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Cysteine Metabolism-Related Genes and Bacterial Resistance to Potassium Tellurite▽†

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Tellurite exerts a deleterious effect on a number of small molecules containing sulfur moieties that have a recognized role in cellular oxidative stress. Because cysteine is involved in the biosynthesis of glutathione and other sulfur-containing compounds, we investigated the expression of *Geobacillus stearothermophilus* V cysteine-related genes *cobA*, *cysK*, and *iscS* and *Escherichia coli* cysteine regulon genes under conditions that included the addition of K_2TeO_3 to the culture medium. Results showed that cell tolerance to tellurite correlates with the expression level of the cysteine metabolic genes and that these genes are up-regulated when tellurite is present in the growth medium.

Sulfur is an essential element that is required for the biosynthesis of proteins, enzyme cofactors, and other important biomolecules. In bacteria, this element can be assimilated into sulfur-containing amino acids through enzymatic fixation from inorganic sources, such as sulfate and/or thiosulfate (15, 38). Although tellurium shares several chemical properties with sulfur, no biological function for Te is known to date. Conversely, some tellurium compounds, like the oxyanion tellurite (TeO_3^{2-}), are extremely toxic for most forms of life, especially microorganisms (34).

It has been proposed that K_2TeO_3 toxicity could be due to the oxidation of cellular thiols such as glutathione (37) or the generation of superoxide radical during tellurite reduction, which would cause a redox imbalance resulting in intracellular oxidative stress (5, 23, 26, 33, 34, 36).

Maintenance of cell redox balance is one of the most important processes involving molecules synthesized from reduced sulfur taken from the environment. Glutathione (GSH) is one of the major nonprotein thiols in living organisms, including humans, yeast, and bacteria (6, 10). GSH has been involved in resistance to osmotic and oxidative stress as well as in *Escherichia coli* resistance to the toxic effects of electrophiles like methylglyoxal (6, 11, 31). A protective effect of GSH against oxidative stress has also been described for *Lactococcus lactis* and *Rhodobacter capsulatus* (17, 18).

Three genes involved in tellurite resistance have been described previously for the thermotolerant gram-positive rod *Geobacillus stearothermophilus* V (27, 33, 41). The genes that are involved in the metabolism of cysteine are *cysK*, *iscS*, and *cobA*, and they encode a cysteine synthase (CysK), a cysteine desulfurase (IscS), and a uroporphyrinogen-III C-methyltransferase (SUMT), respectively. CysK catalyzes the last step of inorganic sulfur fixation into L-cysteine, while SUMT is involved in the biosynthesis of siroheme, an essential sulfite reductase cofactor that participates in the inorganic assimilation of sulfur (15). We recently demonstrated that *cobA* and *ubiE* genes from *G. stearothermophilus* V confer increased tolerance to oxyanions of selenium and tellurium when expressed in *E. coli* (1, 32). Finally, IscS, which yields sulfur and L-alanine from L-cysteine, has been shown to be involved, along with IscA and IscU, in the recovery of [Fe-S] clusters (9, 29).

The purpose of this study was to evaluate the responsiveness of *cysK*, *iscS*, and *cobA* from *G. stearothermophilus* and some genes of the *E. coli* Cys regulon in medium containing potassium tellurite. Results indicate that bacterial tolerance to tellurite involves, at least in part, several components of the cysteine metabolic pathway.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and stress induction. *E. coli* strains and plasmids used in this work are listed in Table 1. Cells were grown routinely in LB medium (28) at 37°C with shaking. Experiments were initiated with the addition of 1:100 dilutions of overnight cultures to the medium. Cell cultures reaching an optical density at 600 nm (OD_{600}) of ~0.6 were amended with K_2TeO_3 (0.5 μg/ml), H_2O_2 (125 μg/ml), diamide (850 μg/ml), or paraquat (250 μg/ml), as required. Controls received equal volumes of sterile water.

Geobacillus stearothermophilus V was grown in ATTC medium with or without potassium tellurite (50 μg/ml) at 65°C as described previously (39). All experiments were carried out at least in triplicate.

Antimicrobial disk assay and determination of MICs. Overnight cultures of *E. coli* or its derivatives were diluted 100-fold with LB medium, and 100-μl aliquots were spread on LB-agar (2%) plates. Ten microliters of K_2TeO_3 (10 μg), H_2O_2 (1.25 mg), or paraquat (3.0 mg) was then spotted independently onto sterile filter paper disks that were placed in the center of the plates. Growth inhibition zones were determined after incubation at 37°C for 24 h. To determine MICs, cells were grown with shaking at 37°C in LB medium supplemented with appropriate concentrations of the compounds under study (7).

