R Factors from *Proteus mirabilis* and *P. vulgaris*

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SUMMARY

Eighty-nine R factors were transmitted by conjugation to *Escherichia coli* K12 from isolates of *Proteus hauseri* (*P. mirabilis* plus *P. vulgaris*). More than half were non-selftransmissible. The remainder included plasmids assigned to the previously defined groups FII, A-C complex, J, N and P, as well as some not belonging to any known compatibility groups. R factors from strains isolated in India, Thailand and Japan carried plasmids whose inheritance was extremely unstable in *E. coli* K12. All belonged to a new compatibility group, V.

INTRODUCTION

Swarming *Proteus* isolates are regarded either as constituting a single species (*Proteus hauseri*) (Kauffmann, 1951) or two distinct species (*P. mirabilis* and *P. vulgaris*) (Hauser, 1885). The question of their separability was discussed by Coetzee (1972). Clearly, even if two species are distinguished they are closely related: they share antigens including phage and bacteriocin receptors (Coetzee, 1972); some, at least, of their enzymes are serologically indistinguishable (Guo & Liu, 1965; Smit & Coetzee, 1967); and they show extensive homology in DNA:DNA hybridization tests (Brenner & Falkow, 1971; Coetzee, 1972).

Thus, the group may be considered as a single species or as two closely related species, perhaps constituting a subgenus (Kauffman, 1956). For the purpose of this paper no distinction will be drawn between *P. mirabilis* and *P. vulgaris*, and strains will be referred to as '*P. mirabilis/P. vulgaris*'.

Several reports of natural isolates of *P. mirabilis/P. vulgaris* carrying R factors appeared during the mid 1960s. These are listed by Coetzee (1972). This work was undertaken to compare the R factor set from *P. mirabilis/P. vulgaris* with those of *P. rettgeri* (Coetze, Datta & Hedges, 1972), *P. morganii* (Hedges, Datta, Coetzee & Dennison, 1973) and *Providencia* (Hedges, 1974).

METHODS

Proteus mirabilis/P. vulgaris. About 1000 cultures, donated by laboratories from many different countries, were tested as R factor donors.

Pm13 (Coetzee & Sacks, 1960; Coetzee, 1963) was selected as a standard host for compatibility determination in this species. Two strains were selected for this purpose, Pm13-A which carries a spontaneous mutation to nalidixic acid resistance, and Pm13-B which carries a spontaneous rifampicin resistance mutation.

Escherichia coli K12 strains J53-1 (F^- *pro met nal^R*), J53-2 (F^- *pro met rif^R*) and J62 (F^- *pro his trp lac*) were used (Hedges *et al.* 1973).

Phage was phage PRRI (Olsen & Shipley, 1973).

Table 1. *Standard plasmids*

Plasmid	Com- patibility group	Genetic markers*	Reference
<i>Flac</i>	FI	<i>lac</i> ⁺	Jacob & Adelberg (1959)
R386	FI	T	Dennison (1972)
R1 <i>drd</i> 16	FII	K	Meynell & Datta (1967)
R1-1	FII	A, S, C, Su	Hedges <i>et al.</i> (1973)
R136	FII	T	Lawn, Meynell, Meynell & Datta (1967)
ColB-K98	FIHI	<i>colB</i>	Hedges & Datta (1972)
R124	FIV	T	
Folac	FV	<i>lac</i> ⁺	Datta (1974)
RA1	A	T, Su	Hedges & Datta (1971)
RA1-1b	A	C, Su	Datta & Hedges (1973)
R57b	C	A, C, Gk, Su	Datta & Hedges (1972a)
R57b-1	C	C, Su	
R40a	C	A, K, Su	
R27	H	T	Grindley, Grindley & Anderson (1972)
R726	H	S, T, C, Su	Datta & Olarte (1974)
R726-1	H	S, C, Su	
R64	I α	S, T	Hedges & Datta (1973)
R144	I α	T, K, <i>colIb</i>	
R648	I α	A, S, K	
R483	I β	S, Tp, <i>colIa</i>	
R621a	I γ	T	
R621a-1	I γ	A, T, C, K	Hedges (1974)
R621a-1a	I γ	A, C, K	Segregant of R621a-1
R391	J	K	Coetzee <i>et al.</i> (1972)
R387	K	S, C	Hedges & Datta (1971)
R472	L	A	Hedges, Rodriguez-Lemoine & Datta (1974b)
R831	L	S, K	
R471	L	A, C, K	
R446b	M	S, T	Hedges <i>et al.</i> (1973)
R1P69	M	A, T, K	Witchitz & Gerbaud (1972)
R1P135	M	S, T, G, Su	
R447b	N	A, K	Hedges <i>et al.</i> (1973)
N3T	N	T	Hedges (1972)
R390	N	A, S, T, C, Su	Coetzee <i>et al.</i> (1972)
R16	O	A, S, T, Su	Datta & Olarte (1974)
R16-1	O	S, Su	Hedges (1974)
R724	O	S, T, C, Su	Datta & Olarte (1974)
RP4	P	A, T, K	Datta <i>et al.</i> (1971)
R751	P	Tp	Jobanputra & Datta (1974)
R906	P	A, S, Su	Hedges, Jacob & Smith (1974a)
R478	S	T, C, K	Hedges <i>et al.</i> (1974b)
R477-1	S	S, T, Su	
Rts1	T	K	Coetzee <i>et al.</i> (1972)
R401	T	A, S	
R388	W	Tp, Su	Datta & Hedges (1972b)
R6K	X	A, S	Kontomichalou, Mitani & Clowes (1970)
P1CM	Y	C	Ikeda & Tomizawa (1968); Smith (1972);
ϕ Amp	Y	A	J. R. Scott & R. W. Hedges (unpublished); M. Yarmolinsky (unpublished).

