Characteristics of RP4 Tellurite-resistance Transposon Tn521

By DAVID E. BRADLEY,¹* KANWAL K. GREWAL,¹ DIANE E. TAYLOR²
AND JEANETTE WHELAN¹

¹Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland A1B 3V6, Canada

²Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

(Received 16 November 1987; revised 22 February 1988)

A restriction map of the tellurite-resistance (Te^r) transposon Tn521 (parent plasmid RP4Te^r) was prepared. Five sites from RP4Te^r, including the *Eco*RI origin, were found in pIN25::Tn521. Tn521 was inserted into a transferable 27.5 kb vector (pCU109) to make three different insertion mutants, in which the size of Tn521 was measured accurately at 4.5 kb. Unlike the Te^r of IncHI2 plasmids, that of Tn521 in RP4Te^r was non-inducible. Te^r was expressed in five widely differing bacterial species to which RP4Te^r was transferred from *Escherichia coli*. Electron micrographs of bacteria expressing the Te^r of RP4Te^r, H complex plasmids, and chromosomal mutants, all revealed similar tellurium metal crystallites when the bacteria were grown in potassium tellurite medium. No other Te^r determinants were found amongst 54 plasmids representing most incompatibility groups (excluding the H complex).

INTRODUCTION

Although low concentrations of tellurium are widespread in the environment (Schroeder *et al.*, 1967), bacterial resistance to the element (in the form of tellurite) is comparatively rare, and generally plasmid-mediated (Summers & Jacoby, 1977). In *Escherichia coli*, plasmids determining transferable tellurite resistance (Te^r) have been found to belong to incompatibility group Pa (not IncPβ; Bradley, 1985), and some groups of the H complex (Taylor & Summers, 1979). Te^r is usually expressed constitutively, but in the case of IncPa plasmids it requires the activation of a silent gene by plating bacteria carrying the plasmids on medium containing a normally lethal level of tellurite; a Te^r variant of RP4 was obtained in this way (RP4Te^r; Bradley, 1985). The Te^r determinant of RP4Te^r is located in a transposable segment of DNA (Tn521) approximately 4.5 kb in size, its estimated location spanning the single *Eco*RI site of the plasmid (Bradley & Taylor, 1987; Taylor & Bradley, 1987). This paper defines these parameters more accurately by means of a detailed restriction map of Tn521. We have also investigated fundamental aspects of its biology including the level of Te^r expressed, its expression in bacteria other than *E. coli*, and the structure of metallic tellurium produced by the resistance mechanism. These results are compared with corresponding data for the Te^r determinant of the IncHI2 plasmid pMER610 (Jobling & Ritchie, 1987).

METHODS

Bacterial strains, bacteriophages and plasmids. *E. coli* K12 background strains (relevant markers only cited) were JE2571 (Leu⁻Thr⁻Str^rSpc^rPil⁻Fla⁻) and J53 (Pro⁻Met⁻Str^rSpc^rPil⁺Fla⁺). Other bacterial species were *Acinetobacter calcoaceticus* C91 (Divers *et al.*, 1985), *Alcaligenes odorans* AO (St John's General Hospital), *Pseudomonas aeruginosa* PAO1150.1 (Bradley, 1980a), *Pseudomonas putida* PpS388 (Fennewald *et al.*, 1978) and *Serratia marcescens* SM13880 (ATCC 13880). Where appropriate, mutants resistant to nalidixic acid, rifampicin or spectinomycin (suffixes -1, -2, -4, respectively) were constructed. We tested 54 plasmids, which are not cited individually, for the generation of Te^r; most were from the list of Jacob *et al.* (1977). Other plasmids used are

Table 1. *Plasmids used*

Plasmid	Inc group	Resistance markers*	Reference
F	FI	Nil	Jacob <i>et al.</i> (1977)
F::Tn521	FI	Te	Bradley & Taylor (1987)
RP4	P	Ap Km Tc	Jacob <i>et al.</i> (1977)
RP4Te ^r	P	Ap Km Tc Te	Bradley (1985)
pIN25	T	Ap Km	Bradley & Whelan (1985)
pIN25::Tn521	T	Ap Km Te	Bradley & Taylor (1987)
pCU109	Nil	Cm	Thatte <i>et al.</i> (1985)
pMN705	Nil	Te	This paper
pMN706	Nil	Cm Te	This paper
pMN808	Nil	Cm Te	This paper

* Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline; Te, tellurium as potassium tellurite.

recorded in Tables 1 and 2. Those in Table 2 are listed by Jacob *et al.* (1977) and Jacoby (1977) save for pMG110 (Wolfson *et al.*, 1982) and pMUR162 (Datta & Hughes, 1983). Phage PR4 (Stanisich, 1974) was used to check for the constitutive synthesis of N pili.

