

## Tellurite Susceptibility and Non-Plasmid-Mediated Resistance in *Escherichia coli*

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Tellurite ( $\text{TeO}_3^{2-}$ ) is highly toxic toward *Escherichia coli* (MIC,  $\sim 1 \mu\text{g ml}^{-1}$ ). Mutants (Tel) that were resistant to low levels of  $\text{TeO}_3^{2-}$  (MIC,  $\sim 10 \mu\text{g ml}^{-1}$ ) and collaterally resistant to arsenate were isolated. These Tel mutants were unable to grow on media containing low levels of  $\text{P}_i$ , which supported growth of the parent strain. When grown at much higher  $\text{P}_i$  levels they exhibited derepressed levels of the outer membrane *phoE* protein and the periplasmic *phoS* protein, as well as several other proteins indicative of  $\text{P}_i$  starvation. Tel mutants were markedly defective in  $^{32}\text{P}_i$  transport, and  $\text{TeO}_3^{2-}$  was shown to be a potent competitive inhibitor of  $^{32}\text{P}_i$  transport in the parent strain. The Tel phenotype could be complemented by an  $\text{F}'$  plasmid harboring the *phoR*, *phoB*, and *phoA* loci, and curing of the  $\text{F}'$  plasmid completely restored  $\text{TeO}_3^{2-}$  resistance. Of a variety of well-characterized  $\text{P}_i$  transport mutants, only *phoB* mutants were equally resistant to  $\text{TeO}_3^{2-}$ , and susceptibility could also be restored in strains carrying an  $\text{F}'$  plasmid for the *phoB* region and lost once more after  $\text{F}'$  curing. The *tel* and *phoB* loci were equally cotransducible with *lac*. Tel mutants still synthesized alkaline phosphatase, the *phoA* gene product, after  $\text{P}_i$  starvation, suggesting that the *phoB* locus per se was not involved because *phoB* is a positive regulatory gene for *phoA* expression. The results indicate that  $\text{TeO}_3^{2-}$  is transported into *E. coli* by a phosphate transport system and that resistance to  $\text{TeO}_3^{2-}$  specifically selects for as yet uncharacterized mutants in the *phoB-phoA* region of the chromosome.

Tellurium oxyanions, such as  $\text{TeO}_3^{2-}$ , are highly toxic to both gram-negative and -positive bacteria. Resistance to  $\text{TeO}_3^{2-}$  has been primarily ascribed to plasmids, especially in various species of *Klebsiella*, *Citrobacter*, and *Pseudomonas* (14, 21, 22). The natural resistance to  $\text{TeO}_3^{2-}$  has also been used in the identification of *Corynebacterium* species (5). However, the molecular basis of either the acquired or natural resistance is unknown. In some cases the reduction of  $\text{TeO}_3^{2-}$  to Te and alkylation of Te has been suggested as possible mechanisms (23).  $\text{TeO}_3^{2-}$  has been recently shown to interact with the *Escherichia coli* and *Klebsiella pneumoniae* cell surface to enhance bacteriophage P1 vir absorption (J. M. Tomás, M. Regue, R. Pares, and J. Jofre, Can. J. Microbiol., in press).

To try to understand the basis of  $\text{TeO}_3^{2-}$  resistance, whether chromosomal or plasmid encoded, we felt it was important to first understand the molecular basis of this susceptibility. Most strains of *E. coli* and *Salmonella typhimurium* are highly susceptible to  $\text{TeO}_3^{2-}$ , and plasmids conferring resistance in these strains have been described (22). Spontaneous mutants resistant to  $\text{TeO}_3^{2-}$  can be obtained relatively easily; thus, we decided to use this system to examine the molecular basis of susceptibility and resistance in non-plasmid-bearing strains. The results of this study demonstrate that  $\text{TeO}_3^{2-}$  is transported on the phosphate transport pathway in *E. coli* and also provide an excellent selection method for unusual mutants of this pathway.

