The tellurite-resistance determinants *tehAtehB* and *klaAklA**telB* have different biochemical requirements

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The *tehAtehB* operon from the *Escherichia coli* chromosome (32.3 min) mediates resistance to potassium tellurite (K_2TeO_3) when expressed on a multicopy plasmid such as pUC8 (pTWT100). An MIC of 128 $\mu g\ ml^{-1}$ is observed when *tehAtehB* is expressed in a wild-type host and grown on rich media. In this study, the *tehAtehB* determinant was transformed into mutants deficient in electron transport processes and/or thiol redox coupling within *E. coli*. These mutants included *ubi*, *nad*, *cys*, *nar*, *trx*, *grx*, *gsh* and *sod*. MICs of *tehAtehB* transformed into these mutants ranged from 1–16 $\mu g\ K_2TeO_3\ ml^{-1}$ compared to 0.03–2 $\mu g\ ml^{-1}$ for strains transformed with a control plasmid. The tellurite-resistance determinant locus *kilA* cloned from the IncP α plasmid RK2Te^r (pDT1558) was also investigated in these strains. This tellurite-resistance determinant showed little or no dependency on the host genotype. The ability of *tehAtehB* to mediate resistance in wild-type hosts is limited to rich medium. Rich medium may provide a key unidentified cofactor required by TehAtehB that is not provided under minimal conditions. Again, the ability of the *kilA* determinant to mediate tellurite resistance was independent of medium conditions. These data suggest that either a reducing environment or electron-reducing equivalents are required for *tehAtehB* to mediate high levels of resistance to potassium tellurite. Therefore, the two resistance determinants studied here possess two very different biochemical mechanisms of resistance. Our data also suggest a mechanism for endogenous resistance to tellurite which involves nitrate reductase, superoxide dismutase, and thiol redox processes.

Keywords: tellurite resistance, glutathione, *tehAtehB*, *kilA*, thiol redox

INTRODUCTION

Tellurite (TeO_3^{2-}) is toxic to most micro-organisms, especially Gram-negative bacteria. Bacteria exposed to tellurite turn black as a result of internal deposition of elemental tellurium (Bradley *et al.*, 1988; Lloyd-Jones *et al.*, 1994; Taylor *et al.*, 1988). The toxicity of tellurite has long been considered to be due to its oxidizing ability. However, the standard reduction potential of tellurite reduction is quite negative, $E_{m,7}^* = -1.186\ V$ for the reaction $TeO_3^{2-} + 3H_2O + 4e^- \leftrightarrow Te^0 + 6OH^-$. Thus the specific mechanism of cellular oxidation and toxicity is unknown. Resistance to tellurite (Te^{VI}) is usually mediated by conjugative plasmids and the determinants encoded on these plasmids are usually highly specific for tellurite (Walter & Taylor, 1992).

RK2 is a 60 kb plasmid of incompatibility group P α , which can be stably maintained in a broad range of Gram-negative bacteria (Thomas & Smith, 1987). This plasmid encodes a network of co-regulated genes known as the *kil-kor* regulon (Figurski *et al.*, 1982; Pansegrau *et al.*, 1994). Within this regulon is a tellurite-resistance determinant which is normally cryptic or expressed at very low levels in wild-type RK2 (Bradley, 1985). The tellurite-resistance determinant was mapped to the *kilA* locus and was cloned into a pUC8 plasmid giving pDT1558 from a resistant variant of RK2, RK2Te^r (Walter & Taylor, 1989). The operon comprises three genes, *klaA*, *klaB* and *klaC*, based on the gene designations of Pansegrau *et al.* (1994). The tellurite-resistant variant has been previously referred to as *kilA*, *telA* and *telB* (Turner *et al.*, 1994a, b; Walter *et al.*, 1991b), and all three genes were found to be

necessary for resistance (Turner *et al.*, 1994b). In order to maintain the designation between the Te^r and Te^s variants [which arises from a point mutation in *klaC* (Walter *et al.*, 1991b)], the third gene of the Te^r operon retains the original nomenclature of *telB*. Independent expression of each individual gene gives varying degrees of growth inhibition (Turner *et al.*, 1994a) which is the phenotype originally associated with the operon. The genes encode polypeptides of 28 kDa (KlaA), 42 kDa (KlaB) and a predicted integral membrane protein of 32 kDa (TelB) (Walter *et al.*, 1991a).

The operon *tehAtebB* specifies tellurite resistance when overexpressed (Walter & Taylor, 1989; Walter *et al.*, 1991b). The operon was originally cloned as a 6.8 kb *SalI* fragment into pUC8, when cloning the resistance determinant from pHH1508a (Walter *et al.*, 1991b). Subsequent analysis placed the two genes within a single operon located at 32.3 min on the *E. coli* chromosome (Taylor *et al.*, 1994). These genes, *tehA* and *tehB*, encode proteins of 36 kDa (putative integral membrane protein) and 23 kDa, respectively (Walter *et al.*, 1991b).