Media, antibiotics, culture methods, matings. These, including drug concentrations and cross-streak mating, were as described previously (Bradley, 1984, 1985; Bradley *et al.*, 1981). Difco Nutrient Broth was used for induction experiments, and Brain Heart Infusion Broth or Agar (BBL) for routine purposes. Te^r was selected using potassium tellurite (K₂TeO₃) at 5 µg ml⁻¹ (= 2 × 10⁻⁵ M-Te) in Brain Heart Infusion agar. Mueller Hinton Broth (BBL) was used to determine minimum inhibitory concentrations of potassium tellurite (see below). Overnight plate matings were as described by Bradley *et al.* (1980) save that plates were incubated overnight instead of for 1 h.

Determination of mutation frequencies for drug and tellurite resistances. The chromosomal mutation frequencies for Str^r, Spc^r and Te^r, and the Te^r generation frequency of plasmid RP4 to RP4Te^r, were estimated by growing a shake culture of the strain under test to OD₆₂₀ 1.0, then spreading 110 ml of the culture on 15 cm diameter nutrient plates containing the agent under test. After 1–2 d incubation at 37 °C, only one or two colonies were usually obtained, so the values given are approximate.

Transpositions. The general approaches advocated by Foster (1984) and Bennett (1984) were used. Detection of transpositions of Tn521 to other replicons has been found difficult due to an apparent very low transposition frequency (not measured) combined with the ability of *E. coli* to form colonies with transient Te^r. Tn521 selection was therefore by means of derepressed Te^r transfer (Bradley & Taylor, 1987), using a transfer-derepressed vector such as pCU109 (27.5 kb). It carries the transfer operon of a wild-type IncN plasmid together with the chloramphenicol-resistance marker and multicopy characteristics of pACYC184 (Thatte *et al.*, 1985). A non-transferable variant of F::Tn521 was the best donor plasmid since it had no potentially transposable ampicillin resistance, although it did contain Tn1000 (no resistance or other markers; Guyer, 1978). A strain containing both the transposon donor plasmid and the target plasmid was constructed, occasionally being stored on Dorset egg medium (Bennett, 1984). This strain was crossed with JE2571-1 (NaI^r) by overnight plate mating (Bradley *et al.*, 1980). A large loopful (about 10¹⁰ cells) from this mating was spread on selective agar (nalidixic acid/tellurite). Antiserum to donor conjugative pili was also added to broth mating mixtures (equal volumes) to prevent the transfer of Tra⁺ revertant plasmids. Up to 500 transconjugants were cross-streak mated with JE2571-2 (rifampicin/tellurite selection) without purification. Transconjugants of strains giving derepressed transfer of Te^r were purified and then screened for plasmid content by agarose gel electrophoresis (see below). In a first screening, clones giving clear DNA bands after linearizing with *Eco*RI (unique site) were selected. We chose plasmids which were not unresolved cointegrates (with F::Tn521). Further selection was based on restriction patterns with other enzymes. It proved extremely difficult to obtain cells containing pCU109::Tn521 alone without copies of either pCU109::Tn1000.

Preparation of DNA and restriction enzyme analysis. Plasmid DNA was prepared as described by Taylor & Brose (1985) and Whiteley & Taylor (1983), or by a modification of the method of Birnboim & Doly (1979). Restriction enzymes were used according to the manufacturers' recommendations (BRL and United States Biochemical Corporation). The size standard was phage λ DNA digested with various enzymes. Gel illustrations have not been included as experimental evidence, but reviewers have seen key photographs, which are identified by '(see Methods)'.