### MATERIALS AND METHODS

**Bacterial strains, bacteriophages, and growth conditions.** The strains used in this study, along with their properties and

origins, are listed in Table 1. Phages  $\lambda$ , P1, T4, T6, and  $\phi 80$  as well as colicin V were lab stock. Phages TU1A and TP1 were gifts of M. Schwartz, Paris. These phages were always grown on *E. coli* C600. Luria broth (LB) was used as rich medium, and LB agar was prepared by the addition of 1.5% agar or 0.6% agar for soft agar. The  $\text{P}_i$  content of  $\text{P}_i$ -free minimal media (18) was adjusted to between 41  $\mu\text{M}$  (derepressed conditions) and 660  $\mu\text{M}$  (repressed conditions).

**Isolation and characterization of  $\text{TeO}_3^{2-}$ -resistant mutants.** Log phase cells ( $10^8$ ) grown in LB were spread on LB agar containing 5  $\mu\text{g}$  of  $\text{K}_2\text{TeO}_3$  per ml. After 24 h at 37°C the clearly resistant colonies were purified by restreaking twice on the same media. All  $\text{TeO}_3^{2-}$ -resistant (Tel) mutants were routinely tested for original amino acid auxotrophic markers, phage and colicin susceptibility by spot testing, and their ability to ferment lactose on MacConkey plates. MICs for heavy metal salts and antibiotics were determined in LB broth after 18 h of incubation at 37°C. Cultures were inoculated at 1% with freshly grown cells.

Sugar fermentation tests were carried out with eosine-methylene blue broth, with sugars sterilized separately as 10% solutions and added at 0.1% final concentration. Overnight cultures of cells grown in LB were used as 1% inocula.

Phosphate growth requirements were determined in minimal medium (19) adjusted to various  $\text{P}_i$  levels with sterile  $\text{K}_2\text{HPO}_4$ - $\text{KH}_2\text{PO}_4$  buffer (pH 7.0). Cells were first grown on minimal medium containing 660  $\mu\text{M}$   $\text{P}_i$  to mid-log phase, washed three times with  $\text{P}_i$ -free medium, and inoculated at 1%. Cultures were incubated at 37°C for 18 h and scored for growth.

**Cell surface isolation and analyses.** Periplasmic proteins were released by osmotic shock (26). Cell envelopes were prepared by lysis with a French pressure cell at 16,000 lb/in<sup>2</sup>, followed by removal of unbroken cells at 10,000  $\times g$  for 10 min and finally by sedimentation at 100,000  $\times g$  for 2 h. Cytoplasmic membranes were solubilized twice with sodium *N*-lauryl sarcosinate (7), and the remaining outer membrane

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TABLE 1. *E. coli* strains used in this study

Strains	Description	MIC ( $\mu\text{g ml}^{-1}$ ) for $\text{TeO}_3^{2-}$	Source
CSH30	F <sup>-</sup> <i>trpC</i>	1	Lab stock
C600	F <sup>-</sup> <i>lacY1 leuB6 supE44 thr-1 tonA21</i> $\lambda^-$	1	Lab stock
KT86	F <sup>-</sup> <i>lacY1 leuB6 supE44 Tel thr-1 tonA21</i> $\lambda^-$	10	This study
KT87	F <sup>-</sup> <i>lacY1 leuB6 supE44 Tel thr-1 tonA21</i> $\lambda^-$	10	This study
E15	Hfr <i>fadL701 fep ompF637 <math>\Delta</math>phoA8 pit-10 purE relA1 spoT1 tonA22</i>	1	B. Bachmann
C31	Hfr <i>fep ompF627 phoS25 pit-10 purE relA1 spoT1 tonA22</i>	1	B. Bachmann
S26	Hfr <i>lip ompF627 phoA4 purE relA1 tonA22</i>	1	B. Bachmann
LEP-1	F <sup>-</sup> <i>azi-6 lacI22 lacZ73 mtl-1 phoB23 proC34 purE42 rpsL109 thi-1 trpE38 tsx-67 xyl-5</i>	10	B. Bachmann
C72	Hfr <i>fep ompF627 phoS27 pit-10 purE relA1 spoT1 tonA22</i>	1	B. Bachmann
C90	Hfr <i>fep ompF627 phoT9 pit-10 purE relA1 spoT1 tonA22</i>	1	B. Bachmann
10B5	Hfr <i>fadL701 glpD3 glpR2 lip ompF627 phoA8 pit-1 pst-2 purE relA1 spoT1 tonA22</i>	2	B. Bachmann
6S5	F <sup>-</sup> <i>his-53 lacY1 metB1 nalA12 pit-1 proC24 pst-2 pyrF30 rpsL97 thyA25</i>	0.25	B. Bachmann
ORF4/KLF251	F <sup>-</sup> 254 F' point of origin: P059 of Hfr OR11 Chromosome markers: <i>ara-14 azi-6 lacZ36 leu-6 metE-70 mtl-1 proC32 purE-32 recA1 rpsL109 supE44 thi-1 tonA23 trpE-38 tsx-6 xyl-5</i> $\lambda^-$	1	R. Harkness, Victoria, B.C.
KT201	KT86 + F <sup>-</sup> 254	1	This study
KT202	KT201 cured of F <sup>-</sup> 254	10	This study

