R Factors from *Proteus morganii*

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SUMMARY

Twenty-eight R factors transmissible to *Escherichia coli* K12 were derived from 178 naturally occurring strains of *Proteus morganii* and evidence was found of non-transmissible resistance plasmids in some strains. Nine plasmids were assigned to group N; one *P. morganii* strain carried two N plasmids which were incompatible in reference strains of *E. coli* or *P. morganii*. Six *fi*⁺ R factors of group FII were found. One was unique among F-like plasmids in conferring *hspII* restriction. Entry exclusion by these R factors had a specificity distinct from that of FII plasmids previously described. Three R factors of compatibility group FI were the first recognized plasmids of this group with repressed pilus synthesis. Two *fi*⁺ R factors incompatible with R6K (and with this plasmid constituting group X) were identified, as were one plasmid of group P and an R factor compatible with plasmids of all groups so far described and therefore assigned to a new group (M). Similarities and differences between the R factor sets of *P. morganii* and *P. rettgeri* are discussed.

INTRODUCTION

The relationship of *Proteus morganii* to other *Proteus* species is uncertain. Ewing (1958) suggested that *P. morganii*, *P. rettgeri* and *Providence* should be united as a genus distinct from *P. mirabilis* and *P. vulgaris* which are closely related in biochemical properties (Smit & Coetzee, 1967) and in DNA homology (Brenner & Falkow, 1971). Smit & Coetzee (1967) suggested that *P. morganii* should be raised to generic status as *Morganella*. *Proteus morganii* differs from the other members of the *Proteus* tribe in that the GC% of its DNA is > 50 whereas the GC% for other members of the tribe is about 40 (Hill, 1966). Despite this and other differences, most bacteriologists consider the *Proteus* tribe to be a natural group (Coetzee, 1972). We have been sampling the sets of plasmids from taxa within the *Proteus* tribe to find out whether there are significant differences or relationships. Among *P. rettgeri* R factors (Coetzee, Datta & Hedges, 1972), N plasmids (Datta & Hedges, 1971) were the most abundant. Plasmids of groups J and T (Coetzee *et al.* 1972) and one of group W (Hedges & Datta, 1971) were also observed. This R factor set is quite distinct from those of other groups of bacteria. For example, in *Shigella flexneri* a large majority of

R factors are fi^+ , probably of group FII (Watson, 1967; Yoshikawa, Nagashima & Matsushima, 1971), whilst R factors from *Salmonella* are predominantly fi^- (Meynell, Meynell & Datta, 1968), some I-like, some of the N group and some, conferring streptomycin and sulphonamide resistances, not yet assigned to any compatibility group (Lawn, Meynell, Meynell & Datta, 1967).

The selective pressures operating to establish and maintain plasmid differences between the taxa are largely unknown but some clues are available. For example many fi^+ R factors are unstable in *Salmonella typhimurium* (Watanabe & Ogata, 1970) and in *Proteus mirabilis* (e.g. Kopecko & Punch, 1971); I-like plasmids are unstable in *Shigella* (Yoshikawa *et al.* 1971). Failure of certain types of R factors to establish themselves in bacteria of particular species seems likely to influence distributions of various classes. For example I-like R factors fail to transfer to the *Proteus* tribe (Datta & Hedges, 1972*a*) and hence we do not expect to find any I-like plasmids in this group. Even more severe is the restriction of transfer of plasmids to *Pseudomonas aeruginosa*. So far as is known, only plasmids of groups P (Datta *et al.* 1971) and C (Datta & Hedges, 1972*b*) can be transferred into *P. aeruginosa*, and plasmids of these two classes constitute the R factor set of this species.

METHODS

Proteus morganii. Wild-type *P. morganii* strains (167) were isolated in South Africa in 1966–7 under the same conditions as those for the *P. rettgeri* collection (Coetzee *et al.* 1972). Eleven wild-type *P. morganii* strains (which we designated M201–211) were isolated in New Haven (U.S.A.) by Dr A. von Graevenitz in 1972. *Proteus morganii* 2815 is an R⁻ laboratory strain (Coetzee, 1972).

