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RP4 Fertility Variants in *Acinetobacter calcoaceticus*

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SUMMARY

The resistance plasmid RP4 can be used to mediate conjugation in *Acinetobacter calcoaceticus*. Transfer of chromosomal genes occurs only on solid surfaces and not in liquid matings. Two RP4-carrying donor strains which donate the chromosome in different directions from different origins have been isolated. As a result it is possible to demonstrate that the linkage map of *A. calcoaceticus* is circular.

The fate of RP4 in recombinants was examined. Although frequent loss of the complete plasmid was detected, segregation of plasmid antibiotic resistances was not observed.

A possible mechanism by which RP4 could interact with the *A. calcoaceticus* chromosome is discussed.

1. INTRODUCTION

The resistance plasmid RP4 is the prototype of the P incompatibility group (Datta *et al.* 1971). Although not reported in either *Escherichia coli* or *Pseudomonas aeruginosa*, RP4 has recently been shown to mediate conjugation in *Acinetobacter calcoaceticus* (Towner & Vivian, 1976). Recombinants are formed 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 provided meaningful data on linkage relationships in this species, with all the genetic markers so far isolated being shown to map on a single linkage group.

Linkage relationships amongst the more distal markers are difficult to investigate owing to the rarity of these classes of recombinants. Recent reports of fertility variants in plasmids closely related to RP4 (Olsen & Gonzalez, 1974; Unger, Sokatch & Martin, 1975; Haas & Holloway, 1976) therefore led us to look for RP4-carrying strains of *A. calcoaceticus* with different donor characteristics.

In this paper we report on the isolation of a new fertility variant which donates the chromosome from a different origin in a different direction to that originally reported (Towner & Vivian, 1976). The fate of the plasmid in recombinants and the importance of the conditions of mating are also examined.

2. MATERIALS AND METHODS

(*1*) *Strains and media*

Bacterial strains used are listed in Table 1. Media and mating techniques were as described previously (Towner & Vivian, 1976). Following mating on Millipore

filters the cells were washed at least once in quarter-strength Ringer's solution before plating out. This enabled selection to be made for the more leaky markers.

(ii) *Isolation of donor strains*

Donor strains of *A. calcoaceticus* were constructed by mating directly with *E. coli* K12 J53 (RP4). Selection was made for *A. calcoaceticus* colonies which had acquired resistance to tetracycline. Colonies which appeared were then purified by streaking on tetracycline-containing medium.

(iii) *Maintenance of donor cultures*

RP4 is lost spontaneously from *A. calcoaceticus* at a frequency of up to 2% following short periods of incubation on non-selective media, and more readily following longer periods of incubation (Towner & Vivian, 1976). Donor strains were therefore maintained on plates of minimal medium supplemented with 5 μg/ml tetracycline HCl (Sigma) kept at 4 °C. Single colonies were then used to produce exponential cultures in aerated nutrient broth. This procedure has been found to be consistently successful in producing an efficient donor culture of any *A. calcoaceticus* strain carrying RP4.

Table 1. *Bacterial strains used*

	Auxotrophs of *A. calcoaceticus* EBF65/65
Strain no.	Markers
C48	*ile-1 met-1*
C426	*trp-2 his-1*
C467	*ile-1 cys-3*
C469	*ile-1 trp-2 his-1*
C471	*cys-3 his-1*
C478	*phe-1 thi-2*
C480	*trp-2 ile-1*
C486	*his-1 ile-1*
C493	*phe-1 trp-2*
C494	*thi-2 trp-2*
C495	*phe-1 his-1*
C497	*ile-I thi-2*
E. coli K12 J53 (RP4)	F$^-$ *pro met nal*^{r}

