The tellurite-resistance determinants \texttt{tehAtehB} and \texttt{klaAklaBtelB} have different biochemical requirements

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The \texttt{tehAtehB} operon from the \textit{Escherichia coli} chromosome (32.3 min) mediates resistance to potassium tellurite (K$_2$TeO$_4$) when expressed on a multicopy plasmid such as pUC8 (pTWT100). An MIC of 128 \textmu g ml$^{-1}$ is observed when \texttt{tehAtehB} is expressed in a wild-type host and grown on rich media. In this study, the \texttt{tehAtehB} determinant was transformed into mutants deficient in electron transport processes and/or thiol redox coupling within \textit{E. coli}. These mutants included \texttt{ubi}, \texttt{nsd}, \texttt{cys}, \texttt{nar}, \texttt{trx}, \texttt{grx}, \texttt{gsh} and \texttt{sod}. MICS of \texttt{tehAtehB} transformed into these mutants ranged from 1-16 \textmu g K$_2$TeO$_4$ ml$^{-1}$ compared to 0.93-2 \textmu g ml$^{-1}$ for strains transformed with a control plasmid. The tellurite-resistance determinant locus \texttt{MA} cloned from the IncPa plasmid \textit{RK2Ter} (pDT1558) was also investigated in these strains. This tellurite-resistance determinant showed little or no dependency on the host genotype. The ability of \texttt{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 \texttt{kiaA} 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 \texttt{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, \texttt{tehAtehB}, \texttt{kiaA}, thiol redox

**INTRODUCTION**

Tellurite (TeO$_5^{2-}$) 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, $E_{\text{m,Te}^2+/TeO_5^{2-}} = -1.186$ V for the reaction $\text{TeO}_5^{2-} + 3\text{H}_2\text{O} + 4e^- \leftrightarrow \text{Te}^0 + 6\text{OH}^-$. Thus the specific mechanism of cellular oxidation and toxicity is unknown. Resistance to tellurite (Te$^+$) 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 Pa, 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 \texttt{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 \texttt{kiaA} locus and was cloned into a pUC8 plasmid giving pDT1558 from a resistant variant of RK2, RK2Te$^+$. The tellurite-resistance determinant was referred to as \texttt{kiaA}, \texttt{telA} and \texttt{telB} (Turner et al., 1994a, b; Walter et al., 1991b), and all three genes were found to be...
necessarily for resistance (Turner et al., 1994b). In order to maintain the designation between the Te and Te* variants [which arises from a point mutation in klaC (Walter et al., 1991b)], the third point of the Te* operon retains the original nomenclature of teB. 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., 1991b).

The operon tebAteB gives specific tellurite resistance when overexpressed (Walter & Taylor, 1989; Walter et al., 1991b). The operon was originally cloned as a 6.8 kb Sall fragment into pUC8, when cloning the resistance determinant from pH11508a (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, tebA and tebB, 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 klaAklaBtelB and tebAteB 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); AN95, AN120 (uncA401 argE3 thi-1 gall2 xyl-3 metl-1 rplL704), LE392 (F' supE44 supF58 bsdR514 gall2 galT22 metB1 trpR55 lacY1), RG51 (LE392 ΔuncB-uncD Δr::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 Bacterienne, CNRS, Marseille, France); JA199 (ΔtrpE5 leu-6 thi-1 bsdR bsdM*), EC1214 (JA199 cyc) and plasmid pJYW2, which overexpresses CysJH (sulfite reductase), and pJSR102, which overexpresses CyspJH (sulfite reductase with excess sirohaem synthesis) (Wu et al., 1991), provided by Dr N. M. Kedich (Duke University Durham, NC, USA); QC4468, QC1712, QC1725 and QC1799 as well as plasmids pDT1-5 which overexpress sodA (Touati, 1983) and pHS1-6 expressing sodB (Carllov 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' hisd52 trpF606 lys4 trpA164 lacY1 proA22 rpoD24 xyl-5 metl-1 recA13 merB] (Boyer & Roulland-Dussoix, 1969) and MC4100 (Casabianca, 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 tebAteB 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 tebAteB (Walter et al., 1991a). pRT6 is the same as pDT1364 with the ampicillin-resistance marker replaced with a kanamycin-resistance cassette from pK118 (Pharmacia). pDT1558 contains the klaAklaBtelB operon from the IncPa plasmid RK2TTe cloned into pUC8 (Walter et al., 1991a). Similarly, pRT4 is pDT1558 with the kanamycin cassette. The derivatives pRT3 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 μg ml⁻¹), chloramphenicol (30 μg ml⁻¹), kanamycin sulfate (42 μg ml⁻¹) and tetracycline (125 μg ml⁻¹).

