R Factors from *Proteus rettgeri*

By J. N. COETZEE

Department of Microbiology, University of Pretoria, Republic of South Africa

NAOMI DATTA * AND R. W. HEDGES

Department of Bacteriology, Royal Postgraduate Medical School,
Hammersmith Hospital, London, W12 0HS

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SUMMARY

Of 12 R factors transferred from wild strains of *Proteus rettgeri* to *Escherichia coli* K12, all were *fi*⁻. Five were of the N-compatibility group; three belonged to a newly defined group T, of which Rts1 is the prototype; one belonged to group W. The other three constituted at least one compatibility group, not previously described. The replication of two of the T plasmids, like that of Rts1, was temperature sensitive. This set of R factors is significantly different from those derived from bacteria of other genera.

INTRODUCTION

Plasmids transferable to *Escherichia coli* have been identified in several naturally occurring *Proteus* strains (Falkow, Wohlhieter, Citarella & Baron, 1964; Datta & Kontomichalou, 1966; Smith & Armour, 1966; Mitsuhashi, Hashimoto, Egawa, Tanaka & Nagai, 1967; Terawaki, Takayasu & Akiba, 1967; Naide, Kawamura, Makino, Tamura & Watanabe, 1967; Dobozy & Hammer, 1968; Pitton, 1970; Poty, Goze & Gagnon, 1971; Odakura, Tanaka & Mitsuhashi, 1971). R factors of several different compatibility groups can be transferred from *E. coli* to members of the *Proteus* tribe. However, transfer of I-like plasmids did not occur at a detectable frequency and the rate of transfer of several other types of plasmid was lower than that in crosses between strains of *E. coli* (Datta & Hedges, 1972*a*).

Strains of *Proteus rettgeri* are often resistant to a broader spectrum of antibiotics than other members of the group (Odakura *et al.* 1971). We decided to look for R factors in this species, to determine their compatibility properties and the extent to which the *Proteus* R factor set resembles sets of other genera.

METHODS

Bacteria. One hundred and forty-four strains of *Proteus rettgeri* were isolated during 1967 from human faeces in Pretoria. They all had the phenotypic characteristics of *P. rettgeri*. Many strains were resistant to 100 µg/ml tetracycline, ampicillin, streptomycin and chloramphenicol. Resistance to sulphonamides varied and only 16 strains were resistant to 40 µg/ml of kanamycin. In addition, one R factor, R7K, derived from a strain of *P. rettgeri* isolated in Athens, was received from Dr P. Kontamichalou (Kontomichalou, 1971).

Escherichia coli K12 strains: J53 F⁻ *lac*⁺, *pro*, *met* (Clowes & Hayes, 1968); J53-1, a nalidixic acid-resistant mutant of J53; J53-2, a rifampicin-resistant mutant of J53; J62 F⁻ *lac*, *pro*,

* To whom reprint requests should be addressed.

Table 1. 'Standard' R factors used in tests for compatibility

Factor	Compati- bility group	fi	Resistance and restriction determinants*	Reference
R1-1†	F II	+	ASCSu	Hedges & Datta (1972)
R1drd16‡	F II	—	K	Frydman & Meynell (1969)
R386	F I	+	T	Dennison (1972)
R124	F IV	+	T	Hedges & Datta (1972)
R64	I	—	ST	Lawn, Meynell, Meynell & Datta (1967)
R144	I	—	TK	Lawn <i>et al.</i> (1967)
R46	N	—	ASTSu	Datta & Hedges (1971)
R15	N	—	SSuM <i>hsp</i> II	Datta & Hedges (1971)
R269 N-1	N	—	ASTK <i>hsp</i> II	Hedges (1972)
RP4	P	—	ATK	Datta <i>et al.</i> (1971)
RP4-4§	P	—	AT	.
S-a	W	—	SCKSu	Hedges & Datta (1971)
RA3	W	—	SCSu	Hedges & Datta (1971)
R388	W	—	SuTp	Datta & Hedges (1972b)
RA1	.	—	TSu	Hedges & Datta (1971)
R300	.	—	SSu	Lawn <i>et al.</i> (1967).

* Symbols designate resistance to: A, ampicillin; S, streptomycin; T, tetracycline; C, chloromphenicol; K, kanamycin; Su, sulphonamides; M, mercury salts; Tp, trimethoprim.

† Spontaneous segregant of R1, lacking K-resistance.

‡ Mutant of R1: constitutive pilus synthesis and lacking all resistances except K-resistance.