3. RESULTS

(i) *Importance of mating conditions*

We have found the conditions of mating to be extremely important in RP4-mediated conjugation. Dennison & Baumberg (1975) investigated the transfer of RP4 in *E. coli* and showed that mating on a solid surface greatly increased the efficiency of DNA transfer. Although we have previously demonstrated transfer of RP4 from *E. coli* to *A. calcoaceticus* using a plate-mating technique (Towner & Vivian, 1976), we have been unable to observe any transfer of chromosomal genes between strains of *A. calcoaceticus* by this method. This suggests either that the

mating unions are extremely fragile or that there is a low efficiency of pair formation, transfer of chromosomal DNA only occurring when the cells are forced into close contact in the pores of a Millipore filter. Previous failures to observe chromosomal gene transfer by RP4 in other genera may therefore be due to physical rather than genetical factors.

(ii) *Selection for a new donor type*

Our previous work with *A. calcoaceticus* demonstrated that the ability to donate from a particular origin was a property of RP4 rather than the host strain. This was confirmed by transferring RP4 from the original donor strain to other *A. calcoaceticus* auxotrophs which then acquired identical donor characteristics.

The original type of donor, now designated DO, donated *his-1* as a proximal marker and *ile-1* as a distal marker (Towner & Vivian, 1976). As the primary object of this work was to attempt to isolate a donor which would transfer the distal markers at a higher frequency, *E. coli* K12 J53 (RP4) was crossed with C426 (*trp his*) and selection made as described in Methods for C426 colonies which had acquired RP4. These potential new donors were then crossed with C48 (*ile met*), selection being made for the transfer of the distal *ile*$^+$ marker from C426. The lack of a plate-mating technique sensitive enough to detect chromosomal recombination meant that each isolate had to be tested individually on a Millipore filter. One particular isolate (of a donor type now designated D5) was found to donate *ile*$^+$ at a frequency up to ten times that of the original donor DO. All other isolates tested have so far been found to belong to either the DO or D5 types.

(iii) *Different patterns of donor ability*

Crosses were performed to compare the different frequencies of the various classes of recombinants using the two donor types. These results are presented in Table 2 and confirm that D5 donates the DO distal markers at a higher frequency than DO. Some occasional variation has been found in recombination frequencies (probably as a result of physical factors), but relative frequencies are reproducible in a particular mating.

As it is not possible to deduce the order of the various markers simply from the different frequencies of recovery, we next attempted to determine linkage relationships between the markers studied using the two different donor types. The most striking feature of the results obtained (Table 3) is that with D5, *his-1* is shown to be linked to *thi-1* and *ile-1* while at the same time showing only slight linkage to *trp-2*. Co-transfer of *trp-2* with other markers also gives strikingly different results to those obtained using DO.

Considering all the evidence we conclude that D5 donates the chromosome predominantly in one direction from an origin situated between *thi-2* and *trp*-2 (with *trp-2* as a distal marker), while DO donates predominantly in the opposite direction from an origin situated between *ile-1* and *his-1* (with *ile-1* as a distal marker). Combining the two sets of linkage data it is also possible to show that

A. calcoaceticus has a circular linkage map with the markers and origins arranged as shown in Fig. 1.

(iv) *Stability of donor characteristics*

A number of cycles of mating have been performed between different *A. calcoaceticus* auxotrophs in which recombinants from one mating have been used as new donors in another. The two types of donor characteristics have remained stable during these matings with no change in donor type being observed. It therefore appears that the initial interaction of RP4 with the *A. calcoaceticus* chromosome must largely determine its donor origin when transferred to a new donor stain, although there remains the possibility that these different properties of RP4 were present before any chromosome mobilization event had occurred.