MIC determinations. For MIC determination, overnight cultures grown in LB broth were diluted 10⁵ in Penassay broth or LB broth and 10–25 μl 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 tebAteB and klaAklaBtelB 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 tebAteB in the pUC8 vector (pTWT100) results in a culture with an MIC of 128–256 μg ml⁻¹, and the klaAklaBtelB operon gives an MIC of 256 μg 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 klaAklaBtelB, was also utilized and gave the same or higher MICs as pDT1558. This was expected since reduced expression of klaAklaBtelB 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 tebAteB 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)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description of relevant genotype</th>
<th>MIC of tellurite (µg ml⁻¹) with plasmids:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pUC8 (Control)</td>
</tr>
<tr>
<td>JM83</td>
<td>Δlac-pro 80dlacZ ΔM15 rpsL</td>
<td>Wild-type</td>
<td>2</td>
</tr>
<tr>
<td>AT2427</td>
<td>F⁻ cysJ43 rel-A1 spoT1 thi-1 pheM510 2</td>
<td>Sulfite reductase β</td>
<td>2</td>
</tr>
<tr>
<td>AT2455</td>
<td>Hfr thi-1 cysG44 mal-18 rel-A1 ž  spoT1</td>
<td>Sirohaem synthesis</td>
<td>2</td>
</tr>
<tr>
<td>DG37</td>
<td>Hfr rel-A1 Δ(pas-cys-A)127 spoT1</td>
<td>Sulfate permease and O-acetylserine sulfhydrylase B</td>
<td>2</td>
</tr>
<tr>
<td>EC2524</td>
<td>araD139 ΔlacU169 thi trp rpsL rel-A</td>
<td>Sulfite reductase α and β, adenylsulfate reductase</td>
<td>2</td>
</tr>
<tr>
<td>JM246</td>
<td>F⁻ cysI53 ΔQ(rrnD–rrnE)</td>
<td>Sulfite reductase α</td>
<td>2</td>
</tr>
<tr>
<td>JM457</td>
<td>F⁻ pro-50 his-97 fpk-1 cysI53    metB1 lac galT47 mtl strA192 tsc</td>
<td>Sulfite reductase α, serine acetyltransferase</td>
<td>2</td>
</tr>
<tr>
<td>JT14</td>
<td>cysB214 trpE9829 pyrF</td>
<td>Positive regulator</td>
<td>1–2</td>
</tr>
<tr>
<td>NK1</td>
<td>trpE5 leu-6 thi cysB rbs⁺ r c m⁺</td>
<td>Positive regulator</td>
<td>1–2</td>
</tr>
<tr>
<td>RL165</td>
<td>thr-1 leuB6 trp-1 his-61 cysK111 argH1 thi-1 lacY1 gal-6 cycL-7 malA1(p) mtl-2 rpsL9 tonA2 supE44 ž</td>
<td>O-Acetylserylne sulfhydrylase A</td>
<td>2</td>
</tr>
</tbody>
</table>

ND, Not determined.

pTWT100 ranged from 0.5 to 8 µg ml⁻¹. Little or no effect was observed for the MICs that the klaAklkBteB determinant was able to mediate in these strains. MICs of 1–2 µg K₂TeO₃ 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 anc (FIFO-ATPase). No effects on wild-type MICs were observed in this type of host, with or without a resistance determinant.
R. J. TURNER, J. H. WEINER and D. E. TAYLOR