Escherichia coli K12 strains: J53 F⁻ *lac*⁺, *met*, *pro* (Clowes & Hayes, 1968); J53–I, a nalidixic acid-resistant mutant of J53; J62 F⁻ *lac*, *pro*, *his*, *trp* (Clowes & Hayes, 1968); J62–I, a nalidixic acid-resistant mutant of J62; HfrC, Hfr *met* (Clowes & Hayes, 1968).

Salmonella typhimurium LT2 strain M329 *trp*.

Plasmids. Standard plasmids are listed in Table 1, R factors derived from the *Proteus morganii* strains in Table 3.

Phages. λ vir (Jacob & Wollman, 1954). MS2 (Davis, Strauss & Sinsheimer, 1961).

Media. Nutrient broth was Oxoid no. 2 (CM67); nutrient agar was the same solidified with 1.2 % New Zealand agar; for sensitivity tests, 4 % (v/v) lysed horse blood was incorporated in nutrient agar; MacConkey agar was Oxoid CM7b. Minimal salts agar was as described by Clowes & Hayes (1968).

Detection of antibiotic resistance. In both wild strains and strains of *Escherichia coli* K12, the resistance spectrum to eight drugs was scored using Oxoid 'multidisks' (code no. 30-44K) and individual discs placed on plates of lysed blood agar.

Transfer and compatibility of plasmids, fi determination, isolation of segregants were as described by Coetzee *et al.* (1972).

Bacteriophage increase. Multiplication of phage MS2 in R⁺ cultures (for evidence of the presence of F pili) was measured as described by Datta *et al.* (1966).

Restriction and modification of λ vir. The *hspII* restriction by R factors was determined as described by Hedges (1972).

Table 1. 'Standard' plasmids of various compatibility groups

Plasmid	Characteristics*	Compatibility group	Reference
<i>Flac</i>	Lac ⁺	FI	Jacob & Adelberg (1959)
R386	T	FI	Dennison (1972)
Rldrd16	K	FII	Meynell & Datta (1967)
RI-1	ASCSu	FII	Coetzee <i>et al.</i> (1972)
ColB-K98	Col B ⁺	FIII	Frydman & Meynell (1969)
R124	T	FIV	Hedges & Datta (1972)
R144	TK ColI ⁺	I	Lawn, Meynell, Meynell & Datta (1967)
R64	ST	I	Lawn, Meynell, Meynell & Datta (1967)
R15	SSu, <i>hspII</i>	N	Datta & Hedges (1971)
R45	ATSu	N	Hedges (1972)
R269N-1	ASTK, <i>hspII</i>	N	Hedges (1972)
R245	T	N	Hedges (1972)
RP4	ATK	P	Datta, Hedges, Shaw, Sykes & Richmond (1971)
R751	Tp	P	Datta & Jobanputra (unpublished)
S-a	SCKSu	W	Hedges & Datta (1971)
R388	SuTp	W	Datta & Hedges (1972c)
R7K	AS	W	Coetzee <i>et al.</i> (1972)
R57b	ACSuGk	C	Witchitz & Chabbert (1972), Datta & Hedges (1972b)
R391	K	J	Coetzee <i>et al.</i> (1972)
RA1	TSu	A	Hedges & Datta (1971)
R300	SSu	Undesignated	Lawn <i>et al.</i> (1967)
R387	SC	Undesignated	Hedges & Datta (1971)
R6K	AS	X	Kontomichalou, Mitani & Clowes (1970)
R483	STp	B	Hedges, Datta & Fleming (1972)
R401	AS	T	Coetzee <i>et al.</i> (1972)
Rtsl	K	T	Coetzee <i>et al.</i> (1972)

* Abbreviations for resistance markers: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Gk, gentamicin (and low-level kanamycin); Su, sulphonamides; Tp, trimethoprim.

RESULTS AND DISCUSSION

Resistance phenotypes of the Proteus morganii strains. Of the 167 strains from South Africa, all were resistant to polymixin, 80 to tetracycline, 69 to streptomycin, 66 to sulphonamides, 63 to chloramphenicol, 51 to ampicillin, 11 to kanamycin and one to nitrofurantoin (Table 2).

