

SHORT PAPER

Mobilization of the non-conjugative IncQ plasmid RSF1010

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(Received 18 November 1980)

SUMMARY

The broad host-range non-conjugative IncQ plasmid RSF1010 was mobilised with 100% efficiency in membrane filter matings, both in *E. coli* K12 and *P. aeruginosa* PAO, by broad host-range conjugative IncP plasmids. No homology between RSF1010 and an IncP plasmid could be detected. In *E. coli*, IncI α and IncX plasmids, but not IncF, IncN or IncW plasmids, were also relatively efficient at mobilising RSF1010, while in *P. aeruginosa*, R91-5 (IncP-10) was highly efficient, but pMG5 (IncP-2) and FP2 (IncP-8) were very inefficient. IncP plasmids also mobilised several plasmids derived from RSF1010 for use as in vectors in *in vitro* recombination experiments very efficiently, and pSC101 quite efficiently: this reduces the level of biological containment possible with these plasmids.

1. INTRODUCTION

The prototype IncQ group plasmid NTP2 which determines resistance to streptomycin and sulphonamides, was described by Anderson & Lewis (1965; reviewed by Anderson, 1968). Many other similar plasmids have since been identified in a wide range of bacterial hosts and shown to belong to the same incompatibility group (Smith, Humphreys & Anderson, 1974; Grinter & Barth, 1976). All are small multicopy plasmids, and where tested, share a high proportion of DNA sequence homology (Milliken & Clowes, 1973; Smith *et al.*, 1974; Barth & Grinter, 1974; Guerry, van Embden & Falkow, 1974). One IncQ plasmid was originally isolated from *Pseudomonas aeruginosa* (Bryan, van den Elzen & Tseng, 1972; Barth & Grinter, 1974), and more recently the IncQ plasmid RSF1010 has been shown to replicate stably in this organism (Nagahari & Sakaguchi, 1978). This is probably a general property of IncQ plasmids, which fall into the IncP-4 group of *P. aeruginosa* (Jacoby, 1977). The combination of small size, high copy number, and broad host range has led to efforts to develop cloning vectors from these plasmids (Nagahari & Sakaguchi, 1978; Bagdasarian *et al.*, 1979; Barth, 1979; Gautier & Bonewald, 1980).

Our interest lies in the conjugal transmission of IncQ plasmids. The early studies of Anderson (reviewed, 1968) showed that NTP2 could be mobilised by the conjugative IncI plasmid Δ that was present in the same *S. typhimurium* 29 strain. Guerry *et al.* (1974) found that the prototype IncI plasmid ColIb could transfer the IncQ plasmid RSF1010 with moderate efficiency (about 0.5% of the level of ColIb transfer), and that several IncF plasmids were much less efficient. A third IncI plasmid, R144*drd3*, was used by Grinter & Barth (1976) to mobilise several IncQ plasmids. In this paper, we report that IncP plasmids are extremely efficient at mobilizing IncQ plasmids. Both IncP and co-

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existing IncQ plasmids were transferred with similar efficiencies of approximately one when the matings were carried out on solid medium in either *E. coli* K12 or *P. aeruginosa* PAO strains. In addition, IncI α and IncX plasmids mobilized RSF1010 relatively efficiently in *E. coli*, and the IncP-10 plasmid R91-5 mobilized it efficiently in *P. aeruginosa*.

2. MATERIALS AND METHODS

(i) *Bacterial strains and plasmids*

The *E. coli* K12 strains were C600 (*thr leu*), ED2196 (*his trp nal*), JC3272 (*his trp lys str*) and its *recA56* derivative JC6310 (Willetts & Maule, 1979). *P. aeruginosa* strains were PAO5 (*trp-54 rif-5*; Chandler & Krishnapillai, 1977) and PAO12 (*pur-136 leu-8 chl-3*; Pemberton & Holloway, 1972).

The bacterial plasmids are listed in Tables 1 and 2. Further information about them is provided by Bukhari, Shapiro & Adhya (1977) and Bradley (1980).

(ii) *Media*

The media described by Willetts and Finnegan (1970) were used.