RNA extraction, plasmid construction, and cell transformation. RNA purifications used the RNeasy kit (QIAGEN). Briefly, cultures of *E. coli* K-12 or its derivatives (OD_{600} of ~0.6) were split in two and one was amended with 0.5 μg/ml K_2TeO_3 and incubated for 10 min. Cells were sedimented at 13,000 × *g* for

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)[a]	Source or reference
Strains		
E. coli TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*Δ*M15* Δ*lacX74 deoR nupG recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL*(Str^r) *endA1* λ$^-$	Invitrogen
E. coli BW25113	*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δ*araBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$	Datsenko and Wanner (8)
JW2415	*E. coli* BW25113 Δ*cysA*	Baba et al. (3)
JW2720	*E. coli* BW25113 Δ*cysC*	Baba et al. (3)
JW2722	*E. coli* BW25113 Δ*cysD*	Baba et al. (3)
JW3582	*E. coli* BW25113 Δ*cysE*	Baba et al. (3)
JW3331	*E. coli* BW25113 Δ*cysG*	Baba et al. (3)
JW2732	*E. coli* BW25113 Δ*cysH*	Baba et al. (3)
JW2407	*E. coli* BW25113 Δ*cysK*	Baba et al. (3)
JW2414	*E. coli* BW25113 Δ*cysM*	Baba et al. (3)
JW2418	*E. coli* BW25113 Δ*cysP*	Baba et al. (3)
JW2416	*E. coli* BW25113 Δ*cysW*	Baba et al. (3)
JW3888	*E. coli* BW25113 Δ*sbp*	Baba et al. (3)
JW2514	*E. coli* BW25113 Δ*iscS*	Baba et al. (3)
JW2515	*E. coli* BW25113 Δ*iscR*	Baba et al. (3)
E. coli AB734	F$^-$ *lac-6(del)*	Shapiro and Baneyx (30)
E. coli ADA110	AB734 λϕ*ibp*::*lacZ*	Shapiro and Baneyx (30)
E. coli ADA310	AB734 λϕ*cspA*::*lacZ*	Shapiro and Baneyx (30)
E. coli ADA410	AB734 λϕP3*rpoH*::*lacZ*	Shapiro and Baneyx (30)
E. coli ADA510	AB734 λϕ*sulA*::*lacZ*	Shapiro and Baneyx (30)
G. stearothermophilus V	Wild-type Tel^r	C. Vásquez (39)
Plasmids		
pBR322	Cloning vector, Ap^r Tet^r	Bolivar et al. (4)
pBluescript-SK	Cloning vector, Ap^r	Stratagene
pBR*cobA*	*G. stearothermophilus cobA* gene cloned in pBR322, Ap^r	This study
pBR*cysK*	*G. stearothermophilus cysK* gene cloned in pBR322, Ap^r	This study
pBR*iscS*	*G. stearothermophilus iscS* gene cloned in pBR322, Ap^r	This study
pSK*cobA*	*G. stearothermophilus cobA* gene cloned in pBluescript-SK, Ap^r	This study
pSK*cysK*	*G. stearothermophilus cysK* gene cloned in pBluescript-SK, Ap^r	This study
pSK*iscS*	*G. stearothermophilus iscS* gene cloned in pBluescript-SK, Ap^r	This study

[a] Tel^r, tellurite resistance; Tet^r, tetracycline resistance; Ap^r, ampicillin resistance.

3 min and used for RNA extraction. The $OD_{260/280}$ ratio for the purified RNAs was determined with an Agilent 8453 UV-visible spectrophotometer.

G. stearothermophilus V *cobA*, *cysK*, and *iscS* genes containing 300 bp upstream of their ATG initiation codons were amplified by PCR using primers listed in Table 2. PCR conditions included an initial denaturation at 95°C for 5 min, followed by 30 amplification cycles (95°C for 30 s, 55°C for 30 s, and 72°C for 30 s). A final incubation at 72°C for 10 min was included to ensure complete extension of the amplified fragments. PCR products were cloned in pGEMT-Easy (Invitrogen). Recombinant plasmids were digested with HindIII, and the released fragments were purified and cloned independently into the medium-copy-number vector pBR322 or the high-copy-number vector pBluescript-SK (Table 1).

E. coli TOP10 cells were used in all transformations. Cells were made competent by electroporation (MicroPulser, Bio-Rad) using 0.2-cm cuvettes and a 2.5-kV pulse.

β-Galactosidase assay. *E. coli* cultures (1.5 ml) harvested at different time intervals were sedimented by centrifugation at 13,000 × *g* for 3 min. Cells were permeabilized by the addition of 1.5 ml ice-cold buffer Z (40 mM Na_2HPO_4, 60 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, and 50 mM β-mercaptoethanol, pH 7.5) and assayed for β-galactosidase activity. The *o*-nitrophenyl-β-D-galactopyranoside (ONPG) substrate was used as described previously by Miller (20). Protein concentration was estimated using the Bradford reagent (Sigma).

Bioinformatics and computation analysis. Sequence analysis and primer design were performed using Vector NTI 8.0 (InforMax, Inc.). Nucleotide sequences of the *G. stearothermophilus* V *cobA*, *cysK*, and *iscS* genes and those of the *E. coli cys* regulon (*cysA*, *cysB*, *cysC*, *cysE*, *cysG*, *cysI*, *cysK*, *cysM*, and *cbl*) were obtained from GenBank (accession numbers AY426747, AF533655, AF198621, and NC_000913). Analysis of variance statistical analyses were performed with a 0.05 level of confidence.

