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Relationship Between R and FP Plasmids in *Pseudomonas aeruginosa*

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The plasmid FP110 possessing chromosome mobilizing ability for *Pseudomonas aeruginosa* but carrying no determinants for antibiotic resistance, is found to be related by incompatibility, entry exclusion, and other criteria to the independently isolated R plasmids R18-1 and R56Be which carry resistance determinants for carbenicillin. The frequency of FP plasmid appearance in clinical isolates of *P. aeruginosa* suggests the possibility that they may be a source of R plasmids in this bacterium.

One of the features of recent work with *Pseudomonas aeruginosa* is the finding that plasmids are frequently a component of the genome of this organism. Studies from our laboratory (5, 14) and data presented in this paper have shown that 20 to 30% of strains of *P. aeruginosa* isolated both in Australia and Japan carry FP plasmids, distinguished by their ability to promote the transfer of host chromosome. The frequency of transmissible R plasmids is much less (2) and was only 2% in a survey of nearly 1,000 strains of *P. aeruginosa* in Australia. More recent work (Sinclair, Morgan, and Holloway, unpublished data) shows that nontransmissible plasmids may be more frequent than transmissible plasmids in this organism, a view supported by the data of Mitsuhashi (*in* S. Mitsuhashi, L. Rosival, and V. Krcmery (ed.), *Fourth International Symposium on Antibiotic Resistance,* in press). In view of the frequency of FP plasmids, it is possible that they have an evolutionary relationship to R plasmids. To date, little work has been done to classify the FP plasmids, although recently it has been shown by entry exclusion tests that up to five groups of FP plasmids can be identified (J. Finger and V. Krishnapillai, personal communication).

This paper reports the isolation of a new FP plasmid, FP110, and demonstrates its relationship to two previously isolated and characterized R plasmids, R18-1, which is thought to be identical to RP1-1 (6), and R56Be (10).

(A preliminary account of these results was given at the Fourth International Symposium on Antibiotic Resistance, Smolenice, June 1979.)

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The bacterial strains and plasmids used in this study are shown in Table 1. Phage F116L (8) was used for transductions. Phages B39, G101, and E79 (9) were used to test for their multiplication in *P. aeruginosa* strains carrying plasmids. Phage PRD1 (12) was used for isolating transfer-deficient mutants of R18 and RP4::Tn7.

Media. Nutrient broth (NB), nutrient agar (NA), and minimal medium (MM) have been described previously (15). The antibiotics carbenicillin (as Pyopen, Beecham), rifampin (as Rifadin, Lepetit), streptomycin (Sigma Chemical Co.) and trimethoprim (Sigma) were added at the concentration indicated in the text.

Amino acids, purines, and pyrimidines were added to MM as required to a final concentration of 1 mM, except for isoleucine, which was used at 0.5 mM.

Plate matings and transductional procedures. Plate matings and transductional procedures were as previously described (16).

RESULTS

Isolation of FP110. A survey was undertaken to identify FP plasmids in hospital isolates of *P. aeruginosa.* The techniques used were essentially those of Pemberton and Holloway (14), and the aim was to find plasmids with chromosome-mobilizing properties different from that of FP2, so that mapping of the whole *P. aeruginosa* PAO chromosome could be achieved. In the present study, 780 isolates of *P. aeruginosa* were screened for the ability to transfer host chromosome, and 148 were found to have chromosome mobilizing ability (Cma). One of these was selected for further study and carried the plasmid FP110. This plasmid has properties of chromosome transfer considerably different from FP2 which will be reported in detail elsewhere (P. Royle and B. Holloway, manuscript in preparation). FP110 has a major origin of chromosome transfer at about 25 min on the PAO chromosome map, from which it transfers chromosome in a direction opposite to that found with FP2.

