R factors from Providence

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SUMMARY

Of more than 100 R factors transferred from naturally occurring Providence strains, approximately 60% belonged to the A-C compatibility complex. Plasmids of groups F₁, J, N, P and X, and the prototype of a new group G were also transferred. The Providence R factor set differs from those of *Proteus rettgeri* and *P. morganii* most strikingly in the abundance of plasmids of the A-C group and the infrequency of N group plasmids.

INTRODUCTION

The bacteria currently assigned to the genus Providence were previously described as members of *Proteus inconstans*. Although removed from the genus *Proteus* they are generally considered to be related to this group, especially to *Proteus rettgeri* (Coetzee, 1972).

Although Providence bacteria are usually considered normal intestinal commensals they have been implicated as being the causative agents of diarrhoea (Brown, 1952; Ewing, Tanner & Dennard, 1954; Bhat, Myers & Feldman, 1971). They are a cause of urinary-tract infections (Janis, Evans & Hoeprich, 1968; Li & Miller, 1970; Dobrey, 1971) and other parenteral infections, including septicaemia (e.g. Schmitt & Neussel, 1970; Janis *et al.* 1968).

Hospital isolates of Providence are among the Gram-negative bacilli resistant to the widest range of antibiotics (Tomaschoff, 1969) and it has been suggested that this resistance may favour the evolution of Providence as a major pathogenic group (Li & Miller, 1970). Since plasmids are transferable from *Escherichia coli* and other enterobacteria into Providence (Coetzee, 1964) it is to be expected that R factors should be present in naturally occurring strains, but there have been rather few demonstrations of R factors in Providence (Chabbert, Baudens & Bouanchaud, 1967; Dobrey, 1971; Lowbury, Babb & Roe, 1972; Villemon-Lemosquet, Morel & Freymuth, 1973).

In this paper we describe 103 R factors from strains of Providence obtained from a wide geographic range.

METHODS

Providence strains are listed in Table 1.

Escherichia coli K12 strains used were J53 (F⁻, *pro*, *met*), J62 (F⁻, *pro*, *his*, *trp*, *lac*), J53.2, J62.2 (rifampicin resistance mutants of J53 and J62) and Hfrc (*met*).

Standard plasmids representing the various compatibility groups are listed in Table 2.

Bacteriophages were MS2 (Davis, Strauss & Sinsheimer, 1961) and λ vir (Jacob & Wollman 1954).

Techniques were as described by Coetzee, Datta & Hedges (1972) and Hedges, Datta, Coetzee & Dennison (1973).

Table 1. *Sources of Providence strains, arranged geographically in order of longitude*

Source	Strains	Plasmids
Dr P. Bhat, Vellore, India	18	0
Dr C. H. Teoh-Chan, Hong-Kong	2	0
Dr S. Mitsuhashi, Maebashi, Japan	7	0
Dr G. Buckle, Prahran, Australia	1	0
Dr H. C. W. Shott, Dunedin, New Zealand	2	0
Mrs R. Dobrey, Vancouver, Canada	53	9
Dr W. H. Cockroft, Vancouver, Canada	9	6
Mrs C. A. Johnston, Victoria, B.C., Canada	5	0
Dr S. Falkow, Seattle, U.S.A.	1	0
Dr S. Finegold, Los Angeles, U.S.A.	15	0
Dr J. E. Rosenblatt, Los Angeles, U.S.A.	16	0
Dr Y. Goodman, Edmonton, Canada	5	0
Dr J. N. Wilfert, Salt Lake City, U.S.A.	15	4
Dr F. A. Holden, Saskatoon, Canada	1	1
Dr T. McMahon, Winnipeg, Canada	14	6
Dr D. J. Blazevic, Minneapolis, U.S.A.	2	0
Dr J. Davies, Madison, U.S.A.	1	0
Dr S. Cohen, Chicago, U.S.A.	4	1
Dr W. H. Ewing, Atlanta, U.S.A.	28	2
Dr L. A. Hatch, London, Ontario, Canada	9	2
Dr J. Parker, London, Ontario, Canada	15	3
Dr W. D. Leers, Toronto, Canada	5	1
Dr M. B. Graves, Ottawa, Canada	8	0
Dr H. Abramovitch, Montreal, Canada	12	1
Mr B. Martineau, Montreal, Canada	20	0
Dr H. Pharand, Montreal, Canada	7	4
Dr H. Starkey, Montreal, Canada	27	20
Dr F. Sabatelli, Bloomfield, U.S.A.	20	0
Dr H. Isenberg, Long Island, U.S.A.	17	0
Dr A. A. Medeiros, Boston, U.S.A.	4	1
Dr Sabbath, Boston, U.S.A.	3	1
Dr E. Moorhouse, Dublin, Eire	12	3
Dr K. J. Harrison, Bristol	2	0
Dr E. J. L. Lowbury, Birmingham	6	8
Dr R. Howden, Sheffield	4	0
Dr L. Greenbury, Aylesbury	20	14
Dr F. Anderson, London	3	2
Dr I. Phillips, London	7	0
National Collection of Type Cultures, London	1	1
Dr B. Brisou, Toulon, France	9	2
Dr E. Tomaschoff, Dusseldorf, Germany	20	9
Dr H. Brede, Tiervlei, Republic of South Africa	49	0
Dr J. N. Coetzee, Pretoria, Republic of South Africa	61	2
Total	540	103