§ Spontaneous segregant/mutant of RP4, lacking K-resistance.

|| Factor not assigned to any compatibility group yet defined.

his, *trp* (Clowes & Hayes, 1968); J62-1, a nalidixic acid-resistant mutant of J62; HfrC *met* (Clowes & Hayes, 1968). *Salmonella typhimurium* LT2, SL329 *trp*. *Klebsiella aerogenes* B9, a wild, drug-sensitive strain.

Phages. MS2 (Davis, Strauss & Sinsheimer, 1961). λ vir (Jacob & Wollman, 1964).

R factors. Standard R factors are listed in Table 1. R factors transferred from *Proteus rettgeri* strains are listed in Table 2.

Media. Nutrient broth was Oxoid No. 2 (CM 67); nutrient agar was the same solidified with 1.2 % New Zealand agar; MacConkey agar was Oxoid CM7b. Minimal agar salts was as described in Clowes & Hayes (1968).

Transfer of R factors. Methods were as described by Datta, Hedges, Shaw, Sykes & Richmond (1971) and Datta & Hedges (1972a).

Compatibility of R factors. Relative frequencies of R transfer by conjugation (in mixtures incubated for 1 h) into a strain of K12 with and without a second plasmid under test were determined. This gave a measure of exclusion (Meynell, Meynell & Datta, 1968). Recipient colonies were purified by restreaking on MacConkey agar containing the appropriate drug (and 5 % lysed horse blood, where the drug was sulphonamide or trimethoprim) and tested for resistance markers of each R factor, resident and newly acquired. Where both factors were present, a clone was grown overnight in drug-free broth, plated on non-selective medium and 20 colonies tested for the continued carriage of both factors. R factors transferred from *Proteus rettgeri* to *Escherichia coli* K12 were tested in this way against appropriate 'standard' R factors, listed in Table 1, and against one another.

Transfer from 'doubles'. Where resistance markers of both donor and recipient cultures were identified in J53 cultures after transfer, the 'double' was tested to see whether the two plasmids were transferable separately or always together. A clone was grown in broth and used as donor in a second mating with J62(R-) as recipient. Mixtures were plated on

Table 2. R factors from *Proteus rettgeri*

<i>Proteus rettgeri</i>	R factor	<i>fi</i>	Characters determined by R factor	Compatibility group
55	R390	—	ASSpTCSu <i>hsp</i> II	N
107	R391	—	K	J
105	R392	—	K	?J
122	R394	—	AK	T
134	R395	—	ASSpTCSu <i>hsp</i> II	N
135	R396	—	ASSpTCSu <i>hsp</i> II	N
149	R397	—	K	?J
169	R398	—	ASSpTCSu <i>hsp</i> II	N
137	R400	—	ASSpTCSu <i>hsp</i> II	N
148	R401	—	AS	T
175	R402	—	ASSp	T
7KR	R7K	—	AS	W

The symbol Sp indicates spectinomycin resistance. Others as in Table 1.

selective media to measure the frequency of transfer of resistance genes from either plasmid. Recipient clones (20 from each selective medium) were purified and tested for the presence of selected and unselected resistance markers.

fi character. R factors were transferred to HfrC. An undiluted lysate of phage MS2 (titre approximately 10^{11} plaque-forming units/ml) was spotted on the R⁺ culture. Visible lysis indicated that the plasmid was *fi*⁻.

Restriction of phage λ vir. Phage λ *vir* lysates of J53 and J53(N3) were plated in agar layer on nutrient agar plates with J53 or J53 carrying the R factors to be tested and the plaques counted. The concentration of phage was chosen to allow plaque counts to be made on restricting and non-restricting cultures (Table 4).

Segregants of R390. Spontaneous segregants, sensitive to streptomycin, tetracycline or chloramphenicol, were isolated by replica plating. Segregants sensitive to sulphonamides appeared as small colonies on nutrient agar containing 5 % lysed horse blood and 25 µg sulphadimidine/ml. Approximately 10000 colonies were examined for loss of resistance markers.

RESULTS

Phenotypic characters conferred on J62-1 by R factors from Proteus rettgeri

R factors were transferred from *Proteus rettgeri* to *Escherichia coli* K12 J62-2 and thence to other lines of K12 to test their *fi* character and their compatibility with other R factors. All 12 R factors were *fi*⁻. Some belonged to previously defined compatibility groups. Others were used to define new groups. Evidence for their classification by compatibility is given below. Their characters are summarized in Table 2.