Te^r inducibility tests. The experimental approach was that of Silver *et al.* (1981). We used a long exposure of strains carrying RP4 or RP4Te^r to sublethal molarities of tellurium (see Results) since short-term exposure had no

Fig. 1. Restriction map of *Tn521* with reference to the region where it is located in the parent plasmid *RP4Te⁺* (*RP4* mutant plasmid expressing *Te⁺*; see Bradley, 1985). The map is based on a 60 kb *RP4* replicon (Lanka *et al.*, 1983), restriction sites, etc., being extrapolated from the 56.4 kb size used elsewhere (Figurski *et al.*, 1982; Taylor & Bradley, 1987). + and - identify the transposon orientations in Fig. 2. Abbreviations for restriction enzymes: Ec, *EcoRI*; Sm, *SmaI*; Sp, *SphI*; St, *SstII*; Xh, *XhoI*. This array is quite different from that found by Jobling & Ritchie (1987) for the *Te⁺* determinant of the *IncH12* plasmid *pMER610* (*BglII*, *BglII*, *PstI*, *BamHI*, *HindIII*, *SalI*, *EcoRI*).

effect. Attempted induction was by static overnight growth, followed by 10:1 dilution and 7 h shaking, then a repeat overnight static culture, all in potassium tellurite medium.

Determination of minimum inhibitory concentration (MIC) of potassium tellurite. The method of Waterworth (1978) was based on visually observing the end-point of bacterial growth in Mueller Hinton Broth containing stepwise dilutions of potassium tellurite. The lowest potassium tellurite concentration without bacterial growth was recorded as the MIC.

Electron microscopy. Bacteria were grown overnight in Brain Heart Infusion Broth containing 50 µg potassium tellurite ml⁻¹, equivalent to 10 µg Te metal ml⁻¹. They were mounted on electron microscope specimen support grids and negatively stained by standard methods.

RESULTS

Restriction map of *Tn521*

The size of the *RP4* replicon used was 60 kb (Lanka *et al.*, 1983). We employed two approaches to map *Tn521* and locate it accurately on its *RP4Te⁺* parent plasmid. In the first, we determined which *RP4Te⁺* restriction sites in the vicinity of the *Te⁺* determinant (*SmaI*, *XhoI*, *EcoRI*, *SphI*, *SstII*; see Taylor & Bradley, 1987 and Fig. 1) were acquired by the 132 kb *IncT* plasmid *pIN25* after transposition of *Tn521* to form *pIN25::Tn521*. We found that all had been added to the *pIN25* replicon. An additional 3.2 kb *EcoRI* fragment (unique to the recombinant) was cut in *EcoRI* + *SstII* double digests to give two fragments of 1.96 kb and 1.24 kb (see Methods), demonstrating the acquisition of an *SstII* site; this result located one end of the transposon (see below).

In the second approach we used *F::Tn521* as donor plasmid to obtain three *Tn521* insertions into *pCU109* at different loci. These were used to give an accurate size measurement and to locate the transposon ends. The first plasmid (*pMN705*) was found in the course of screening for loss of the *pCU109* *Cm^r* marker caused by transposon insertion. It contained *Tn1000* rather than *Tn521* in the *Cm^r* determinant, although both transposons were carried by this plasmid. The exact location of *Tn1000* was found by *HindIII* digestion, which gave fragments of 2.5, 2.7, 4.5 and approximately 30 kb (Fig. 2a; see Methods). The transferability of the *Te⁺* of *pMN705* remained derepressed, indicating *Tn521* insertion outside the transfer operon. An *XhoI* digest giving fragments of 2.7, 3.1, 4.5 and about 28 kb (see Methods) located *Tn521* and gave a size estimate of 4.5–6.0 kb (Fig. 2a). An extra *SstII* site was acquired in addition to that present in *Tn1000*. Its location at one end of *Tn521* was indicated by restriction enzyme digests of two other *pCU109::Tn521* recombinants, *pMN706* and *pMN808*, both lacking *Tn1000*. They no longer determined transferability in spite of being selected for it; the apparent *Tra⁺* phenotype

Fig. 2. (a) pMN705 showing insertions of Tn1000 and Tn521. (b) Locations of Tn521 insertions in pMN706 and pMN808. + and - define the orientation of Tn521 with reference to Fig. 1. Abbreviations: E, *EcoRI*; H, *HindIII*; S, *SstII*; X, *XhoI*; Cm', chloramphenicol resistance; Rep, replication; Tra, transfer.

found during initial screening (Methods) was due to efficient mobilization of the plasmids by pCU109. Tn521 insertion into the transfer operon of pCU109 permitted its location using *SstII*, *EcoRI* and *XhoI* digests (Fig. 2b). With *SstII*, pMN706 gave only three fragments, the same number as the vector. This showed that the *SstII* site acquired by pIN25::Tn521 and pMN705 was absent (see below). Two of the three *SstII* fragments were the same size in pMN706 and the vector pCU109 (15.5 and 4.1 kb), but the third fragment was 12.4 kb in pMN706 and 7.9 kb in pCU109 (see Methods). This gave an accurate size of 4.5 kb for Tn521, the same as the original estimate (Bradley & Taylor, 1987). pMN808 also had only three *SstII* sites, Tn521 inserting into a 15.6 kb fragment of pCU109 increasing its size to about 20 kb (see Methods). An *EcoRI* digest of pMN808 gave a 16.0 kb doublet, which clearly located the Tn521 insertion point in a different position from that in pMN706 (Fig. 2b).