(iii) *Membrane filter matings and Southern hybridisations.*

These were carried out as described by Willetts, Crowther & Holloway (1981).

3. RESULTS

(i) *Mobilization of RSF1010 in E. coli K12*

Strains carrying both RSF1010 and a conjugative plasmid were constructed, and the donor ability of each plasmid measured in membrane filter matings with the recipient strain ED2196. In order to accomplish this, conjugative plasmids were chosen that carried only one at most of the Su^R and Sm^R markers of RSF1010, and in addition determined resistance to a further antibiotic. Bradley (1980) has classified the conjugative pili encoded by enterobacterial plasmids of different incompatibility groups into three main morphological groups; at least one member of each group was tested, although no attempt was made to cover all the different pilus or transfer system types. Matings on solid medium were chosen since it is only under these conditions that many plasmids (including those belonging to the IncP group) transfer efficiently (Bradley, Taylor & Cohen, 1980).

The results are presented in Table 1(a). The most striking observation was that the two IncP plasmids, RP1 and R751, mobilized RSF1010 very efficiently. The frequencies of RSF1010 transfer were similar to those of transfer by the IncP plasmid itself. The transfer-derepressed IncI α plasmid R64-11 and two IncX plasmids were also relatively efficient, RSF1010 transfer being 4-10 times less than that of the mobilizing plasmid. Plasmids belonging to the IncF groups (the three tested were chosen because of their different *oriT* specificities; Everett & Willetts, 1980) and to the IncN and IncW groups were all very inefficient at transferring RSF1010; this took place at frequencies 10³-10⁵ fold less than transfer of the mobilizing plasmid.

Representative IncP, IncI and IncX group plasmids were also tested in a RecA⁻ donor strain. In all cases RSF1010 was transferred from these at frequencies similar to those measured in Rec⁺ strains. Mobilization as a result of covalent union between RSF1010 and the mobilizing plasmid *via* regions of homology is therefore ruled out. In fact the extent of DNA-DNA duplex formation between RP4 and RSF1010 has been shown to be less than 1% (Falkow *et al.* 1974), and in a Southern (1975) transfer hybridisation

Table 1. Mobilization of *RSF1010*

Conjugative plasmid	Incompatibility group	Transfer frequencies		
		Conjugative plasmid	<i>RSF1010</i>	Ratio
(a) In <i>E. coli</i> ^a				
F <i>lac</i>	FI	0.96	1.8×10^{-5}	1.9×10^{-5}
R1-16	FII	0.99	8.0×10^{-6}	8.1×10^{-6}
R136i ⁻ -1	FII	0.44	2.0×10^{-6}	4.5×10^{-6}
R64-11	I α	0.52	3.1×10^{-2}	6.0×10^{-2}
R64-11 ^b	I α	1.4	0.22	0.16
pED902 ^c	N	0.62	4.7×10^{-5}	7.6×10^{-5}
pED912 ^d	N	1.1	7.4×10^{-6}	6.7×10^{-6}
RP1	P	0.99	1.01	1.0
RP1 ^b	P	0.67	0.60	0.90
R751	P	0.20	0.19	0.95
R7K	W	0.057	5.0×10^{-5}	8.8×10^{-4}
R388	W	0.75	1.0×10^{-4}	1.3×10^{-4}
TP231	X	0.021	6.8×10^{-3}	0.32
RTEM	X	0.43	4.8×10^{-2}	0.11
RTEM ^b	X	0.88	0.18	0.20
(b) In <i>P. aeruginosa</i>				
R18	P-1	0.055	0.052	0.95
R68	P-1	0.13	0.15	1.2
pMG5	P-2	0.065	2.0×10^{-6}	3.1×10^{-5}
FP2	P-8	2.3×10^{-3}	1.4×10^{-7}	6.1×10^{-5}
R91-5	P-10	1.04	0.60	0.58

^a Unless noted, donor strains were derivatives of JC3272, and the recipient strain was ED2196. A *Nae*^R contraselection was used.

^b Donor strains were derivatives of JC6310 (JC3272 *recA56*).