In the present study, the tellurite-resistance determinants *klaAklAtebB* and *tehAtebB* were transformed into various *E. coli* mutants deficient in electron transport processes and/or redox coupling in order to determine if their resistance mechanisms are coupled to these cellular processes.

METHODS

Bacterial strains and plasmids. *E. coli* strains used in this study were: JM83, AT2427, AT2455, DG37, JM246, JM457, JT14, NK1, RL165 and EC2254, provided by Dr B. Bachmann (*E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, CT, USA); AN59, AN120 (*uncA401 argE3 thi-1 galK2 xyl-5 mtl-1 rpsL704*), LE392 (*F' supE44 supF58 hsdR514 galK2 galT22 metB1 trpR55 lacY1*), RG51 (LE392 Δ uncB-uncD *src::Tn10 recA56*) and BPR100, provided by Dr B. Rosen (Wayne State University, Detroit, MI, USA); JF420, JF432, JF1070, JF2062, JF2200 and JF2201, provided by Dr J. Fuchs (University of Minnesota, St Paul, MN, USA); GR70N, GR71N and GR75N, provided by Dr R. B. Gennis (University of Illinois, Urbana, IL, USA); JHC1113, JHC1096 and JHC1078 provided by Dr T. Nunoshiba (Harvard School of Public Health, New Haven, CT, USA); LCB2048, provided by Dr G. Giordano (Laboratoire de Chimie Bactérienne, CNRS, Marseille, France); JA199 (Δ trpE5 *leu-6 thi hsdR hsdM⁺*), EC1124 (JA199 *cysI*) and plasmid pJYW2, which overexpresses CysJIIH (sulfite reductase), and pJRS102, which overexpresses CysGJIIH (sulfite reductase with excess sirohaem synthesis) (Wu *et al.*, 1991), provided by Dr N. M. Kredich (Duke University Durham, NC, USA); QC4468, QC1712, QC1725 and QC1799 as well as plasmids pDT1-5 which overexpress *sodA* (Touati, 1983) and pHS1-8 expressing *sodB* (Carlioz *et al.*, 1988) were obtained from Dr D. Touati (Institut Jacques Monod, CNRS, Paris, France). Other *E. coli* strains used in this study were HB101 [*F' hsdS20 (r_B⁻ m_B⁻) leu supE44 ara-14 galK2 lacY1 proA2 rpsL20 xyl-5 mtl-1 recA13 mcrB*] (Boyer & Roulland-Dussoix, 1969) and MC4100 (Casadaban, 1976). The genotypes of the different strains not listed here are reported in Tables 1-6.

The tellurite-resistance plasmids used in this study include pTWT100 which contains the *tehAtebB* as the 3.3 kb fragment from pDT1364 (Taylor *et al.*, 1994; Turner *et al.*, 1995).

pDT1364 is the original pUC8-based clone of a 6.8 kb fragment from the *E. coli* chromosome containing *tehAtebB* (Walter *et al.*, 1991b). pRT6 is the same as pDT1364 with the ampicillin-resistance marker replaced with a kanamycin-resistance cassette from pKIXX (Pharmacia). pDT1558 contains the *klaAklAtebB* operon from the IncP α plasmid RK2Te^r cloned into pUC8 (Walter *et al.*, 1991a). Similarly, pRT4 is pDT1558 with the kanamycin cassette. The derivatives pRT4 and pRT6 were used in the cases where the strain tested contained an ampicillin-resistance marker.

Growth conditions. All overnight bacterial cultures were grown in Luria-Bertani (LB) broth (Sambrook *et al.*, 1989). Experiments with minimal media used Min A broth (Miller, 1992) supplemented with thiamine and the required amino acids based on the strain genotype. Carbon sources were added to a final concentration of 2% (w/v). All media experiments were performed in 1-2 ml total volume in a 30 ml culture tube, and were incubated at 37 °C in a shaker bath. Antibiotics were used at the following concentrations: ampicillin (100 μ g ml⁻¹), chloramphenicol (30 μ g ml⁻¹), kanamycin sulfate (42 μ g ml⁻¹) and tetracycline (12.5 μ g ml⁻¹).

MIC determinations. For MIC determination, overnight cultures grown in LB broth were diluted 10⁴ in Penassay broth or LB broth and 10-25 μ l aliquots were dropped onto medium plates containing serial dilutions of potassium tellurite (Sigma). Bacterial growth media used included Brain Heart Infusion (Oxoid), LB (Becton Dickinson), and Mueller-Hinton (Oxoid). The most reproducible and consistent data was obtained using LB agar and it is with this medium that the MIC values are reported in Tables 1-6. All reported MIC values are the result of at least three independent determinations. If variability of the MIC was observed, the ranges are reported. All MIC determinations were performed under aerobic growth conditions.