* A = ampicillin, S = streptomycin, T = tetracycline, C = chloramphenicol, K = kanamycin, Gk = gentamicin and kanamycin, G = gentamicin, Su = sulphonamides, Tp = trimethoprim.

Table 2. Drug sensitivity patterns of *P. mirabilis*/*P. vulgaris* isolates

Boston		London		Vancouver		Phenotype resistances*
No.	Percentage	No.	Percentage	No.	Percentage	
118	59	324	81	45	28	Sensitive
10	5	27	7	24	15	A
8	4	25	6	10	6	Su
2	1	6	1	2	1	S
4	2	1	< 1	42	26	K
23	11	7	2	5	3	S, Su
1	< 1	0	0	1	< 1	S, K
1	< 1	0	0	30	19	K, Su
0	0	2	< 1	0	0	A, S
0	0	3	1	0	0	A, K
6	3	5	1	0	0	A, S, Su
20	10	0	0	1	< 1	S, K, Su
1	< 1	0	0	0	0	A, S, C, Su
5	2	0	0	0	0	A, S, K, Su
3	1	0	0	0	0	A, S, C, K, Su
202		400		160	Total	

* For abbreviations see Table 1.

Plasmids are listed in Table 1.

Techniques were as described by Datta *et al.* (1971), Dennison (1972) and Coetzee *et al.* (1972).

RESULTS AND DISCUSSION

Resistance patterns of P. mirabilis/P. vulgaris isolates

Approximately 1000 separate *P. mirabilis*/*P. vulgaris* isolates from a wide range of geographical sources were tested for drug resistance. Almost all *P. mirabilis*/*P. vulgaris* isolates are insensitive to tetracycline and polymixin, so these drugs were not included in the present investigation. The drugs used were ampicillin, streptomycin, chloramphenicol, kanamycin, gentamicin, trimethoprim and sulphadimidine.

Many of the cultures tested were selected by the bacteriologists who isolated the *P. mirabilis*/*P. vulgaris* strains as showing atypical drug resistance patterns and hence likely to carry R factors. Thus, the resistance patterns of the complete collections are not representative of the group. However, large collections of unselected isolates were available from three sources: Dr H. Gaya, Hammersmith Hospital, London; Professor M. Finland, Boston City Hospital, Massachusetts, U.S.A.; and Mrs R. Dobrey and Miss C. Malcolm, Shaughnessy Hospital, Vancouver, British Columbia, Canada. The resistance phenotypes of the cultures from these collections are listed in Table 2.

The marked differences in the resistance patterns of three groups and the differences between these and the patterns reported from by other authors from other sources (e.g. Tomaschoff, 1969; Chai & Soo-Hoo, 1970; Diky, 1972; von Graevenitz & Nourbakhsh, 1972) indicates that, as is the case with *Serratia marcescens* (Hedges *et al.* 1974*b*) the *P. mirabilis*/*P. vulgaris* populations of different hospitals are often distinct. This is confirmed by the differences between the specificities of R factors from the different sources (Table 3).