fraction was sedimented at  $100,000 \times g$  for 2 h. Outer membranes were suspended in 10 mM Tris hydrochloride–5 mM  $\text{MgCl}_2$  (pH 7.3) after brief sonication.

Membrane proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by a modification of the Laemmli procedure (3) with 12% acrylamide and 0.65% bisacrylamide. Protein gels were routinely stained with Coomassie blue. Protein concentrations were determined by the procedure described by Lowry et al. (12) with bovine serum albumin as a standard.

**Genetic techniques and plasmids.** Conjugation and F' episome curing were carried out by the method of Miller (15). Transduction experiments were carried out with P1 *cmts* (11) as the high frequency transducing phage. P1 *cmts* was grown in soft agar on a *lac*<sup>+</sup> *E. coli* strain and transduced into KT86 (Tel<sup>+</sup> *lac*<sup>-</sup>). *lac*<sup>+</sup> transductants were screened for  $\text{TeO}_3^{2-}$  resistance ( $10 \mu\text{g ml}^{-1}$ ). Plasmid DNA was isolated from whole cells by alkaline extraction, electrophoresed on 0.7% agarose gels, and stained with ethidium bromide (10).

**Phosphate transport and alkaline phosphatase assays.** Cells grown on minimal medium plus  $330 \mu\text{M P}_i$  were harvested at mid-log phase and then suspended in  $\text{P}_i$ -free minimal medium and incubated for 2 h at  $37^\circ\text{C}$  (derepressed conditions). The cells were then harvested once more and washed twice with  $\text{P}_i$ -free minimal medium. These cells (1 ml at  $350 \mu\text{g}$  [dry weight]) were incubated at a concentration chosen to reflect high-affinity transport activity ( $8 \times 10^{-7} \text{ M } ^{32}\text{P}_i$ ;  $0.10 \text{ Ci mol}^{-1}$ ) in the presence or absence of different concentrations of  $\text{P}_i$ ,  $\text{K}_2\text{TeO}_3$  or  $\text{Na}_2\text{HAsO}_4$ . Fractions (0.1 ml) were removed at 30-s intervals for 1 min, filtered (pore size,  $0.45 \mu\text{m}$ ; Schleicher & Schull, Inc., Keene, N.H.), washed with 5 ml of  $\text{P}_i$ -free minimal medium, and assayed for radioactivity by scintillation counting. The rate of  $^{32}\text{P}_i$  transport was calculated from a linear plot of the rate data. In competition experiments, unlabeled  $\text{P}_i$ ,  $\text{TeO}_3^{2-}$ , or  $\text{AsO}_4^{3-}$  was added at different concentrations simultaneously with  $^{32}\text{P}_i$ .

Alkaline phosphatase (EC 3.1.3.1) was measured by the method of Torriani (25).

## RESULTS

**Isolation and properties of Tel mutants.** Spontaneous mutants of *E. coli* C600 occurring at a frequency of  $5 \times 10^{-6}$  were isolated as resistant to  $5 \mu\text{g}$  of  $\text{TeO}_3^{2-}$  per ml. All Tel mutants were identical to the parent strain with respect to