R391, R392 and R397

Three of the R factors, R391, R392 and R397, conferred resistance to kanamycin only. All three were transferred between strains of K12 at low frequency. Each co-existed stably with standard R factors of all compatibility groups (Table 1). Results are shown in Table 3.

R390, R395, R396, R398 and R400

Five R factors, indistinguishable from one another, conferred resistance to ampicillin, streptomycin/spectinomycin, tetracycline, chloramphenicol and sulphonamide. They carried

Table 3. *Transfer and compatibility of R391*

Donor	Recipient	Frequency of transfer	Exconjugant clones tested for presence of each plasmid	Transfer frequency from 'doubles' selection for transfer of:	
				R391	Other
J62.2(R391)	J53	2×10^{-4}			
	J53(RA1)	8×10^{-5}	20/20 both present	3×10^{-5}	9×10^{-3}
	J53(R64)	3×10^{-4}	20/20 both present	2×10^{-5}	4×10^{-3}
	J53(R46)	3×10^{-4}	19/20 both present	1×10^{-5}	1×10^{-3}
	J53(RP4-4)	3×10^{-4}	20/20 both present	6×10^{-5}	9×10^{-2}
	J53(RA3)	1×10^{-4}	20/20 both present	2×10^{-5}	3×10^{-3}
	J53(RA1)	2×10^{-4}	18/20 both present	1×10^{-6}	7×10^{-5}
	J53(R300)	2×10^{-4}	18/20 both present	$< 10^{-7}$	2×10^{-4}
	J53(R390)	6×10^{-5}	20/20 both present	1×10^{-5}	3×10^{-3}

Matings were made in parallel, in conditions described in the text. Frequency of transfer was calculated per donor cell. Results are presented in sequence from left to right. Both R factors were stable in each 'double'. From each 'double' the other plasmid was transferred independently of R391. Figures for R392 and R397 were very similar to those above.

the determinant for host specificity pattern II, *hsp* II (Bannister & Glover, 1968; Hedges, 1972) (Table 4) and belonged to compatibility group N (Table 5) (Datta & Hedges, 1971). Six classes of spontaneous segregants of R390 were identified. The frequency of loss of one or more resistance marker was approximately 10^{-3} . From the characters of the segregants it is possible to construct a deletion map of R390 (Fig. 1).

R390-3, a non-transmissible segregant, was shown still to have the compatibility of the N group, since it was always eliminated by the introduction of R15. J62 (R390-3) was used as a recipient in matings with J53(RA1) and J53(R300), standard R factors which could not be detected in the presence of wild-type R390. R390-3 was compatible with RA1 and R300.

The penicillinase determined by R390 was examined by Drs J. T. Smith and J. W. Dale. It had no appreciable activity against oxacillin.

R401

R401 gave resistance to ampicillin and streptomycin (but not spectinomycin). It was not excluded by, and co-existed stably with, all the standard R factors. As with Rts1 (Terawaki *et al.* 1967), the replication of R401 was temperature sensitive. We therefore tested the compatibility of these two plasmids. Transfer of R401 into a strain carrying Rts1 resulted in the elimination of Rts1 from 17 of 20 clones tested. The other three clones were resistant to ampicillin, streptomycin and kanamycin (i.e. had all the antibiotic resistance markers of both plasmids). That they carried recombinant plasmids was indicated by the coincident loss of all resistance markers during growth at 43 °C (Table 6*a, b*) and by the fact that, when they were used as donors, all three resistance genes were transferred together, no matter which drug was used for selection. The replication of R401 was temperature sensitive not only in *Escherichia coli* K12 but also in *Klebsiella aerogenes* B9 and in *Salmonella typhimurium* LT2 (Table 6*c*). *Proteus rettgeri* 148 failed to grow at 43 °C, so the temperature-sensitivity of R401 could not be tested in its original host.