^c pED902 is a *Tc*^R *Spc*^S *Sul*^S *in vitro* deletion mutant of N3 (Brown and Willetts, unpublished data); the *Tc*^R marker was poorly expressed in ED2196, and ED3818 (JC3272 *nal*) was used as recipient strain instead.

^d pED912 is a *Tc*^R *Spc*^S *Sul*^S *Ap*^S *in vitro* deletion mutant of R46 (Brown and Willetts, 1981).

^e Donor strains were derivatives of PAO12 and the recipient strain was PAO5. Transfer of *RSF1010* was measured using its *Sm*^R marker, and the contraselection was *Rif*^R.

Table 2. Mobilization by *RP1* of plasmids related to *RSF1010*

Non-conjugative plasmid	Plasmid transfer frequencies ^a		
	RP1	Non-conjugative	Ratio
NTP2	0.23	0.54	2.3
R300B	1.3	1.4	1.1
pKT210 ^b	0.79	0.60	0.8
pKT212 ^b	0.62	0.46	0.8
pKT214 ^b	0.81	0.78	1.0
pSC101	0.62 ^c	0.083	0.13

^a The donor strain was JC3272 except for R300B where it was C600. The recipient strain was ED2196, and a *NaI*^R contraselection was used. *Km*^R was used to select for *RP1* transfer.

^b These are derivatives of *RSF1010* constructed by *in vitro* manipulation (Bagdasarian *et al.*, 1979). Their *Cm*^R marker was used to measure their transfer.

^c The mobilizing plasmid was pED770, a *Tc*^R*Km*^R*Ap*^R*Tra*⁺ derivative of *RP1* obtained from a partial *Hae*II digest (J. Watson and N. S. Willetts, unpublished data). The *Tc*^R marker of pSC101 was selected.

experiment, no homology between the IncP plasmid R68 and RSF1010 could be detected (Fig. 1).

RP1 also mobilized the related IncQ plasmids R300B and NTP2 efficiently (Table 2). In addition, three pKT plasmids derived from RSF1010 for use as vectors in *in vitro* recombination experiments (Bagdasarian *et al.* 1979) were efficiently transferred by RP1. The non-IncQ vector plasmid pSC101 (Cohen & Chang, 1977) was mobilized at a lower, but still appreciable, level (Table 2).

(ii) *Mobilization of RSF1010 in P. aeruginosa*

The IncP-1 plasmids R18 (probably identical to RP1; Chandler & Krishnapillai, 1974) and R68, which both belong to the *E. coli* IncP group, mobilized RSF1010 efficiently in a *P. aeruginosa* PAO strain (Table 1(b)). Interestingly, the IncP-10 plasmid R91-5 also mobilized RSF1010 very efficiently while two other *P. aeruginosa* plasmids, pMG5 (IncP-2) and FP2 (IncP-8) were very inefficient.

4. DISCUSSION

These results confirm and extend the findings of other authors. The IncI α plasmid R64-11, like the other IncI plasmids Δ (Anderson, 1968), ColIb (Guerry *et al.* 1974) and R144-3 (Grinter & Barth, 1976) mobilized RSF1010 quite efficiently. But IncP plasmids (IncP-1 in *P. aeruginosa*) were noticeably more efficient: they mobilized the IncQ plasmids RSF1010, NTP2 and R300B with approximately 100% efficiency in both *E. coli* K12 and *P. aeruginosa* PAO strains. Previous reports (Barth, 1979; Gautier & Bonewald, 1980) have mentioned mobilization of IncQ plasmids by IncP plasmids, but without stressing the efficiency of this process. It seems appropriate and significant that the broad host-range IncQ plasmids should have as their optimal mobilizing plasmids those belonging to the broad host-range IncP group. We have ruled out the possibility that homology between IncP and IncQ plasmids is the basis of the mechanism of mobilization. Further possibilities are that IncQ plasmids have an origin of transfer directly recognized by the IncP plasmid conjugation system, or that as in the case of ColE1 transfer by IncF plasmids (Dougan *et al.* 1978; Inselburg & Ware, 1977), they encode mobilization genes that recognize the origin of transfer and link this to the IncP conjugation system. Experiments to distinguish between these alternatives are in progress.

