confer resistance to Sm in combination with Su, Gm and Hg (Bryan *et al.*, 1973), and include plasmids like R38 which can inhibit the fertility of RP1 and recombine with it (Jacoby, 1974; Stanisich, unpublished data). The phenotypic similarity between RP1, R26 and R527 therefore introduces the possibility that the last two may represent the emergence, in the clinical situation, of recombinants between an RP1-like element and another which contributes the additional resistance determinants. The finding that the Sm Su Gm Hg determinants of R26 are encoded in a DNA fragment of molecular weight 14×10^6 daltons supports this view, since R26 and R527 (52×10^6 daltons) and RP1 (38×10^6 daltons) differ by a molecule of this size (Stanisich *et al.*, 1976). Thus, although most *Pseudomonas* plasmids are not readily transferable to *Escherichia*, the ability of the P-group plasmids to achieve this endows them with the potential to acquire new combinations of genetic information and to transmit it to these and other organisms. The biological and clinical implications of this have not yet been assessed.

The generous provision of bacterial strains and phages is gratefully acknowledged. We thank Drs P. M. Chandler and V. Krishnapillai for discussion of their results prior to publication. J.M.O. is grateful to Professor M. H. Richmond for the hospitality of his laboratory. Financial support for this work was from a Medical Research Council grant to M. H. Richmond.

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