R394

R394 conferred resistance to ampicillin and kanamycin. In the first set of experiments this plasmid appeared incompatible with R46 and R390 and therefore to be an N plasmid. But in subsequent experiments R394 and various N plasmids (including R46 and R390)

Table 4. Restriction of phage determined by R factors

Culture	No. of plaques from uniform dose of λ vir grown on:	
	J53	J53(N3)
J53	374	40
J53(R15)	20	44
J53(R390)	9	42
J53(R391)	427	33
J53(R392)	468	39
J53(R394)	470	44
J53(R395)	16	40
J53(R396)	22	56
J53(R397)	488	37
J53(R398)	16	33
J53(R400)	18	46
J53(R401)	421	43
J53(R402)	418	39
J53(R7K)	406	41

Table 5. Transfer and compatibility of R390, R395, R396, R398 and R400

Donor	Recipient	Frequency of transfer	Exconjugant clones tested for presence of each plasmid	Transfer frequency from 'doubles' selection for transfer of:	
				R390	Other
J62-2(R390)	J53	4×10^{-3}	.	.	.
	J53(R1drd16)	2×10^{-3}	20/20 both present	1×10^{-3}	2×10^{-5}
	J53(R144)	5×10^{-3}	20/20 both present	8×10^{-3}	2×10^{-3}
	J53(R269N-1)	2×10^{-5}	0/10 both present	.	.
	J53(RP4)	2×10^{-3}	18/20 both present	1×10^{-3}	3×10^{-5}
	J53(S-a)	3×10^{-3}	20/20 both present	3×10^{-3}	9×10^{-5}
	J53(R15)	2×10^{-4}	0/20 both present	.	.

Method as given in Table 3. Both R factors were stable in each 'double'. From each 'double' R390 was transferred independently. R395, R396, R398 and R400, like R390, were transferred to J53(R15) at reduced frequency compared with their transfer frequency to J53. R15 was eliminated by these factors from all clones tested.

were shown to be compatible (Table 7). In other experiments, not shown in Table 7, elimination of R46 by R394 occurred again. When R394 was transferred to J53 (R390) the 'doubles' carried kanamycin resistance (of R394) chloramphenicol and sulphonamide resistance (of R390), ampicillin resistance (of both factors) but not tetracycline resistance (a marker of R390 present in the recipient at mating). From this double, each plasmid was independently transferred, R390 without tetracycline resistance. In another mating in which R394 was transferred to J53 carrying the N plasmid, R15, (selection being for kanamycin resistance) ampicillin resistance was lost from R394, but R15 was retained unaltered. Each plasmid was transferred independently from the 'double'.

When R394 was transferred to J53(R401) or J53(R402) most kanamycin-resistant exconjugants had lost the resident plasmid. Where resistance genes of both plasmids were present there was evidence for recombination. All resistance markers were co-transferred in all matings and the markers of the temperature-sensitive plasmids, R401 and R402 (see below) were stable during growth at 43 °C (Table 6d).

Fig. 1. Deletion map of R390.

Segregant	Deletion	Selected for sensitivity to:	Transmissibility	<i>hspII</i>	Drug resistance
R390-1	1	Chloramphenicol	+	+	ATSu
R390-2	2	Chloramphenicol	+	+	ASTSu
R390-3	3	Chloramphenicol	+	—	A
R390-4	4	Chloramphenicol	+	—	A
R390-8	8	Sulphonamide	+	+	A
R290-12	12	Tetracycline	—	+	ASCSu

A genetic map of R390 is based on the properties of spontaneous segregants. It is assumed that all deletions arise by elimination of a single continuous segment (shown as arcs). Distances between genetic markers are arbitrary. Symbols: Su^a and Su^b are the two possible sites for the gene determining sulphonamide resistance. *tra* represents the genes determining transferability. *rep* genes are those necessary for replication of the plasmid.

R402

R402 conferred resistance to ampicillin and streptomycin/spectinomycin. It co-existed stably with all standard R factors and with R391. However, its replication was temperature sensitive (Table 6*d*). When R394 was introduced into J53(R402) the resident plasmid was either eliminated or recombined with the incoming plasmid (Tables 7, 6*d*).

R7K

R7K conferred resistance to ampicillin and a low level of resistance to streptomycin, but not spectinomycin. It could not be tested for P compatibility, since all known P plasmids confer ampicillin resistance and the level of streptomycin resistance determined by R7K was too low for use in selection. It co-existed stably with plasmids of all other compatibility groups, except those of group W. Table 8 shows that it belonged to compatibility group W.

