Host Ranges of R Factors

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SUMMARY

All R factors so far described can be transmitted to *Escherichia coli*. F-like R factors are transmissible to the *Proteus* group but not to the Pseudomonadaceae. Among *fi*⁻ R factors, I-like plasmids are not transferable to *Proteus* although they mediate conjugation, allowing transfer of other plasmids to this group. They are not transmissible to the Pseudomonadaceae. P-group plasmids are transmissible to both taxa. Plasmids of groups N and W are transmissible to *Proteus* (transfer to *Pseudomonas* was not tested because of lack of suitable markers).

INTRODUCTION

R factors have been classified into two categories, *fi*⁺ and *fi*⁻, according to their ability to inhibit F-mediated conjugation (Watanabe *et al.* 1964). *fi*⁺ R factors determine sex pili related to those of F (Lawn *et al.* 1967). *fi*⁻ R factors have been described as having certain properties in common; production of I pili (Novick, 1969), mutual incompatibility (Watanabe *et al.* 1964), ability to restrict phage multiplication (Watanabe *et al.* 1964) and replication in *Proteus mirabilis* as single uniformly sized molecules (Falkow, Citarella, Wohlhieter & Watanabe, 1966; Nisioka, Mitani & Clowes, 1969; Goto, Yoshida, Terawaki, Nakaya & Suzuki, 1970).

Among 26 strains of *Escherichia coli* carrying *fi*⁻ R factors, 20 supported multiplication of phage If1 (whose receptor is the I pilus) while six did not (Lawn *et al.* 1967). For the latter group, therefore, there was no evidence for production of I pili. Three of these (R45, R46 and R199) confer susceptibility to another phage, Ike (Khatoon & Iyer, 1971; and R. V. Iyer, personal communication): this phage does not attack R⁻ bacteria nor strains carrying any I-like R factor so far tested. Although *fi*⁻ R factors show mutual incompatibility (Watanabe *et al.* 1964; Meynell, Meynell & Datta, 1968) exceptions have been reported (Watanabe, 1968; Bouanchaud & Chabbert, 1969; Bannister, 1970; Romero, 1970; Datta *et al.* 1971; Datta & Hedges, 1971; Hedges & Datta, 1971; Khatoon & Iyer, 1971).

Restriction and modification of phages by *fi*⁻ R factors was first described by Watanabe *et al.* (1964) in respect of N₃ and R15. Bannister & Glover (1968) and Bannister (1970*b*) have demonstrated phage restriction patterns with *fi*⁻ factors and at least one *fi*⁺ factor. However, neither Bannister (1969) nor we (unpublished observations) have demonstrated restriction and modification of phage by any I-like plasmids (i.e. those which supported the multiplication of phage If1).

Most *fi*⁺ R factors which have been transferred to *Proteus mirabilis* dissociate, in that host, into DNA molecules with different densities and molecular weights (Falkow *et al.* 1966; Nisioka *et al.* 1969). No such dissociation has been found with *fi*⁻ plasmids.

It happens that those *fi*⁻ R factors which have been studied in *Proteus* (N₃, R15 and

R46) are mutually incompatible but we find that these plasmids do not exclude, and do co-exist stably with typical I-like factors such as R144 and R64. They are members of the N group of plasmids (Datta & Hedges, 1971). The only other *fi*⁻ R factor described as being transmissible to *Proteus*, Rts1 (Goto *et al.* 1970) is not a member of either N or I-like groups (R. W. Hedges, unpublished).

In attempts to transfer R factors (with constitutive pilus synthesis) into strains of the *Proteus* group (i.e. *Proteus* and *Providencia*) F-like factors were introduced but all attempts to transfer I-like factors failed. We are unable to find any report of the establishment of I-like plasmids in any member of the *Proteus* group.