Table 3. *R factors from P. mirabilis/P. vulgaris*

Plasmid	Source	Resistances†	Compatibility
R759a, R760a, R771a	Prof. M. Finland (Boston)	A, S, K	N
R761a	Prof. M. Finland (Boston)	A, S	N
R762a, R766a, R767a, R772	Prof. M. Finland (Boston)	K(<i>tra</i> ⁻)	‡
R763, R764a, R765	Prof. M. Finland (Boston)	A, S, T	‡
R759b, R760b, R761b, R764b, R771b	Prof. M. Finland (Boston)	S, Su(<i>tra</i> ⁻)	
R762b, R766b, R768, R767b	Prof. M. Finland (Boston)	Su(<i>tra</i> ⁻)	
R393	Dr H. Gaya (London)	S	N
R492, R493	Dr H. Gaya (London)	A(<i>tra</i> ⁻)	
R494, R842, R843	Dr H. Gaya (London)	A, T, K	FII
R490, R491, R774, R841	Dr H. Gaya (London)	Su(<i>tra</i> ⁻)	
R873, R911	Miss C. Malcolm (Vancouver)	Su(<i>tra</i> ⁻)	
R874	Miss C. Malcolm (Vancouver)	S, Su(<i>tra</i> ⁻)	
R908, R909, R910, R921, R941, R942, R943, R944, R945	Miss C. Malcolm (Vancouver)	K(<i>tra</i> ⁻)	
Plac*	Prof. S. Falkow (Seattle)	Su	A-C complex
Rts1*	Dr M. Yoshikawa (Tokyo)	K	T
R22Ka*	Dr P. Kontomichalou (Athens)	A(<i>tra</i> ⁻)	
R22Kb*	Dr P. Kontomichalou (Athens)	S	N
R436a, R437a, R438, R439, R440	Dr E. J. L. Lowbury (Birmingham)	A, T, K	P
R436b, R437b	Dr E. J. L. Lowbury (Birmingham)	S, Su(<i>tra</i> ⁻)	
R468, R469	Dr F. Anderson (London)	K(<i>tra</i> ⁻)	
R470	Dr F. Anderson (London)	Su(<i>tra</i> ⁻)	
R704a	Dr F. Anderson (London)	A, S, C, K, Su	FII
R704b	Dr F. Anderson (London)	T, Su	A-C complex
R959	Dr F. Anderson (London)	A, S, Su(<i>tra</i> ⁻)	
R645	Dr I. Phillips (London)	A, S, Su(<i>tra</i> ⁻)	
R665	Dr E. Tomaschoff (Dusseldorf)	A, Su	A-C complex
R682, R683, R684	Dr E. Tomaschoff (Dusseldorf)	S, Su(<i>tra</i> ⁻)	
R701	Dr F. Sabatelli (Bloomfield, U.S.A.)	A, S, K	N
R702	Dr F. Sabatelli (Bloomfield, U.S.A.)	S, T, K, Su	P
R705, R706	Prof. J. N. Coetzee (Pretoria)	K	J
R753, R754, R755, R756	Prof. S. Falkow (Ching Mai, Thailand)	A, S, C, Su	V
R757a	Prof. S. Falkow (Ching Mai, Thailand)	A, S, Su	V
R757b, R758	Prof. S. Falkow (Ching Mai, Thailand)	S, Su(<i>tra</i> ⁻)	
R769, R770	Dr C. K. J. Paniker (Kerala, India)	S, C, K, Su	V
R866, R867, R868	Dr M. Lachmajer (Gdansk, Poland)	A(<i>tra</i> ⁻)	
R869, R870	Dr M. Lachmajer (Gdansk, Poland)	A, S, Su(<i>tra</i> ⁻)	
R871	Dr J. N. Wilfert (Salt Lake City)	Su	A-C complex
R901, R902, R903	Prof. S. Mitsuhashi (Maebashi, Japan)	S, C, K, Su	V
R904	Prof. S. Mitsuhashi (Maebashi, Japan)	S, Su(<i>tra</i> ⁻)	
R905	Prof. S. Mitsuhashi (Maebashi, Japan)	S, C, Su	V
R940	Miss M. M. Moody (Bethesda, Md., USA)	A, S, T, K, Su	P

* These plasmids were transferred from *P. mirabilis/P. vulgaris* strains before being sent to Hammersmith.

† *tra*⁻ = non selftransmissible (NST). For other abbreviations see Table 1.