Table 6. *Effect of growth at 43 °C on RtsI, R401, R394 and R402 and recombinant plasmids*

(a) Colonies tested for resistance after 6 h growth in broth at 43 °C (evidence for recombination between R401 and RtsI)					
	Total	S ^r K ^r	S ^s K ^s	S ^r K ^s	S ^s K ^r
J53(R401/RtsI)*	49	11	38	0	0
(b) Colonies tested after growth overnight at 37 °C or 43 °C					
J62(R401)	37 °C	61/61	A ^r S ^r		
	43 °C	61/64	A ^s S ^s : 3/64 A ^r S ^r		
J62(RtsI)	37 °C	57/58	K ^r : 1/58 K ^s		
	43 °C	61/61	K ^s		
JS2(R401/RtsI)	37 °C	62/62	S ^r K ^r		
	43 °C	63/63	S ^s K ^s		
(c) Temperature sensitivity of R401 in hosts other than <i>Escherichia coli</i> . Colonies tested for resistance after overnight growth in broth at 37 °C or 43 °C					
<i>Klebsiella aerogenes</i> B9(R401)					
	37 °C	75/76	A ^r S ^r : 1/76 A ^s S ^s		
	43 °C	125/125	A ^s S ^s		
<i>Salmonella typhimurium</i> LT2(R401)					
	37 °C	45/45	A ^r S ^r		
	43 °C	123/150	A ^s S ^s : 27/150 A ^r S ^r		
(d) Stability of R394, R401 and R402 and recombinants. Colonies tested after growth overnight at 43 °C					
	Total	A ^r K ^r	A ^r S ^r	A ^r K ^r S ^r	A ^s K ^s S ^s (R-)
J53(R394)	43	43	.	.	0
J53(R401)	45	.	6	.	39
J53(R402)	42	.	6	.	36
J53(R394/R401)	44	.	.	43	1
J53(R394/R402)	41	.	.	41	0

* All three clones (see text) in which the kanamycin resistance of RtsI was retained on introduction of R401 behaved in the same way.

As with other members of this group there was little surface exclusion of related plasmids, but elimination of the resident plasmid (Hedges & Datta, 1971).

DISCUSSION

All plasmids so far identified in the *Proteus* group have been *fi*⁻, although *fi*⁺ factors have been transferred to *Proteus* strains in various laboratories (Harada, Suzuki, Kameda & Mitsuhashi, 1960; Datta & Hedges, 1972*a*). No plasmids of the compatibility group which includes collb-P9, R64 and R144 (I-like plasmids) have been found in *Proteus*: I-like plasmids are not transmissible to the *Proteus* group (Datta & Hedges, 1972*a*). The lack of F-like and I-like plasmids is in marked contrast with the R factor sets observed in other genera. In *Escherichia coli* and *Shigella* spp. *fi*⁺ factors are in the majority (Watson, 1967; Meynell *et al.* 1968). In *Salmonella* spp. *fi*⁻ factors predominate, most of which are I-like (Meynell *et al.* 1968 and unpublished data).

Which R factors one finds depends on the bacterial genera examined, not on geographical location. The R factors listed in Table 2 all (except R7K) came from strains of *Proteus*

Table 7. *Transfer and compatibility of R394*

Expt no.	Donor	Recipient	Compati- bility group of resi- dent R factor	Frequency of transfer	Recombinant clones tested for presence of each plasmid	Transfer frequency from 'doubles': selection for transfer of:	
						R 394	Other
1 2 1 2	J62-1(R394)	J53	.	2×10^{-3}	.	3×10^{-3}	.
		J53(R1-1)	F	8×10^{-4}	20/20 both present	7×10^{-4}	1×10^{-2}
		J53(R64)	I	3×10^{-3}	18/18 both present	8×10^{-4}	2×10^{-3}
		J53(R46)	N	4×10^{-3}	1/20 both present	Not tested	Not tested
		J53(R46)	N	2×10^{-3}	18/20 both present	1×10^{-3}	2×10^{-3}
		J53(R390)	N	5×10^{-3}	1/20 both present	Not tested	Not tested
		J53(R390)	N	3×10^{-3}	19/20 both present	1×10^{-3}	9×10^{-3}
		J53(R15)	N	3×10^{-3}	19/20 both present	2×10^{-3}	1×10^{-2}
		J53(RP4-4)	P	3×10^{-3}	20/20 both present	1×10^{-3}	5×10^{-3}
		J53(R388)	W	3×10^{-3}	20/20 both present	6×10^{-4}	3×10^{-3}
		J53(RA1)	.	5×10^{-3}	20/20 both present	2×10^{-3}	6×10^{-5}
		J53(R300)	.	2×10^{-3}	18/20 both present	9×10^{-3}	3×10^{-4}
		J53(R391)	J	7×10^{-4}	No test available	.	.
		J53(R401)	T	1×10^{-3}	3/20 both present	5×10^{-3} *	5×10^{-3} *
		J53(R402)	T	5×10^{-4}	7/20 both present	6×10^{-3} *	6×10^{-3} *

Methods as for Table 3. In Expt 1, R394 appeared to have the compatibility properties of an N plasmid (although there was no exclusion). On retesting, R394 did not eliminate and did co-exist stably with N plasmids (but see text). Co-existence with all other plasmids was unaltered on retesting.

