SHORT COMMUNICATION

Detection of Tellurite-resistance Determinants in IncP Plasmids

By DAVID E. BRADLEY

*Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland,
Canada A1B 3V6*

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Six IncP plasmids were tested for their ability to generate tellurite-resistant variants by plating bacterial strains harbouring them on medium containing potassium tellurite. Four plasmids, three of subgroup IncP α and one not allocated, formed variants that could transfer tellurite-resistance at the same frequency as plasmid-determined drug resistance. This property was not shared by two examples of subgroup IncP β .

INTRODUCTION

Resistance to metal ions in Gram-negative bacteria is frequently plasmid-mediated (for a review see Foster, 1983). In *Escherichia coli* K12, resistance to tellurite (TeO₃) is carried by some plasmids belonging to the H complex of incompatibility groups (Bradley *et al.*, 1982), and by an unclassified plasmid of the 'pre-antibiotic era' (Datta & Hughes, 1983). No others with this phenotype have been reported. Several IncP plasmid variants exhibiting transferable tellurite-resistance (Te^r) are described here. IncP plasmids form two groups based on DNA homologies (Villarroel *et al.*, 1983; Yakobson & Guiney, 1983; Chikami *et al.*, 1985); members of both were tested for their ability to generate Te^r.

METHODS

Bacterial strains, plasmids and bacteriophages. *E. coli* K12 strains were J53 (Pro⁻ Met⁻) and JE2571 (Leu⁻ Thr⁻ Str^r Fla⁻ Pil⁻) with nalidixic acid-resistance (suffix -1) or rifampicin-resistance (suffix -2) cited in Bradley (1984). Plasmids are listed in Table 1. With the exception of pMG22, which was donated by Dr G. A. Jacoby (Jacoby, 1980), they were all supplied by Dr N. Datta and Dr R. W. Hedges from the Hammersmith collection. Detailed information on most IncP plasmids is given by Villarroel *et al.* (1983).

Media and matings. Media, drug concentrations and qualitative and quantitative mating procedures were as described by Bradley (1984). Potassium tellurite was used at a concentration of 5 $\mu\text{g ml}^{-1}$ (2×10^{-5} M).

RESULTS AND DISCUSSION

IncP plasmids carrying Te^r determinants were isolated by chance in the course of other work. For this study, they were obtained by spreading $> 1 \times 10^9$ cells (a large loopful), from fresh overnight plate cultures of strain JE2571 or strain J53, harbouring an IncP plasmid, on tellurite plates in 0.1 ml broth. Most of the numerous colonies which appeared after drying and overnight incubation grew well on subculturing to a similar plate. They were tested by cross-streak mating (detects derepressed, not repressed transfer) for the transferability of Te^r and a plasmid-determined drug resistance (Table 1). Transferable Te^r was generated by RP4, pUZ8, R26 and pMG22, but not by R772 and R751. The latter two plasmids were able to generate a very few Te^r variants, and the phenotype could not be transferred even by the efficient plate mating method (Bradley, 1984). The reason for this is not understood. JE2571 and J53 without IncP plasmids could not be made Te^r with this procedure (Table 1).

Table 1. *Isolation of IncP plasmids with tellurite-resistance*

Strain	Plasmid	IncP subgroup	Drug resistance markers*	Colonies on tellurite medium†	Clones transferring Te ^r /p ^r ‡
JE2571	—	—	—	30§	NT
J53	—	—	—	6§	NT
JE2571	RP4	α	Ap Km Tc	800	16/18
J53-2	pUZ8	α	Km Tc Hg	800	13/14
JE2571	R26	α	Ap Cm Gm Hg Km Sm Su Tc	400	17/19
J53-2	pMG22	—	Gm Hg Km Su Tc	800	9/17
J53-2	R772	β	Km	6	0/3
J53-2	R751	β	Tp	0	NT
JE2571	R751	β	Tp	8	0/2

* Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Hg, mercury; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Tp, trimethoprim.