Coetzee (1964) mated Fredericq's strain CA62 with a strain of *Providencia*. CA62 carried plasmids ColI and ColEI and could transfer both to *Escherichia coli* (P. Fredericq, personal communication). Coetzee was unable to demonstrate transfer of the ColI plasmid to *Providencia*, although ColEI [itself probably non-transmissible (Novick, 1969)] was established in *Providencia*. Thus, the conjugation mechanism of the ColI plasmid apparently effected transfer into *Providencia*. In conformation, we find that an I-like R factor, R144drd3, can introduce a non-transmissible resistance plasmid into *Proteus mirabilis*.

We have failed to transfer any R factor other than RP4 into most strains of *Rhizobium* (Datta *et al.* 1971, and R. W. Hedges, unpublished observations) but an exceptional strain, *Rhizobium lupini* 6.2 (De Ley & Rassel, 1965), acted as recipient for F'*lac* and F-like R factors but, as with the *Proteus* group, no transfer of I-like R factors was detected.

METHODS

Bacteria, plasmids and phages are listed in Table 1.

Media. Nutrient broth was Oxoid No. 2 (CM 67); MacConkey agar was Oxoid CM 7b.

Propagation of If1. If1 was added to the culture under test. After 10 min for adsorption, excess phage was neutralized with antiserum. The phage titre was assayed immediately and again after overnight incubation. Negative and positive control cultures (R⁻ or containing I-like R factors) were tested in parallel (Datta *et al.* 1971).

Intergeneric transfer of R factors. Donors were late log phase unshaken broth cultures. Recipients were late log phase shaken cultures. Mixtures were made as in Datta *et al.* (1971), incubated and plated on selective media. In transfer experiments with members of the *Proteus* groups as recipients, mixtures were plated on MacConkey agar (to prevent swarming) with polymixin B sulphate (Burroughs Wellcome & Co.) 25 µg/ml, to prevent growth of the *Escherichia coli* donor, and an appropriate antibiotic (see Table 1) – streptomycin, chloramphenicol or kanamycin, all at 25 µg/ml, or carbenicillin, 100 µg/ml – to prevent growth of R⁻ bacteria. In transfer experiments to *Pseudomonas aeruginosa* PAO2 mixtures were plated on minimal salts agar, supplemented with serine, 20 µg/ml, and with carbenicillin, 1000 µg/ml.

Mobilization. An *Escherichia coli* PROTO(SSu) late log phase shaken broth culture (0.5 ml) was mixed with a late log phase unshaken broth culture of J53 carrying a derepressed R factor (0.5 ml), 2.5 ml fresh broth was added and this mixture was incubated, unshaken, at 37° for 30 min when 1.0 ml of a late log phase shaken broth culture of the recipient was added. These mixtures were incubated overnight, diluted back 1:2 in fresh broth, incubated for 1 h at 37°, diluted and plated on selective media.

When the recipient was J62-1, nalidixic acid, 25 µg/ml, was used for selection; with the *Proteus* group, polymixin B was used as above.

Sulphonamide resistance. The presence of the SSu plasmid was detected by growth on

Table 1.

Bacteria	Reference or source	
<i>Escherichia coli</i> K12		
PROTO	F- <i>lac</i> ⁺ prototrophic	Smith & Gyles (1970)
J53	F- <i>lac</i> ⁺ <i>pro met</i>	Clowes & Hayes (1968)
J62-1	F- <i>lac</i> ⁻ <i>pro his try</i> <i>nal</i> ^r mutant of J62	Clowes & Hayes (1968).
Proteus group		
<i>Proteus mirabilis</i> PM13	.	Coetzee (1972)
<i>P. vulgaris</i> 69	.	Coetzee (1972)
<i>P. morganii</i> 2815	.	Coetzee (1972)
Providence 29	.	Coetzee (1972)
<i>Pseudomonas aeruginosa</i>		
PAO2 FP- <i>ser</i>	.	Holloway (1969)
<i>Rhizobium lupini</i> 62	.	De Ley & Rassel (1965)
<i>Salmonella typhimurium</i> LT2	.	Sanderson (1967)
Plasmids	Resistance markers*	
F' <i>lac</i>	.	Jacob & Adelberg (1959)
R1 <i>drd</i> 19	ASCKSu	Lawn <i>et al.</i> (1967)
R192 <i>drd</i> 7	STCSu	Lawn <i>et al.</i> (1967)
R538.1 <i>drd</i>	SCSu	Romero & Meynell (1969)
RTEM	AS	Datta & Kontomichalou (1965)
R144 <i>drd</i> 3	TK	Lawn <i>et al.</i> (1967)
R163 <i>drd</i> 7	STK	Lawn <i>et al.</i> (1967)
R64 <i>drd</i> 11-RP4 recombinant	AST	Datta <i>et al.</i> (1971)
RP4	ATK	Datta <i>et al.</i> (1971)
R15	SSu	Watanabe <i>et al.</i> (1964)
N3	STS _u	Watanabe <i>et al.</i> (1964)
R46	ASTSu	Datta & Hedges (1971)
S-a	SCKSu	Watanabe <i>et al.</i> (1968)
SSu	SSu	Smith & Linggood (1970)
Phage	Receptor	
If1	I-pili	Meynell & Lawn (1968)