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description of relevant genotype</th>
<th>MIC of tellurite (µg ml⁻¹) with plasmids:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pUC8 (Control)</td>
</tr>
<tr>
<td>GR70N</td>
<td>F: thi rpsL gal</td>
<td>Wild-type</td>
<td>2</td>
</tr>
<tr>
<td>GR71N</td>
<td>F: nadA50::Tn10 thi rpsL gal</td>
<td>Quinolinate synthetase A</td>
<td>2</td>
</tr>
<tr>
<td>GR75N</td>
<td>F: nadA50::Tn10 thi rpsL gal cydA2</td>
<td>Quinolinate synthetase A, cytochrome d terminal oxidase</td>
<td>1</td>
</tr>
<tr>
<td>W4546</td>
<td>galT23 nadC8 Ω(rrnD–rrnE)</td>
<td>Quinolinate phosphoribosyl transferase</td>
<td>1–2</td>
</tr>
<tr>
<td>W3899</td>
<td>supE44 nadB7</td>
<td>Quinolinate synthetase B</td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description of relevant genotype</th>
<th>MIC of tellurite (µg ml⁻¹) with plasmids:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pUC8 (Control)</td>
</tr>
<tr>
<td>JF1070</td>
<td>AB1157*; proAB lacIPOZYA</td>
<td>Wild-type</td>
<td>2</td>
</tr>
<tr>
<td>JF2062</td>
<td>JF1070; trxA::kan</td>
<td>Thioredoxin</td>
<td>0.5–1</td>
</tr>
<tr>
<td>JF432</td>
<td>JF1070; trxB gal*</td>
<td>Thioredoxin reductase</td>
<td>0.5–1</td>
</tr>
<tr>
<td>BPR100</td>
<td>JF1070; grx::kan</td>
<td>Glutaredoxin</td>
<td>0.5</td>
</tr>
<tr>
<td>JF420</td>
<td>JF1070; gy-A</td>
<td>Glutathione oxidoreductase</td>
<td>0.25</td>
</tr>
<tr>
<td>JF2200</td>
<td>JF1070; gshA::kan</td>
<td>Glutathione synthetase A</td>
<td>0.25</td>
</tr>
<tr>
<td>JF2201</td>
<td>JF1070; gshB::kan</td>
<td>Glutathione synthetase B</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* AB1157: F⁻ thr-1 leuB6 lacY1 supE44 rfbD1 thi-1 malT1 ara-14 galK2 syl-5 mtl-1 mgl-5 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 µg ml⁻¹ (Table 4). When these mutants were transformed with the plasmids carrying tehAtebB, MICs ranged from 2 to 8 µg ml⁻¹ compared to 128–256 µg 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 (ggbA), 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 µg 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 µg ml⁻¹ in the presence of BSO and down to 1 µg 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 cyS (EC1124) hosts harbouring pJRS102 (which over-expresses cysGJIF) gave tellurite MICs in the range of
Tellurite-resistance determinants