Real-time RT-PCR. The induction of gene expression was calculated based on the difference between the crossing points of each quantitative reverse transcription-PCR (RT-PCR) determination (Cp − Cp_{Te}, where Cp and Cp_{Te} are the crossing points in the absence and presence of tellurite, respectively). Crossing points define the cycle at which the amplification becomes exponential, and they are inversely proportional to the amount of the specific RNA template present in the sample.

Approximately 2 μg of RNA from control or tellurite-treated cells was used for real-time RT-PCR experiments using a LightCycler RNA amplification kit (Roche Applied Science) according to instructions of the vendor. The specific oligonucleotide primers used are listed in Table 2. PCR products were visualized and analyzed using a LightCycler 2.0 instrument.

RESULTS

Behavior of *E. coli* strains carrying *G. stearothermophilus* genes in tellurite-containing medium. *G. stearothermophilus* V *cobA*, *cysK*, and *iscS* genes containing their own promoters were separately cloned in the medium- and high-copy-number plasmids pBR322 and pBluescript-SK, respectively. The resulting recombinant plasmids were used to transform *Escherichia coli*, and bacterial tolerance to K_2TeO_3 and other oxidative stress inducers was evaluated by measuring growth inhibition zones in tellurite-amended solid medium. Figure 1 shows that pBR*cobA*, pBR*cysK*, and pBR*iscS* increased *E. coli* resistance approximately 20% for tellurite, 25% for paraquat, 25% for diamide, and 20% for hydrogen peroxide. In turn, *E. coli* cells carrying pSK*cobA*, pSK*cysK*, and pSK*iscS* exhibited an additional 20% tolerance to K_2TeO_3 and hydrogen peroxide (see Fig. S1S in the supplemental material), suggesting a gene dosage effect. This behavior was not observed for the thiol reducer diamide (data not shown).

TABLE 2. Primers used in PCR and quantitative RT-PCR

Primer name	Sequence	PCR product
For *G. stearothermophilus* V:		
*cobA*PH	5′-AAGCTTTTCGCTCCGGATGTTGCCGATTAT-3′	*cobA* gene + promoter
*cobA*H3	5′-AAGCTTCGGTCCCGGCGATGAAAACGTGATTACCGT-3′	*cobA* gene + promoter
*cysK*PH	5′-AAGCTTCGCTTACAGACTATTCCGCCTGTCT-3′	*cysK* gene + promoter
*cysK*H3	5′-AAGCTTGTCTTCGAATTGGTAAAGCGGCGTGCTTAAGTAGC-3′	*cysK* gene + promoter
*iscS*PH	5′-AAGCTTTCGCAGACATTACTAGAACGGTTGCTGTAG-3′	*iscS* gene + promoter
*iscS*H3	5′-AAGCTTATGAATCTTGAACAAATAAGAAAAGATAAC-3′	*iscS* gene + promoter
*gapA*3	5′-GGTGATGTGTTTACGAGCAG-3	*gapA* probe
*gapA*5	5′-GTAAAGTTGGTATTAACGGTTTTGG-3′	*gapA* probe
*scobA*5	5′-GTACCTCTGCTTCTTCCCCG-3′	*cobA* probe
*subiE*5	5′-CTGCACAAAGCGAATCCGTT-3′	*ubiE* probe
*scysK*3	5′-ACTCATCGTATCTGGCATGA-3′	*cysK* probe
*siscS*3	5′-CGAATACACGGTAAAACAAT-3′	*iscS* probe
For *E. coli:*		
*sgapA*3	5′-GGTGATGTGTTTACGAGCAG-3′	*gapA* probe
*sgapA*5	5′-GTAAAGTTGGTATTAACGGTTTTGG-3′	*gapA* probe
*scysA*3	5′-AACCAGCGGGCATATTCGCTTCCACGGCAC-3′	*cysA* probe
*scysA*5	5′-CAAGCAGCAGAATTTGCGGTTCCACAGCCA-3′	*cysA* probe
*scysB*3	5′-CTGCGCGATTAAAGGCAGTATCCAGTTCTG-3′	*cysB* probe
*scysB*5	5′-ACCTGGCCGGATAAAGGTTCACTGTATATCGC-3′	*cysB* probe
*scysC*3	5′-TTTAGCGATGCCGATCGTAAAGAGAATATC-3′	*cysC* probe
*scysC*5	5′-GTTCACCATTGAGATGAATTTCTGCCGATT-3′	*cysC* probe
*scysE*3	5′-TCTGTGACGTTCCAGGTCGATATTCACCCG-3′	*cysE* probe
*scysE*5	5′-GCTGGTCCATATCCATTGATGGCTTATCGCTG-3′	*cysE* probe
*scysG*3	5′-ACCACCACATCTGCCTGCTGAATTTGTTGC-3′	*cysG* probe
*scysG*5	5′-ACTTGAATCACTGCTGCCGTTACATCTGGG-3′	*cysG* probe
*scysI*3	5′-GTGAAAACTCGATGGCTTGCGTGTCATTCC-3′	*cysI* probe
*scysI*5	5′-CCCTATCAGTTCATCAAGCGACGCCAGGAT-3′	*cysI* probe
*scysK*3	5′-CTTTCAGCAGCTTGCGGCGTTCAATACTCA-3′	*cysK* probe
*scysK*5	5′-TGAAGATAACTCGCTGACTATCGGTCACACGC-3′	*cysK* probe
*scysM*3	5′-GGCGAAGGAAAGCTGCTCGATCAGTTCAAT-3′	*cysM* probe
*scysM*5	5′-GATGAATATCCAGCACCTCATCCACCAGAGAAGC-3′	*cysM* probe
*scbl*3	5′-GCCCGCAAAGGTTTGCTGGCAGATATTGTA-3′	*cbl* probe
*scbl*5	5′-TTTCCTCTTCACTGCTTTCCATCACCTGCC-3′	*cbl* probe