Transfer of drug resistance to Escherichia coli K12 and properties of the exconjugants. Transfer of every resistance determinant except polymixin and nitrofurantoin from each *Proteus morganii* strain to *Escherichia coli* J62-1 was attempted. Those cases in which R transfer was observed are recorded in Table 3. Nine of the R factors conferred upon J62-1 restriction specificity *hspII*. Eight R factors, when carried by HfrC, prevented visible lysis by phage MS2, i.e. these were *fi*⁺ (Watanabe, Nishida, Ogata, Arai & Sato, 1964). Three other plasmids, R453, R455 and R456, gave ambiguous answers in the *fi* test, unless the R⁺ HfrC strains were tested in the presence of an antibiotic selecting against R-segregants, when their *fi*⁺ character was evident.

F-pilus production by fi⁺ R factors. Strains carrying *fi*⁺ R factors of three compatibility groups (FI, FII and X) were tested for F-like pili. Strains carrying R444 or R455 supported the multiplication of phage MS2 with an efficiency comparable with that of strains carrying wild-type (repressed for pilus synthesis) RI. R444 and R455, therefore, determine F-like pili. R485⁺ *Escherichia coli* did not support multiplication of MS2.

Table 2. *Resistance spectra of the Proteus morganii strains*

Resistance	Number	Resistance	Number
None	67	ATC	2
C	2	ASC	5
Su	2	STSu	8
A	3	ATCSu	1
T	12	ASTC	1
AT	1	ASTSu	3
SC	2	STCSu	8
TSu	2	STCSuFz	1
TC	6	ASTCSu	24
SSu	6	ASTCSuK	11

* Abbreviations as in Table 1; in addition Fz indicates resistance to furazolidone.

Table 3. *R factors from Proteus morganii*

Strain	Characters transferred* to <i>Escherichia coli</i> K12	Designation	Compatibility group
M6	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R444	FII
M8	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R445	FII
M15	A, <i>fi</i> ⁻	R446a	P
	S, T, <i>fi</i> ⁻	R446b	M
	S, Sp, Su, <i>hspII</i> , <i>fi</i> ⁻	R446c	N
M16	A, S, Sp, T, C, Su, <i>hspII</i> , <i>fi</i> ⁻	R447a	N
	A, K, <i>fi</i> ⁻	R447b	N
M21	A, S, Sp, T, C, Su, <i>hspII</i> , <i>fi</i> ⁻	R448	N
M25	S, Su, <i>fi</i> ⁻	R496	Undesignated
M53	S, Su, <i>fi</i> ⁻	R450	Undesignated
M56	S, Su, <i>fi</i> ⁻	R451	Undesignated
M60	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R452	FII
M66	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R453	FI
M68	A, S, Sp, T, C, Su, <i>hspII</i> , <i>fi</i> ⁻	R454	N
M69	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R455	FI
M73	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R456	FI
M82	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R457	FII
M83	A, S, Sp, T, C, Su, <i>fi</i> ⁺	R458	FII
M111	A, S, Sp, T, C, Su, K, <i>hspII</i> , <i>fi</i> ⁺	R459	FII
M134	S, Sp, Su, <i>fi</i> ⁻	R460	Undesignated
M158	A, S, Sp, T, C, Su, <i>hspII</i> , <i>fi</i> ⁻	R461	N
M159	A, S, Sp, T, C, Su, <i>hspII</i> , <i>fi</i> ⁻	R462	N
M180	S, Su, <i>fi</i> ⁻	R464	Undesignated
M184	S, Sp, T, Su, <i>hspII</i> , <i>fi</i> ⁻	R465	N
M185	S, Su, <i>fi</i> ⁻	R466	Undesignated
M194	A, S, Sp, T, <i>hspII</i> , <i>fi</i> ⁻	R467	N
M203	Su, <i>fi</i> ⁺	R485	X
M206	Su, <i>fi</i> ⁺	R487	X

* Abbreviations as for Table 1. In addition Sp indicates resistance to spectinomycin.