The variability in the appearance on different recipient plasmids of the additional *SstII* site which we have located at the 'minus' end of Tn521 (Fig. 1) may be explained by the transposon having only one constant end (Fig. 1, +). The variable end could have two possible end-points (i.e. direct inverted repeat sequences). Alternatively the sequence at the Tn521 terminus together with the sequence at the insertion site on the recipient plasmid may have generated an *SstII* site present on neither element. However, it seems improbable that such a site would be generated at random while another *SstII* site is known to be present close to one end of the transposable region of the parent plasmid RP4Te^r (Taylor & Bradley, 1987). Furthermore, identical sequences would have to be recognized at the insertion sites in the two very different recipient plasmids pIN25 and pCU109. While the only way to settle the question unequivocally would be to determine the relevant sequences, either explanation pinpoints the transposon end at or near the *SstII* site. The 'plus' end of Tn521 was defined by the 4.5 kb size measurement; the nearest RP4Te^r restriction site to the *SmaI* site at 1 kb was a *PstI* site 5 kb away in a clockwise direction (see Lanka *et al.*, 1983). While these results are not conclusive, they suggested that both *kilA* and *korA* (Figurski *et al.*, 1982; Bechhofer *et al.*, 1986) were included in Tn521. The restriction sites located in the Te^r determinant of the IncHI2 plasmid pMER610 by Jobling & Ritchie (1987) are different from those in Tn521 (see Fig. 1 legend).

Fig. 3. Determination of sublethal Te concentrations. Shake cultures of the strains were grown in different concentrations of potassium tellurite in Difco Nutrient Broth until the control culture without Te had reached OD₆₂₀ 0.68 (5 h). The OD₆₂₀ values of the other cultures were measured and plotted against Te concentration. ○, JE2571; ●, JE2571(RP4); □, JE2571(RP4) after overnight growth in broth containing 5 × 10⁻⁸ M Te; ■, JE2571(RP4Te^r).

Fig. 4. (a) Growth in 1 × 10⁻⁶ M-Te medium of JE2571(RP4) after growth in 5 × 10⁻⁸ M-Te medium (see Methods). JE2571(RP4) control: ○, in plain broth; ●, in Te broth. JE2571(RP4) previously grown in 5 × 10⁻⁸ M-Te medium: □, in plain broth; ■, in Te broth. (b) Growth in 1 × 10⁻⁵ M-Te medium of JE2571(RP4Te^r) after growth in 2 × 10⁻⁷ M-Te medium. JE2571(RP4Te^r) control: ○, in plain broth; ●, in Te broth. JE2571(RP4Te^r) previously grown in 2 × 10⁻⁷ M-Te medium: □, in plain broth; ■, in Te broth.

Non-inducibility of Te^r determinant of *Tn521*

Jobling & Ritchie (1987) demonstrated the inducibility of the Te^r determinant of the IncHI2 plasmid pMER610, a high level of Te^r appearing in the host bacterium after only 30 min exposure to a sublethal level of Te (see Silver *et al.*, 1981). We tested *E. coli* JE2571(RP4Te^r) but used a much longer exposure, since preliminary results with short-term exposure suggested non-inducibility. The maximum sublethal molarities of Te for test strains were determined (Fig. 3): JE2571 and JE2571(RP4), 5 × 10⁻⁸ M; JE2571(RP4Te^r), 2 × 10⁻⁷ M. JE2571(RP4Te^r) was sensitive to 1 × 10⁻⁴ M-Te (Fig. 3). To test the inducibility of the unexpressed Te^r determinant of RP4, we measured the growth rate in 1 × 10⁻⁶ M-Te medium of strain JE2571(RP4) that had been grown in 5 × 10⁻⁸ M-Te through three subcultures over 24 h (see Methods). JE2571(RP4) exposed to a low level of potassium tellurite did not grow any faster than the control JE2571(RP4) (Fig. 4a). To test the inducibility of the Te^r of RP4Te^r, we used a sublethal Te concentration of 2 × 10⁻⁷ M to induce resistance (Fig. 3), and 1 × 10⁻⁵ M for the challenge. The