Transpositions involving FP110. FP110 does not carry any resistance determinants. To allow its selection in transfer experiments, derivatives of FP110 were obtained carrying the anti-

TABLE 1. *Strains of P. aeruginosa and plasmids*[a]

Bacterial strain	Genotype or phenotype	Reference
P. aeruginosa		
PAO2	*ser-3*	13
PAO8	*met-28 ilv-202 str-1*	7
PAO1670	*pur-136 leu-8 chl-3 rif-1*	3
E. coli		
W3110T$^-$	*deoC*	1
Plasmid		
FP110	Cma, Phi (B39), Tra	This paper
R18	Cb, Km/Nm, Tc, Phi (G101), Tra	6
pMO161	Cb, Km/Nm, Tc, Phi (G101), Tra$^-$	This paper (derived from R18)
R18-1	Cb, Phi (B39), Tra	6
R56Be	Cb, Phi (B39), Tra	10
RP4::Tn*C2*	Cb, Km/Nm, Tc, Tp, Sm, Phi (G101), Tra (Tn*C2* has been renamed Tn*7*)	1
pMO171	Cb, Km/Nm, Tc, Tp, Sm, Phi (G101), Tra$^-$	This paper (derived from RP4::Tn*C2*)

[a] Genotype symbols designate the following: *chl*, chloramphenicol resistance; *deoC*, phosphodeoxyriboaldolase; *ilv*, isoleucine plus valine; *leu*, leucine; *met*, methionine; *pur*, adenine; *rif*, rifampin resistance; *str*, streptomycin resistance; *ser*, serine. Plasmid phenotype symbols are used according to proposals in reference 12, except for Cma (chromosome mobilizing ability [3]). Cb, Carbenicillin resistance; Km, kanamycin resistance; Nm, neomycin resistance; Tc, tetracycline resistance; Tp, trimethoprim resistance; Sm, streptomycin resistance; Phi, interference with phage propagation; Tra, mediating conjugation. Abbreviations for antibiotics used in media were carbenicillin (cb), trimethoprim (tp), streptomycin (sm), and rifampin (rd).

biotic resistance transposons Tn*1* (4) or Tn*7* (1). Tn*1* was derived from pMO161, a *tra* mutant of R18 obtained from J. Carrigan. FP110 was transferred to PAO8(pMO161), its presence there being recognized by the inability of the host strain to plate phage B39. PAO8(pMO161) (FP110) was then crossed to PAO2, and selection was made for serine-requiring, carbenicillin-resistant (500 μg/ml) exconjugants. The frequency of such exconjugants was too low to quantitate but one such clone with these properties was isolated. It had all the expected Cma properties of FP110. FP110::Tn*7* was constructed, using an *Escherichia coli* strain W3110T$^-$RP4::Tn*7* obtained from N. Grinter. FP110::Tn*7* was transferred to PAO1670, and a Tra$^-$ mutant was isolated by selecting for PRD1 resistance. FP110 was transferred to this strain, and the double plasmid strain was then mated to PAO2. Exconjugants were selected on MM plus serine, streptomycin (500 μg/ml) and trimethoprim (1,000 μg/ml). The transposition of Tn*7* to FP110 occurred at a frequency of 5×10^{-4} per donor parent cell.

The possibility that FP110, R18-1, and R56Be were related was first indicated by the finding that *P. aeruginosa* PAO strains carrying these plasmids show a marked inhibition of phage multiplication with the bacteriophage B39. A variety of plasmids inhibit the multiplication of phages in *P. aeruginosa*, and this property has been used to indicate relationships between plasmids (9). *P. aeruginosa* PAO strains carrying R18-1, RP1-1, or R56Be do not support the multiplication of phage B39, but they support normal multiplication of E79, G101, and F116 (9, 10). *P. aeruginosa* PAO(FP110) behaves similarly, and this can be taken as prima facie evidence of a relationship between them.

Entry exclusion and incompatibility relationships of FP110 and R plasmids. Two tests which are generally accepted as indicative of plasmid relationship are entry exclusion and incompatibility. It has been shown (10) that, in respect to the response to donor specific phages, entry exclusion and characteristics of their β-lactamase, RP1-1 and R56Be are closely related.

Entry exclusion tests with FP110::Tn*7* and R18-1 and R56Be (Table 2) show a relationship between these plasmids. RP1-1 and R18-1 are thought to be closely related or identical (6).