RESULTS

Plasmids containing the *tehAtebB* and *klaAklAtebB* determinants were transformed into a variety of mutants with important roles in the maintenance of thiol redox balance and electron transport. These recombinant cultures were assessed for their ability to mediate resistance to tellurite (Tables 1-6). The determinant *tehAtebB* in the pUC8 vector (pTWT100) results in a culture with an MIC of 128-256 μ g ml⁻¹, and the *klaAklAtebB* operon gives an MIC of 256 μ g ml⁻¹ in pUC8 (pDT1558) when harboured in wild-type *E. coli* strains (Taylor *et al.*, 1994; Turner *et al.*, 1994b; Walter & Taylor, 1989). In some experiments, pDT1555, a pACYC184-based clone of *klaAklAtebB*, was also utilized and gave the same or higher MICs as pDT1558. This was expected since reduced expression of *klaAklAtebB* gives higher MIC values resulting from a reduced-lethality phenotype (Turner *et al.*, 1994b).

Importance of cysteine, ubiquinone and nicotinamide biosynthesis

When plasmids containing the *tehAtebB* determinant were transformed into mutants in the cysteine (Table 1), ubiquinone (Table 2) or nicotinamide (Table 3) metabolic pathways, the resistance to tellurite was severely depressed. Tellurite MICs in these strains transformed with

Table 1. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in cysteine metabolism (*cys*)

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtehB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
JM83	$\Delta lac-pro \phi 80dlacZ \Delta M15 rpsL$	Wild-type	2	128–256	256
AT2427	$F^- cysJ43 relA1 spoT1 thi-1 phoM510 \lambda^-$	Sulfite reductase β	2	4	256
AT2455	Hfr <i>thi-1 cysG44 mal-18 relA1</i> $\lambda^- spoT1$	Sirohaem synthesis	2	4	256
DG37	Hfr <i>relA1</i> $\Delta(pts-cysA)127 spoT1$	Sulfate permease and O-acetylserine sulfhydrylase B	2	4	256
EC2254	<i>araD139</i> $\Delta lacU169 thi trp rpsL relA cys362::mud1::(\Delta p lac)$	Sulfite reductase α and β , adenylyltransferase	2	8	256
JM246	$F^- cysI53 \lambda^- \Omega(rrnD-rrnE)1$	Sulfite reductase α	2	2	256
JM457	$F^- pro-50 his-97 fpk-1 cysI53 cysE51 metB1 lac galT47 mtl strA192 tsx$	Sulfite reductase α , serine acetyltransferase	2	4	256
JT14	<i>cysB214 trpE9829 pyrF</i>	Positive regulator	1–2	2	256
NK1	<i>trpE5 leu-6 thi cysB rbs+ r- m+</i>	Positive regulator	1–2	4	ND
RL165	<i>thr-1 leuB6 trp-1 his-61 cysK511 argH1 thi-1 lacY1 gal-6 xyl-7 malA1(l^R) mtl-2 rpsL9 tonA2 supE44</i> λ^-	O-Acetylserine sulfhydrylase A	2	8	256

ND, Not determined.

Table 2. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in ubiquinone metabolism (*ubi*)

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtehB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
JM83	$\Delta lac-pro \phi 80dlacZ \Delta M15 rpsL$	Wild-type	2	128–256	256
AN59	<i>thr-1 leuB6 ubiB352 thi-1 lacZ4 rpsL8 supE44</i>	2-Octaprenylphenol $\rightarrow$ 2-octaprenyl-6-methoxyphenol	2	4	128
AN66	<i>thr-1 leuB6 ubiD410 lacZ4 rpsL8 (p1)⁺ supE44</i>	3-Octaprenyl-4-hydroxybenzoate $\rightarrow$ 2-octaprenylphenol	2	1	256
JF496	<i>asnB50::Tn5 ubiF411 nagB2 relA1 spoT1 bglR13 asnA31 rbs-4 metB1</i>	2-Octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone $\rightarrow$ 2-octaprenyl-3-methyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone	2	4	256

pTWT100 ranged from 0.5 to 8 $\mu\text{g ml}^{-1}$. Little or no effect was observed for the MICs that the *klaAklaBtelB* determinant was able to mediate in these strains. MICs of 1–2 $\mu\text{g K}_2\text{TeO}_3 \text{ ml}^{-1}$ were observed in these strains carrying the control plasmid pUC8. These results suggest that the products of these metabolic pathways are utilized in some manner by the *tehAtehB* determinant.

In some of the specific mutants more than one strain was utilized because we were not able to obtain the appropriate

isogenic wild-type strains. Additionally, not all mutants were stable and the possibility of selection of revertants during the experiment which might then contaminate the cultures was a concern. Therefore, utilizing many mutants alleviated these difficulties.

Other energetics mutant strains which were studied included *unc* (F_1F_0 -ATPase). No effects on wild-type MICs were observed in this type of host, with or without a resistance determinant.