Table 2. MIC of potassium tellurite for various bacterial species with and without plasmids

Species	Strain	Plasmid	Inc group	MIC of potassium tellurite ($\mu\text{g ml}^{-1}$)
<i>Escherichia coli</i>	JE2571-1Te*	None	None	325
<i>Escherichia coli</i>	JE2571	None	None	3
		RP4	P	4
		RP4Te ^r	P	325
		pIN25	T	4
		pIN25::Tn521	T	275
		F	FI	4
		F::Tn521	FI	300
		R478	HI1	240
		MIP233	HI3	100
		pMG110†	HII	175
		pMUR162	None‡	175
<i>Serratia marcescens</i>	ATCC 13880	None	None	10
		RP4Te ^r	P	250
<i>Pseudomonas aeruginosa</i>	PAO1150.1	None	None	75
		RP4	P-1	75
		RP4Te ^r	P-1	300
		CAM	P-2	100
		OCT	P-2	100
		RPL11	P-2	90
<i>Pseudomonas putida</i>	PpS388	None	None	6
		RP4Te ^r	P-1	375
<i>Alcaligenes odorans</i>	Clinical	None	None	30
		RP4Te ^r	P	475
<i>Acinetobacter calcoaceticus</i>	C91	None	None	175
		RP4Te ^r	P	300

* A chromosomal Te^r mutant of strain JE2571-1 (see text).

† pMG110 was chosen as the most stable IncHII plasmid.

‡ The only Murray plasmid with Te^r (Datta & Hughes, 1983).

growth rates of pre-exposed and control cultures were compared (Fig. 4*b*). Since the pre-exposed culture of JE2571(RP4Te^r) did not grow as well as the control, it was concluded that neither the Te^r of RP4 nor that of RP4Te^r was inducible.

Generation of Te^r by *E. coli* K12 and plasmids

We searched for a Tn521-like transposon in representative plasmids (usually three examples) of currently recognized incompatibility groups B, C(A), C, D, FI, FII, HI1, II, I1 + B, I2, J, K, M, N, SI, SII, SIII, T, U, W, X and Z by screening for Te^r generation (Bradley, 1985). Stable Te^r derivatives were isolated from strains harbouring one plasmid from groups D (R687; Bradley, 1980*b*), FII (R100), SII (R71; Coetzee *et al.*, 1986) and W (R388), also R1343b and R40a (IncC). Since the Te^r in these mutants was not transferable, it was probably due to chromosomal mutation. This was tested by comparing the mutation frequencies for Te^r, Str^r and Spc^r for host strains: J53, 1.7×10^{-9} Te^r mutations per cell, 9.3×10^{-9} Str^r mutations per cell, and 1.0×10^{-10} Spc^r mutations per cell; JE2571, $<2.0 \times 10^{-10}$ Te^r mutations per cell; JE2571-1, 5.0×10^{-9} Te^r mutations per cell. The difference between JE2571 and JE2571-1 presumably reflected clonal variation. JE2571(RP4) gave 1.7×10^{-8} Te^r mutations per cell [Te^r generation frequency; end product JE2571(RP4Te^r)], which must be compared with JE2571 not JE2571-1.

Expression of Tn521 in different bacterial species

Tn521 expression was demonstrated by comparing the MIC of potassium tellurite for bacterial species with and without the transposon in its broad-host-range parent plasmid RP4Te^r. We found (i) that Te^r was expressed at a higher level than that of both H complex plasmids in *E. coli*, and IncP-2 plasmids in *P. aeruginosa*, and (ii) that it was expressed at high MIC levels in all the bacterial species tested, where it was transferable at derepressed frequencies (Table 2). The single exception was *P. putida* PpS388-2(RP4Te^r), which gave a

transfer frequency of only 2.3×10^{-5} transconjugants per donor h^{-1} by plate mating. We also tested RP4Te^r for determination of transferable resistance to the Te-related elements iodine, arsenic (arsenite and arsenate), antimony and selenium, but could find none (details not given).