From the entry exclusion data in Table 2, it can be concluded that FP110 is related to R18-1 and R56Be. Further evidence to support this relationship was obtained from incompatibility experiments using FP110::Tn*7*, differential selection being made for the streptomycin resistance coded for by this plasmid and the carbenicillin resistance of R18-1 and R56Be.

Plate matings with *P. aeruginosa* PAO strains each carrying one of the three plasmids were made (Table 2). Selection was made on MM + serine + carbenicillin when FP110::Tn*7* was the resident plasmid, and on NA + rifampin (250

TABLE 2. *Entry exclusion and incompatibility relationships between FP110, R18-1, and R56Be*[a]

Bacterial strain with resident plasmid	Bacterial strain with incoming plasmid	Selective medium	Transfer frequency of incoming plasmid/donor parent	Incompatibility status
PAO2 none	PAO1670 (FP110::Tn*1*)	MM + serine + CB	1.0×10^{-1}	—
PAO2 (FP110)	PAO1670 (FP110::Tn*1*)	MM + serine + CB	1.6×10^{-4}	ND
PAO1670 (FP110::Tn*1*)	PAO2 (FP110::Tn*7*)	NA + RD + SM	1.0×10^{-4}	Incompatible (86/100)
PAO2 (FP110::Tn*7*)	PAO1670 (FP110::Tn*1*)	MM + serine + CB	1.0×10^{-3}	Compatible (0/100)
PAO1670 (R18-1)	PAO2 (FP110::Tn*7*)	NA + RD + SM	1.4×10^{-4}	Incompatible (100/100)
PAO2 (FP110::Tn*7*)	PAO1670 (R18-1)	MM + serine + CB	1.3×10^{-4}	Compatible (0/100)
PAO1670 (R56Be)	PAO2 (FP110::Tn*7*)	NA + RD + SM	1.0×10^{-4}	Incompatible (100/100)
PAO2 (FP110::Tn*7*)	PAO1670 (R56Be)	MM + serine + CB	2.0×10^{-3}	Compatible (2/100)

[a] Entry exclusion tests were carried out by mating 0.1 ml of a late-exponential culture of each strain on a warmed NA plate at 37°C. After 3 h, the mating mixture was suspended in saline, and dilutions were made and plated on media to select for the incoming plasmid. Viable counts of the donor strain were made by plating the cell mixture on the appropriately supplemented minimal media. After successive single-colony isolations, 100 clones from each cross were examined for their ability to grow on NA + streptomycin (SM; 500 μg/ml) + trimethoprim (TP; 1,000 μg/ml) + carbenicillin (CB; 500 μg/ml) to indicate maintenance of both plasmids in the host strain. Failure to grow is an indication that the resistance markers of the resident plasmid were lost due to incompatibility, and this is indicated as a proportion in the right hand column. ND, Not done, due to lack of selective marker on resident plasmid.

μg/ml) + streptomycin (250 μg/ml) when FP110 ::Tn7 was to be transferred.

When R56Be was the resident plasmid, of 100 transconjugant clones tested, none retained R56Be indicating incompatibility between that plasmid and FP110::Tn7. By contrast, when FP110::Tn7 was the resident plasmid, 98 of 100 clones tested carried resistance for both streptomycin and carbenicillin which could be taken as evidence that the plasmids FP110::Tn7 and R56Be are compatible. A similar result was obtained with FP110::Tn7 and R18-1. With R18-1, as the resident plasmid, complete incompatibility was found; with FP110::Tn7, none was found. Essentially the same result was obtained with matings between FP110::Tn*1* and FP110::Tn7. In all cases where Tn7 was carried by the resident plasmid, there was retention of both streptomycin and carbenicillin resistance in the transconjugants.

Retention of streptomycin resistance does not necessarily mean that the entire FP110::Tn7 plasmid is retained. To test the possibility that Tn7 was retained without its carrier plasmid FP110::Tn7, eight clones retaining both resistances were selected and the frequency of transfer by selection of either carbenicillin or streptomycin resistance to PAO1670 was measured. For each clone, the coinheritance of the unselected resistance was also measured (Table 3).