phage susceptibility (T4, T6,  $\phi 80$ , TU1A, and TP1), colicin V susceptibility, and auxotrophic markers (*leu*, *thi*, *thr*) and were Lac<sup>-</sup> as well. No changes were found in various antibiotic susceptibilities. The ability to ferment each of 10 different sugars was unchanged. Two independent, but typical, Tel mutants (KT86 and KT87) were selected for heavy metal susceptibility studies. Both strains exhibited higher MICs for  $\text{TeO}_3^{2-}$  (10-fold) and  $\text{AsO}_4^{3-}$  (5-fold) relative to that of parent strain C600, the MIC of which for  $\text{TeO}_3^{2-}$  was  $1 \mu\text{g ml}^{-1}$  and for  $\text{AsO}_4$  was 7 mM; but no changes in the MIC toward  $\text{Na}_2\text{AsO}_2$ ,  $\text{HgCl}_2$ ,  $\text{CdSO}_4$ ,  $\text{AgNO}_3$ ,  $\text{CsCl}_2$ , or  $\text{CuSO}_4$  were found. Both Tel mutants showed a minimum growth requirement for  $\text{P}_i$  of more than three times that of *E. coli* C600 (62 versus  $21 \mu\text{M}$ ). These observations suggest that Tel mutants harbor a defect with respect to the transport of  $\text{P}_i$ .

**Outer membranes and periplasmic proteins.** After growth under conditions which normally completely repress the production of the periplasmic  $\text{P}_i$ -binding protein, the *phoS* gene product ( $38,000 M_r$ ), strain KT86 was found to continue to produce small quantities of a periplasmic protein which was of the approximate molecular weight (MW) of the *phoS* gene product (Fig. 1A). A protein of identical MW was present in  $\text{P}_i$ -deficient cells of the C600 parent (Fig. 1B).

Synthesis of the PhoS protein is indicative of  $\text{P}_i$  starvation. After growth under derepressing conditions ( $40 \mu\text{M P}_i$ ), C600 expressed this protein abundantly (Fig. 1B). As a control, *E. coli* C31 (*phoS*) was specifically defective in the same PhoS protein when grown under derepressed conditions (data not shown). When the outer membrane proteins of C600 and KT86 from cells grown in LB were examined by SDS-PAGE (Fig. 2), a protein of  $45,000 M_r$  was particularly expressed in KT86 but not in C600. However, when C600 was grown under derepressed conditions ( $41 \mu\text{M P}_i$ ) a protein of this MW was produced. The  $46,000 M_r$  protein corresponds to the well known PhoE porin that is produced in response to  $\text{P}_i$  starvation (25). An unknown protein of  $20,000 M_r$  present in LB-grown *E. coli* C600 was inexplicably strongly reduced in LB-grown KT86 and practically disappeared in *E. coli* C600 grown under derepressed conditions. The results of these experiments suggest that Tel mutants, even when grown in LB, are starved for  $\text{P}_i$ .

**Transport of  $^{32}\text{P}_i$ .** Evidence presented above suggests that Tel mutants may have been defective in  $\text{P}_i$  transport. When the transport of  $^{32}\text{P}_i$  was measured directly, after  $\text{P}_i$  starva-

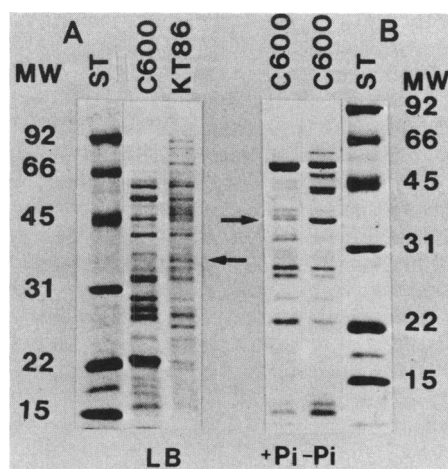


FIG. 1. SDS-PAGE of periplasmic proteins released by osmotic shock (19). (A) MW standards (ST) are indicated in thousands. *E. coli* C600 and KT86 were grown in LB broth. The arrows indicate the 38,000  $M_r$  polypeptide identified as the phosphate periplasmic binding protein (PhoS). (B) *E. coli* C600 was grown under repressed conditions (660  $\mu\text{M}$   $\text{P}_i$  [+ $\text{P}_i$ ]) or derepressed conditions (40  $\mu\text{M}$   $\text{P}_i$  [- $\text{P}_i$ ]).

