

Association of Tellurium Resistance and Bacteriophage Inhibition Conferred by R Plasmids

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Concomitant resistance to tellurium compounds (Ter) and inhibition of coliphage development (Phi) are properties mediated by many H2 incompatibility group R plasmids which have been isolated from diverse bacterial and geographic sources. Ter plasmids from tellurium-resistant bacteria that were isolated from sewage and industrial wastes also mediated phage inhibition. Of these Ter plasmids, three from *Citrobacter freundii* belonged to the H incompatibility group, whereas three from *Klebsiella pneumoniae* did not.

Resistance to tellurium compounds is uncommon among bacteria, except for *Corynebacterium diphtheriae* (1), *Streptococcus faecalis* (15), and many isolates of *Staphylococcus aureus* (6). Summers and Jacoby have recently shown that resistance to the oxyanions of tellurite and tellurate in gram-negative bacteria may be conferred by R plasmids (17). At present, the biochemical basis of plasmid-mediated tellurite resistance is unknown.

S-group plasmids have recently been reclassified into the H incompatibility group, subgroup H2 (23). H1 and H2 plasmids are incompatible with one another (22). The H1-subgroup plasmids are incompatible with the F factor, whereas H2 plasmids are compatible with F (16).

It is possible to differentiate between plasmids of the two H subgroups superficially, because H2 plasmids inhibit the development of some DNA phages, whereas H1 plasmids do not (20). Bacteriophages λ , T1, T5, and T7 are inhibited in development, but T4 and P1 are not (21). Watanabe et al. (26) and Revel and Georgopoulos (13) showed that phage DNA is restricted in cells carrying the H2 plasmid N-1, although DNA modification did not take place.

Tellurium resistance (Ter) and phage inhibition (Phi) are unusual properties (17). Since tellurium resistance was characteristic of H2 plasmids, we looked for both properties in our diverse collection of H2 plasmids.

The H2 plasmids used in this study and their bacterial and geographic origins are shown in Table 1. Resistance to potassium tellurite was tested in the *Escherichia coli* C strains RG176 and RG488 (21). Growth of R⁻ strains was inhibited by 10⁻⁵ M potassium tellurite, as noted previously (17). Each H2 plasmid, except R476b,

conferred upon its host a 100-fold increase in potassium tellurite resistance (Table 2). Moreover, these H2 plasmids also reduced the efficiencies of plating of bacteriophages λ , T1, T5, and T7 (Table 2). Thus, the properties of bacteriophage inhibition and tellurium resistance were concomitantly expressed by most H2 plasmids, except for R476b, which did not express either property.

The incompatibility status of R476b was tested with reference H1 and H2 plasmids and with F42 as described previously (22). R476b belongs to the H2 incompatibility group and has similar properties of incompatibility and entry exclusion to those of other H2 subgroup plasmids.

Of bacteria isolated from film-reprocessing sludge, 95% were multiply resistant to tellurite, mercury, and silver; 10 and 22% bacteria isolated from Boston city sewage and hospital sewage, respectively, were tellurium resistant. The transferability of mercury resistance in such isolates was determined by mating them with *E. coli* K-12 AB1932-1 (19). A single colony of each isolate was inoculated into a well of a microtiter plate containing 0.2 ml of a 1/100 dilution of an overnight culture of AB1932-1 in LB-broth (12) with 0.2% glucose. The microtiter plate was incubated at 30°C overnight, and 0.025 ml of the mating mixture was streaked on MacConkey lactose agar containing 75 μ g of nalidixic acid per ml and 50 mM HgCl₂ to select for transconjugants of AB1932-1. Ten Lac⁻ colonies from each selection were further screened for metal and antibiotic resistance patterns. The metal resistance markers were not always transferred together. The predominant biotypes among strains carrying transmissible plasmids were *Klebsiella*

TABLE 1. Origin of H-group plasmids

Plasmid designation	Incompatibility subgroup	Relevant markers ^a	Origin		Reference
			Bacterial	Geographic	
pRG1251	H1	Ap Cm Sm Sp Su Tc	<i>Salmonella typhi</i>	Thailand	21
pSD114	H2	Cm Km Sm Tc	<i>Salmonella anatum</i>	Canada	22
pAS251-2	H2	Cm Km Sm Tc	<i>Salmonella typhimurium</i>	Canada	21
N-1	H2	Sm Su Tc Hgr	<i>Shigella flexneri</i>	Japan	21
pSD12	H2	Cm Tc	<i>S. typhimurium</i>	Canada	20
pSD16	H2	Km Sm Tc	<i>S. typhimurium</i>	Canada	25
pSD274	H2	Sm Tc	<i>S. typhimurium</i>	Canada	25
TP116	H2	Cm Sm Su Hgr	<i>S. typhi</i>	Spain	2
pWR23	H2	Lac Scr	<i>Salmonella tennessee</i>	U.S.A.	8 ^b
MIP233	H2 ^c	Scr	<i>Salmonella ohio</i>	England	9, 10
MIP235	H2	Lac Cm Sm Su	<i>Salmonella oranienburg</i>	Brazil	9, 10
R476b	H2	Sm Su Tc Asr Hgr	<i>Serratia marcescens</i>	U.S.A.	5
R477-1	H2	Sm Su Tc Asr Hgr Ter	<i>S. marcescens</i>	U.S.A.	5, 23
R478	H2	Cm Km Tc Asr Hgr Ter	<i>S. marcescens</i>	U.S.A.	5, 23
R826	H2	Ap Cm Gm Km Sm Tc Asr Hgr Pmr Ter	<i>S. marcescens</i>	France	5, 17
R828	H2	Ap Cm Gm Km Sm Tc Asr Hgr Pmr Ter	<i>S. marcescens</i>	France	5, 17

