Plasmids Capable of Transfer and Chromosome Mobilization in *Acinetobacter calcoaceticus*

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INTRODUCTION

The P incompatibility group plasmid RP4 (Datta *et al.*, 1971) was recently shown to mediate conjugation in *Acinetobacter calcoaceticus* (Towner & Vivian, 1976*a*). RP4 is probably identical to the plasmids designated R1822, R18 and RP1 (Holloway & Richmond, 1973). Recombinants are formed in *A. calcoaceticus* at a frequency of 10^{-6} to 10^{-8} per recipient cell. In spite of this relatively low frequency of gene transfer, the system has already provided meaningful data on linkage relationships in this species, including the demonstration that the linkage map of *A. calcoaceticus* is circular (Towner & Vivian, 1976*b*). However, the fact that recombination only occurs on a solid surface means that genetic analysis is time consuming and would be greatly facilitated if the frequency of recombinant formation could be increased.

In *Pseudomonas aeruginosa*, the plasmid R1822 (alias RP4) was inefficient in promoting chromosome mobilization (Stanisich & Holloway, 1971), while closely related plasmids, for example R9169 (alias R91), mobilized the chromosome efficiently. Other plasmids of the P incompatibility group might, therefore, be more efficient than RP4 in mediating chromosome transfer in *A. calcoaceticus*.

In this paper we report on the ability of a range of P incompatibility group plasmids to transfer chromosomal genes in *A. calcoaceticus*. Experiments were also performed to test for transfer to *A. calcoaceticus* of members of the C, F, I, and W incompatibility groups from *Escherichia coli* and the P2 incompatibility group from *P. aeruginosa* in the hope that other groups of plasmids might also act as efficient sex factors in this species.

METHODS

Bacterial strains. The strains of *E. coli* K12 (Clowes & Hayes, 1968) used were: J53 (F^{-} , *pro*, *met*); J53.1 *nal^r* mutant of J53); J53.2 (*rif^r* mutant of J53); J62 (F^{-} , *pro*, *his*, *trp*, *lac*); J62.1 (*nal^r* mutant of J62); and C600 (F^{-} , *thr*, *leu*, *thy*, *lac*). The *P. aeruginosa* strain used was PAO1 (FP^{-} , prototroph) (Holloway, 1969). The strains of *A. calcoaceticus* EBF65/65 (Towner & Vivian, 1976*a*) used were: C417 (*trp*, *str^r* [high-level]); C426 (*trp*, *his*); and C478 (*phe*, *thi*).

Plasmids used are listed in Table 1.

Media used were as described previously (Towner & Vivian, 1976*a*).

Antibiotics were obtained from Sigma (except where indicated) and were added to media as freshly prepared solutions at the following final concentrations ($\mu\text{g ml}^{-1}$): tetracycline hydrochloride, 5; sodium ampicillin (Beecham Research Laboratories), 500; trimethoprim, 500; sulfadiazine, 500; gentamicin sulphate, 10; and dihydrostreptomycin sulphate, 2000.

Millipore mating technique. This technique was used for all matings as described by Towner & Vivian (1976*a*). After resuspension from the filter the cells were sedimented and resuspended in the same volume of quarter-strength Ringer's solution before plating out. This procedure eliminated any background growth due to carry-over of auxotrophic requirements.

Test for plasmid stability. The stabilities of the different plasmids transferred to *A. calcoaceticus* were compared by picking a single colony from antibiotic-containing medium into nutrient broth and incubating for 24 h at 28 °C on a rotary shaker. The resulting suspension was plated out for single colonies on minimal medium and then replicated to plates of minimal medium containing the appropriate selective antibiotic.

Table 1. *Plasmids*

Plasmid*	Incompatibility group	Resistance markers†	Reference
RP4	P	A,Tc,K	Datta <i>et al.</i> (1971)
R751	P	Tp	Jobanputra & Datta (1974)
R1033	P	A,Tc,K,Ge,S,Su,C	Smith <i>et al.</i> (1975)
R91	P	A,Tc,K	Stanisich & Holloway (1971)
R702	P	Tc,K,S,Su	Hedges & Jacob (1974)
R _{GN823}	P	A,Tc,K,S,Su,C	Sawai <i>et al.</i> (1970)
R68.45	P	A,Tc,K	Haas & Holloway (1976)
R388	W	Su,Tp	Datta & Hedges (1972 <i>a</i>)
S-a	W	K,Su,S,C	Watanabe, Furuse & Sakaizumi (1968)
R40a	C	A,K,Su	Datta & Hedges (1972 <i>b</i>)
R57b	C	A,Ge,Su,C	Datta & Hedges (1972 <i>b</i>)
RPL11	P ₂	A,Tc,K,Ge,S,Su,C	Korfhagen & Loper (1975)
R1-16	F _{II}	A,K,S,Su,C	Meynell & Cooke (1969)
R64	I _α	Tc,S	Lawn <i>et al.</i> (1967)