To circumvent the repression of cysteine biosynthesis in rich medium, the same experiments were conducted in M9 minimal plates. Results showed that all strains exhibited increased tolerance levels (~10 to 20%) to K_2TeO_3, paraquat, and H_2O_2 compared to those observed with LB medium (not shown). Differences in tellurite tolerance observed in solid medium were also assessed by MIC determinations (Table 3). Curiously, the MICs for pSK*cobA* and pBR*cobA* increase by twofold after 24 h, while the others remain the same. Although we do not have a definitive explanation for this result, it was systematically observed. In this context, a physiological adaptation phenomenon cannot be ruled out.

Response of *E. coli* stress reporter strains to potassium tellurite. *E. coli* ADA strains induce the expression of the β-galactosidase gene when exposed to different stress conditions (30). These strains carry single-copy fusion genes consisting of the *lacZ* reporter gene fused to the promoter regions of the highly inducible cytoplasmic small heat shock proteins IbpA and IbpB (ADA110), the major cold shock protein CspA (ADA310), the P3 promoter of the *rpoH* gene (activated upon periplasmic protein misfolding) (ADA410), or the SOS-inducible *sulA* promoter (ADA510) (30).

E. coli ADA cells were challenged with potassium tellurite to determine promoter activation in vivo. The parental isogenic *E. coli* AB734 was used as a control. Sublethal concentrations of K_2TeO_3 had very little effect on *P3rpoH* and *sulA* activation, while *E. coli* ADA110 and ADA310 showed increased β-galactosidase activities, suggesting that tellurite affects cytoplasmic proteins and has no evident effect on DNA (not shown). When *E. coli* ADA110 and ADA310 cells carrying *G. stearothermophilus* V *cobA*, *cysK*, or *iscS* genes were challenged with K_2TeO_3, β-galactosidase activity was lower than that observed for control cells transformed with pBluescript (Fig. 2A and B), suggesting that expression of *cobA*, *cysK*, and *iscS* protects against the denaturation of cytoplasmic proteins. Similar results were obtained when diamide, paraquat, and hydrogen peroxide were used (not shown).

Induction of *G. stearothermophilus* V *cobA*, *cysK*, and *iscS* gene promoters by K_2TeO_3. Alignment of nucleotide sequences upstream of the ATG initiation codon of *cobA*, *cysK*, and *iscS* showed similarities of 40 to 44%. No binding motifs to known *E. coli* or *Bacillus subtilis* sigma factors such as σ^{70} and σ^{43} were found.

Real-time RT-PCR was used to monitor the functional capability of the *G. stearothermophilus* V *cobA*, *iscS*, and *cysK* promoters in *E. coli* grown in the presence or in the absence of K_2TeO_3. The housekeeping gene *gapA* was used as a control. Induction of gene expression was expressed as the difference between the crossing points of each RT-PCR determination ($Cp - Cp_{Te}$). Positive numbers reflect larger amounts of the particular, specific mRNA in cells exposed to the toxic condition. The calculated Cp values were 2.49 for *cobA*, 3.51 for