Group FI plasmids

R453, R455 and R456, which all conferred the same resistance pattern, gave ambiguous results in the *fi* test because they were unstable in HfrC. All three were incompatible with *Flac*. Thus these R factors belong to group FI. Various segregants of R455 were isolated and their compatibility properties tested. R455-2 (a tetracycline-sensitive segregant) was incompatible with R386, thus confirming R455 as an FI plasmid. Appropriate segregants were compatible with standard plasmids of all other groups.

Table 4. *Compatibility and exclusion properties of FII plasmids from Proteus morganii*

Donor	Recipient	Antibiotic resistance selected	Efficiency of transfer	Character of recombinant
J53 (R1drd16)	J62-1	K	2×10^{-1}	—
	J62-1 (R444)	K	1×10^{-1}	10 of 10 $K^R C^S$
J62-1 (R444)	J53	C	4×10^{-2}	—
	J53 (R1drd16)	C	2×10^{-2}	10 of 10 $C^R K^S$
J62-1 (R444)	J53 (R444-5)	C	3×10^{-3}	—

* The superscripts R, S denote resistance and sensitivity respectively, to the drugs concerned. Other abbreviations as in Table 1.

The presence of R453, R455 or R456 in a strain of J53 reduced the plaque titre of λ vir; the level of reduction was very variable but usually approached 10^{-4} . The phage was not modified, but inhibited by an unknown mechanism.

These R factors were unlike the FI plasmids so far described (Dennison, 1972) in that their pilus production was repressed.

Group FII plasmids

Most, but not all, fi^+ R factors belong to the FII compatibility group (Watanabe *et al.* 1964; Lawn *et al.* 1967; Dennison, 1972; Hedges & Datta, 1972). Therefore, the five fi^+ R factors R444, R445, R452, R457 and R458 were tested for compatibility with R1drd16. All these R factors were incompatible with R1drd16 but were unusual among FII plasmids in not significantly excluding this R factor (Watanabe *et al.* 1964; Meynell & Datta, 1969).

R444, taken as the representative of this apparently homogeneous group, was excluded by R1drd16 very slightly, if at all. To test whether R444 was totally incapable of exclusion or had a different exclusion specificity from R1drd16 (and hence from that of all FII plasmids previously tested) segregants having lost particular resistance markers were sought. None was found among about 10^4 clones of J62-1 (R444) but, as is common with fi^+ R factors, segregants were found in an R444⁺ strain of *Salmonella typhimurium* LT2 (see Watanabe & Ogata, 1970). No $C^R S^S$ or $C^S S^R$ segregants were found but 10 % of clones were $C^S S^S$. Of 57 such clones tested 51 were $A^R T^R$, two were resistant to ampicillin only and four had lost all resistance markers. The segregants conferring resistance to ampicillin only were non-transmissible but the $A^R T^R$ segregants were transferred to *Escherichia coli* J53. Two such segregants, R444-4 and R444-5, excluded wild-type R444 (Table 4).

Typical fi^+ , FII R factors exclude one another very efficiently (Watanabe *et al.* 1964; Meynell *et al.* 1968). Thus the FII plasmids of *Proteus morganii* are not typical members of their group but appear to have undergone a significant evolutionary divergence from the homologous plasmids of *Escherichia coli*, *Salmonella* and *Shigella*. They might either be incapable of effecting or suffering exclusion or they might confer and be susceptible to a new specificity of exclusion distinct from that of R1drd16. The exclusion of wild-type R444 by its segregants, R444-4 and R444-5 confirmed the latter model (Table 4). This is the first reported example of two R factors of a single compatibility group showing distinct exclusion specificities.