RESULTS AND DISCUSSION

The Providence isolates

The sources of the strains tested are listed in Table 1. Each isolate was tested against discs impregnated with ampicillin, carbenicillin, tetracycline, streptomycin, chloramphenicol, sulphonamide, kanamycin, gentamicin and trimethoprim. Isolates which showed resistance to any of these drugs were tested for ability to transfer that resistance to a strain of *Escherichia coli* K12; usually J53.2, although J62.2 was used for the transfer of R470.

For reasons of space, no detailed account is given of the resistance patterns, but it was evident that the highly resistant strains mentioned in the introduction were common only

Table 2. *Standard plasmids*

No.	Compatibility group	Genetic markers*	Reference
<i>Flac</i>	F _I	<i>fi</i> ⁻	Jacob & Adelberg (1959)
R386	F _I	<i>fi</i> ⁺	Dennison (1972)
R1	F _{II}	ASCSuK	Lawn, Meynell, Meynell & Datta (1967); Hedges, Datta, Coetzee & Dennison (1973)
R1 <i>drd</i> 16	F _{II}	K	
R1-1	F _{II}	ASCSu	
ColB K98	F _{III}	<i>fi</i> ⁺	Hedges & Datta (1972)
R124	F _{IV}	T	
RA1	A-C†	TSu	Datta & Hedges (1972 <i>b</i>); Datta & Hedges (1973 <i>a</i>)
R57b	A-C	ACSuGk	
R57b-1	A-C	CSu	
<i>Plac</i>	A-C‡	Su <i>lac</i>	Coetzee (1973); Hedges & Datta (unpublished)
<i>PlacR1drd</i> 19-2	A-C	ASCSu	
<i>PlacR447b</i>	A-C	KSu	
R64	I _z	ST	Hedges & Datta (1973)
R144	I _α	TK	Hedges & Datta (1973)
R483	I _β	STp	Hedges & Datta (1973)
R621a	I _γ	T	Hedges & Datta (1973)
R721	I _δ	Tp	Datta (unpublished)
RM413	I _ε	A	Hedges, Datta, Kontomichalou & Smith (1974)
JR66a	I _ω	SK	Hedges & Datta (1973)
R27	H	T	Grindley, Grindley & Anderson (1972)
R726	H	STCSu	Datta (unpublished)
R726-1	H	SCSu	
R391	J	K	Coetzee, Datta & Hedges (1972)
R387	K	SC	Hedges & Datta (1971)
R446b	M	ST	Hedges, Datta, Coetzee & Dennison (1973)
R69(IP)	M	ATK	Dhabbert <i>et al.</i> (1972)
R15	N	SSu	Catta & Hedges (1971)
N3	N	STSu	
N3T	N	T	Hedges (1972)
R ¹⁶	O	ASTSu	Hedges, Datta, Kontomichalou & Smith (1973)
R ¹⁶ -1	O	SSu	Hedges & Datta (unpublished)
RP4	P	ATK	Datta <i>et al.</i> (1971)
RP4-4	P	AT	Coetzee, Datta & Hedges (1972)
R751	P	Tp	Datta (unpublished)
Rts1	T	K	Coetzee, Datta & Hedges (1972)
R401	T	AS	
R388	W	SuTp	Datta & Hedges (1972 <i>b</i>)
R6K	X	AS	
R485	X	Su	

* Abbreviations: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Gk, gentamicin and kanamycin; Su, sulphonamide; Tp, trimethoprim.