* Resistance markers: A = ampicillin/carbenicillin; S = streptomycin; T = tetracycline; C = chloramphenicol; K = kanamycin; Su = sulphonamides.

streptomycin plates as described above. Colonies from these plates were purified by streaking first on MacConkey agar plus streptomycin, then on plain MacConkey agar. Colonies from these plates were tested for sulphonamide resistance by streaking out for isolated colonies on MacConkey agar (to prevent swarming) with 4 % lysed horse blood [to facilitate sulphonamide activity (Harper & Cawston, 1954)] and sulphadimidine 100 µg/ml.

RESULTS

Propagation of If1

No increase in titre of phage If1 occurred in cultures of J53 carrying the R factors R15, N3 or R46 of the N group (Datta & Hedges, 1971), RP4 of the P group (Datta *et al.* 1971) or S-a of the W group (Hedges & Datta, 1971).

Intergeneric transfer of R factors

Transfer of drd R factors from J53 to the Proteus group. Results of attempts to transfer *drd* F-like and I-like R factors to members of the Proteus group are presented in Table 2.

Transfer of fli⁻ R factors of various compatibility groups to Proteus mirabilis PM13. R factors of

Table 2. *Frequency of transfer of derepressed I-like and F-like R factors to the Proteus group*

Frequencies of transfer calculated per donor cell, expressed to nearest order of magnitude.

Recipient	Donor: J53 carrying R factor				
	R144drd3	R163drd7	R1drd19	R192drd7	R538-1drd
<i>Proteus mirabilis</i> PM13	o*	o*	10 ⁻⁴	10 ⁻⁴	10 ⁻³
<i>P. vulgaris</i> 69	o*	o*	10 ⁻⁴	10 ⁻⁸ †	o*
<i>P. morganii</i> 2815	o*	o*	10 ⁻⁴	10 ⁻³	10 ⁻⁴
Providence 29	o*	o*	10 ⁻⁵	10 ⁻³	10 ⁻⁴
<i>Escherichia coli</i> J62-1	1	> 1	> 1	> 1	> 1

* Not detected, < 10⁻⁸.† *P. vulgaris* (R192drd7) did not express streptomycin resistance, although the determinant was still present and transmissible to K12.Table 3. *Transfer of fi⁻ R factors from Escherichia coli J53 to Proteus mirabilis PM13*

R factor (compatibility group in parenthesis)

Selective agent	R144drd3(I)	R163drd7(I)	R64drd11- RP4(I)	RP4(P)	R46(N)	S-a(W)
	Kan	Kan	Carb	Kan	Str	Str
Frequency of transfer per donor cell to						
PM13	< 10 ⁻⁹	< 10 ⁻⁹	< 10 ⁻⁹	8.2 × 10 ⁻⁷	2.5 × 10 ⁻⁸	1.8 × 10 ⁻⁶
J62-1	> 1	> 1	> 1	1.3 × 10 ⁻³	2.0 × 10 ⁻³	1.1 × 10 ⁻³

Mating mixtures (see Methods) were incubated for 2 h and plated on MacConkey agar containing polymixin sulphate 25 µg/ml and aminoglycoside 25 µg/ml or carbenicillin 100 µg/ml.

groups, P, N and W were transmitted from J53 to PM13 (at frequencies lower than between lines of K12) but I-like R factors were not transmitted to this strain (Table 3).