Electron microscopy of bacteria grown in tellurite medium

Bacteria grown in or on potassium tellurite medium produce a black colour due to the reduction of potassium tellurite to tellurium metal (Tucker *et al.*, 1962). We used electron microscopy of whole cells to ascertain if the form and location of the metal particles, not previously illustrated, was the same for all sources of the Te^r determinant. *P. aeruginosa* PAO1150.1(RP4Te^r) grown overnight in broth containing 50 µg potassium tellurite ml⁻¹ had dense crystallites in most of its cells (Fig. 5a). Sometimes these appeared to extend outside the cell (Fig. 5a, arrowed), but were surrounded by a single membrane (Fig. 5b), which we believe to be the outer membrane; reduction to Te metal presumably occurred at the inner membrane. *E. coli* JE2571-1Te^r, JE2571(MIP233) and JE2571(RP4Te^r), and *S. marcescens* SM13880(RP4Te^r), all produced crystallites, varying in size from the relatively large ones of PAO1150.1(RP4Te^r) to a finer form synthesized by SM13880(RP4Te^r) (Fig. 5c).

DISCUSSION

The expression of a normally silent Te^r determinant on plasmid RP4 was detected on potassium tellurite medium at 1.7×10^{-8} mutations per cell, which was consistent with a point mutation in an as yet unspecified regulatory gene. This might conceivably be *korA*, which is an autorepressor (Young *et al.*, 1985) and is probably included in Tn521 (see below). The Te^r system is not inducible, so Te^r expression might simply require a mutation in a repressor. Alternatively, the frequency of generation of Te^r is consistent with the reversion of a previous mutation that inactivated the Te^r structural gene.

Constitutive Te^r expression due to Tn521 appears possible in bacterial species included in the RP4 host range (Olsen & Shipley, 1973), and in all cases studied here it involved the reduction of potassium tellurite to crystallites of metallic tellurium. The level of Te^r determined by Tn521 was much higher than that determined by H complex and other plasmids, and this might be considered to reflect a difference in Te^r mechanisms. However, the metallic tellurium end-product was the same in all cases and the variations in Te^r levels probably reflected differences in the efficiencies of the same or similar metabolic pathways.

Our data suggest that both *kilA* and *korA* are present on Tn521. *kilA*, which has a lethal action on the host bacterium, is controlled by *korA* together with *korB* and *korE* (Figurski *et al.*, 1982; Young *et al.*, 1985, 1987); *korB* and *korE* are outside Tn521. The possible inclusion of *kilA* and *korA* in Tn521 is based solely on restriction data and cannot be regarded as conclusive. However, if substantiated later, the important suggestion that plasmid-borne transposons could include genes determining essential replication functions will have to be examined. We have not yet attempted to detect *kilA* function determined by plasmids containing the transposon. Both *kilA* and *korA* have been found in all other IncPα plasmids (Figurski *et al.*, 1982; listed by Villarroel *et al.*, 1983), most of which have been shown to generate transferable Te^r (Bradley, 1985). We therefore consider it likely that some or all of these plasmids harbour Tn521-like transposons, although we have not been able to demonstrate this. This apparent lack of transposition could well have been due to a frequency too low to be detected. Indeed, Tn521 was very difficult to transpose from RP4Te^r to other plasmids and could not be transposed to the bacterial chromosome; a low transposition frequency or other limiting characteristic is not surprising, otherwise transposable Te^r would be expected to occur in other plasmids; our tests, which are of a provisional nature, suggest that this is not the case.

The Te^r determinants of RP4Te^r (Tn521) and the IncHI2 plasmid pMER610 are distinct: (i) the restriction sites in Tn521 are different from those in the Te^r determinant of pMER610 (see legend to Fig. 1), (ii) the Te^r of RP4Te^r is constitutive whereas that of pMER610 is inducible, (iii) the level of Te^r expressed by RP4Te^r is consistently higher than that expressed by H complex plasmids, (iv) the Te^r of H complex plasmids does not appear to be transposable (D. E. Bradley & D. E. Taylor, unpublished results). However, the structural genes of the elements may or may

Fig. 5. Negatively stained cells grown in potassium tellurite medium. (a) Dividing cell of *P. aeruginosa* PAO1150.1(RP4Te⁻) with a tellurium crystallite (arrowed) extending out of the cell. Bar 1.0 µm. (b) detail of a similar crystallite. Bar 100 nm. (c) *S. marcescens* SM13880(RP4Te⁻). Bar 1.0 µm.

not be related. We propose that the wild-type transposon in RP4, which does not express Te^r , be designated Tn522.