Table 2. *Relative frequencies of different recombinant classes*

Cross		Marker selected	Average no. of recombinants/10^8 recipient cells	
Donor	Recipient	from donor	D0	D5
C48 (RP4)	C426	*his-1*+	146	22
	C478	*thi-2*+	100	41
	C426	*trp-2*+	44	9
	C478	*phe-1*+	12	13
C426 (RP4)	C48	*met-1*+	4	5
	C467	*cys-3*+	2	18
	C467	*ile-1*+	2	18

Table 3. *Linkage between markers*

Marker selected from donor	Unselected marker	% Co-transfer	
		D0	D5
his-1	*thi-2*	86	90
	ile-1	3	29
	trp-2	24	2
	met-1	1	4
thi-2	*his-1*	82	64
	ile-1	1	10
trp-2	*his-1*	57	3
	ile-1	3	0
phe-1	*met-1*	17	42
	ile-1	2	14
cys-3	*trp-2*	73	23
	ile-1	75	91
ile-1	*his-1*	40	54
	trp-2	78	11
	cys-3	71	55

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(v) *Fate of RP4 in recombinants*

Recombinants were examined for the presence of RP4 on the basis of tetracycline resistance by replicating on to plates of suitably supplemented minimal medium containing 5 μg/ml tetracycline HCl. Of a total of 1729 recombinants examined only 1033 (59 %) retained resistance to tetracycline. The presence of the ampicillin marker was also tested for in a similar fashion, but was found to be difficult to quantify owing to a variable delay in expression.

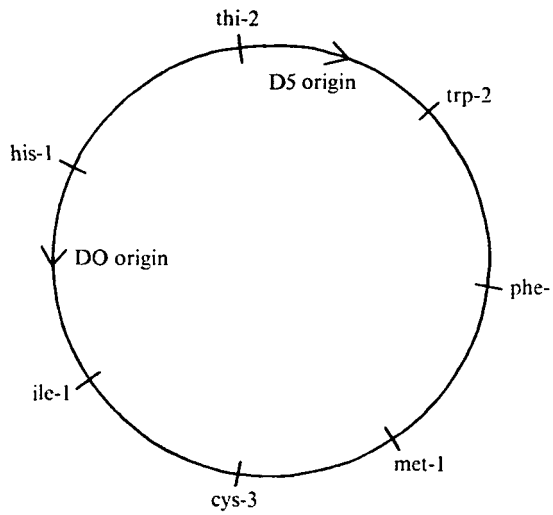

Fig. 1. Linkage map of *Acinetobacter calcoaceticus*. Markers are arbitrarily spaced.

We have been unable to detect any correlation between different recombinant classes and the presence or lack of the plasmid. Lack of the plasmid also results in the loss of chromosome donor ability by the recombinants.

4. DISCUSSION

Haas & Holloway (1976) reported that the related plasmid R68.45 became unstable after it had been involved in the transfer of the *P. aeruginosa* chromosome, resulting in partial or complete loss of the plasmid. With RP4 we have not detected any loss of resistance markers in *A. calcoaceticus* unless involving the loss of the entire plasmid. Some recombinants appeared at first to have lost ampicillin resistance while retaining the resistance to tetracycline. However, during the subsequent purification procedures the ampicillin resistance became expressed. This variable expression phenomenon has been described previously for the P group plasmid R1822 (Olsen & Shipley, 1973). It therefore appears from our results that although RP4 becomes more unstable and is readily lost following chromosome transfer, loss of the ampicillin or tetracycline markers could only occur as a very rare event.

Very little is known about any possible physical association between RP4 and

the *A. calcoaceticus* chromosome. However, there is much evidence that plasmids of the P incompatibility group have the capacity to interact with the chromosome of enterobacteria (Hedges & Jacob, 1974; Olsen & Gonzalez, 1974; Unger, Sokatch & Martin, 1975; Dixon, Cannon & Kondorosi, 1976). The F factor of *E. coli* mobilizes the chromosome owing in part at least to the interaction of insertion sequences located both on the plasmid and the chromosome (Hu, Ohtsubo & Davidson, 1975). Such interaction can occur at a number of different sites (Saedler & Heiss, 1973). In view of our observation that chromosome transfer in *A. calcoaceticus* can occur from at least two distinct origins, the possibility of regions of genetic homology between RP4 and the *A. calcoaceticus* chromosome would be worthy of investigation.

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