R444 resembled several other fi^+ R factors in being unstable in *Salmonella typhimurium* LT2. Watanabe & Ogata (1970) showed that the *recA* gene of LT2 is necessary for this instability. Thus plasmid deletions are mediated by the bacterial recombination system in

contrast to deletions of the *Escherichia coli* chromosome whose occurrence is independent of the recombination machinery (Franklin, 1967; Inselburg, 1967; Hedges, 1969; Anderson, 1970; Spudich, Horn & Yanofsky, 1970). The commonest type of segregant produced from R444 in *S. typhimurium* conferred resistance to ampicillin and tetracycline only. We failed to find any segregants sensitive to chloramphenicol but resistant to streptomycin, or vice versa, in about 10^4 clones replica-plated. Thus, apparently R444 carried sites highly susceptible to attack by the *S. typhimurium* recombination system (though not that of *E. coli*) located so as to permit dissociation of the genes conferring resistance to chloramphenicol, streptomycin and sulphonamides from those responsible for the other resistances, plasmid replication and transmissibility.

R459 appeared to be both *fi*⁺ and *hspII*⁺. It was, thus, necessary to exclude the possibility that R459⁺ strains actually carried two plasmids, one of group FII and one of group N. Transfer of chloramphenicol or tetracycline resistance from *Proteus morganii* MIII to *Escherichia coli* K12 usually involved transfer of all genetic characters including *hspII*⁺ and *fi*⁺ markers but about 10 % of exconjugants were non-restricting and lacked kanamycin resistance. The plasmids in these clones were *fi*⁺ and incompatible with R1drd16.

All kanamycin-resistant exconjugants were resistant to so many agents (A, S, Sp, T, C, K, Su and Hg²⁺ ions) that their compatibility with standard R factors could not be tested. Therefore clones of K12 (R459) carrying segregants lacking one or more resistance markers were isolated. From the patterns of markers lost we were able to construct a genetic map (Fig. 1).

One of the segregants, R459-13, lacked resistance to ampicillin, streptomycin and kanamycin but retained *hspII* specificity. Matings in which R1drd16 (group FII) or R45 (group N) were transferred to this strain showed that the *hspII* restriction was carried by a plasmid with FII compatibility properties. Other crosses demonstrated that all the antibiotic resistance markers associated with R459 were carried on the FII plasmid.

R459 is the first exception to the generalization (Hedges, 1972) that all plasmids conferring *hspII* specificity are members of the N compatibility group. Perhaps this plasmid has been derived by recombination between an *fi*⁺ R factor (similar to R444, etc) and an N plasmid. We have observed one example of recombination between an N and an *fi*⁺ R factor (Hedges, 1972) and suggested that chloramphenicol resistance of N plasmids such as R390 (Coetzee *et al.* 1972) may have originated from F-like R factors. W. V. Shaw (personal communication) has found that the chloramphenicol transacetylase produced by R390 is indistinguishable electrophoretically from that produced by *fi*⁺ R factors.

Group N plasmids

Since all previously described plasmids conferring *hspII* specificity are members of the N compatibility group (Datta & Hedges, 1971; Hedges, 1972) the eight *Proteus morganii* R factors R446c, R447a, R448, R454, R461, R462, R465 and R467 all conferring *hspII*, were tested for compatibility with N plasmids. All these R factors displaced and were displaced by known N plasmids.

R447b, conferring resistance to ampicillin and kanamycin, excluded and was excluded by R15 and the two plasmids were incompatible. R447b was therefore an N plasmid (Table 5).

R447a, derived from the same culture, *Proteus morganii* M16, also belonged to compatibility group N (see above). R447a and R477b were incompatible in both *Escherichia coli* K12 and *P. morganii* 2815 (Table 5). With *P. morganii* M16 as donor and *E. coli* K12 J53-1 as recipient, chloramphenicol resistance was transferred with an efficiency greater

Fig. 1. Deletion map of R459. A genetic map of R459 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.

Segregants	A	S	T	C	Su	K	M	<i>hspII</i>
4	+	+	+	+	+	—	+	—
5	—	—	+	—	—	—	—	—
7	+	—	+	+	+	—	+	—
13	—	—	+	+	+	—	—	+

A, Ampicillin resistance; S, streptomycin resistance; T, tetracycline resistance; C, chloramphenicol resistance; Su, sulphonamide resistance; K, kanamycin resistance; M, mercury resistance; *rep*, replication functions; *hspII*, restriction genes; (C Su)^a and (C Su)^b, the two possible locations for these genes.