We are grateful to Elisa Brose, who isolated pIN25 DNA for restriction enzyme analysis. This work was supported by the Medical Research Council of Canada (grants MT5608 for D.E.B. and MT6200 for D.E.T.).

REFERENCES

- BECHHOFFER, D. H., KORNACK, J. A., FIRSHEIN, W. & FIGURSKI, D. H. (1986). Gene control in broad host range plasmid RK2: expression, polypeptide product, and multiple regulatory functions of *korB*. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 394–398.
- BENNETT, P. M. (1984). Detection of transposable elements on plasmids. *Methods in Microbiology* **17**, 227–231.
- BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513–1523.
- BRADLEY, D. E. (1980a). A function of *Pseudomonas aeruginosa* polar pili: twitching motility. *Canadian Journal of Microbiology* **26**, 146–154.
- BRADLEY, D. E. (1980b). Morphological and serological characteristics of conjugative pili. *Plasmid* **4**, 155–169.
- BRADLEY, D. E. (1984). Characteristics and function of thick and thin conjugative pili determined by transfer-derepressed plasmids of incompatibility groups I₁, I₂, I₅, B, K and Z. *Journal of General Microbiology* **130**, 1489–1502.
- BRADLEY, D. E. (1985). Detection of tellurite-resistance determinants in IncP plasmids. *Journal of General Microbiology* **131**, 3135–3137.
- BRADLEY, D. E. & WHELAN, J. (1985). Conjugation systems of IncT plasmids. *Journal of General Microbiology* **131**, 2665–2671.
- BRADLEY, D. E. & TAYLOR, D. E. (1987). Transposition from RP4 to other replicons of a tellurite-resistance determinant not normally expressed by IncPα plasmids. *FEMS Microbiology Letters* **41**, 237–240.
- BRADLEY, D. E., TAYLOR, D. E. & COHEN, D. R. (1980). Specification of surface mating systems among conjugative drug resistance plasmids in *Escherichia coli* K-12. *Journal of Bacteriology* **143**, 1466–1470.
- BRADLEY, D. E., COETZEE, J. N., BOTHMA, T. & HEDGES, R. W. (1981). Phage t: a group T plasmid-dependent bacteriophage. *Journal of General Microbiology* **126**, 397–403.
- COETZEE, J. N., BRADLEY, D. E., HEDGES, R. W., HUGHES, V. M., MCCONNELL, M. M., DU TOIT, L. & TWEEHUYSEN, M. (1986). Bacteriophages F₀lac h, SR, SF: phages which adsorb to pili encoded by plasmids of the new S-complex. *Journal of General Microbiology* **132**, 2907–2917.
- DATTA, N. & HUGHES, V. M. (1983). Plasmids of the same Inc groups in enterobacteria before and after the medical use of antibiotics. *Nature, London* **306**, 616–617.
- DIVERS, M., CRAVEN, P. L. & VIVIAN, A. (1985). Molecular analysis of an antibiotic resistance plasmid, pAV5, and its derivative plasmids in *Actinobacter calcoaceticus*. *Journal of General Microbiology* **131**, 3367–3374.
- FENNEWALD, M. W., PREVATT, W., MEYER, R. & SHAPIRO, J. (1978). Isolation of IncP-2 plasmid DNA from *Pseudomonas aeruginosa*. *Plasmid* **1**, 164–173.
- FIGURSKI, D. H., POHLMAN, R. F., BECHHOFFER, D. H., PRINCE, A. S. & KELTON, C. A. (1982). Broad host range plasmid RK2 encodes multiple *kil* genes potentially lethal to *Escherichia coli* host cells. *Proceedings of the National Academy of the United States of America* **79**, 1935–1939.
- FOSTER, T. J. (1984). Analysis of plasmids with transposons. *Methods in Microbiology* **17**, 197–226.
- GUYER, M. S. (1978). The gamma delta sequence of F is an insertion sequence. *Journal of Molecular Biology* **126**, 347–365.
- JACOB, A. E., SHAPIRO, J. A., YAMAMOTO, L., SMITH, D. I., COHEN, S. N. & BERG, D. (1977). Plasmids studied in *Escherichia coli* and other enteric bacteria. In *DNA Insertion Elements, Plasmids and Episomes*, pp. 607–638. Edited by A. I. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor Laboratory.
- JACOBY, G. A. (1977). Classification of plasmids in *Pseudomonas aeruginosa*. In *Microbiology – 1977*, pp. 119–126. Edited by D. Schlessinger, Washington, DC: American Society for Microbiology.
- JOBLING, M. G. & RITCHIE, D. A. (1987). Genetic and physical analysis of plasmid genes expressing inducible resistance of tellurite in *Escherichia coli*. *Molecular and General Genetics* **208**, 288–293.
- LANKA, E., LURZ, R. & FÜRST, J. P. (1983). Molecular cloning and mapping of *SphI* restriction fragments of plasmid RP4. *Plasmid* **10**, 303–307.
- OLSEN, R. H. & SHIPLEY, P. (1973). Host range and properties of the *Pseudomonas* R factor R1822. *Journal of Bacteriology* **113**, 772–780.
- SCHROEDER, H. A., BUCKMAN, J. & BALASSA, J. J. (1967). Abnormal trace elements in man: tellurium. *Journal of Chronic Diseases* **20**, 1147–1161.
- SILVER, S., BUDD, K., LEAHY, K. M., SHAW, W. V., HAMMOND, D., NOVICK, R. P., WILLSKY, G. R., MALAMY, M. H. & ROSENBERG, H. (1981). Inducible plasmid-determined resistance to arsenate, arsenite, and antimony(III) in *Escherichia coli* and *Staphylococcus aureus*. *Journal of Bacteriology* **146**, 983–996.
- STANISICH, V. A. (1974). The properties and host range of the male-specific bacteriophages of *Pseudomonas aeruginosa*. *Journal of General Microbiology* **84**, 332–342.
- SUMMERS, A. O. & JACOBY, G. A. (1977). Plasmid-determined resistance to tellurium compounds. *Journal of Bacteriology* **129**, 276–281.
- TAYLOR, D. E. & BRADLEY, D. E. (1987). Location on RP4 of a tellurite resistance determinant not normally expressed by IncPα plasmids. *Antimicrobial Agents and Chemotherapy* **31**, 823–825.
- TAYLOR, D. E. & BROSE, E. C. (1985). Restriction endonuclease mapping of R27 (TP117), an incompatibility group HI subgroup 1 plasmid from *Salmonella typhimurium*. *Plasmid* **13**, 75–77.