Table 3. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in nicotinamide metabolism (*nad*)

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtebB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
GR70N	F ⁻ <i>thi rpsL gal</i>	Wild-type	2	128	256
GR71N	F ⁻ <i>nadA50::Tn10 thi rpsL gal</i>	Quinolinate synthetase A	2	4	256
GR75N	F ⁻ <i>nadA50::Tn10 thi rpsL gal cydA2</i>	Quinolinate synthetase A, cytochrome <i>d</i> terminal oxidase	1	4	256
W4546	<i>galT23 nadC8 Ω(rrnD-rrnE)1</i>	Quinolinate phosphoribosyl transferase	1–2	4	128
W3899	<i>supE44 nadB7</i>	Quinolinate synthetase B	2	4	256

Table 4. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in thiol redox enzymes and metabolites

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtebB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
JF1070	AB1157*; <i>proAB lacIPOZYA</i>	Wild-type	2	256	256
JF2062	JF1070; <i>trxA::kan</i>	Thioredoxin	0.5–1	8	256
JF432	JF1070; <i>trxB gal⁺</i>	Thioredoxin reductase	0.5–1	4–8	256
BPR100	JF1070; <i>grx::kan</i>	Glutaredoxin	0.5	4	256
JF420	JF1070; <i>gor-1</i>	Glutathione oxidoreductase	0.25	4	256
JF2200	JF1070; <i>gshA::kan</i>	Glutathione synthetase A	0.25	4	256
JF2201	JF1070; <i>gshB::kan</i>	Glutathione synthetase B	0.25	4	256

* AB1157: F⁻ *thr-1 lenB6 lacY1 supE44 rfbD1 thi-1 malT1 ara-14 galK2 xyl-5 mtl-1 mgl-51 hisG4 (gpt-pro)62 kdgK51 argE3 tsx-33*.

Importance of thiol redox balance components

Mutants in thiol redox, carrying the control plasmid, displayed hypersensitivity to tellurite, with MIC values ranging from 0.25 to 1 $\mu\text{g ml}^{-1}$ (Table 4). When these mutants were transformed with the plasmids carrying *tehAtebB*, MICs ranged from 2 to 8 $\mu\text{g ml}^{-1}$ compared to 128–256 $\mu\text{g ml}^{-1}$ for the wild-type. Again, no effect was observed on the ability of the *klaAklaBtelB* operon to mediate resistance in these strains.

The results in Table 4 suggested that thiol redox is involved both in host resistance and *tehAtebB*-mediated tellurite resistance. To test if glutathione is involved in the resistance mechanism of *tehAtebB*, glutathione inhibitors were employed. Buthione sulfoximine (BSO), an inhibitor of γ -glutamylcysteine synthetase (*gshA*), was added to cultures at a concentration of 10 mM. This concentration would be expected to eliminate all glutathione synthesis (Griffith & Meister, 1979). A scavenger of glutathione and free cysteine, diethylmaleate (DEM), was also utilized, at a concentration of 5 mM. Wild-type host strains exposed to these compounds had a reduction of their MIC from 2 to 1 $\mu\text{g ml}^{-1}$. No effect on the MIC with HB101 (pDT1558) was observed. However, the MIC with HB101(pDT1364) or HB101(pTWT100) decreased from

128 to 64 $\mu\text{g ml}^{-1}$ in the presence of BSO and down to 1 $\mu\text{g ml}^{-1}$ in the presence of DEM. The failure of BSO to have a significantly large effect on *E. coli* glutathione concentration has been reported by others (Moore *et al.*, 1989; Romero & Canada, 1991). Consistent with these previous findings, we also only observed a minor effect, but the results with the scavenger DEM confirm the mutant data, suggesting that glutathione is involved both in the host resistance as well as with the resistance mediated by *tehAtebB*.

Importance of nitrate and sulfite reductases

Recent studies by C. Avazeri and co-workers have shown that nitrate reductase (NarG) has a tellurite reductase activity (unpublished). Additionally, overexpression of the *narG* operon behind an inducible *tac* promoter gives rise to low levels of tellurite resistance under aerobic conditions (C. Avazeri and others, unpublished). We tested whether overexpression of sulfite reductase had similar properties, or if this enzyme would modulate tellurite resistance in the presence or absence of overexpressed *tehAtebB* or *klaAklaBtelB*. The wild-type JA199 or *cysI* (EC1124) hosts harbouring pJRS102 (which overexpresses *cysGJIH*) gave tellurite MICs in the range of

Table 5. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in nitrate reductase

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtehB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
MC4100	F ⁻ <i>araD139</i> Δ (<i>argR-lac</i>)U169 <i>ftbB5301 ptsF25 relA1 rpsL150</i> <i>deoC1 rpsR</i>	Wild-type	2	128–256	256–512
LCB2048	<i>thi-1 thr-1 leu6 lacY1 supE44 rpsL</i> Δ <i>nar-25(narG-narH) narZ::\Omega</i>	Nitrate reductases (NarGHJI and NarZYWV)	0.015–0.03	16	64

Table 6. Effect of determinants on tellurite MICs in strains of *E. coli* deficient in antistress response