Table 5. Compatibility properties of R447a and R477b

Donor	Recipient	Antibiotic resistance selected	Efficiency of transfer	Character of recombinants
153 (269N-1)	162-1	K	1×10^{-4}	—
	162-1 (R447a)	K	9×10^{-6}	10 of 10 K ^R C ^S
162-1 (R447b)	153	K	1×10^{-3}	—
	153 (R15)	K	2×10^{-5}	18 of 18 K ^R S ^S
153 (R15)	162-1	S	2×10^{-3}	—
	162-1 (R447b)	S	1×10^{-4}	19 of 19 K ^S S ^R
153 (R447b)	162-1	K	3×10^{-3}	—
	162-1 (R447a)	K	4×10^{-5}	17 of 17 K ^R C ^S
162-1 (R447a)	153	C	6×10^{-4}	—
	153 (R447b)	C	1×10^{-4}	17 of 17 K ^S C ^R
162-1 (R447a)	<i>Proteus morganii</i> 2815	S	1×10^{-5}	—
	<i>P. morganii</i> 2815 (R447b)	S	1×10^{-6}	10 of 10 K ^S C ^R

Abbreviations as in Table 4.

than 10^{-3} , kanamycin resistance with an efficiency of about 10^{-5} /donor cell, but no *E. coli* exconjugant simultaneously resistant to both antibiotics was observed: the efficiency of co-transfer of the two resistance genes was $< 10^{-7}$. In *P.morganii* M16, either the two plasmids existed as a single entity or M16 was unusual in permitting stable coexistence of the two incompatible plasmids. Since attempts to transfer genetic markers from both plasmids to cells of *E. coli* K12 consistently failed one must either reject the notion that R447a and R447b exist as a single structure or postulate that this plasmid is obligatorily broken during transfer. A mutant of *E. coli* K12 in which two incompatible F plasmids can coexist stably has been described (Palchoudhury & Iyer, 1971). It may be that *P.morganii* M16 is a naturally occurring strain with analogous properties.

R447b is the first naturally occurring N plasmid so far described which confers kanamycin resistance. An N plasmid conferring resistance to kanamycin, R269N-I, has however been observed in the laboratory, produced by recombination between an N plasmid and an *fi*⁺ R factor (Hedges, 1972).

Group P plasmid

R446a, conferring resistance to ampicillin, was incompatible with R751, which confers resistance to trimethoprim and belongs to compatibility group P (N. Datta and R. S. Jobanputra, unpublished). R446a could not be tested for compatibility with the prototype P plasmid, RP4 (Datta *et al.* 1971), since no segregant of RP4, lacking ampicillin resistance genes, was available. R446a was compatible with plasmids of all other defined groups.

Group X plasmids

R485 and R487, apparently identical, conferred resistance to sulphonamides only. They were *fi*⁺ and even reduced the transfer rate of R386 (a naturally occurring FI plasmid derepressed for F pilus synthesis and not susceptible to fertility inhibition by F-like R factors). Thus their *fi* character was different from that of normal F-like R factors. They resemble two non-F-like but *fi*⁺ R factors JR66a (Datta & Hedges, 1973) and R62 (Meynell, 1973). Perhaps some non-F-like plasmids have evolved independently a mechanism for reducing the F-piliation of cells carrying F-like plasmids, operating at a different level from the repression mechanism of F-like plasmids. The prevalence of phages which adsorb to F pili (Loeb, 1960; Marvin & Hohn, 1969; Miyake, Shiba, Sakurai & Watanabe, 1969) and the occurrence of plasmids derepressed for F-pilus synthesis (F, *Folac*, ColV and R386) (and of physiological derepression of plasmids whose F pilus production is genetically repressed) makes it an advantage for a non-F-like plasmid to be able to repress F-pilus synthesis by a co-habiting plasmid, so that the *fi*⁺ phenotype is, in effect, a resistance determinant.

R485 and R487 were compatible with all tested plasmids except R6K, the only plasmid previously allocated to group X. R6K exists in *Escherichia coli* K12 in multiple copies (Kontomichalou *et al.* 1970). Since it is possible to eliminate R6K by introduction of an incompatible plasmid, incompatibility cannot be interpreted as operating through the mechanism which imposes stringent control on plasmid replication.