Like IncI α plasmids, IncX plasmids mobilized RSF1010 with a somewhat lower efficiency than did IncP plasmids. IncF, IncN and IncW plasmids mobilized very inefficiently, and this was probably by an entirely different mechanism – perhaps co-integrate formation during transposition of a DNA sequence from one plasmid to the other (Crisona *et al.* 1980; Willetts *et al.* 1981). IncI α plasmids synthesize thin flexible pili, IncX and IncF plasmids synthesize thick flexible pili, and IncP, IncN and IncW plasmids synthesize rigid pili (Bradley, 1980). There was therefore no correlation between ability to mobilize IncQ plasmids and pilus morphology. Also, although *P. aeruginosa* IncP-1 plasmids and the IncP-10 plasmid R91-5 confer sensitivity to the phage PR4 (Chandler & Krishnapillai, 1977), this cannot be correlated with RSF1010-mobilizing ability since IncN and IncW plasmids also give sensitivity to this phage (Bradley & Rutherford, 1975), while IncI α and IncX plasmids do not. IncP-1 and IncP-10 plasmids do not share DNA homology (Morris & Broda, 1979), and their conjugation

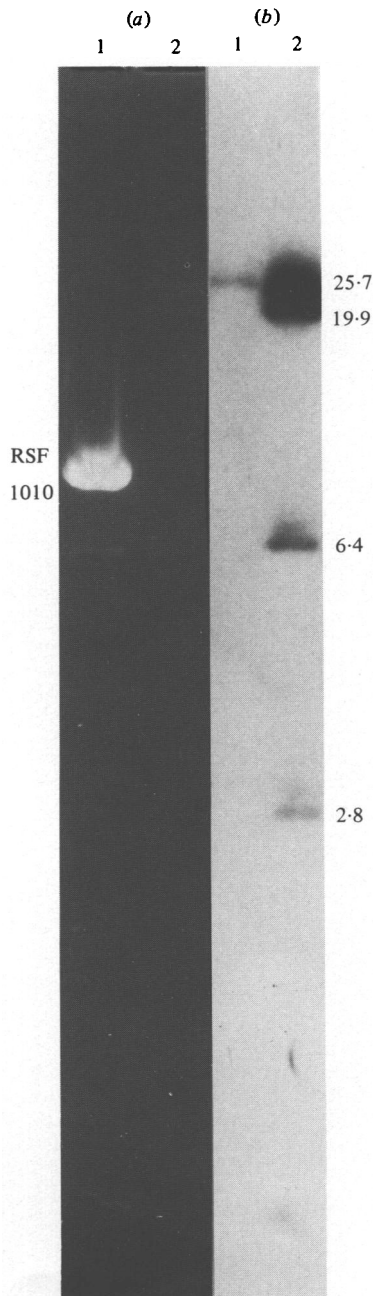


Fig. 1. Lack of homology between RSF1010 and R68. (a) Horizontal agarose gel of restriction enzyme cleavage fragments produced by: 1, *Eco*RI digestion of 0.5 μ g RSF1010 DNA, giving a single 8.3 kb fragment; 2, *Pst*I digestion of 0.02 μ g R68 DNA to provide size standards. (b) Autoradiogram produced after transfer of the fragments to a nitrocellulose filter followed by hybridization to 32 P-labelled R68 DNA. Note that a contaminating large DNA molecule in the RSF1010 DNA hybridized weakly to R68.

systems are genetically unrelated (H. Stokes, R. Moore and V. Krishnapillai, personal communication).

The efficient mobilization of IncQ plasmids and of cloning vectors derived from them (Bagdasarian *et al.* 1979), is an important consideration with regard to their biological containment, and it will clearly be an advantage to delete that part of the plasmid which is essential for this process.

We are grateful to Wendy Smith for excellent technical assistance, and to Stan Falkow, Peter Barth, Henry Smith, Ken Timmis, Naomi Datta, Richard Pinney, Bruce Holloway and Tony Brown for providing bacterial plasmids. This work was supported by an Australian Research Grants Committee grant to Professor B. W. Holloway, and by MRC grant G978/88 to N. S. W.

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