Strain	Genotype	Description of relevant genotype	MIC of tellurite ($\mu\text{g ml}^{-1}$) with plasmids:		
			pUC8 (Control)	pTWT100 (<i>tehAtehB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
QC4468	F ⁻ Δ (<i>lac</i>)U169 <i>rpsL</i>	Wild-type	2	128	256
QC1712	QC4468; Δ <i>sodA3 rhu::Tn5</i> <i>zdh::Tn10</i>	Superoxide dismutase [Mn]	0.125–0.25	128	128
QC1725	QC4468; Δ <i>sodA3 zdh::mini-tet</i>	Superoxide dismutase [Mn]	0.125–0.25	128–256	256
QC1799	QC4468; Δ <i>sodA3</i> Δ <i>sodB2</i>	Superoxide dismutases [Mn] and [Fe]	0.125–0.25	128	128
JHC1113	QC4468; <i>marR1 xdd-2207::Tn10</i>	Repressor multi-antibiotic resistance	4	256	256
JHC1096	QC4468; <i>zdd-239::Tn9 del1738</i> (Δ <i>mar</i>)	Deletion in MarRAB	2–4	256	256
JHC1078	QC4468; <i>soxR105 zjc-2204::Tn10</i>	Repressor of redox stress response	4	256	256

2–4 $\mu\text{g ml}^{-1}$. No $\text{TeO}_3^{2-} \rightarrow \text{Te}^0$ activity was observed in cell-free extracts of JA199(pUC8), JA199(pJRS102) or JA199(pJYW2) using a qualitative aerobic assay (assessed by blackening of the extract/culture as measured by ΔA_{600}). Hosts harbouring both pJRS102 and pRT6 (*tehAtehB* clone) or pRT4 (*klaAklaBtelB* clone) had MICs of 128 and 256 $\mu\text{g ml}^{-1}$, respectively. These results suggest that sulfite reductase alone does not interact with tellurite directly and does not enhance the activity of both resistance determinants. However, the absence of sulfite reductase is detrimental to the ability of the *tehAtehB* determinant to mediate resistance (Table 1).

Tellurite resistance of nitrate reductase mutants is severely depressed, with MICs of 0.015–0.03 $\mu\text{g ml}^{-1}$ (Table 5). The resistance level for the *tehAtehB* determinant was lowered in this host but not to the same extent as with other mutants studied. However, unlike the other mutants tested, the tellurite resistance mediated by the *klaAklaBtelB* determinant was also reduced in nitrate reductase mutants.

Importance of oxidative stress response

We also investigated the MICs of tellurite in mutants linked to oxidative stress (Table 6). Superoxide dismutase

mutants were hypersensitive to tellurite, with MICs ranging from 0.125 to 0.25 $\mu\text{g ml}^{-1}$. Little or no effect was observed on the MICs of the tellurite-resistance determinants in hosts lacking either or both superoxide dismutases (SodA, SodB). Additionally, overexpression of SodA did not give rise to enhanced resistance to tellurite compared to the wild-type host.

Mutants unable to elicit a stress response (*sox* or *mar*) (Ariza *et al.*, 1994) were also tested. As these proteins regulate a large number of genes, we considered it possible that one of them may be vital to the resistance mechanism mediated by the two tellurite-resistance determinants. However, as seen by the data in Table 6, this was not the case. The MICs for the determinants were comparable to the wild-type or slightly increased.

Effect of media conditions

The effect of stress from medium conditions was also assessed based on the ability of the determinants to facilitate growth in the presence of 25 $\mu\text{g K}_2\text{TeO}_3 \text{ ml}^{-1}$ using the wild-type host HB101 (Table 7). This concentration is well below the MIC and in rich media both resistance determinants allow the host to grow to normal cell densities. However, when only a minimal salts

Table 7. Dependency on medium conditions for the tellurite-resistance determinants to confer resistance to 25 µg K₂TeO₃ ml⁻¹

—, No growth; +, ++, +++, increasing amounts of growth; ~, observed darkening of the broth as a result of TeO₃²⁻ → Te⁰ reduction but little or no viable cells present.

Medium	Plasmid		
	pUC8 (Control)	pTWT100 (<i>tehAtebB</i>)	pDT1558 (<i>klaAklaBtelB</i>)
Luria-Bertani broth	—	+++	+++
Terrific Broth	—	+++	+++
Mueller-Hinton	—	+++	+++
MinA + glucose	—	—	+++
MinA + glycerol	—	—	++
MinA + succinate	—	—	+
MinA + lactate	—	~	++
MinA + glycerol + fumarate	—	—	++
MinA + glycerol + DMSO	—	—	++
Peptone + glucose + fumarate	—	++	++
MinA + glucose + 1 % yeast extract	—	++	++
MinA + glucose + 1 % tryptone	—	++	++
MinA + glucose + 1 % Casamino acids	—	+	++
MinA + glucose + 0.1 % cystine	—	~	++
MinA + glucose + 0.1 % oxidized glutathione	—	~	++
MinA + glucose + 10 mM L-2-oxothiazolidine	—	+	+++