These results show that there are two types of clones carrying both resistances. Numbers 1 through 5 very likely have an insertion of Tn7 into the chromosome or some other replicon so that transfer of streptomycin resistance can only occur by further transposition of Tn7 to FP110 ::Tn*1*. This view is supported by the fact that where selection for streptomycin resistance resulted in a low (ca. 10^{-5}) transfer frequency streptomycin resistance and high (100%) coinheritance of carbenicillin resistance, the transconjugant strains now cotransferred streptomycin and carbenicillin resistance at high frequency (10^{-2}), with no transfer of single resistance. Numbers 6 through 8 probably carry FP110 with both transposons Tn*1* and Tn7 inserted. The lower frequency of recovery of streptomycin resistance relative to that of carbenicillin resistance from donors 6 through 8 probably represents only differences in expression between the two resistances. In either case, considering the relative transfer frequency of each antibiotic resistance and their frequency of coinheritance, there is no evidence to support the view that when FP110::Tn7 is in the recipient, it coexists with the incoming FP110::Tn*1*. We infer that a similar situation occurs in the FP110 × R18-1 and FP110 × R56Be crosses. It is concluded that FP110, R18-1, and R56Be are mutually incompatible and this, combined with the phage susceptibility and entry exclusion data, points to a strong relationship between these plasmids. The results with Tn7-loaded plasmids in recipients stress the need for doing reciprocal incompatibility tests.

DISCUSSION

The data reported above comprise the first evidence that FP plasmids are related to R plasmids. In view of the relative ease with which FP110 can acquire Tn7, there is now a basis for further examination of FP plasmids as a source of R plasmids in clinical strains of *P. aeruginosa*. It is probably fortuitous that the first FP plasmid to show this type of relationship has somewhat different properties of chromosome mobilization, for example, its different site of origin for chromosome transfer compared with other FP plasmids. It should be stressed that significant differences between the R plasmids and FP110 have been shown. FP110, FP110::Tn*1*, and FP110::Tn7 all show Cma with a maximum marker transfer frequency of 5×10^{-4} per donor cell, whereas R18-1 and R56Be do not possess

TABLE 3. *Carbenicillin and streptomycin resistance transferability from CB^r SM^r exconjugants*[a]

Clone no.	Transfer frequency SM^r	% Coinheritance, CB^r	Transfer frequency CB^r	% Coinheritance SM^r
1	8.0×10^{-6}	100	9.1×10^{-2}	0
2	1.0×10^{-5}	100	9.3×10^{-2}	0
3	4.3×10^{-6}	100	7.4×10^{-2}	0
4	6.8×10^{-6}	100	5.4×10^{-2}	0
5	8.0×10^{-6}	100	8.6×10^{-2}	0
6	2.2×10^{-3}	100	8.6×10^{-2}	100
7	1.5×10^{-3}	100	7.6×10^{-2}	88
8	3.7×10^{-4}	100	1.8×10^{-2}	100

[a] Eight exconjugants from the mating PAO1670 (FP110::Tn*1*) × PAO2 (FP110::Tn7), randomly chosen after selection on MM + serine + 500 μg of carbenicillin per ml, were each patch mated to PAO1670 on NA for 3 h at 37°C. Exconjugants were then selected on NA + rifampin (250 μg/ml) + streptomycin (500 μg/ml) or NA + rifampin (250 μg/ml) + carbenicillin (500 μg/ml). A total of 100 exconjugants from each selection were then scored for the other, nonselected resistance.

Cma. R18-1 and R56Be are transducible by phage F116L, whereas FP110::Tn*1* is not, suggesting that FP110::Tn*1* is larger than the other two plasmids. F116L is thought to transduce plasmids up to a maximum deoxyribonucleic acid size of about 44×10^6 daltons (V. Stanisich, personal communication). Further evidence to support the relationship could come from a molecular study of these three plasmids, for example, comparison of restriction endonuclease maps or heteroduplex analysis.

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