SHORT COMMUNICATION

Mobilization of the *Proteus morganii* Chromosome by R Plasmids

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R plasmids R702, R711b, R1, D, Rip69, R447b, R471 and R394, belonging to different incompatibility groups, mobilized the *Proteus morganii* 2815 chromosome. Matings employing plasmids R711b or R702 as sex factors with doubly auxotrophic recipients produced recombinants characterized by the obligatory inheritance of *ser-1*⁺, irrespective of the selected marker.

INTRODUCTION

Proteus morganii occupies a unique position in the *Proteus* group. It differs in biochemical aspects as well as in its G+C mole percentage (see Coetzee, 1972). The chromosome of *P. mirabilis* is mobilized by various R plasmids (Coetzee, 1978*a, b*, 1979*a, b*) and a map of the chromosome is available (Coetzee, 1979*b*). This paper reports attempts to mobilize the *P. morganii* chromosome with R plasmids.

METHODS

Bacteria and plasmids. These are listed in Table 1.

Media. Nutrient broth and agar were as described by Coetzee (1978*a*). MacConkey agar was from Difco. Minimal medium (MM) was that of Grabow & Smit (1967) containing methionine (40 µg ml⁻¹) and calcium pantothenate (5 µg ml⁻¹) and was supplemented with amino acids (40 µg ml⁻¹) when necessary. Incubation temperature was 37 °C.

Antibacterial drugs. Ampicillin, chloramphenicol, tetracycline and kanamycin (each 30 µg ml⁻¹), nalidixic acid (75 µg ml⁻¹) and streptomycin (1 mg ml⁻¹) were added to MM or MacConkey agar when required.

Plate matings. These were done by the method of Coetzee (1978*a*). Briefly, equal volumes (0.4 ml) of the partners were mixed and filtered through Millipore membranes which were then placed on nutrient agar and incubated overnight. Growth was harvested and washed with saline and suitable dilutions were plated on selective media and incubated for 4 d. Recombinants were tested for expression of plasmid antibiotic resistance markers by the method of Coetzee (1978*a*, 1979*b*).

Unselected marker analysis. Recombinants (50 to 500) selected for the transfer of a single marker on appropriately supplemented MM were replicated to MM to score prototrophs.

RESULTS AND DISCUSSION

Plasmids R702, R711b, R1, D, Rip69, R447b, R471 and R394 were stably maintained in *P. morganii* 2815, had intra-strain transfer frequencies of 10⁻³ to 10⁻² per donor (not shown) and mobilized the *P. morganii* chromosome. Plasmids Rip69, R447b, R471 or

Table 1. *Bacteria and plasmids*

Bacteria	Relevant properties	Reference
<i>Proteus morganii</i>		
2815	Wild-type strain resistant to ampicillin	Coetzee (1972)
2815-2	<i>str-1 nal-1</i> serially selected spontaneous mutant of 2815	Beck & Coetzee (unpublished)
YB10	$\left. \begin{array}{l} arg-1 \\ leu-1 \\ his-1 \\ ser-1 \end{array} \right\}$ NTG*-induced mutants of 2815-2	Beck & Coetzee (unpublished)
YB16		
YB57		
YBJ176		
YBJ176-6	$\left. \begin{array}{l} ser-1 arg-2 \\ ser-1 ilv-2 \\ ser-1 his-2 \\ ser-1 ade-3 \\ ser-1 leu-4 \end{array} \right\}$ NTG-induced mutants of YBJ176	Beck & Coetzee (unpublished)
YBJ176-39		
YBJ176-53		
YBJ176-59		
YBJ176-73		
Plasmids†		
R702	IncP-1. Markers‡: Km Tc Sm Su	Hedges & Jacob (1974)
R711b	IncX. Reassigned to IncD. Marker: Km	Hedges (1974); R. W. Hedges (personal communication)
R1	IncFII. Markers: Ap Cm Km Sm Su	Hedges <i>et al.</i> (1973)
D	Symbol for hybrid plasmid P-lacR1d rd 19. Markers: Lac Ap Cm Km Sm Su	Coetzee (1978a)
Rip69	IncM. Markers: Ap Km Tc	Witchitz & Gerbaud (1972)
R447b	IncN. Markers: Ap Km	Hedges <i>et al.</i> (1973)
R471	IncL. Markers: Ap Cm Km	Hedges <i>et al.</i> (1975)
R394	IncT. Markers: Ap Km	Coetzee <i>et al.</i> (1972)

* N-Methyl-N'-nitro-N-nitrosoguanidine.

† Plasmids were contained in *P. morganii* 2815.

‡ Symbols designate resistance to: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulphonamide; Tc, tetracycline. Lac, lactose utilization.

R394 produced recombinants for markers *arg-1*⁺, *leu-1*⁺, *his-1*⁺ or *ser-1*⁺ at frequencies of about 6×10^{-7} to 9×10^{-7} per donor. The similar recombination frequencies observed with each of these plasmids for various markers were indicative of a random mode of transfer (Coetzee, 1979a). Chromosome mobilization by plasmid R711b produced recombinants for the above-mentioned markers at frequencies of 9×10^{-7} to 4.5×10^{-6} (*arg-1*⁺), 2×10^{-6} to 4.5×10^{-6} (*leu-1*⁺), 3×10^{-8} to 1.1×10^{-6} (*his-1*⁺) and 1.6×10^{-6} to 1.5×10^{-5} (*ser-1*⁺) per donor in repeat experiments. The great experimental variation encountered for each individual marker could possibly be attributed to plasmid interaction with mobile IS sequences on the host chromosome (see Starlinger & Saedler, 1976). Similar results were obtained for plasmids R702, R1 and D. The array of plasmids capable of mobilizing the *P. morganii* chromosome is generous and compares well with the number of R plasmids reported to mobilize the *P. mirabilis* chromosome (Coetzee, 1979b).