Transfer of plasmids from J53 to Pseudomonas aeruginosa PAO2. Failure to transfer F-like or I-like factors, repressed or derepressed for pilus synthesis, has previously been reported. The P factor RP4 is transmissible to this strain (Datta *et al.* 1971). A recombinant between RP4 and R64drd11 carrying the penicillinase gene of RP4 and the constitutive I pilus production of R64drd11 was tested for transfer to PAO2, as was RTEM, an R factor whose penicillinase is indistinguishable from that of RP4 (Fullbrook, Elson & Slocombe, 1970, Sykes & Richmond, 1970). No transfer was detected in either case.

Transfer of plasmids to Rhizobium lupini 6.2. F[']lac was transferred from *Salmonella typhimurium* LT2 and the derepressed F-like R factors R1drd19 and R192drd7 were transferred from J53 to *Rhizobium lupini* 6.2, in each case with an efficiency of approximately 10⁻⁷ per donor cell. I-like R factors, R144drd3 and R163drd8 could not be transferred into this *Rhizobium*. Kanamycin was used to select recombinants in all R-factor crosses.

Intergeneric mobilization of SSu plasmid by R144drd3

The SSu plasmid was not self-transmissible to J62-1 or PM13. R1drd16 was transferred efficiently from J53 to J62-1 and to PM13 at a lower, but easily measurable rate. R1drd16 mobilized the SSu plasmid at low frequency to both recipients.

R144drd3 was transferred efficiently from J53 to J62-1, but no transfer was detected to PM13. R144drd3 effected the transfer of the SSu plasmid into both J62-1 and PM13 with comparable efficiency (greater than that of R1drd16).

Table 4. Mobilization of non-transmissible SSu plasmid

Frequencies of transfer are calculated per input resistant cell, i.e. per cell of J53(R+) for kanamycin resistance transfer and per cell of PROTO(SSu) for SSu resistance transfer.

Donor	Recipient			
	<i>Escherichia coli</i> J62-1		<i>Proteus mirabilis</i> PM13	
	Kan	Str, Sul*	Kan	Str, Sul*
PROTO(SSu)	.	Not detected†	.	Not detected†
PROTO(SSu)+J53(R1drd16)	6×10^{-2}	2×10^{-9}	10^{-6}	4×10^8
PROTO(SSu)+J53(R144drd3)	2×10^{-2}	3×10^{-5}	Not detected†	8×10^{-5}

* Primary selection of recombinants was with streptomycin. Sulphonamide resistance was demonstrated subsequently (see Methods).

† $< 10^{-9}$.

Table 5. Intergeneric transmissibility of plasmids

References to information tabulated here are given in the text.

Plasmid classes ...	F	F-like R factors	I-like plasmids R144, R64, ColI	P plasmids RP4	N plasmids R46	W plasmids S-a
Plasmids used ...	F' <i>lac</i>	R _I , R _{I92}				
Host strains						
<i>Escherichia</i> , <i>Salmonella</i> , <i>Shigella</i>	+	+	+	+	+	+
<i>Proteus</i> group	+	+	—	+	+	+
<i>Pseudomonas aeruginosa</i>	—	—	—	+	N.T.*	N.T.*
<i>Rhizobium lupini</i> 6.2	+	+	—	+	N.T.*	N.T.*
Other rhizobia and agrobacteria	—	—	—	+	N.T.*	N.T.*

* Not tested because of lack of suitable markers.

DISCUSSION

Bacterial sex factors, including transmissible R factors, have a wide host range (Harada, Suzuki, Kameda & Mitsuhashi, 1960; Kuwabara, Akiba, Koyama & Arai, 1963; Abe, Goto & Kuwabara, 1966; Knapp & Lebek, 1967) and have been described as promiscuous (Falkow, Haapala & Silver, 1969). There are limits, however, to their ability to infect bacteria even among the Enterobacteriaceae and other aerobic Gram-negative bacilli.