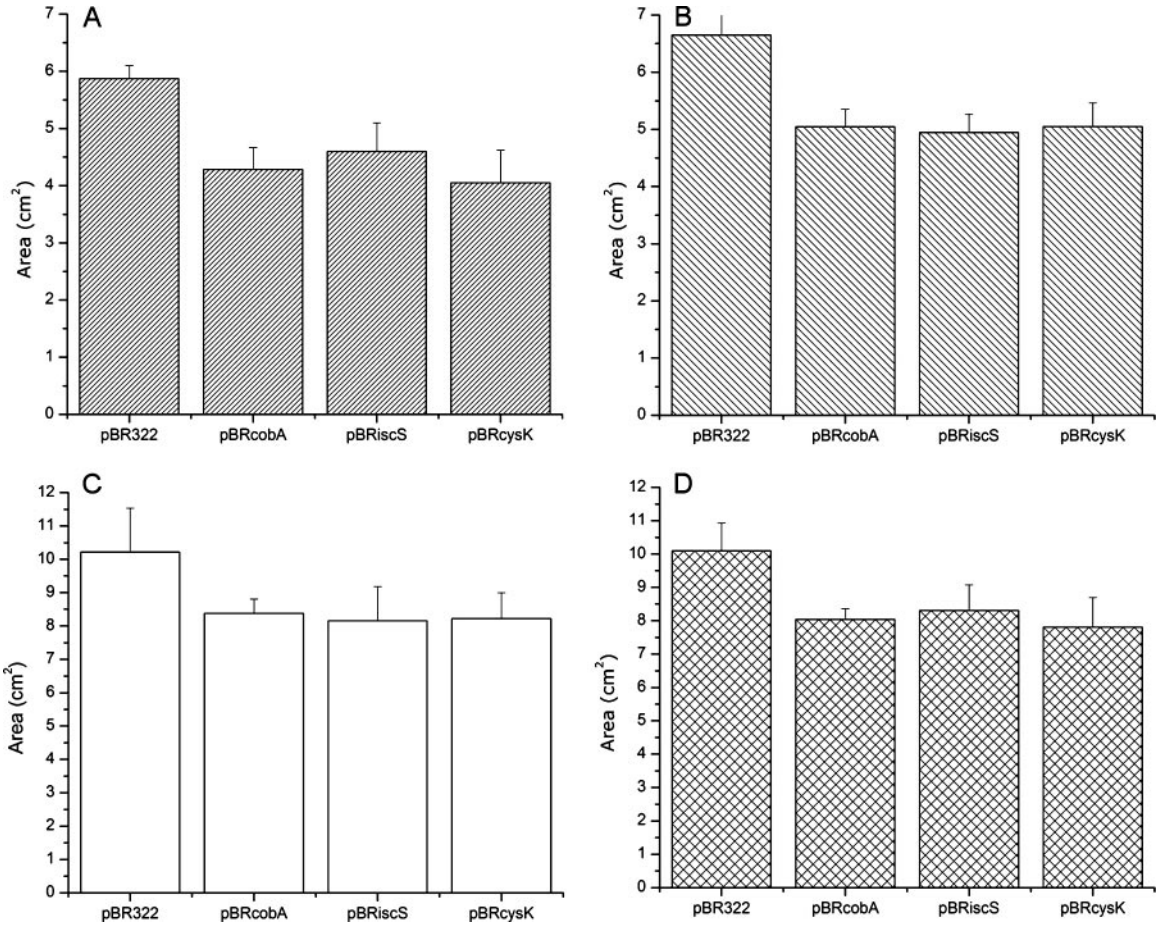

FIG. 1. Growth inhibition zones of *E. coli* cells expressing *G. stearothermophilus* V *cobA*, *cysK*, and *iscS* genes cloned in pBR322 when exposed to the following toxic substances: paraquat (A), diamide (B), hydrogen peroxide (C), and potassium tellurite (D). Cells were grown overnight in plates of LB-ampicillin at 37°C. Error bars represent standard deviations ($n = 8$). See Materials and Methods for details.

cysK, 2.54 for *iscS*, 5.02 for *ubiE*, and −0.95 for *gapA*. Thus, while expression of the *G. stearothermophilus* V genes increased in the presence of potassium tellurite, that of the *gapA* housekeeping gene decreased by approximately 10% (Fig. 3).

Expression of *E. coli* cysteine metabolism-related genes in cells exposed to potassium tellurite. An experimental approach similar to that described in the previous section was followed to evaluate the effect of potassium tellurite on the expression of *E. coli* cysteine metabolism genes. The participation of Cys regulon genes in tellurite tolerance was investigated using *E. coli* BW25113 and its isogenic derivatives *cysA*, *cysC*, *cysE*, *cysG*, *cysI*, *cysK*, *cysM*, and *cbl.* The loss of cysteine metabolism-related genes is paralleled with an increase of growth inhibition areas for all toxic compounds included in this study. In general, strains Δ*cysA*, Δ*cysK*, Δ*cysM*, Δ*cbl*, and Δ*cysC* exhibited the highest sensitivity to the reactive oxygen species (ROS) generators paraquat and hydrogen peroxide as well as to potassium tellurite (Fig. 4).

TABLE 3. MICs of K_2TeO_3 for *E. coli* cells expressing *cobA*, *cysK,* and *iscS* genes from *G. stearothermophilus* V

Plasmid	K_2TeO_3 MIC (μg/ml)[a] at:	
	12 h	24 h
pBR322	1	1
pBluescript SK⁻	1	1
pSKcobA	6.25	12.5
pSKcysK	12.5	12.5
pSKiscS	12.5	12.5
pBRcobA	3.125	6.25
pBRcysK	6.25	6.25
pBRiscS	6.25	6.25

[a] Values are the means of three independent determinations.

Total RNA from untreated cells and cells treated with potassium tellurite was used, along with specific primers for each gene of the regulon, as templates for performing real-time RT-PCR (Fig. 5). Most of the *E. coli* cysteine metabolism-related genes showed increased expression when cells were grown in the presence of potassium tellurite. The ΔCp values were 0.92 for *cysA*, 0.32 for *cysB*, 0.19 for *cysC*, 1.06 for *cysE*, 0.35 for *cysG*, 1.06 for *cysI*, 1.17 for *cysM*, 2.04 for *cysK*, 2.94 for *cbl*, and −1.3 for *gapA*.