† $>1 \times 10^9$ bacteria were spread in 0.1 ml broth on plates containing potassium tellurite ($5 \mu\text{g ml}^{-1}$). The colonies (given to nearest 50) were counted after drying and incubating overnight at 37°C .

‡ Twenty clones were subcultured from those originally obtained, but not all grew on tellurite medium. Those that did were mated by cross-streaking with strain JE2571 or J53 (or -2 derivatives) selecting for resistance to tellurite (Te^r) and plasmid-determined resistances (p^r) to kanamycin (R772), trimethoprim (R751) or tetracycline (remainder) on separate plates. Three clones of J53-2(R772), and two of JE2571(R751) also failed to transfer Te^r by plate-mating. NT, Not tested.

§ None of these colonies grew on subculturing to tellurite medium.

Table 2. *Comparison of transfer frequencies for tellurite-resistant IncP variant plasmids using tellurite and kanamycin selection*

Plasmid	Transfer frequencies for plasmid-selecting markers*	
	Km ^r	Te ^r
RP4Te ^r	2.7	2.7
pUZ8Te ^r	2.7	2.3
R26Te ^r	1.5	1.6
pMG22Te ^r	1.5	1.7

* Transconjugants per donor h⁻¹. Isogenic matings in host strain JE2571, the recipient organism being JE2571-1.

Abbreviations: Km^r, kanamycin resistance; Te^r, tellurite-resistance.

The transfer frequencies for Te^r and Km^r (kanamycin-resistance) were compared by plate mating (Bradley, 1984) for RP4Te^r, pUZ8Te^r, R26Te^r and pMG22Te^r in isogenic matings using strain JE2571-1 as recipient. In all cases both markers transferred at almost the same derepressed frequency (Table 2), close to that obtained previously for RP4 (1.4 transconjugants per donor h⁻¹; Bradley, 1984). The genetic changes permitting Te^r expression were therefore stable in all four cases.

There are two possible reasons why this 'hidden' Te^r has not been detected previously in IncP plasmids: it could either require induction, or expression could be regulated under repression. Induction was tested by the method of Rouch *et al.* (1985), using strain JE2571(RP4), without success. Taken in conjunction with the observation that single Te^r colonies were obtained in the experiments summarized in Table 1, this indicated that Te^r determinants existed in a repressed state. A spontaneous mutation would be required to lift repression and allow full expression of Te^r.

In the course of this work it was often found that strains produced mucoid colonies on receiving plasmids carrying Te^r. Mucoid colonies have also been found (on nutrient media) for *E. coli* K12 strain C600 and derivatives, JE2571 being one of them (V. M. Hughes, personal communication). Mucoid colonies are caused by polysaccharide production due to low

temperature growth, and other factors (see Davis *et al.*, 1969). They behaved like normal ones in matings, etc., and apart from some inconvenience other workers should not be concerned about them.

It is of particular interest that the ability or otherwise to generate T_e^r variant plasmids is reflected in the grouping of IncP plasmids as RP4-like (IncP α) or non-RP4-like (IncP β ; Villarroel *et al.*, 1983; Jakobson & Guiney, 1983; Chikami *et al.*, 1985). RP4, pUZ8 and R26, belong to group IncP α , and easily generate T_e^r , whereas R772 and R751 (IncP β) do not. On this basis, pMG22, which has not been allocated to a subgroup, is probably IncP α .

The possibility exists that the T_e^r determinants described are carried on a transposon, although preliminary attempts to demonstrate this have not been successful. However, T_e^r was translocated to an IncT plasmid from a variant of RP4 that was obtained after passage through a clinical isolate of *E. coli* carrying non-transferable T_e^r (unpublished results). It is thus quite possible that the IncP plasmids described here carry T_e^r transposons.

The use of T_e^r determinants in other bacterial species to which IncP plasmids can be transferred would be valuable. It is also quite likely that T_e^r variant plasmids could be isolated from members of other incompatibility groups; a survey is in progress.

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