medium was utilized with a single carbon source, the determinant *tehAtebB* did not protect the host from tellurite. In contrast, the *klaAklaBtelB* determinant was able to protect the host under all medium conditions studied. Because the addition of tryptone or Casamino acids was able to restore growth for hosts harbouring *tehAtebB*, it is possible that the missing factor supplied by the medium is an amino acid or a peptide. These medium components do not act on tellurite to reduce its effective concentration (Turner *et al.*, 1992b). Based on the mutant data, we suggest that this may be a cysteine-based peptide, possibly glutathione. Unfortunately, the addition of reduced thiol (cysteine or glutathione-SH) causes direct reduction of the tellurite to elemental tellurium and could not be tested. Addition of excess amounts of the oxidized forms (cystine and glutathione-S-S-glutathione), showed some TeO₃²⁻ → Te⁰ reduction with only a trace amount of growth. Furthermore, addition of L-2-oxothiazolidine, which has been shown to increase intracellular glutathione-SH levels (Romero & Canada, 1991), was able to restore some growth.

DISCUSSION

A number of possible mechanisms can be evoked to explain tellurite resistance. These include reduced uptake and increased efflux, detoxification through reduction or chemical modification, sequestration, repair of cellular damage, production of a compensating enzyme, modification of the target of toxicity, regulation of host genes, and modification of host enzymic function and/or specificity. We have ruled out increased efflux and/or

reduced uptake as a mode of resistance for both the *klaAklaBtelB* and *tehAtebB* tellurite-resistance determinants based on tellurite accumulation studies (Turner *et al.*, 1995). However, the precise biochemical mechanism has yet to be identified for these tellurite-resistance determinants.

In the present study we have addressed the metabolic state of the host necessary for the tellurite-resistance determinants to mediate resistance. We have found that the *tehAtebB* determinant is dependent almost exclusively on the host cell to provide a particular metabolic state. Resistance mediated by the *tehAtebB* determinant requires a functional electron transport chain with a functional quinone pool. Additionally, a cysteine metabolic pathway is required, probably for the synthesis of glutathione which was also found to be required. Other thiol redox metabolites were also found to be required for *tehAtebB* to mediate full resistance. In contrast, the *klaAklaBtelB* tellurite-resistance determinant required none of these host systems. It is clearly evident from these studies that the tellurite-resistance determinants *tehAtebB* and *klaAklaBtelB* have two very different biochemical mechanisms. Additionally, there appears to be a synergy between the hosts resistance mechanisms and that of *tehAtebB*.

Two other tellurite-resistance determinants have been identified. The *ter* determinant from the IncHI2 plasmid also mediates tellurite resistance (Hill *et al.*, 1993; Lloyd-Jones *et al.*, 1994). Although the mechanism of resistance has not been determined for this system, reduced uptake and/or efflux has been ruled out (Lloyd-Jones *et al.*, 1994;

Turner *et al.*, 1995). Clones of the *ter* determinant from both the pMER610 and R478 plasmids have been transformed into some of the mutant strains studied here (unpublished results). These preliminary results suggest that the biochemical resistance mechanism of the *ter* determinant is different from that of the *tebAtebB* and *klaAklAtebB* determinants described in the present paper. Additionally, the oxyanion efflux pump *arsABC* from the FI plasmid has been shown to mediate moderate levels of resistance towards tellurite (Turner *et al.*, 1992a). There are also a variety of organisms which have demonstrated reduction of inorganic oxyanions including TeO_3^{2-} (reviewed by Turner *et al.*, 1995, and Walter & Taylor, 1992). The data suggests that there may be as many as five distinct mechanisms of tellurite resistance.

The MarRAB and SoxRAB responses regulate a large number of genes (Ariza *et al.*, 1994; Rosner & Slonczewski, 1994). We tested the hypothesis that the stress of exposure to tellurite may induce such a response. However, this was not observed in our experiments.

Results from the minimal medium experiments suggest two possibilities: (i) the increased stress of growing under minimal medium conditions is beyond the energetic capabilities for the *tebAtebB* determinant; (ii) there is a requirement in the growth medium for a factor, possibly amino acid/peptide-based, that either acts on tellurite to bring it to the cell in a less toxic form or acts as a cofactor to TeHAtebB.

Our study also provides information on the host's mechanism of processing tellurite. Cultures of *E. coli* without a resistance determinant will turn black upon exposure to tellurite as a result of tellurium deposition. Most of the mutant strains studied have an MIC between 1 and 2 $\mu\text{g ml}^{-1}$ without any plasmid or when harbouring a control plasmid pUC8. However, a strain with nitrate reductases mutations (*narG* and *narX*) showed a marked hypersensitivity to tellurite with an MIC of 0.03 $\mu\text{g ml}^{-1}$. This suggests that the membrane-bound nitrate reductase may be the primary defence of the cell against tellurite in agreement with the observations of C. Avazeri and others (unpublished). Mutants in the thiol redox coupling activity in the cell had reduced MICs, from 0.25 to 0.5 $\mu\text{g ml}^{-1}$, indicating that these thiol agents are also used by *E. coli* as a defence against tellurite. Glutathione has been implicated in the resistance of other heavy metals in *E. coli* including arsenate (Oden *et al.*, 1994) and cis-platinum (Salles & Calsou, 1992) resistance. Defects in glutathione synthesis in many micro-organisms result in increased susceptibility to a variety of heavy metals including cadmium, mercury, copper, lead, zinc, bismuth and silver (Penninckx & Elskens, 1993). Selenite (SeO_3^{2-}) reacts with glutathione-SH to give Glt-S-Se-S-Glt which is then acted on by glutathione reductase to give oxidized glutathione and Se^0 (Ganter, 1971). Although the chemistry of selenium and tellurium is slightly different, it is still possible that this activity occurs to some extent in *E. coli*. Since TeO_3^{2-} reacts readily with free thiols, other targets in the cell for tellurite attack could include CoA-SH and lipoamide dehydrogenase. We have further experiments underway to test this hypothesis.