*RP4 and R68.45 were obtained from Dr J. E. Beringer, John Innes Institute, Norwich. All other plasmids were obtained from Dr R. W. Hedges, Royal Postgraduate Medical School, London W12.

†A, ampicillin; S, streptomycin; Tc, tetracycline; K, kanamycin; C, chloramphenicol; Su, sulphonamides; Tp, trimethoprim; Ge, gentamicin.

RESULTS

Ability of plasmids to transfer to A. calcoaceticus

The P incompatibility group plasmids used in this study were all transferable from *E. coli* K12 to *A. calcoaceticus* using the Millipore mating method and selecting for acquisition of the indicated antibiotic resistance marker (Table 2). Other antibiotic resistance markers carried by these plasmids (except for kanamycin resistance) were also expressed in *A. calcoaceticus* and levels of resistance were comparable to those found in the R⁺ *E. coli* donor. It was not possible to measure accurately the level of resistance conferred by the kanamycin plasmid marker because *A. calcoaceticus* EBF65/65 already carries a chromosomal marker specifying resistance to this antibiotic.

Crosses were also performed in an attempt to detect transfer of the C group plasmids R40a and R57b, the F-like plasmid R1-16, the I-like plasmid R64 and the W group plasmids R388 and S-a from their *E. coli* hosts to *A. calcoaceticus*. Jacoby (1975) has reported that C and W plasmids are transferable from *E. coli* to *P. aeruginosa*. Transfer of R1-16 and R64 was tested because these plasmids transfer at high frequency between *E. coli* strains and have been used to mediate chromosomal transfer between members of the Enterobacteriaceae (Meynell & Cooke, 1969; Dixon & Postgate, 1971). However none of these plasmids was transferable to *A. calcoaceticus* at a detectable level (> 1 in 10⁹).

RPL11 is a plasmid from *P. aeruginosa* belonging to incompatibility group P₂ (Korfhagen & Loper, 1975). Plasmids of this incompatibility group can be transferred between different species of *Pseudomonas* but cannot be transferred to members of the Enterobacteriaceae (Shahrabadi, Bryan & Van Den Elzen, 1975). The ability of RPL11 to transfer to *A. calcoaceticus* was tested by mating *P. aeruginosa* PAOI(RPL11) with the streptomycin-resistant (high-level) strain C417, and then selecting for tetracycline-resistant and streptomycin-resistant (high-level), tryptophan-requiring cells. Transfer was not observed.

Transfer of P incompatibility group plasmids and chromosomal genes between strains of A. calcoaceticus

Mating experiments were performed between auxotrophic mutants of *A. calcoaceticus* to determine the frequency of transfer of P incompatibility group plasmids in *A. calcoaceticus* and also the frequency of transfer of chromosomal genes (Table 3). Most of these plasmids promoted chromosomal gene transfer in *A. calcoaceticus* at detectable frequencies. The

Table 2. *Transfer of P incompatibility group plasmids between E. coli and A. calcoaceticus*

Escherichia coli donor strains were mated with c478 (*phe thi*) using the Millipore mating technique as described in Methods. Selection was made for c478 cells which had acquired the indicated plasmid antibiotic resistance marker. Frequencies of transfer are expressed as R⁺ recipients per recipient. The abbreviations are defined in Table 1.

Donor	Selected marker	Mean frequency of transfer
J53(RP4)	Tc	1.0×10^{-5}
J62(R751)	Tp	3.2×10^{-8}
J53.2(R1033)	Tc	3.4×10^{-6}
J53.2(R91)	Tc	6.2×10^{-6}
J62(R702)	Tc	3.9×10^{-5}
J53.2(R _{GN823})	Tc	5.0×10^{-4}
J53(R68.45)	Tc	1.1×10^{-6}

Table 3. *Transfer of A. calcoaceticus chromosomal genes by P incompatibility group plasmids*

Strain c478 (*phe thi*) carrying the different plasmids was mated with strain c426 (*his trp*) using the Millipore mating technique. Selection was made against both auxotrophic markers contained in the donor and for the acquisition of either *his*⁺ or *trp*⁺ by the recipient. Frequencies of plasmid transfer are expressed as R⁺ recipients per recipient.