† The two compatibility groups A and C are closely related (see text). Because the presence of a plasmid of either group in the recipient markedly reduces the efficiency of transfer of any other plasmid of either group, a misleading suggestion of incompatibility may be produced even though the two plasmids under investigation are compatible (Datta & Hedges, 1972*a*). We believe that not all the plasmids can be assigned uniquely into the two compatibility groups previously defined but that these R factors constitute a complex analogous to that constituted by the plasmids determining I pili (Hedges & Datta, 1973). We have therefore assigned all these R factors to 'the A-C complex'.

‡ It appears (Datta & Hedges, unpublished) that the behaviour of *Plac* is more satisfactorily accounted for by including it in the A-C complex than in the P group, as formerly.

in hospital isolates, especially from urinary infections. It was almost exclusively from such isolates that the R factors listed in Table 2 were derived.

Strains from hospital parenteral infections in general differed markedly from those from other sources. The former were resistant to a wide range of drugs whereas the latter were

normally sensitive to all except tetracycline and polymixin. The latter strains were exemplified by strains from the collections of Professor Prema Bhat and Professor H. D. Brede. Professor Bhat's strains were from human faeces and Professor Brede's were from various sources including faeces.

The proportion of strains demonstrated to carry R factors is not necessarily a reflexion of the character of the genus. On the one hand, there were a substantial number of strains whose resistance spectra suggested the presence of plasmids but from which transfer was never demonstrated, while on the other hand it is likely that I received larger numbers of Providence isolates from hospitals where multiple resistant Providence strains were a frequent cause of parenteral infection, whilst hospitals with sensitive strains only would not be so concerned about this group and not have made collections. The generous response of Canadian bacteriologists and personal communications from researchers at several hospitals there, many of whose strains were R⁺, may suggest a notable abundance of Providence in that part of the world.

Intrinsic resistance

Almost all strains of Providence were resistant to polymixin, as is typical of the *Proteus* group.

A proportion of strains were resistant to ampicillin but sensitive to carbenicillin. From ampicillin-sensitive strains, mutants resistant to ampicillin were obtained. Such mutants were never resistant to carbenicillin, nor could carbenicillin-resistant mutants be isolated from them. Dr J. T. Smith (School of Pharmacy, University of London) examined mutants of this type and found them to contain no more β -lactamase activity than the sensitive strains.

When tested for chloramphenicol resistance by the agar disc (30 μ g chloramphenicol) diffusion technique, Providence strains exhibited three phenotypes. Strains with a zone of inhibition of 10 mm were judged to be fully sensitive whereas isolates giving no zones whatsoever were clearly resistant. In addition, however, several strains tested under identical conditions yielded zones of intermediate diameter (7 to 8 mm), suggesting a level of resistance greater than the fully sensitive strains but substantially less than the fully resistant isolates. Whereas the strains showing the intermediate resistance phenotype gave rise to fully resistant mutants, no resistant derivatives were isolated from the fully sensitive strains. A possible explanation for this observation was suggested by tests for the presence of the enzyme chloramphenicol acetyltransferase (CAT) which is responsible for plasmid-mediated chloramphenicol resistance and presumably chromosomal chloramphenicol resistance in *Proteus* species (Shaw, 1971). Two of the fully sensitive Providence strains, one each from South Africa and India, contained no detectable CAT activity, whereas the strains demonstrating intermediate zones of inhibition (two from Canada, one from South Africa) or complete resistance contained easily measurable CAT (L. Sands and W. V. Shaw, University of Miami, unpublished experiments). Taken together, the sensitivity studies and the enzyme assay data suggest two broad subgroups of Providence strains: those which contain CAT and those which do not. The degree of chloramphenicol resistance of the former could therefore be a function of the quantity or catalytic properties of the enzyme responsible for resistance.