Group M plasmid

R446b, conferring resistance to tetracycline and streptomycin, was compatible with plasmids of all *fi*⁻ groups (R144, R45, R388, Rts1, RP4, R57b, R391, RA1, R300, R483, R6K and R387). It was therefore the prototype of a new compatibility group which we designate M.

Streptomycin-sulphonamide resistance factors

R450, R451, R466 and R496 conferred resistance to streptomycin (but not spectinomycin) and sulphonamides. R466, chosen as apparently typical, was shown to be compatible with all the R factors tested with R460 (see below). R466 resembled R300 in all tested exclusion, compatibility, and drug-resistance characteristics except that it neither excluded R483 nor was excluded by it (see Hedges, Datta & Fleming, 1972).

R460 conferred resistance to spectinomycin, as well as streptomycin and sulphonamides. Thus, presumably, it determines streptomycin/spectinomycin adenylate synthetase (Davies, Brzezinska & Benveniste, 1971). It was compatible with R1drd16 and with members of all *fi*⁻ groups tested (R144, R245, RP4, R388, R401, R57b, R391, RAI, R6K, R387, R483 and R446b). It could not be tested with R300.

Numerous streptomycin-sulphonamide R factors have been reported from other genera (e.g. Lawn *et al.* 1967). None of these is incompatible with any testable plasmid and it is not known whether they constitute a single group. To obtain genetic markers that could be tested we have looked for segregants of R300 and R466 lacking either streptomycin or sulphonamide resistance and have attempted to translocate the kanamycin resistance of Rts1 on to R300 (see Yokata, Kanamaru, Mori & Akiba, 1969) but neither procedure was successful, so we have been unable to determine the compatibilities of these plasmids.

Strains of Proteus morganii not transferring resistances to Escherichia coli

Two strains M52 and M94, both resistant to A, S, T, C, Su and K, were never observed to transfer any of these resistances to *Escherichia coli*. Segregants lacking chloramphenicol resistance were sought. Of sixteen such segregants isolated from M52, thirteen retained all other resistances whilst three retained resistance only to A, T and K. Of four segregants from M94, three had lost only C resistance whilst one retained resistance only to A, T and K. The coincident loss of several resistances implies that the genes responsible were organized into a unit, presumably a non-transmissible plasmid.

CONCLUSIONS

The resistance patterns of the *Proteus morganii* strains, shown in Table 2, have several features of interest. Whereas *P. mirabilis* and *P. rettgeri* are normally resistant to tetracycline, *P. morganii* is naturally sensitive to this drug (Turck, 1967; Tomaschoff, 1969; von Graevenitz & Nourbakhsh, 1972).

Many strains showed resistance to A, S, T, C and Su and a high proportion of the transmissible R factors from *Proteus morganii* (13 of 28) (Table 2) conferred this resistance pattern. A similarly high proportion of R factors from *P. rettgeri* conferred the same resistance pattern (Coetzee *et al.* 1972) but all of these belonged to the N compatibility group whereas the A, S, T, C, Su R factors from *P. morganii* include plasmids of three different compatibility groups, N, FI and FII. So far as we know, plasmids of the latter groups are the first F-like plasmids detected in naturally occurring *Proteus* strains. As was the case with R factors from *P. rettgeri*, none of those from *P. morganii* belonged to the I compatibility group. It now seems that plasmids of this compatibility group are almost exclusive to *Salmonella* (Datta & Hedges, 1971; Yoshikawa *et al.* 1971). The commonest R factors in both *P. rettgeri* and *P. morganii* were N plasmids. In both species the most frequent N plasmids conferred A, S, T, C and Su resistances and *hspII* specificity. Chloramphenicol resistance has not been observed in N plasmids from any other genera.

The set of R factors found in strains of *Proteusmorganii* resembled that of *P. rettgeri* (Coetzee *et al.* 1972) in that N was the most common compatibility group and in the high proportion of plasmids conferring resistance to A, S, T, C and Su. It differed from the latter set in including a high proportion of *fi*⁺ plasmids.

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