Hypersensitivity was also observed in *sod* hosts with MICs in the 0.125–0.25 $\mu\text{g ml}^{-1}$ range. This sensitivity may be an indication that O_2^- is produced as a by-product of the $\text{TeO}_3^{2-} \rightarrow \text{Te}^0$ reduction within the cell. Metabolic superoxide production is considered to be due to leakage of electrons from the respiratory chain dehydrogenases (Imlay & Fridovich, 1991). It is possible that the tellurite reduction activity catalysed by nitrate reductase produces excessive amounts of O_2^- .

Further protection can be afforded by defects in phosphate transport which can mediate low levels of resistance to tellurite (Tomas & Kay, 1986). Thus we can envisage a model in which tellurite enters the cell by the phosphate uptake system and is acted on at the cytoplasmic side of the inner membrane by nitrate reductase. Tellurite which circumvents this line of defence is then acted on by glutathione and other reduced thiols in the cytoplasm which would give rise to further $\text{TeO}_3^{2-} \rightarrow \text{Te}^0$ reduction. Superoxide dismutase would then act on the O_2^- produced as a result of the TeO_3^{2-} reduction. The result of eliminating components of this cascade would severely damage thiol redox. Subsequently, the consequences of tellurite exposure would be the shutting down of cellular processes involved in DNA synthesis, protein synthesis, as well as most reductases.

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REFERENCES

- Ariza, R. R., Cohen, S. P., Bachhawat, N., Levy, S. B. & Demple, B. (1994). Repressor mutations in the *marRAB* operon that activate oxidative stress genes and multiple antibiotic resistance in *Escherichia coli*. *J Bacteriol* **176**, 143–148.
- Boyer, H. W. & Roulland-Dussoix, D. (1969). A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J Mol Biol* **41**, 459–472.
- Bradley, D. E. (1985). Detection of tellurite-resistance determinants in IncP plasmids. *J Gen Microbiol* **131**, 3135–3137.
- Bradley, D. E., Grewal, K., Taylor, D. E. & Whelan, J. (1988). Characteristics of RP4 tellurite-resistance transposon Tn521. *J Gen Microbiol* **134**, 2009–2018.
- Carliz, A., Ludwig, M. L., Stallings, W. C., Fee, J. A., Steinman, H. M. & Touati, D. (1988). Iron superoxide dismutase: nucleotide sequence of the gene from *Escherichia coli* K12 and correlations with crystal structures. *J Biol Chem* **263**, 1555–1562.
- Casadaban, M. J. (1976). Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and mu. *J Mol Biol* **104**, 541–555.
- Figurski, D. H., Pohlman, R. F., Bechhofer, 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. *Proc Natl Acad Sci USA* **79**, 1935–1939.