tion for 2 h, KT86 exhibited only 6% of the transport activity of the parent strain (Fig. 3). The incorporation of [ $^{14}\text{C}$ ]D-glucose and [ $^3\text{H}$ ]proline was not affected in K86 (data not shown), indicating a specific transport defect. This suggests that  $\text{TeO}_3^{2-}$  enters *E. coli* by one of the two major  $\text{P}_i$  transport systems (18). This was confirmed in the parent strain C600 by competition assays in which  $\text{P}_i$ ,  $\text{TeO}_3^{2-}$ , and the known phosphate analog  $\text{AsO}_4^{3-}$  were compared as unlabeled competitive inhibitors (Fig. 4).  $\text{TeO}_3^{2-}$  was approximately a 200-fold stronger inhibitor of  $^{32}\text{P}_i$  uptake than was  $\text{AsO}_4^{3-}$  and was equally as competitive as  $\text{P}_i$  (data not shown). Thus,  $\text{TeO}_3^{2-}$  is a strong competitive inhibitor of  $\text{P}_i$  transport in *E. coli*, suggesting that it enters the cell via this transport system(s).

**Location of the genetic locus for Tel mutants.** Evidence

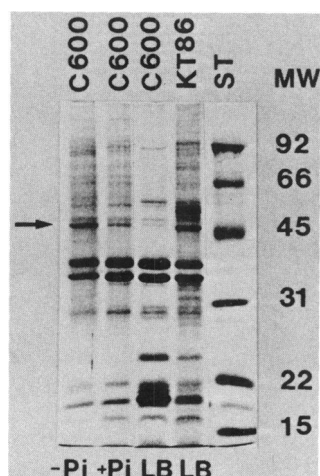


FIG. 2. SDS-PAGE of outer membrane proteins of *E. coli* strains. Outer membranes were prepared by the method of Filip et al. (7). *E. coli* C600 was grown under derepressed conditions (40  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  [ $\text{P}_i$ ]) or repressed conditions (660  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$  [+ $\text{P}_i$ ]) or in LB. *E. coli* KT86 was grown in LB broth. MW standards (ST) are indicated in thousands. The arrow indicates the 46,000  $M_r$  band.

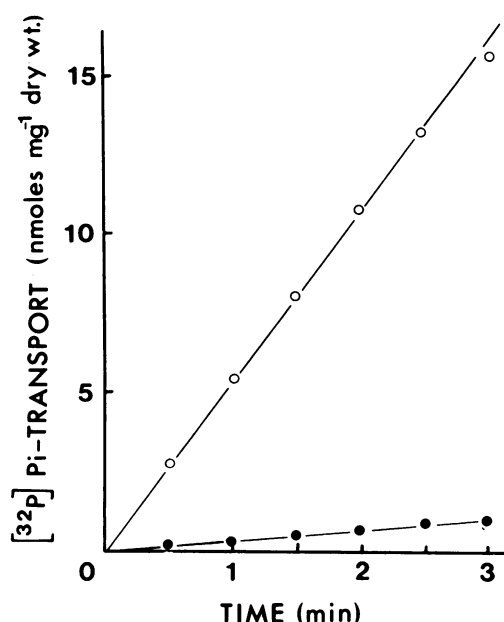


FIG. 3.  $^{32}\text{P}_i$  transport in wild-type and a Tel mutant of *E. coli*. Cells (0.35 mg [dry weight]  $\text{ml}^{-1}$ ) were starved for  $\text{P}_i$  for 2 h and then incubated with 0.8  $\mu\text{M}$   $^{32}\text{P}_i$  for the times indicated and collected by filtration. Symbols: O, *E. coli* C600; ●, *E. coli* KT86.

presented above indicates that Tel mutants are possibly defective either in some component or in the synthesis of the  $\text{P}_i$  transport system of *E. coli*. When a series of well-characterized  $\text{P}_i$  transport mutants were assayed for  $\text{TeO}_3^{2-}$  susceptibility (Table 1), only *phoB* mutants showed an MIC