Most of the Providence isolates were resistant to tetracycline but of several sensitive strains tested none gave rise to resistant mutants. Thus the relationship between the resistant and sensitive types is obscure.

The strains showed a range of susceptibilities to the various aminoglycosides. By analogy

with the findings from various enterobacteria some of this resistance was probably chromosomal whilst some may have been due to non-self-transmissible plasmids.

Plasmids assigned to the A-C complex. Evidence has already been presented that compatibility groups A and C are closely related. Recombination between RA1 (the prototype plasmid of group A) and R57b (a member of group C) has been reported, and the presence in a culture of an R factor of either group reduces the efficiency with which that culture can act as a conjugal recipient for plasmids of either group (Datta & Hedges 1972*a*; Datta & Hedges 1973*a*).

This exclusion is so effective that a large proportion of matings between two strains each carrying an R factor of group A or C, probably involves transfer of a plasmid into a recipient which has previously suffered spontaneous loss of its original R factor. Thus, transconjugants are produced in which the resident plasmid has been lost even though the two plasmids may be compatible. In the course of this work we have also discovered that several of our R factors are apparently completely incompatible with plasmids of both group A and group C.

This may be analogous to the behaviour of JR66a which is incompatible with plasmids of group I α and I β (Datta & Hedges, 1973*b*) in which case they should be designated a third compatibility type within the A-C complex. We hope to investigate this question, using transduction, a type of genetic transfer not susceptible to exclusion (Watanabe, 1969) and DNA homology studies. In this paper we shall assign all R factors which exclude R57b, R57b-1, R707 or R715b and which are eliminated in matings leading to the transfer of these plasmids, to the A-C complex.

R480, which confers resistance to sulphonamides and the ability to use lactose, was transferred from the Providence strain to *Escherichia coli* J62.2. It was incompatible with Plac-R1drd19-2 and hence belongs to the A-C complex.

Plasmids of this complex constitute about 60% of those studied. The R factor transferred from Providence by Chabbert *et al.* (1967) has been shown to belong to the same compatibility group as R40a, designated *com6* by Chabbert *et al.* (1972), i.e. it is also a member of this complex, whilst the R factors from Providence strains identified by Villemon-Lemosquet *et al.* (1973) determine resistance to ampicillin and sulphonamides, a pattern shown by several of the plasmids of the A-C complex described in this paper, e.g. R667.

Plasmids of group P. The providence R factors described by Lowbury *et al.* (1972), and a similar R factor, R690, obtained from a Providence isolate from Stoke Mandeville Hospital, Aylesbury, Buckinghamshire, were incompatible with R751 and are thus members of group P. They closely resemble RP4 (Datta *et al.* 1971).

Plasmids of group N. Five plasmids of group N were identified: R497, R728, R731, R732 and R825. Only one of these, R497, determined *hspII* restriction (Hedges, 1972).

Plasmids of group FI. R714a and R715a are unstable in HfrC and incompatible with *Flac*. Introduction of *Flac* into a strain of J62 carrying either R factor rapidly eliminates the resident R factor whereas introduction of R714a or R715a into an *Flac*⁺ cell led to elimination of the F' only after many cell generations. This non-reciprocity seems characteristic of the FI group (MacFarren & Clowes, 1967).

R811. This plasmid, which confers resistance to ampicillin, is compatible with plasmids of all the defined groups (Table 3). The only group which could not be tested directly was I ϵ since the only known member, RM413, also confers ampicillin resistance. RM413, however, both excludes and is excluded by R721 and transfer of either plasmid to a strain carrying the other usually leads to transconjugants from which the resident plasmid has been eliminated.