^a Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulfonamides; Tc, tetracycline; Asr, sodium arsenate; Hgr, mercuric chloride; Pmr, phenylmercuric acetate; Lac, lactose fermentation; Scr, sucrose fermentation; Ter, potassium tellurite.

^b pWR23 was originally designated *lac scr* (24).

^c MIP233 was recently reclassified as the single member of the H3 subgroup by its lack of DNA homology with H1 and H2 plasmids (14).

TABLE 2. Tellurite resistance and bacteriophage inhibition mediated by plasmids of the H2 incompatibility group^a

Plasmid	Minimal inhibitory concn for K ₂ TeO ₃ (M)	Efficiency of plating with phage:			
		λ	T1	T5	T7
None	10 ⁻⁵	1	1	1	1
pSD114	10 ⁻³	10 ⁻⁴	10 ⁻¹	<10 ⁻⁸	10 ⁻¹
pAS251-2	10 ⁻³	10 ⁻⁴	10 ⁻¹	<10 ⁻⁸	10 ⁻¹
N-1	10 ⁻³	10 ⁻⁴	10 ⁻¹	<10 ⁻⁸	10 ⁻¹
pSD12	10 ⁻³	10 ⁻⁴	10 ⁻¹	<10 ⁻⁸	10 ⁻¹
pSD16	10 ⁻³	10 ⁻¹	10 ⁻¹	10 ⁻³	10 ⁻¹
pSD274	10 ⁻³	0.5	10 ⁻¹	10 ⁻⁴	10 ⁻¹
TP116	10 ⁻³	10 ⁻¹	10 ⁻¹	<10 ⁻⁸	0.5
pWR23	10 ⁻³	10 ⁻⁴	10 ⁻²	10 ⁻¹	10 ⁻¹
MIP233	10 ⁻³	10 ⁻⁴	10 ⁻¹	10 ⁻⁴	10 ⁻¹
MIP235	10 ⁻³	10 ⁻⁴	10 ⁻¹	10 ⁻¹	10 ⁻¹
R476b	10 ⁻⁵	1	1	1	1
R477-1	10 ⁻³	10 ⁻¹	0.5	0.5	10 ⁻¹
R478	10 ⁻³	10 ⁻¹	0.5	0.5	10 ⁻¹
R826	10 ⁻³	10 ⁻⁴	10 ⁻⁴	<10 ⁻⁸	0.5
R828	10 ⁻³	10 ⁻⁴	10 ⁻⁴	<10 ⁻⁸	0.5

^a *E. coli* C strain RG176 or R488 was used in all experiments.

pneumoniae in the case of both hospital and city sewages and *Citrobacter freundii* in the case of film-reprocessing sludge (18).

Six plasmids mediating tellurium resistance,

referred to in this paper as Ter plasmids, with the H2 plasmid pSD114 were chosen for further study. All six of these plasmids conferred phage inhibition on their host, *E. coli* AB1932-1 (Table 3). This host is a λ lysogen; therefore, the efficiency of plating for phage λ was not tested. The level of phage T1 inhibition was greater in strain AB1932-1 than in strain RG176 (Tables 2 and 3).

TABLE 3. Bacteriophage inhibition mediated by tellurite resistance plasmids

Plasmid	Resistance markers ^a	Efficiency of plating ^b with phage:		
		T1	T5	T7
None		1	1	1
pSD114	Ter Cm Km Sm Tc	10 ⁻⁶	<10 ⁻⁸	10 ⁻¹
RAS5002 ^c	Ter Hgr Km Tc Sm	10 ⁻⁶	<10 ⁻⁸	10 ⁻³
RAS5003 ^c	Ter Hgr Cm Km Tc Sm	10 ⁻⁶	<10 ⁻⁸	10 ⁻³
RAS5005 ^d	Ter	10 ⁻⁶	<10 ⁻⁸	10 ⁻¹
RAS5007 ^e	Ter Hgr	10 ⁻⁶	<10 ⁻⁸	10 ⁻³
RAS5009 ^e	Ter Hgr Pmr	<10 ⁻⁸	<10 ⁻⁸	<10 ⁻⁸
RAS5012 ^e	Ter Hgr Pmr	10 ⁻⁶	<10 ⁻⁸	10 ⁻⁴

^a For abbreviations, see footnote a Table 1.