In this paper we show that I-like R factors as well as ColI (Coetzee, 1964) are not transferred to organisms of the *Proteus* group. F'*lac* (Falkow, Wohlhieter, Citarella & Baron, 1964) and F-like R factors (Harada *et al.* 1960) are transmissible to *Proteus*.

Various *fi*⁻ R factors have been reported either to be transmissible to *Proteus* or to have been first detected in naturally occurring *Proteus* strains. Examples are N₃, R₁₅ (Falkow *et al.* 1966) and R₄₆ (Smith, 1969) which have been transferred to *Proteus mirabilis*, and Rts₁ (Terawaki, Takayasu & Akiba, 1967) which came from a strain of *P. vulgaris* and has been studied in *Escherichia coli* and *P. mirabilis* (Goto *et al.* 1970). N₃, R₁₅ and R₄₆ all belong to the N compatibility group (Datta & Hedges, 1971). Rts₁ has not yet been assigned to any compatibility group but does not belong to the I-like group nor to the N or W groups (N. Datta & R. W. Hedges, unpublished results).

We have demonstrated the transfer of R factors of groups P and W into *Proteus mirabilis*. RP₄, a P plasmid from *Pseudomonas aeruginosa* (Datta *et al.* 1971), has an exceptionally wide host range. Only plasmids of this class have been shown to be transmissible between *Escherichia coli* and *P. aeruginosa* (Table 5).

I-like plasmids are the only group of R factors which have not been transferred to *Proteus*. Transferability must depend upon characters of both recipient and plasmid since a particular transfer may be possible for one sex factor but impossible for another. Thus, I-like plasmids, transmitted efficiently between *Escherichia coli* strains, are incapable of transfer to *Proteus mirabilis*. An alternative explanation for our failure to detect I-like plasmids in *Proteus* group hosts is that they are unable to express their genetic markers in these hosts. This implies that each testable genetic function (various drug-resistance markers and colicine production) is unable to be expressed, but all the genes necessary for establishment, maintenance and replication are expressed. This explanation is inherently unlikely and is disproved by the case of the recombinant R64drd11-RP4, which behaves in every way like an I plasmid incorporating the penicillinase gene (but no other character) of RP4. RP4 expresses this gene in both *Proteus* and *Pseudomonas* (Datta *et al.* 1971).

Clark (1967), in describing the isolation of *rec*⁻ mutants of *Escherichia coli* K12, refers to the occurrence of mutants 'incapable of mothering merodiploid progeny when crossed with an F' strain'. These mutants may have failed either in the transfer process or in the establishment ('replication') of the plasmid. In the case of I-like plasmids and *Proteus* it must be the latter function which is deficient, since I-mediated genetic transfer of the non-transmissible plasmids SSu and ColEI has been demonstrated.

In the other instances, in Table 5, of failure to accept R factors (e.g. F-like plasmids by *Pseudomonas aeruginosa*) we have no information on the nature of the deficiency.

That establishment depends on genetic characters of the transferred plasmid as well as of the recipient is indicated by properties of some transfer-defective (*traI*) mutants of an F'*lac* episome, which determine apparently normal pilus formation (Willetts, 1971). F'*lac* episomes carrying *traI* mutations are not self-transferring between strains of *Escherichia coli* K12 but can mobilize a non-transmissible ColEI (N. S. Willetts, personal communication) and this seems analogous to our own findings with I-like factors and *Proteus*. The plasmids, in both cases, seem able to bring about conjugation but not to establish themselves in the recipient. It might be possible to isolate mutants of the recipient in which the plasmid could express its establishment functions. It seems likely that *Proteus mirabilis* is unable to recognize genetic sequences which act as promoters in *E. coli* (Dale & Smith, 1971); perhaps one or more operons of the I-like plasmid cannot be transcribed (or translated) in *P. mirabilis*.

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