DISCUSSION

Living organisms require sulfur for the biosynthesis of proteins and other essential enzymatic cofactors and reducing

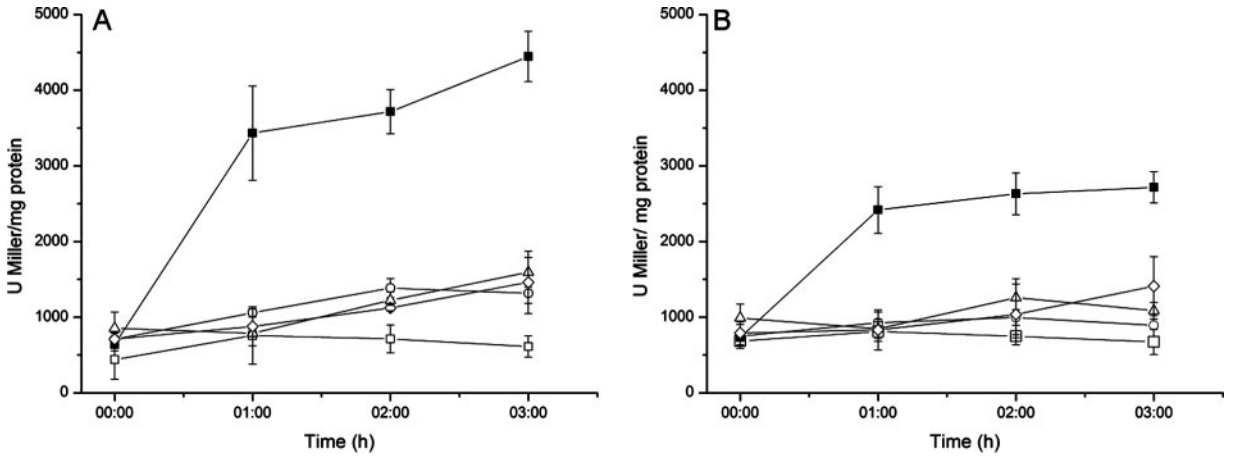

FIG. 2. Determination of β-galactosidase activity in *E. coli* ADA110 (A) and ADA310 (B) in the presence of potassium tellurite. Cells harboring pSK*cobA* (△), pSK*cysK* (○), pSK*iscS* (◇), or the control plasmid pBluescript SK$^-$ (■) were grown at 37°C in LB medium until an OD_{600} of about 0.6 (time zero), when tellurite was amended at 0.5 μg/ml. Control cells were grown in the absence of potassium tellurite (□). Error bars represent standard deviations ($n = 9$).

agents. After incorporation into the cell, sulfur is reduced to sulfide that then reacts with *O*-acetyl-L-serine to form L-cysteine (15). Cysteine has several vital functions in the catalytic cycle of many enzymes, is part of important reducing agents like glutathione, and is required for the biosynthesis and repair of [Fe-S] centers of several essential proteins, including cytochromes, fumarases, and aconitases.

Cysteine-containing molecules like glutathione and thioredoxin play a major role in maintaining an intracellular reducing environment and protecting the cell from oxidative damage (6, 12, 22). It has been reported that GSH is ubiquitous in most gram-negative bacteria and is absent in most gram-positive organisms examined so far (10, 18). These observations suggest that glutathione is not unique in protecting against oxidative stress or maintaining the redox balance of cells. It has been shown that thioredoxin 1 (TrxA), thioredoxin 2 (TrxB), and thioredoxin reductase (TrxC) are not essential for survival in *E. coli*. *trxA* and *trxB* cells exhibit increased sensitivity to H_2O_2, a phenotype that is not observed in *trxC* cells (25). *E. coli* strains with mutations in the GSH system are also viable. For example, *gshA* and *gshB* mutants and *gorA* mutants, encoding the two enzymes of glutathione biosynthesis and glutathione reductase, respectively, exhibit increased sensitivity to the GSH-oxidizing agent diamide (2). Prinz et al. (24) determined that *E. coli* requires the thioredoxin or the GSH/Grx system to grow aerobically and that mutants in these systems were incapable of reducing cytoplasmic disulfide bonds.