Table 3. *R* factors from Providence

No.	Genetic markers	Donor	Compatibility group	Evidence for assignment
R441, R442, R681 R480	AK <i>fi</i> ⁻ Su <i>lac fi</i> ⁻	Dr E. Moorhouse —	Undesignated A-C	Incompatibility with <i>Plac R1drd19-2</i>
R497	ASTK <i>fi</i> ⁻ <i>hspII</i>	Dr L. D. Sabath	N	Incompatibility with R15
R666, R671	Su <i>fi</i> ⁻	Dr E. Tomaschoff	A-C	Incompatibility with R714b
R667, R668, R669, R670, R672, R673, R674	ASu <i>fi</i> ⁻	Dr E. Tomaschoff	A-C	Incompatibility with R57b-1
R686, R688, R689, R691, R694	TSu <i>fi</i> ⁻	Dr C. L. Greenbury	A-C	Incompatibility with R57b-1
R692	ATSu <i>fi</i> ⁻	Dr C. L. Greenbury	A-C	Incompatibility with R57b-1
R698b	ASu <i>fi</i> ⁻	Dr C. L. Greenbury	A-C	Incompatibility with R57b-1
R687, R693, R695, R696, R697, R698a R690	AK <i>fi</i> ⁻ ATK <i>fi</i> ⁻	Dr C. L. Greenbury Dr C. L. Greenbury	Undesignated P	— Incompatibility with R571
R699a	ATKSu <i>fi</i> ⁻	Dr F. Anderson	A-C	Incompatibility with R57b-1
R699b R700, R703	AK <i>fi</i> ⁺ Su <i>fi</i> ⁻	Dr F. Anderson Dr Ewing	Undesignated A-C	— Incompatibility with R714b
R707	ASSpTCKSu <i>fi</i> ⁻	Dr S. Cohen	A-C	Incompatibility with R57b
R708, R709, R710, R711a, R712a, R713 R711b, R712b	ATK <i>fi</i> ⁻ K <i>fi</i> ⁻	Dr E. J. L. Lowbury Dr E. J. L. Lowbury	P X	Incompatibility with R751 Incompatibility with R778b
R714a, R715a	ASSpTCSu <i>fi</i> ⁻	Dr J. N. Wilfert	F _I	Incompatibility with <i>Flac</i>
R714b, R715b	SK <i>fi</i> ⁻	Dr J. N. Wilfert	A-C	Incompatibility with R57b-1
R716, R722, R747	Su <i>fi</i> ⁻	Mrs J. Parker	A-C	Incompatibility with R714b
R717, R718, R719, R752	AKSu <i>fi</i> ⁻	Dr H. Pharand	A-C	Incompatibility with R57b
R728, R731, R732	A <i>fi</i> ⁻	Dr H. Starkey	N	Incompatibility with R15
R722, R729, R730, R739	Su <i>fi</i> ⁻	Dr H. Starkey	A-C	Incompatibility with R714b
R733, R734, R735, R736, R737, R738, R740, R741, R744, R745	AKSu <i>fi</i> ⁻	Dr H. Starkey	A-C	Incompatibility with R57b-1
R742, R743, R746	ATKSu <i>fi</i> ⁻	Dr H. Starkey	A-C	Incompatibility with R57b-1
R748, R749	K <i>fi</i> ⁻	Professor J. N. Coetzee	?J	Compatibility with all testable groups
R750 R775, R776, R777, R778a	SSu <i>fi</i> ⁻ AK <i>fi</i> ⁻	Dr A. A. Medeiros Dr T. McMahon	Undesignated Undesignated	— —
R778b, R779	Su <i>fi</i> ⁻	Dr T. McMahon	X	Incompatibility with R6K
R780	Su <i>fi</i> ⁻	Dr F. A. Holden	A-C	Incompatibility with R714b
R789, R792, R794, R798	KSu <i>fi</i> ⁻	Mrs R. Dobrey	A-C	Incompatibility with R714b
R790	Su <i>fi</i> ⁻	Mrs R. Dobrey	A-C	Incompatibility with R714b

Table 3 (cont.)

No.	Genetic markers	Donor	Compatibility group	Evidence for assignment
R791, R795	TSu <i>fi</i> ⁻	Mrs R. Dobrey	A-C	Incompatibility with R714b
R793, R796	ASu <i>fi</i> ⁻	Mrs R. Dobrey	A-C	Incompatibility with R714b
R799	ACK <i>fi</i> ⁻	Dr W. D. Leers	Undesignated	—
R807, R808, R809, R810, R812	ATSu <i>fi</i> ⁻	Dr W. H. Cockroft	A-C	Incompatibility with R57b-1
R811	A <i>fi</i> ⁻	Dr W. H. Cockroft	G	Compatibility with all other groups
R816, R817	ACSuGK	Dr B. Brisou	A-C	Incompatibility with R707
R824	KSu <i>fi</i> ⁻	Dr L. A. Hatch	A-C	Incompatibility with R57b-1
R825	A <i>fi</i> ⁻	Dr L. A. Hatch	N	Incompatibility with R15
R840	AK <i>fi</i> ⁻	Dr H. Abramovich	Undesignated	—

R811 showed no such interaction with R721 and we therefore designate this the prototype of a new compatibility group, G.