‡ See text. No attempt has been made to determine the incompatibility relationships of the NST R factors.

*Transfer of resistance determinants from P. mirabilis/P. vulgaris
to Escherichia coli K12*

Natural isolates of *P. mirabilis/P. vulgaris* showing resistance to any of the antibiotics under investigation were grown with cultures of *E. coli* K12 J53-1 or J53-2 and any R⁺ *E. coli* transipients were purified on MacConkey agar. The transferred resistance factors are listed in Table 3.

Plasmid-carrying strains of J53-1 and J53-2 were incubated with cultures of J62. Plasmids which either were not transferable to J62, or which did transfer to this strain but at least 10% of whose transipients were not capable of transferring the resistances to further *E. coli* K12 strains, were considered to be non-selftransmissible (NST).

One of the most striking features of the R factor set from *P. mirabilis/P. vulgaris* is the remarkably high proportion of NST plasmids; of 89 R factors transferred from *P. mirabilis/P. vulgaris* strains 48 were NST. This contrasts with a single NST plasmid among 105 from *Providencia* isolates (Hedges, 1974), 5 of 28 from *Proteus morganii* (Hedges *et al.* 1973) and none of 28 from *Serratia marcescens* (Hedges *et al.* 1974*b*).

The high proportion of NST plasmids among the set transferred from *P. mirabilis/P. vulgaris* implies the presence of transfer factors (not, themselves, conferring drug resistances) in an appreciable proportion of *P. mirabilis/P. vulgaris* isolates. In conformity with this conclusion, Mitsuhashi (1969) reported the detection of transfer factors in several isolates of *Proteus* (species not recorded).

Numerous authors (for references and discussion see Coetzee, 1972) have concluded that certain R factors, which in *E. coli* exist as single DNA molecules (composites), dissociate in *P. mirabilis/P. vulgaris* strains into transfer factors (Cohen & Miller, 1970) and presumably into non-selftransmissible plasmids, carrying the resistance genes, which replicate under relaxed control. It is tempting to postulate that the NST plasmids and transfer factors are derived from such dissociated R factors. Studies on DNA homologies of such plasmids may provide evidence.

Since investigation of the compatibility properties of NST plasmids has been undertaken by Smith, Humphreys & Anderson (1974), compatibility properties of NST plasmids from *P. mirabilis/P. vulgaris* will be described only in those cases where an NST plasmid showed interaction with self-transmissible factors.

The DNA molecules of several of the NST *P. mirabilis/P. vulgaris* plasmids have been investigated (Barth & Grinter, 1974; S. Falkow, personal communication). They have been found to be small (molecular weight less than 10⁶ daltons), and similar to those described by Milliken & Clowes (1973), Smith *et al.* (1974) and Barth & Grinter (1974).

R22Ka, an NST plasmid determining a β -lactamase very similar to that of the *E. coli* chromosome, is an exception. This is a plasmid of molecular weight 6.05 $\times$ 10⁷ whose replication is subject to stringent control. Its properties have been described by M. M. Bobrovski and others (unpublished).

Properties of the self-transmissible plasmids

R494, R842, R843 and R704a. These plasmids are all members of group FII. The first three are apparently identical R factors, all from Hammersmith Hospital. They have surface exclusion of the same specificity as R444 (Hedges *et al.* 1973). R704a, from The West Middlesex Hospital, London, closely resembles R1.

Table 4. *Reduction of rate of transfer of P group plasmids by R factors of group Ia*

The recipient was always J62.

Donor	Drug used in selection*	Efficiency of transfer
J53(RP4)	T	1×10^{-3}
	K	1×10^{-3}
J53(RP4) (R64)	K	1×10^{-5}
J53(RP4) (R648)	T	2×10^{-5}
J53(R702)	T	7×10^{-4}
	K	9×10^{-4}
J53(R702) (R64)	K	1×10^{-5}
J53(R702) (R648)	T	1×10^{-5}
J53(R940)	K	4×10^{-3}
J53(R940) (R64)	K	7×10^{-5}

* For abbreviations see Table 1.

In that their R factor set includes FII plasmids, *P. mirabilis*/*P. vulgaris* resembles *P. morganii*.

R22Kb, R393, R701, R759a, R760a, R761a and R771a. *Proteus mirabilis*/*P. vulgaris* isolates carrying R factors of group N have been obtained from Athens, London, Boston and New Jersey. Plasmids of this group were, thus, well represented in the R factor set from *P. mirabilis*/*P. vulgaris*. They were abundant among plasmids from *P. rettgeri* and *P. morganii* and were also observed in isolates of *Providencia*.