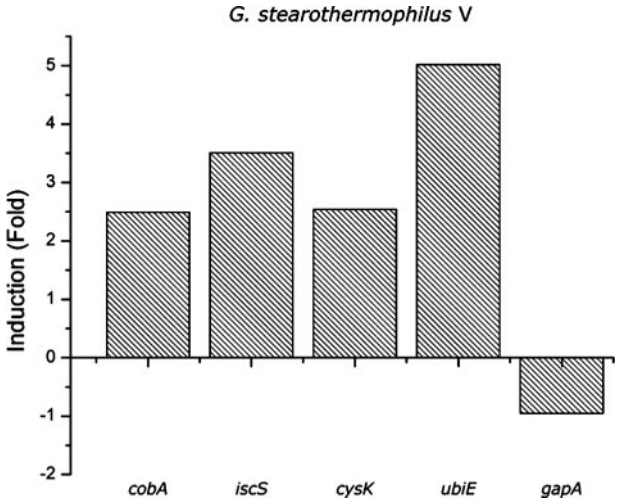

FIG. 3. Expression of *G. stearothermophilus* V genes in LB medium containing potassium tellurite (50 μg/ml). Cells were grown at 65°C for 24 h as described in Materials and Methods. Values are the means of two independent trials.

We have previously identified three *G. stearothermophilus* V genes (*cobA*, *cysK*, and *iscS*) that encode enzymes involved in cysteine metabolism and whose expression mediates tellurite resistance in *E. coli* (33, 40, 41). The participation of *cysK*, *cysM*, and *iscS* genes in tellurite resistance as well as in oxidative stress has been also documented with other microorganisms (19, 21, 26).

Tellurite toxicity is thought to result from the oxidizing character of this molecule. More recently, the idea that tellurite would damage the cell through the establishment of oxidative stress has emerged. This stress condition could be a consequence of the drastic decrease of the concentration of cellular antioxidants and/or could be associated with the presence of some kind of ROS. The observation that superoxide generation results from enzymatic tellurite reduction supports this assumption (5, 23).

The above considerations allow the speculation that an increase in cellular antioxidants would result in higher tolerance to ROS elicitors. Expression of *G. stearothermophilus* V genes containing their own promoters in medium- or high-copy-number plasmids supports this idea (Fig. 1; see Fig S1S in the supplemental material).

To date, most studies concerning bacterial response to oxidative stress have been focused on hydrogen peroxide-, alkylhydroperoxide-, or superoxide-induced stress. Little is known about disulfide stress, a subcategory of oxidative stress that causes the accumulation of nonnative disulfide bonds in the