Plasmid	Frequency of plasmid transfer	No. of recombinants per 10 ⁸ recipient cells	
		<i>his</i> ⁺	<i>trp</i> ⁺
RP4	2.0×10^{-5}	146	44
R751	6.3×10^{-5}	45	28
R1033	8.2×10^{-6}	17	17
R91	8.8×10^{-6}	0	0
R702	2.5×10^{-3}	57	64
R _{GN823}	7.6×10^{-3}	229	89
R68.45	5.3×10^{-7}	0	0

number of recombinants obtained varied slightly between experiments but, in general, for each individual plasmid the frequency of chromosomal gene transfer was enhanced by an increased frequency of independent plasmid transfer.

The relative frequencies of the two types of recombinants varied for the different plasmids used (Table 3). Since RP4 donates the chromosome from at least two different sites (Towner & Vivian, 1976b), some of these plasmids may be of value in mapping studies if, as seems possible, they have different donor properties.

Of particular interest is the result obtained with R68.45. This plasmid is a variant of R68 and was reported to be efficient in transferring chromosomal genes of both *P. aeruginosa* (Haas & Holloway, 1976) and *Rhizobium leguminosarum* (Beringer & Hopwood, 1976). However, in *A. calcoaceticus* R68.45 was only transferred at the comparatively low frequency of 5.3×10^{-7} and transfer of chromosomal genes was not detected.

Stability of P incompatibility group plasmids in A. calcoaceticus

The frequency of plasmid transfer might be governed by the instability of the plasmid in the donor strain. Towner & Vivian (1976a) showed that RP4 was slightly unstable in *A. calcoaceticus*. The stabilities of the different P group plasmids used in this study were therefore compared. However, although all the plasmids tested were slightly unstable in *A. calcoaceticus* (normally about 5% instability), the degree of stability of each individual

plasmid varied between experiments and there was no correlation between this and the frequency of plasmid transfer. The factors controlling P plasmid stability in *A. calcoaceticus* remain to be determined.

DISCUSSION

The object of this investigation was to examine a variety of plasmids belonging to different incompatibility groups in an attempt to find one which would transfer the *A. calcoaceticus* chromosome at a higher frequency than the original system using RP4. However, of the plasmids tested, only members of the P incompatibility group were capable of transfer to *A. calcoaceticus*, and none of these showed great variation from the levels of chromosome transfer obtained with RP4. Indeed some plasmids (R91 and R68.45) which were more efficient than RP4 in promoting chromosome transfer in *P. aeruginosa* (Stanisich & Holloway, 1971; Haas & Holloway, 1976), were unable to do so at a detectable level in *A. calcoaceticus*. As R68.45 is also an efficient promotor of chromosome mobilization in *R. leguminosarum* (Beringer & Hopwood, 1976), it seems that the ability of P incompatibility group plasmids to mobilize chromosomal markers may be species specific.

The frequency of chromosome transfer using a particular plasmid was related to the frequency of independent plasmid transfer, but varied considerably with the mobilizing plasmid (Table 3). One possible way to increase the frequency of plasmid transfer (and hence the frequency of chromosome mobilization) would be to isolate a derepressed mutant. However Dennison & Baumberg (1975), working with *E. coli*, suggested that P incompatibility group plasmids were already naturally derepressed, but that this was masked by the incompetence in liquid matings of donors carrying them. In *A. calcoaceticus*, RP4 transfer occurs at a low frequency in broth matings and is increased if matings are carried out on a solid surface, while chromosome transfer can only be detected on a solid surface (Towner & Vivian, 1976*b*). A similar situation therefore appears to exist. Other attempts to increase the frequency of recombinant formation using ultraviolet light and mitomycin C at the levels of survival used by Evenchik, Stacey & Hayes (1969) had no detectable effect in *A. calcoaceticus* (K. J. Towner, unpublished results).

Thus, it is probable that any future attempt to produce a high frequency conjugation system in *A. calcoaceticus* would benefit from a search for any self-transmissible plasmids that might already be naturally present. The demonstration that some strains of *Bacterium anitratum* (*A. calcoaceticus*) contain extrachromosomal circular DNA molecules (Christiansen *et al.*, 1973) suggests that such a search could be profitable.

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