- Ganther, H. E. (1971). Reduction of selenotrisulfide derivative of glutathione to persulfide analog by glutathione reductase. *Biochemistry* **10**, 4089–4098.
- Griffith, O. W. & Meister, A. (1979). Potent and specific inhibitors of glutathione synthesis by buthione sulfoximine (*S*-*N*-butyl homocysteine sulfoximine). *J Biol Chem* **254**, 7558–7560.
- Hill, S. M., Jobling, M. G., Lloyd, B. H., Strike, P. & Ritchie, D. A. (1993). Functional expression of the tellurite resistance determinant from the IncHI-2 plasmid pMER610. *Mol & Gen Genet* **241**, 203–212.
- Imlay, J. A. & Fridovich, I. (1991). Assay of metabolic superoxide production in *Escherichia coli*. *J Biol Chem* **266**, 6957–6965.
- Lloyd-Jones, J., Osborn, A. M., Ritchie, D. A., Strike, P., Hobman, J. L., Brown, N. L. & Rouch, D. A. (1994). Accumulation and intracellular fate of tellurite in tellurite-resistant *Escherichia coli*: a model for the mechanism of resistance. *FEMS Microbiol Lett* **118**, 113–120.
- Miller, J. F. (1992). *A Short Course in Bacterial Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Moore, W. R., Anderson, M. E., Meister, A., Murata, K. & Kimura, A. (1989). Increased capacity for glutathione synthesis enhances resistance to radiation in *Escherichia coli*: a possible model for mammalian cell protection. *Proc Natl Acad Sci USA* **86**, 1461–1464.
- Oden, K. L., Gladysheva, T. B. & Rosen, B. P. (1994). Arsenate reduction mediated by the plasmid-encoded ArsC protein is coupled to glutathione. *Mol Microbiol* **12**, 301–306.
- Pansegau, W., Lanka, E., Barth, P. T., Figurski, D. H., Guiney, D. G., Haas, D., Helinski, D. R., Schwab, H., Stanisich, V. A. & Thomas, C. M. (1994). Complete nucleotide sequence of Birmingham IncP α plasmids. Compilation and comparative analysis. *J Mol Biol* **239**, 623–663.
- Penninckx, M. J. & Elskens, M. T. (1993). Metabolism and functions of glutathione in micro-organisms. *Adv Microb Physiol* **34**, 239–301.
- Romero, M. J. M. & Canada, A. T. (1991). The evaluation of *Escherichia coli* as a model for oxidant stress in mammalian hepatocytes: role of glutathione. *Toxicol Appl Pharmacol* **111**, 485–495.
- Rosner, J. L. & Slonczewski, J. L. (1994). Dual regulation of *inaA* by the multiple antibiotic resistance (Mar) and superoxide (SoxRS) stress response systems of *Escherichia coli*. *J Bacteriol* **176**, 6262–6269.
- Salles, B. & Calsou, P. (1992). Involvement of glutathione in cisplatin toxicity in *Escherichia coli* K12. *Toxicology* **72**, 341–350.
- Sambrook, J., Fritsch, E. R. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Taylor, D. E., Walter, E. G., Sherburne, R. & Bazett-Jones, D. P. (1988). Structure and location of tellurium deposited in *Escherichia coli* cells harboring tellurite resistance plasmids. *J Ultrastruct Mol Struct Res* **99**, 18–26.
- Taylor, D. E., Hou, Y., Turner, R. J. & Weiner, J. H. (1994). Location of a potassium tellurite resistance operon (*tebAtebB*) within the terminus of *Escherichia coli* K-12. *J Bacteriol* **176**, 2740–2742.
- Thomas, C. M. & Smith, C. A. (1987). Incompatibility group P plasmids: genetics, evolution, and use in genetic manipulation. *Annu Rev Microbiol* **41**, 77–101.
- Tomas, J. M. & Kay, W. W. (1986). Tellurite susceptibility and non-plasmid-mediated resistance in *Escherichia coli*. *Antimicrob Agents Chemother* **30**, 127–131.
- Touati, D. (1983). Cloning and mapping of the manganese superoxide dismutase gene (*sodA*) of *Escherichia coli* K-12. *J Bacteriol* **155**, 1078–1087.
- Turner, R. J., Hou, Y., Weiner, J. H. & Taylor, D. E. (1992a). The arsenical ATPase efflux pump mediates tellurite resistance. *J Bacteriol* **174**, 3092–3094.
- Turner, R. J., Weiner, J. H. & Taylor, D. E. (1992b). Use of diethyldithiocarbamate for quantitative determination of tellurite uptake by bacteria. *Anal Biochem* **204**, 292–295.
- Turner, R. J., Weiner, J. H. & Taylor, D. E. (1994a). Characterization of the growth inhibition phenotype of the *kelAtelAB* operon from IncP α plasmid RK2Te^r. *Biochem Cell Biol* **72**, 333–342.
- Turner, R. J., Weiner, J. H. & Taylor, D. E. (1994b). *In vivo* complementation and site-specific mutagenesis of the tellurite resistance determinant *kelAtelAB* from IncP α plasmid RK2Te^r. *Microbiology* **140**, 1319–1326.
- Turner, R. J., Weiner, J. H. & Taylor, D. E. (1995). Neither reduced uptake nor increased efflux is encoded by tellurite resistance determinants expressed in *Escherichia coli*. *Can J Microbiol* **41**, 92–98.
- Walter, E. G. & Taylor, D. E. (1989). Comparison of tellurite resistance determinants from the IncP α plasmid RP4Te^r and the IncHII plasmid pHH1508a. *J Bacteriol* **171**, 2160–2165.
- Walter, E. G. & Taylor, D. E. (1992). Plasmid-mediated resistance to tellurite: expressed and cryptic. *Plasmid* **27**, 52–64.
- Walter, E. G., Thomas, C. M., Ibbotson, J. P. & Taylor, D. E. (1991a). Transcriptional analysis, translational analysis, and sequence of the *kelA*-tellurite resistance region of plasmid RK2Te^r. *J Bacteriol* **173**, 1111–1119.
- Walter, E. G., Weiner, J. H. & Taylor, D. E. (1991b). Nucleotide sequence and overexpression of the tellurite resistance determinant from the IncHII plasmid pHH1508a. *Gene* **101**, 1–7.
- Wu, J.-Y., Siegel, L. M. & Kredich, N. M. (1991). High-level expression of *Escherichia coli* NADPH-sulfite reductase: requirement for a cloned *cysG* plasmid to overcome limiting siroheme cofactor. *J Bacteriol* **173**, 325–333.

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