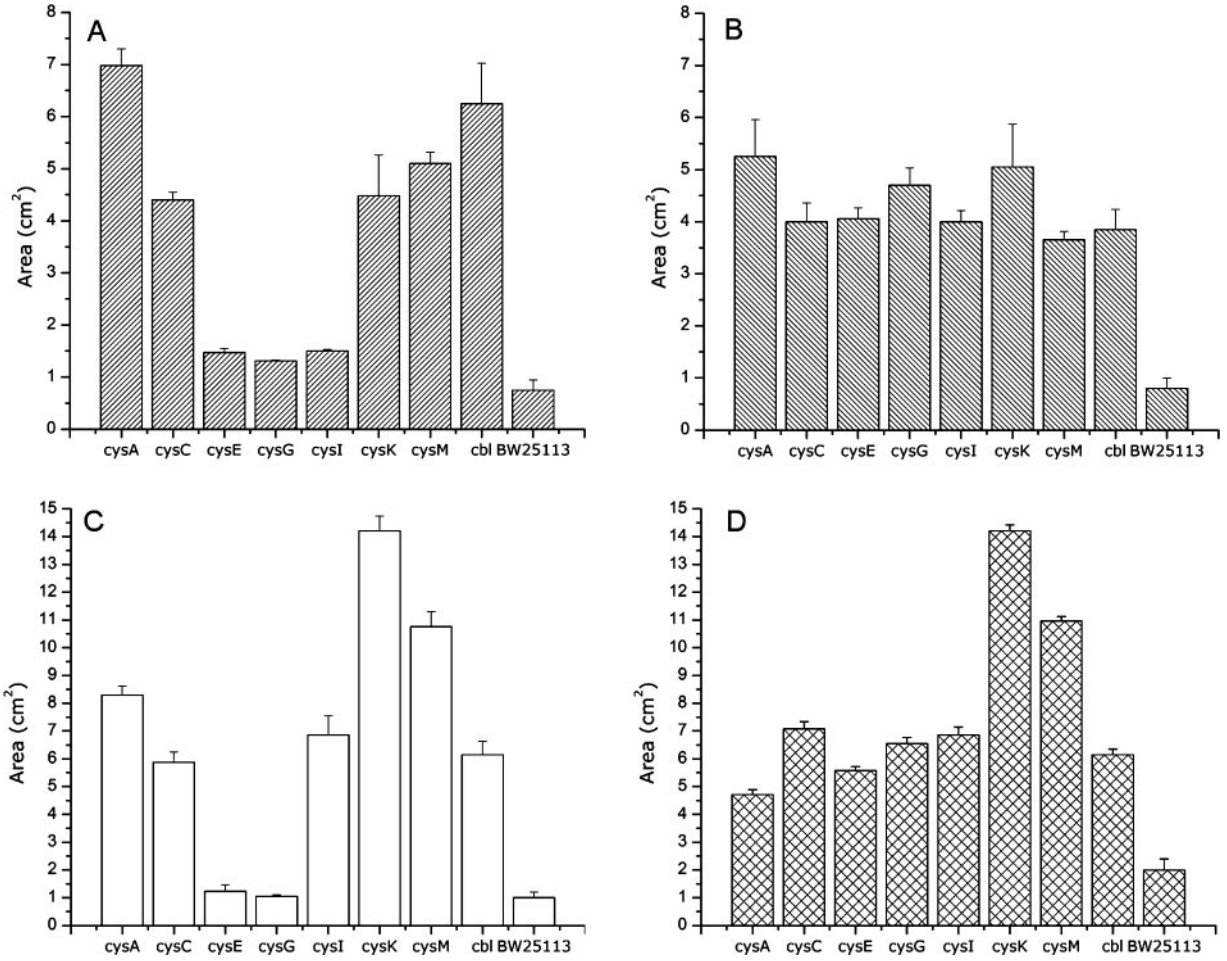

FIG. 4. Growth inhibition zones of *E. coli* defective in the indicated cysteine metabolism-related genes. Cells were grown in LB medium in the presence of paraquat (A), diamide (B), hydrogen peroxide (C), and potassium tellurite (D). Results for the parental, isogenic, wild-type BW25113 strain are shown to the right of each histogram. Error bars represent standard deviations ($n = 6$).

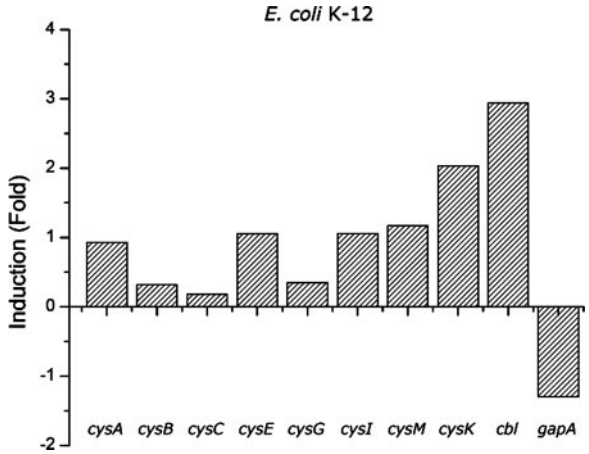

FIG. 5. Expression of *E. coli cys* genes in medium containing potassium tellurite. Cells were inoculated in LB medium containing 0.5 μg/ml of potassium tellurite and cultivated at 37°C with shaking for 24 h. Values are the means of two independent trials.

cytoplasm. *E. coli* cells were also exposed to the thiol-specific oxidant diamide. *E. coli* cells harboring the *G. stearothermophilus* V *cobA*, *cysK*, and *iscS* genes exhibited smaller growth inhibition zones than controls that did not express these genes, suggesting that they have a protective effect against the toxic effect of diamide. Leichert et al. (16) reported that *B. subtilis* isolates independently exposed to hydrogen peroxide or diamide showed similar gene expression profiles, suggesting that they share the same response mechanism. These results agree with our observation that the expression of *G. stearothermophilus* V genes protects *E. coli* against the toxic effects of hydrogen peroxide and diamide.

This protective effect was further observed in transformed *E. coli* reporter strains ADA110 and ADA310 (30), which exhibited important reductions of β-galactosidase activity when exposed to K_2TeO_3. The levels of β-galactosidase in these *E. coli* ADA strains expressing *Geobacillus* genes were indistinguishable from those of controls grown in the absence of tellurite (Fig. 2).

It is well known that living organisms have evolved defense mechanisms to maintain the cell's homeostasis under adverse conditions. In *Escherichia coli*, for example, temperature up-

shifts and other kinds of stress induce the synthesis of heat shock proteins when misfolded proteins accumulate in the cytoplasm (13). In this context, gene induction by tellurite poisoning has been reported for *E. coli* and *Proteus mirabilis*. The *E. coli gutS* gene and *P. mirabilis terZABCDE* operon are positively regulated by tellurite (14, 35). The presence of sequences similar to OxyR binding motifs in the *ter* operon of *P. mirabilis* suggested that such induction would be dependent on this transcriptional regulator. However, no OxyR-like binding motifs were found within regulatory regions of the *G. stearothermophilus* V genes (not shown), suggesting that the K_2TeO_3-induced positive regulation observed in Fig. 3 is not dependent on this regulator.

We further studied the involvement of the *E. coli* Cys regulon genes in tellurite-amended medium. Results showed that strains defective in cysteine metabolism-related genes exhibited higher sensitivity to K_2TeO_3 than did the isogenic, parental, wild-type strain (Fig. 4), suggesting that most of the elements of the cysteine biosynthetic pathway are required to manage the tellurite-induced stress. In addition, all the Cys regulon genes studied here were turned on in the presence of tellurite even in rich medium like LB, where transcription of the *cysB* and *cysE* regulatory genes is repressed (Fig. 5).

In conclusion, we have shown that genes of cysteine biosynthesis are induced in the presence of potassium tellurite. The behavior of the Cys regulon elements could reflect a tellurite-mediated derepression, which in turn could be explained by the depletion of cellular thiols or sulfur-containing molecules such as glutathione.

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