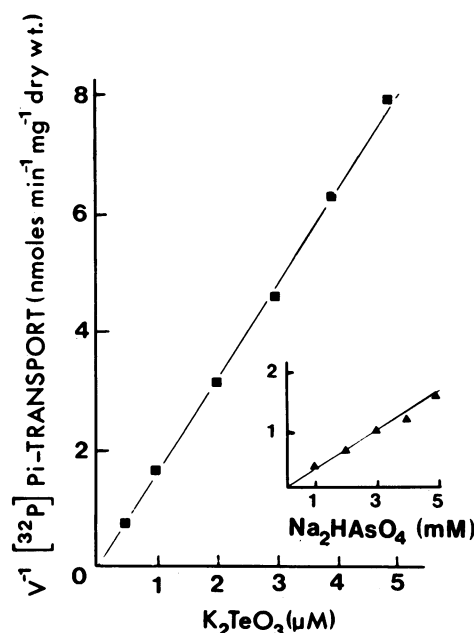


FIG. 4.  $^{32}\text{P}_i$  transport in wild-type *E. coli*. Symbols: ■,  $^{32}\text{P}_i$  transport in the presence of different concentrations of  $\text{K}_2\text{TeO}_3$ ; ▲, inset,  $^{32}\text{P}_i$  transport in the presence of different concentrations of  $\text{Na}_2\text{HAsO}_4$ . Note that the rate of  $^{32}\text{P}_i$  transport is plotted as a reciprocal. Competition for  $^{32}\text{P}_i$  transport by unlabeled  $\text{P}_i$  gave a curve that was superimposable on the  $\text{TeO}_3^{2-}$  inhibition curve.

TABLE 2. Alkaline phosphatase levels of *E. coli* Tel and mutants

Strain	Relevant phenotype	P <sub>i</sub> growth concn (μM)	Sp act (U of enzyme mg of protein <sup>-1</sup> )
		— <sup>a</sup>	
C600		40	0.55
C600		660	<0.002
		—	
KT86	Tel	62	0.47
KT86	Tel	660	0.01
		—	
KT87	Tel	62	0.49
KT87	Tel	660	0.02
LEP-1	PhoB <sup>-</sup>	62	0.01
LEP-1	PhoB <sup>-</sup>	660	<0.002

<sup>a</sup> —, limited amount of P<sub>i</sub> was required for growth.

for TeO<sub>3</sub><sup>2-</sup> equivalent to those of strains KT86 and KT87. By conjugating strains ORF4 and KLF251 carrying the episome F'254 (F' *lac*), as a donor for the wild-type *phoB* locus, and strain KT86 (Lac<sup>-</sup>), as recipient and selecting for Lac<sup>+</sup> transconjugants, we obtained strain KT201, a merodiploid for the 6- to 10-min region of the chromosome. The MIC for TeO<sub>3</sub><sup>2-</sup> of KT201 was found to be reduced to that of *E. coli* C600 (Table 1). After curing the F' from strain KT201 by treatment with acridine orange and selecting for Lac<sup>-</sup> colonies, we obtained strain KT202, which was once again resistant to TeO<sub>3</sub><sup>2-</sup> (Table 1). Thus, both *phoB* and the genetic locus for Tel reside in the *phoR phoB phoA* region covered by F'254. As a confirmation, the transducing phage P1 *cmts* was grown on the donor strain CSH30 (Lac<sup>+</sup>), and the recipient strain KT86 (Tel<sup>+</sup> Lac<sup>-</sup>) was transduced to Lac<sup>+</sup> on lactose minimal plates. Twenty-three percent (46 of 200 colonies) of the Lac<sup>+</sup> transductants were also TeO<sub>3</sub><sup>2-</sup> resistant. Essentially the same frequency of transduction of *phoB* with *lac* was found when the same transduction procedure was used and when strain LEP-1 (*phoB*) was transduced to Lac<sup>+</sup> (44 of 200 colonies became susceptible to TeO<sub>3</sub><sup>2-</sup>). Thus, by the three criteria of equal susceptibility to TeO<sub>3</sub><sup>2-</sup>, the phosphate transport defect, and a similar genetic location, the *tel* locus was indistinguishable from the *phoB* locus.