- TAYLOR, D. E. & SUMMERS, A. O. (1979). Association of tellurium resistance and bacteriophage inhibition conferred by R plasmids. *Journal of Bacteriology* **137**, 1430–1433.
- THATTE, V., BRADLEY, D. E. & IYER, V. N. (1985). N conjugative transfer system of plasmid pCU1. *Journal of Bacteriology* **163**, 1229–1236.
- TUCKER, F. L., WALPER, J. F., APPLEMAN, M. D. & DONOHUE, J. (1962). Complete reduction of tellurite to pure tellurium metal by microorganisms. *Journal of Bacteriology* **83**, 1313–1314.
- VILLARROEL, R., HEDGES, R. W., MAENHAUT, R., LEEMANS, J., ENGLER, G., VAN MONTAGU, M. & SCHELL, J. (1983). Heteroduplex analysis of P plasmid evolution: the role of insertion and deletion of transposable elements. *Molecular and General Genetics* **189**, 390–399.
- WATERWORTH, P. M. (1978). Quantitative methods for bacterial sensitivity testing. In *Laboratory Methods in Antimicrobial Therapy*, pp. 30–40. Edited by D. S. Reeves, I. Phillips, J. D. Williams & R. Wise. Edinburgh: Churchill Livingstone.
- WHITELEY, M. & TAYLOR, D. E. (1983). Identification of DNA homologies among H incompatibility group plasmids by restriction enzyme digestion and Southern transfer hybridization. *Antimicrobial Agents and Chemotherapy* **24**, 194–220.
- WOLFSON, J. S., HOOPER, D. C., SWARTZ, M. N. & MCHUGH, G. L. (1982). Antagonism of the B subunit of DNA gyrase eliminates plasmids pBR322 and pMG110 from *Escherichia coli*. *Journal of Bacteriology* **152**, 338–344.
- YOUNG, C., PRINCE, A. S. & FIGURSKI, D. H. (1985). *korA* function of promiscuous plasmid RK2: an autorepressor that inhibits expression of host-lethal gene *kilA* and replication gene *trfA*. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 7374–7378.
- YOUNG, C., BURLAGE, R. S. & FIGURSKI, D. H. (1987). Control of the *kilA* gene of the broad-host-range plasmid RK2: involvement of *korA*, *korB*, and a new gene, *korE*. *Journal of Bacteriology* **169**, 1315–1320.