Plasmids of group X. R778b and R779 are *fi*⁺ R factors determining sulphonamide resistance. They are incompatible with R6K. R711b and R712b are *fi*⁺ R factors conferring kanamycin resistance. They are incompatible with R778b.

Plasmids assigned to group J. R748 and R749 were shown to be compatible with plasmids of all the compatibility groups listed in Table 3, except group J (because of identity of the resistance markers they could not be tested against this group). They were also compatible with R811 (group G).

Since the Providence strains that carried R748 came from the same hospital as the *Proteus rettgeri* strains that were the source of the plasmids which constitute group J, it seems plausible that R748 and R749 also belong to this group.

R750. This plasmid confers resistance to streptomycin and sulphonamides. It is non-self-transmissible and may resemble the streptomycin and sulphonamide resistance factor described by Milliken & Clowes (1973); it may have been mobilized for transfer to *Escherichia coli* by some other plasmid of the Providence strain. Its molecular structure and replication pattern are under investigation (Barth & Grinter, 1973).

R799. This plasmid was transferred from J53.2 to J62 but from this strain was non-self-transmissible. Presumably the J53.2 strain carried two plasmids, one mobilizing the resistance factor. The postulated mobilizing factor has not been investigated.

The ampicillin-, chloramphenicol- and kanamycin-resistance-bearing J62 strain was mated with J53 strains carrying R factors of all compatibility groups listed in Table 2, except J and I_c. In all cases the R factor of the J53 R⁺ strain was transferred to J62(R799) and coexisted with the resident plasmid.

The mating mixtures were also incubated overnight at 37 °C and tested for transfer of R799. In most cases there was no transfer, but in those mixtures where the J53 strain carried an R factor determining I pilus synthesis (R64, R483, R621a, R721 and JR66a) transfer of R799 (presumably mobilized by I pilus-forming plasmids which had entered the R799⁺ strain) was observed.

Strains carrying R64, R483, R721 or JR66a retained their plasmid as well as the newly acquired R799 but J53(R621a) behaved differently. Of ten R799⁺ transconjugants, nine had

Table 4. *Comparison of the transfer of R621a and R799 from a double with transfer of the recombinant plasmid*

Donor	Recipient	Selective medium	Efficiency of transfer	Character of transconjugants
J62(R621a)(R799)	J53	pro, met, T pro, met, K	1×10^{-2} $< 10^{-6}$	30/30 T ^R K ^S 9T ^R K ^S : 1T ^R K ^R
J53(R621a-R799)	J62	pro, his, trp, T pro, his, trp, K	8×10^{-3} 1×10^{-2}	30/30 T ^R K ^R 30/30 T ^R K ^R

lost tetracycline resistance (the marker of R621a). The one strain which retained tetracycline resistance carried a recombinant plasmid having the tetracycline resistance and transmissibility of R621a and the ampicillin, chloramphenicol and kanamycin resistance determinants of R799.

The reciprocal conjugation J53(R621a) → J62(R799) produced transconjugants all of which showed all the resistances of both plasmids. In subsequent matings these strains behaved as doubles, transferring the two plasmids separately (Table 4).

J62(R621a)(R799) transferred R621a efficiently but R799 was only very rarely mobilized: no such transfer was detected in the 1 h matings (Table 4). After the mating mixtures had been incubated overnight a few R799⁺ transconjugants were detected. Of ten tested, nine were sensitive to tetracycline (the mobilizing R621a not having been transferred, or more probably, having been eliminated by the R799). The one tetracycline resistant transconjugant was found to be a stable double, transferring the two plasmids separately.