Each R factor (including those of group N, but excluding both R401 and R402) was transferred separately from 'doubles' with R394.

* Recipients from 'doubles' selected for R394, R401, or R402 carried all resistances of both factors (20/20 clones tested from each selection).

Table 8. *Transfer and compatibility of R7K*

Donor	Recipient	Frequency of transfer	Recombinant clones tested for presence of each plasmid
J62-1(R7K)	J53	5×10^{-3}	
	J53(R388)	1×10^{-3}	0/20 both present
J53(S-a)	J62-1	2×10^{-4}	
	J62-1(R7K)	1×10^{-4}	0/20 both present

Method as given in Table 3.

rettgeri isolated in South Africa, but among South African *Shigella* strains, Watson (1967) found only *fi*⁺ R factors. Plasmids of the N group (Datta & Hedges, 1971) were the commonest among those described here. They carried resistance to chloramphenicol which had not previously been found on any N plasmid. This suggests that genes from *fi*⁺ R factors have become incorporated into N plasmids: an example of recombination between an N and an F-like R factor is known. An N plasmid, R269N (which confers resistance to ampicillin, streptomycin and tetracycline), acquired in the laboratory the determinant for kanamycin resistance from an *fi*⁺ plasmid, R269F, present in the same culture (Hedges, 1972).

The penicillinases determined by R390 differed from that determined by R46, the prototype N plasmid. The penicillinase of R46 differs from most other β -lactamases in rapidly hydrolysing methicillin and cloxacillin (Datta & Kontomichalou, 1965) and oxacillin (Dale, 1971). Similar β -lactamases are determined by several other N plasmids (R. W. Hedges,

R. B. Sykes, P. Kontomichalou, N. Datta & M. H. Richmond, unpublished) but the enzyme from R390 lacked this distinctive property.

R401 resembled Rts1 in belonging to the same compatibility group, which we propose to designate T, and in that its replication was temperature sensitive. The temperature sensitivity of replication of a plasmid may be determined by the plasmid itself or the host chromosome (Jacob, Brenner & Cuzin, 1964). Since the replication of R401 was temperature sensitive in *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella aerogenes* (Table 6c) the sensitivity was probably determined by the plasmid.

R394 also belonged to compatibility group T. Unlike other members of this group its replication was not temperature sensitive (Table 6d). This R factor gave initially confusing results in tests for compatibility since, with imperfect reproducibility, it dislodged resident R factors of the N group. However, R394 and N plasmids can stably co-exist and be transferred independently from 'doubles'. We have observed dislodgment of compatible *fi*⁻ R factors (including N plasmids) by Rts1 (Hedges, unpublished). The mechanism of dislodgment is not known; perhaps 'repliconation' (Clark, 1967) of T plasmids requires an enzyme, such as a recombinase, which interacts with other plasmids. The same mechanism may account for the deletion particular drug resistance genes in 'doubles' newly infected with R394.

R402 also belonged to the T compatibility group. Its replication was temperature sensitive (Table 6d). R402 was not identical with R401, since it conferred resistance to spectinomycin as well as to streptomycin and presumably therefore determined the production of streptomycin/spectinomycin adenylate synthetase, whereas R401 presumably determined the synthesis of streptomycin phosphotransferase (Davies, Brezezinska & Benveniste, 1971). Three of the 12 R factors from *Proteus rettgeri* belonged, like Rts1, to the T group. Rts1 was derived from a strain *Proteus vulgaris*. Thus all T plasmids so far identified occurred in bacteria of the *Proteus* tribe.

R7K was compatible with all plasmids tested except R factor S-a (Hedges & Datta, 1971) and R388 (Datta & Hedges, 1972b), members of the W compatibility group.

The three R factors from *Proteus rettgeri* which did not belong to compatibility groups N, T or W co-existed with our 'standard' plasmids and thus cannot be assigned to any defined group. We propose to designate R391 as the prototype for a new group, J. R392 and R397 resemble R391 in conferring resistance only to kanamycin and in having a low rate of transfer: we cannot test their compatibility with R391.

Plasmids found in naturally occurring strains of the *Proteus* group thus seem to constitute a set significantly different from those found in *Escherichia coli*, *Shigella* or *Salmonella* spp.

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