Plac, R665, R704b and R871. Plasmids of the A-C complex (Hedges, 1974) were transferred from *P. mirabilis*/*P. vulgaris* isolates from Germany, U.K., U.S.A. and Canada. They therefore seem widespread in this species, although much less abundant than in *Providencia*. It may be significant that three of the A-C plasmids came from sources which had supplied *Providencia* isolates carrying A-C plasmids (West Middlesex Hospital, London; Shaughnessy Hospital, Vancouver, Canada; University of Utah Medical Center, Salt Lake City, U.S.A.).

R436a, R437a, R438, R439, R440, R702 and R940. Plasmids of group P were reported in *P. mirabilis*/*P. vulgaris* by Roe, Jones & Lowbury (1971). The properties of R702 have been described by Hedges & Jacob (1974). Although plasmids of this group have been detected in a wide range of genera, *P. mirabilis*/*P. vulgaris* is the first species in which plasmids with three distinct resistance patterns have been observed.

As with RP4 (Datta *et al.* 1971) the efficiency of transfer of these plasmids was reduced by the presence of R64 (or other members of compatibility group Ia) in the donor (Table 4).

Rts1. This plasmid, transferred from an isolate of *P. mirabilis*/*P. vulgaris* (Terawaki, Takayasu & Akiba, 1967), is one of the prototypes of group T. Other plasmids of this group have been detected only in *P. rettgeri* (Coetzee *et al.* 1972).

R705 and R706. These two plasmids, conferring resistance to kanamycin, are apparently identical with R factors such as R391 and R748, transferred from *P. rettgeri* (Coetzee *et al.* 1972) and *Providencia* (Hedges, 1974), respectively. All came from strains isolated in Pretoria. All are provisionally assigned to group J, but because of identity of genetic markers direct testing has not been possible.

R753, R754, R755, R757a, R769, R770, R901, R902, R903 and R905. These R factors were all transmissible into *E. coli* K12 strains but were very unstably inherited, often being lost during replication of an R⁺ *E. coli* culture unless selection against R⁻ segregants was

Table 5. *Compatibility properties of representative plasmids of group V*

The selective medium was MacConkey agar+rifampicin+kanamycin. The donor was Pm13A (R769-1), a segregant of R769 which had lost the chloramphenicol resistance determinant (i.e. it conferred resistance to streptomycin, kanamycin and sulphonamides).

Recipient	Frequency of transfer*	Unselected marker phenotypes†	
		Chromosomal	Plasmid
Pm13-B	10^{-6}	24/24 N ^s	23 A ^s C ^s :1 A ^R C ^R ‡
Pm13-B(R753)	$< 10^{-8}$	24/24 N ^s	23 A ^s C ^s :1 A ^R C ^R ‡
Pm13-B(R905)	$< 10^{-8}$	24/24 N ^s	21 C ^s :3 C ^R ‡

* Frequencies quoted refer to 1 h matings by the method of Dennison (1972). Where no transfer was noted in these matings, overnight mating mixtures were sampled.

† N, nalidixic acid. For other abbreviations see Table 1.

‡ These were stable recombinants conferring all resistances determined by the two parental plasmids.

maintained. On average, an overnight culture in drug-free broth (started from a single colony on agar containing a drug selective for R⁺ clones) contained about 10 % R⁺ cells. It was therefore impossible to test whether these plasmids could be eliminated from an *E. coli* host by entry of an incompatible plasmid. It was, however, possible to demonstrate that plasmids of this group could be transferred into strains of *E. coli* K12, carrying representative plasmids of all compatibility groups so far defined, without elimination of the resident plasmid. The doubles were stable so long as selection was maintained for the unstably inherited plasmid and both plasmids were separately transferable. Thus, these plasmids did not belong to any known compatibility group.

From *E. coli* hosts, the plasmids were transferred to strains of Pm13. In this host (as in the natural *P. mirabilis*/*P. vulgaris* isolates in which they were originally detected) the R factors were stably inherited; overnight cultures in drug-free broth usually contained 99 to 100 % R⁺ cells.

The results of experiments on the conjugal transfer of plasmids between R⁺ Pm13 strains showed that all these plasmids constitute a single compatibility group (named V, in honour of Dr Vyvyan Salisbury). Table 5 shows examples of the results for matings involving three of these R factors, one from Japan, one from Thailand and one (a spontaneous segregant, lacking chloramphenicol resistance, of a plasmid) from India. These crosses show incompatibility, surface exclusion and recombination between plasmids of group V.