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description of relevant genotype</th>
<th>MIC of tellurite (µg ml⁻¹) with plasmids:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pUC8 (Control)  pTWT100 (tehAtehB)  pDT1558 (klaAklakBt)</td>
</tr>
<tr>
<td>MC4100</td>
<td>F⁻ araD139 Δ(aroR–lac)U169 fthB301 ptaF25 relA1 rpsL150 deoC1 rpsR</td>
<td>Wild-type</td>
<td>2 128–256 256–512</td>
</tr>
<tr>
<td>LCB2048</td>
<td>thi-1 thr-1 leu6 lacY1 supE44 rpsL Δnar-25(narG–narH) narZ::Ω</td>
<td>Nitrate reductases (NarGHJ1 and NarZYWV)</td>
<td>0.015–0.03 16 64</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Description of relevant genotype</th>
<th>MIC of tellurite (µg ml⁻¹) with plasmids:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pUC8 (Control)  pTWT100 (tehAtehB)  pDT1558 (klaAklakBt)</td>
</tr>
<tr>
<td>QC4468</td>
<td>F⁻ Δ(lac)U169 rpsL</td>
<td>Wild-type</td>
<td>2 128 256</td>
</tr>
<tr>
<td>QC1712</td>
<td>QC4468; ΔsodA3 rhu::Tn3 zdh::Tn10</td>
<td>Superoxide dismutase [Mn]</td>
<td>0.125–0.25 128 128</td>
</tr>
<tr>
<td>QC1725</td>
<td>QC4468; ΔsodA3 zdh::mini-tet</td>
<td>Superoxide dismutase [Mn]</td>
<td>0.125–0.25 128–256 256–512</td>
</tr>
<tr>
<td>QC1799</td>
<td>QC4468; ΔsodA3 ΔsodB2</td>
<td>Superoxide dismutase [Mn] and [Fe]</td>
<td>0.125–0.25 128 128</td>
</tr>
<tr>
<td>JHC1113</td>
<td>QC4468; marR1 xdd–2207::Tn10</td>
<td>Repressor multi-antibiotic resistance</td>
<td>4 256 256</td>
</tr>
<tr>
<td>JHC1096</td>
<td>QC4468; zdd-239::Tn9 del1738 (Δmar)</td>
<td>Deletion in MarRAB</td>
<td>2–4 256 256</td>
</tr>
<tr>
<td>JHC1078</td>
<td>QC4468; spoR105 zje-2204::Tn10</td>
<td>Repressor of redox stress response</td>
<td>4 256 256</td>
</tr>
</tbody>
</table>

2–4 µg ml⁻¹. No TeO₂⁻⁻→Te⁰ 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 ΔA₆₀₀). Hosts harbouring both pJRS102 and pRT6 (tehAtehB clone) or pRT4 (klaAklakBt clone) had MICs of 128 and 256 µg 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 µg 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 klaAklakBt 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 µg 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 µg K₂TeO₃ ml⁻¹ using the wild-type host HBlOl (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
medium was utilized with a single carbon source, the determinant \( \text{tehAtehB} \) did not protect the host from tellurite. In contrast, the \( \text{klaAklaBtelB} \) 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 \( \text{tehAtehB} \), 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 \textit{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 \( \text{TeO}_2^{2-} \) to \( \text{Te}^0 \) 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 \( \text{klaAklaBtelB} \) and \( \text{tehAtehB} \) tellurite-resistance determinants based on tellurite accumulation studies (Turner \textit{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 \( \text{tehAtehB} \) determinant is dependent almost exclusively on the host cell to provide a particular metabolic state. Resistance mediated by the \( \text{tehAtehB} \) 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 \( \text{tehAtehB} \) to mediate full resistance. In contrast, the \( \text{klaAklaBtelB} \) tellurite-resistance determinant required none of these host systems. It is clearly evident from these studies that the tellurite-resistance determinants \( \text{tehAtehB} \) and \( \text{klaAklaBtelB} \) have two very different biochemical mechanisms. Additionally, there appears to be a synergy between the hosts resistance mechanisms and that of \( \text{tehAtehB} \).

Two other tellurite-resistance determinants have been identified. The \( \text{ter} \) determinant from the IncH1 plasmid also mediates tellurite resistance (Hill \textit{et al.}, 1993; Lloyd-Jones \textit{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 \textit{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 tehAtehB and klaAklAtehB determinants described in the present paper. Additionally, the oxyanion efflux pump arrABC 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$_3^-$ (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 tehAtehB 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 TehAtehB.

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 tellurite deposition. Most of the mutant strains studied have an MIC between 1 and 2 µg ml$^{-1}$ without any plasmid or when harbouring a control plasmid pUC8. However, a strain with nitrate reductases mutations (narG and narZ) showed a marked hypersensitivity to tellurite with an MIC of 0.05 µg 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 µg 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 cisplatinum (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$_3^-$) 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$^0$ (Ganther, 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$_3^-$ reacts readily with free thiol, 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 µg ml$^{-1}$ range. This sensitivity may be an indication that O$_2^-$ is produced as a by-product of the TeO$_3^-$ $\rightarrow$ 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 TeO$_3^-$ $\rightarrow$ Te$^0$ reduction. Superoxide dismutase would then act on the O$_2^-$ produced as a result of the TeO$_3^-$ 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


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