The very low efficiency of production of recombinants in this experiment, no recombinant having been produced and transferred from perhaps 10^9 donor cells, stands in marked contrast to the rather efficient production of recombinants after the transfer of R799 into R621a⁺ recipient cells (one recombinant in ten tested transconjugants). This contrast strongly suggests that newly-introduced R799 produces enzymes but synthesis is normally repressed once the plasmid is established. One of these activities could be a recombinase, perhaps associated with the circularization of the R factor, which is transferred as a linear molecule.

The mobilization of R799 by plasmids which determine I pili, and the elimination of recombination with R621a, both indicate a relationship between R799 and the I pilus-determining plasmids, especially I (the group of which R621a is the prototype). On the other hand R799 is compatible with R621a, producing stable doubles when R621a is transferred into an R799⁺ culture. Thus the precise relationship between the I complex and R799 is uncertain.

Since I_α plasmids are not transmissible to *Proteus* or *Providencia* (Coetzee, 1964; Datta & Hedges, 1972) the presence of an R factor apparently related to this group is surprising.

We presume that either R799 is unlike the tested I_α plasmids in respect of its interaction with *Providencia* strains or that the deficiencies are made good by the mobilizing plasmid whose presence we have been forced to postulate.

Plasmids conferring resistance to ampicillin and kanamycin

There were 15 such plasmids in the R factor set. Such R factors came from five different hospitals and since the plasmids from each hospital seemed homogeneous we took a single plasmid as the exemplar of each group. Those chosen were R441, R696, R699b, R776 and R840. These were tested for compatibility with plasmids of all designated groups where

suitable markers are available. A segregant of R696 lacking kanamycin resistance was isolated and this was shown to be compatible with R491. The other R factors were not testable for membership of group J.

None of the R factors was testable with members of groups G or I_e nor could the ampicillin-kanamycin R factors (amp-kan R factors) from different sources be tested against one another.

There were numerous differences between the different amp-kan R factors; for example, R696 and R699b were *fi*⁺ whereas the other three were *fi*⁻, R699b determined a much lower level of kanamycin resistance than did the others, and R696 produced spontaneous segregants lacking kanamycin resistance whereas similar segregants could not be obtained from the other R factors. The rates of transfer of different amp-kan R factors were also different, ranging from 5×10^{-3} for R441 to about 100-fold lower for R696. Thus, the Providence amp-kan R factors may well represent several undescribed compatibility groups though the point was not testable.

*Comparison of the R factor sets of Providence with those of
Proteus rettgeri and Proteus morganii*

The abundance of R factors of the A-C complex is the most remarkable feature of the Providence R factor set. Whereas in *Proteus morganii* and *P. rettgeri* plasmids of group N were the most abundant type, they were quite rare in the Providence set.

F-like R factors were not found in *Proteus rettgeri* but plasmids of groups FI and FII were rather frequent in *P. morganii* strains. In Providence strains no FII plasmids were found and only two of group FI. These two conferred resistance to ampicillin, streptomycin, tetracycline, chloramphenicol and sulphonamides. This is the same resistance spectrum as that shown by the FI R factors of *P. morganii* but there are quantitative differences, most notably the level of tetracycline resistance conferred by the Providence R factors being markedly lower than that of the *P. morganii*. The rarity of R factors conferring ampicillin, streptomycin, tetracycline, chloramphenicol and sulphonamide resistance among Providence R factors (2 among 103) contrasts with the frequency of such R factors in *P. rettgeri* (5 of 12) and *P. morganii* (14 of 28).

The frequency of amp-kan R factors in the Providence R factor set is notable; 15 R factors confer these two resistances whilst another 22 confer resistance to these two drugs and to one other. The R factor sets from *Proteus rettgeri* and *P. morganii* contain one amp-kan R factor each and neither contains a plasmid conferring resistance to three drugs including ampicillin and kanamycin.

Thus, both the compatibility types and the resistance patterns of the Providence R factor set are clearly differentiated from those of the other non-swarming members of the Proteaceae.

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Note added in proof

An isolate of Providence from Hammersmith Hospital, London, was found to carry two R factors. R414a, which confers resistance to tetracycline and sulphonamides, is a member of the A-C complex. R414b, which confers resistance to ampicillin and streptomycin, is a member of group T. Neither plasmid was lost very frequently during growth at 43 °C.

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