Only two known regulatory mutations in this region of the chromosome, either *phoR* or *phoB*, could be candidates for the Tel phenotype. Because the synthesis of alkaline phosphatase is normally constitutive in *phoR* strains and, in contrast, the *phoB* gene product is required for the synthesis of this enzyme (9), we attempted to distinguish these candidates by assaying for the level of alkaline phosphatase in the Tel mutants when grown under both high and low P<sub>i</sub> conditions (Table 2). The results indicate that alkaline phosphatase activity is still present in the mutants and can be derepressed to the same degree as that in the parent strain C600. The degree of repression by P<sub>i</sub> with the mutants was somewhat less than that with the parent, probably because of the P<sub>i</sub> transport defect in these strains. As a control LEP-1, a *phoB* mutant, was largely unable to synthesize alkaline phosphatase even under derepressed growth conditions. Because *phoB* is positively required for *phoA* expression, the Tel mutants could not be simply defective in *phoB*, even though *phoB* mutants were resistant to TeO<sub>3</sub><sup>2-</sup>.

### DISCUSSION

The mechanism of TeO<sub>3</sub><sup>2-</sup> toxicity and resistance in *E. coli* has not been well understood, yet resistance can be

mediated either by a chromosomal mutation or by a plasmid. In either case resistance would presumably be due to the inability to transport TeO<sub>3</sub><sup>2-</sup>, the acquisition of an efflux mechanism, or the detoxification of the inhibitor (8). Detoxification has been suggested, but not clearly shown, for TeO<sub>3</sub><sup>2-</sup> resistance plasmids (8, 21).

In the case of the strains studied here, the primary mechanism of resistance in *E. coli* was at the transport level. Tel mutants required higher P<sub>i</sub> levels for growth; they exhibited apparent P<sub>i</sub> starvation properties (production of *phoS*, *phoE*, and *phoA*, as well as other P<sub>i</sub> starvation indicator proteins) under conditions of normally repressible levels of P<sub>i</sub>; they were collaterally resistant to both TeO<sub>3</sub><sup>2-</sup> and AsO<sub>4</sub><sup>3-</sup>, with the latter being a well-known inhibitor of P<sub>i</sub> transport (18); they were unable to transport P<sub>i</sub> even under derepressed conditions; and they harbored a defect which mapped in the *phoB-phoR* region at 9 min. Also, because TeO<sub>3</sub><sup>2-</sup> is an unusually strong competitive inhibitor of the transport of P<sub>i</sub> in the parent cells, the conclusion that TeO<sub>3</sub><sup>2-</sup> enters *E. coli* via a P<sub>i</sub> transport system seems inescapable.

The precise locus affected in Tel mutants is not entirely certain; the nature and mechanism of P<sub>i</sub> transport and the regulation of these systems are still unclear at present. This lack of clarity stems from an unusual complexity of these systems, especially the *pho* regulon. Results of recent studies (1, 2, 6, 9, 13, 24) have shown that there are several loci involved in the *pho* regulon. Three of these loci (*phoR*, *phoB*, and *phoA*) map in the same general location as the Tel mutants described herein, and they consist of two independent but closely linked loci (*phoB-phoR* and *phoA*). Characterization of the *tel* locus depends on which of two prevalent views of the nature of regulation of the *pho* regulon one embraces. An earlier view (17, 27) presents the *phoB* gene product as a positive regulator of *phoA* (alkaline phosphatase) expression and the *phoR* gene product as an inhibitor of *phoA* expression. From this perspective the *tel* locus cannot be *phoB* because *phoA* was still expressed in Tel mutants; also, the *tel* locus could not be identical to *phoR* because *phoA* expression was not constitutive. Accordingly, the *tel* locus either may be unique or a special class of *phoB* mutations. Another, more recent, view (2, 13, 19, 24) suggests that *phoB* is a positive regulator as well but that *phoR* can act as an autogenous positive or negative regulator of the *phoB-phoR* operon, depending on the intracellular level of P<sub>i</sub>. From this perspective the *tel* locus again cannot be simply *phoB*; therefore, it is either a unique *phoR* mutation or perhaps a new locus. We have not sought to further characterize either *phoB* or *phoR* as the locus responsible for the Tel phenotype, primarily because the results would more or less be irrelevant to the major issue here, which is that the P<sub>i</sub> transport system is the permeation route for TeO<sub>3</sub><sup>2-</sup> and that transport deficiency is a means to resistance. It will be of further interest to determine whether plasmid-mediated TeO<sub>3</sub><sup>2-</sup> resistance has the same molecular basis or whether another mechanism, such as inhibitor efflux, mediates resistance, as recently demonstrated for AsO<sub>4</sub> resistance plasmids (16, 20).

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