Plasmids of group V have not been observed in natural isolates of any species other than *P. mirabilis*/*P. vulgaris*. They are clearly adapted to these species to the extent that they cannot efficiently replicate and undergo segregation in *E. coli* K12. The geographical origins of the V⁺ *P. mirabilis*/*P. vulgaris* are remarkable; all came from Asia (India, Thailand and Japan) and among the strains isolated in this continent V plasmids are the most abundant group. Yet not a single V⁺ *P. mirabilis*/*P. vulgaris* strain was found among the large numbers of isolates from Europe, North America or South Africa. It will be interesting to map the geographical distribution pattern of V plasmid carrying strains.

R762a, R766a, R767a, R772, R763, R764a and R765. R763, R767a and R765 were self-transmissible plasmids conferring resistance to ampicillin, streptomycin and tetracycline. R763, selected as a representative plasmid of this group, was compatible with plasmids of all groups testable (no suitable plasmids of groups FIV, G or X were available).

R762a, R766a, R767a and R772 were NST plasmids conferring resistance to kanamycin. Plasmids of all testable groups (no suitable plasmid of group J was available) were trans-

Fig. 1. Cells from a single colony of J53-2(R762a) (R763) were transferred to prewarmed, drug-free nutrient broth and incubated at 37 °C without shaking. By repeated dilution with fresh, prewarmed broth the concentration of bacterial cells was maintained between 5×10^6 and 5×10^7 /ml for 8 h. After this the culture was allowed to attain stationary phase by incubation overnight without further dilution. The kinetics of increase in cell numbers is shown by curve A and the decline in the proportion of kanamycin-resistant (R762a⁺) cells is shown by curve B. No cells which had lost R763 were detected in these experiments

ferred into J53-2(R762a). None of the 'standard' plasmids caused elimination of R762a, but entry of R763, R764a or R765 did. When R763 was transferred into J53-2(R762a) and transciipients selected on medium containing both ampicillin and kanamycin, the transciipient clones were found to carry both plasmids. These doubles were stable so long as kanamycin was present (i.e. selection for retention of R762a was maintained). During growth in drug-free media, however, R762a was rapidly eliminated. Regardless of the presence of antibiotics, R793 was inherited stably.

Figure 1 shows the kinetics of loss of kanamycin resistance (i.e. R762a) from a double during growth in drug-free nutrient broth. The most striking feature of this process is the fact that no significant loss of the plasmid occurred during the first 3 h of growth (immediately after inoculation of the broth from a single colony on an agar plate containing kanamycin), although exponential growth began after less than 1 h. The fact that more than 30% of the cells in the culture were kanamycin sensitive from the outset shows that loss of R762a had taken place during the development of the colony despite the presence of kanamycin in the plate. So the failure to observe increase in the proportion of R762a⁻ segregants cannot be explained by selective effects of very small amounts of kanamycin carried from the plate to the broth. The most plausible interpretation of these results is that the replication of R762a resembles that of R6K (Kontomichalou *et al.* 1970). In exponentially-growing culture, the plasmid replicates under stringent control; when the culture approaches the stationary phase, the regulation of plasmid replication is relaxed and the number of copies per cell increases. Suppose the tendency of R762a to replicate faster than cell division in cells which are entering stationary phase overcomes the tendency of R763 to eliminate R762a during this phase of growth. Then the cells of the colony transferred to broth would have to undergo several rounds of replication to reduce the abundance of R762a plasmids sufficiently to permit the formation of R762a⁻ segregants.

This model is confirmed by the observation that although R762a⁻ segregants were produced while the culture was regularly diluted so as to keep the cell concentration below 10⁸/ml, very few were formed when the culture was subsequently left to grow to saturation over night.

It is not clear whether these plasmids should be regarded as constituting a new compatibility group. The unilateral elimination of R762a (R763 was never eliminated in any of the experiments) could be explained as an extreme example of hierarchy among pairs of incompatible plasmids (MacFarren & Clowes, 1967; Datta, Hedges, Becker & Davies, 1974). However, the absolute failure to obtain recombinants between R762a and R763 suggests that there is no substantial region of genetic homology between these two plasmids. It therefore seems premature to designate a new compatibility group to contain these plasmids.

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