^b Host was *E. coli* AB1932-1.

^c Original host was *K. pneumoniae* isolated from raw sewage effluent of Massachusetts General Hospital, Boston.

^d Original host was *K. pneumoniae* isolated from Boston city sewage (anaerobic fermentation step).

^e Original host was *C. freundii* from an industrial photographic film-reprocessing sludge obtained in New Bedford, Mass.

Since temperature-sensitive transfer is characteristic of H plasmids, transfer frequencies of the Ter plasmids were tested at 26 and 37°C as described previously (20). The plasmid RAS5003 was nontransmissible from *E. coli* AB1932-1. Plasmids from *C. freundii* (RAS5007, RAS5009, and RAS5012) were highly thermosensitive and had a 10,000-fold greater frequency of transfer at 26 than at 37°C. All the transmissible plasmids showed 100% cotransfer frequencies of the Ter and Phi markers.

The six Ter plasmids were tested to determine if they belonged to the H2 incompatibility subgroup. Two of the plasmids from *C. freundii*, RAS5007 and RAS5012, were strongly incompatible with the reference H2-subgroup plasmids and weakly incompatible with the reference H1 plasmid. This type of response has been observed previously for H2 plasmids (22). The plasmids RAS5007 and RAS5012 can therefore be assigned to the H2 subgroup. The other *C. freundii* plasmid, RAS5009, was also incompatible with the H1 plasmid when tested in AB1932-1 but was compatible with both H2 plasmids tested. Unlike H1 plasmids (16) RAS5009 does not show one-way incompatibility with the F factor.

Plasmid DNA was isolated from cells of AB1932-1 containing the Ter plasmids by the method of Hansen and Olsen (4) and separated by electrophoresis on vertical 0.7% agarose gels as described by Meyers et al. (11). A photograph of one such gel is shown in Fig. 1. The H2 plasmids TP116 of 143×10^6 daltons and R478 of 166×10^6 daltons which were used as molecular weight standards also code for both properties. The approximate molecular weights of RAS5002, RAS5003, RAS5007, and RAS5012 were 166×10^6 , and that of RAS5005 was 130×10^6 . Strain AB1932-1 (RAS5009) contained two plasmids, with molecular weights of 46×10^6 and 66×10^6 . Our results show unequivocally that the properties of Ter and Phi are carried by a single plasmid for five of the six Ter plasmids. In the case of RAS5009, the presence of two plasmid DNA bands of lower molecular weight could be explained as dissociation of a large plasmid into two smaller ones, or a cryptic plasmid may accompany a Ter plasmid of uncharacteristically low molecular weight.

We have shown that the unusual properties of phage inhibition and tellurium resistance are often conferred together on plasmids, many of which belong to the H2 incompatibility groups. In some geographic locations, tellurium resistance may be useful as a method of screening for H2 plasmids. These two markers are not, however, specific for H2 plasmids and may also be

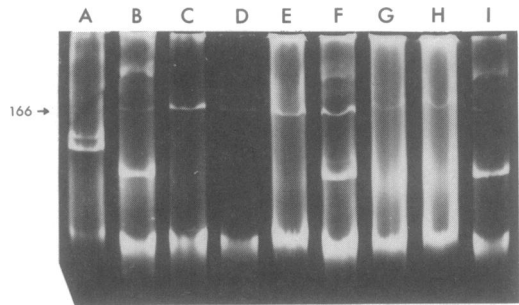


FIG. 1. Agarose gel electrophoresis of Ter plasmid DNA. Ter plasmid DNAs were extracted from *E. coli* AB1932-1. Molecular weight standards were isolated from *E. coli* RG192 (21). All methods were as described previously (4). Samples of plasmid-enriched DNA solution (25 μ l) and tracking dye (10 μ l) were subjected to electrophoresis for 6 h at 100 V through a 0.7% agarose slab gel at 18°C. Bands were visualized on an Ultra-Violet Products transilluminator with shortwave bulbs (254 nm), after staining with ethidium bromide (11). Photographs were taken with a Wratten 23A filter. The slots contained DNA as follows: (A) RAS5009; (B) R478 (molecular weight, 166×10^6 [p. 133 of reference 7]) and S-a (molecular weight, 23×10^6 [11]); (C) RAS5012; (D) RAS5007; (E) RAS5005; (F) TP116 (molecular weight, 143×10^6 [2]), RP1 (molecular weight, 38×10^6 [3]), and S-a; (G) RAS5003; (H) RAS5002; (I) R478 and S-a.

found associated on other, probably unrelated, plasmids.

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