The experimental variation observed in single marker transfer was circumvented by studying linkages in crosses employing plasmids R711b or R702 with doubly auxotrophic recipients derived from strain YBJ176 (see Table 2). The *ser-1* locus was used as one of the markers because the wild-type allele was often transferred at a higher frequency than other markers (see above). Selection was for either donor marker and co-inheritance of unselected markers was scored. Plasmid R711b transferred the *ser-1*⁺, *leu-4*⁺, *ade-3*⁺, *ilv-2*⁺, *arg-2*⁺ and *his-2*⁺ markers at frequencies of 4×10^{-6} to 8.2×10^{-6} (*ser-1*⁺) to 1.2×10^{-7} (*his-2*⁺) per donor (Table 2). Similar frequencies were encountered for R702. Recombinants produced by both plasmids expressed all plasmid markers (not shown). Control crosses using parental strains without R plasmids produced no recombinant clones. Co-inheritance of the unselected allele *ser-1*⁺ in recombinants mediated by either plasmid was always 100%, irrespective of the selected marker. When R711b-promoted recombinants were selected

Table 2. *Co-inheritance of unselected markers in crosses using plasmids R711b and R702*

Crosses were done as described in Methods. MM was supplemented with the growth requirement of the unselected marker and contained streptomycin and nalidixic acid for donor counter-selection. Recombinants were tested for the presence of the unselected marker as described in Methods. Results are the average of three experiments. Experimental variation was less than twofold.

Cross	Selected marker	Recombination frequency per donor	Co-inheritance of unselected marker (%)
2815(R711b) × YBJ176-73	<i>ser-1</i> ⁻	4×10^{-6}	88
	<i>leu-4</i> ⁺	2.8×10^{-6}	100
2815(R711b) × YBJ176-59	<i>ser-1</i> ⁻	7.7×10^{-6}	49
	<i>ade-3</i> ⁺	1.4×10^{-6}	100
2815(R711b) × YBJ176-39	<i>ser-1</i> ⁻	5×10^{-6}	16
	<i>ilv-2</i> ⁺	5.6×10^{-7}	100
2815(R711b) × YBJ176-6	<i>ser-1</i> ⁻	6×10^{-6}	5
	<i>arg-2</i> ⁺	3.2×10^{-7}	100
2815(R711b) × YBJ176-53	<i>ser-1</i> ⁺	8.2×10^{-6}	1.2
	<i>his-2</i> ⁺	1.2×10^{-7}	100
2815(R702) × YBJ176-73	<i>ser-1</i> ⁺	6.6×10^{-6}	89
	<i>leu-4</i> ⁺	3×10^{-6}	100
2815(R702) × YBJ176-59	<i>ser-1</i> ⁺	5.2×10^{-6}	69
	<i>ade-3</i> ⁺	1.4×10^{-6}	100
2815(R702) × YBJ176-39	<i>ser-1</i> ⁺	2.9×10^{-6}	13
	<i>ilv-2</i> ⁺	3.3×10^{-7}	100
2815(R702) × YBJ176-6	<i>ser-1</i> ⁺	4.7×10^{-6}	7
	<i>arg-2</i> ⁺	3.1×10^{-7}	100
2815(R702) × YBJ176-53	<i>ser-1</i> ⁺	3.4×10^{-6}	1.6
	<i>his-2</i> ⁺	6×10^{-8}	100

for *ser-1*⁺, unselected markers showed a relative gradient of co-inheritance from 88% (*leu-4*⁺) to 1.2% (*his-2*⁺). A similar gradient was observed with the use of strain 2815-(R702) as donor.

The obligatory inheritance of *ser-1*⁺ in all recombinant progeny promoted by plasmids R711b and R702 could indicate a special mode of plasmid interaction with the *P.morganii* chromosome. This resembles the obligatory inheritance of the NF chromosome 9 o'clock region by progeny of *Streptomyces coelicolor* A3(2) NF × UF matings (Hopwood *et al.*, 1969) which has been attributed to an association of the NF endogenous plasmid, SCP1, with this region (see Hopwood *et al.*, 1973). Alternatively, the obligatory inheritance of *ser-1*⁺ could indicate an orientated mobilization of the *P.morganii* chromosome by either of these plasmids, with *ser-1*⁺ a proximal marker. However, with this mode of transfer, inheritance of proximal markers is not absolute (Coetzee, 1975; Juliot & Boistard, 1979). The finding that recombinants express the complete set of plasmid markers as well as the *ser-1*⁺ marker is inconsistent with an *Escherichia coli* Hfr-mediated type transfer (Hayes, 1968) and is similar to that observed in *S. coelicolor* A3(2) NF × UF matings where all progeny express the NF donor property (Hopwood *et al.*, 1969). Furthermore, calculations of the mobilizing efficiency of the *ser-1*⁺ marker by these two plasmids yield values of 10^{-3} to 10^{-4} . This frequency of mobilization makes the possibility of R-prime plasmids playing a role in the transfer unlikely, but it cannot be excluded (see Johnston *et al.*, 1978; Coetzee, 1979*b*). Whatever the mode of transfer, the co-inheritance frequencies of markers in relation to *ser-1*⁺ could indicate relative distances from this locus. However, a bidirectional gradient of transfer (Hopwood *et al.*, 1969) cannot be excluded and the order of the loci is undetermined.

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