The *Escherichia coli* BtuE Protein Functions as a Resistance Determinant against Reactive Oxygen Species

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**Abstract**  
This work shows that the recently described *Escherichia coli* BtuE peroxidase protects the bacterium against oxidative stress that is generated by tellurite and by other reactive oxygen species elicitors (ROS). Cells lacking *btuE* (*ΔbtuE*) displayed higher sensitivity to K₂TeO₃ than cells with the wild-type allele. *btuE* was also induced under oxidative stress conditions. Nevertheless, the elimination of *btuE* from cells deficient in catalases / peroxidases (Hpx) resulted in impaired growth and resistance to these toxicants only in aerobic conditions, suggesting that BtuE is involved in the defense against oxidative damage. Genetic complementation of *E. coli* *ΔbtuE* restored toxicant resistance to levels exhibited by the wild type strain. As expected, *btuE* overexpression resulted in decreased amounts of oxidative damage products as well as in lower transcriptional levels of the oxidative stress-induced genes *ibpA*, *soxS* and *katG*.

**Introduction**  
Although the tellurium oxyanion, tellurite (TeO₄⁻²⁻), is toxic to most microorganisms, the ultimate basis of its toxicity has remained elusive. Available evidence from *Escherichia coli* [1], *Pseudomonas pseudalcaligenes* KF707 [2] and *Rhodobacter capsulatus* [3] supports the idea that bacterial tellurite toxicity is related to oxidative stress. In particular, *E. coli* exposed to K₂TeO₃ exhibits increased levels of cytoplasmic reactive oxygen species (ROS), mainly superoxide (O₂⁻⁻) [4]. In turn, increased O₂⁻⁻ levels can trigger a number of metabolic effects, including protein and membrane oxidation, induction of antioxidant enzymes and inactivation of [4Fe-4S] clusters from certain dehydratases [5–9].  

Aerobic organisms protect themselves from ROS by synthesizing antioxidant enzymes as well as low molecular weight molecules such as ascorbate and glutathione [7,10]. *E. coli* contains several antioxidant enzymes, including catalases (*katG* and *katE*) [11–13], superoxide dismutases (*MnSOD*, *FeSOD*, and *CuznSOD*) [13–15], alkylhydroperoxidase [13] and thiol peroxidase [16]. To cope with oxidative stress, the genes encoding these enzymes are often induced by ROS, whether it is produced in different compartments of the bacterial cell or at different growth stages [13].  

Glutathione peroxidases (GPXs) are another kind of antioxidant enzyme that in eukaryotes plays an important role in defending the cell against hydroperoxides and lipid peroxides [17,18]. Conversely, in prokaryotes the available information about GPXs is still very limited. However, a recent report identified and characterized the Se-independent GPX BtuE from *E. coli*, which in *vitro* can catalyze the decomposition of a variety of peroxides, mainly lipid peroxides, using thioredoxins A or C as the reducing agent. It was also shown that, like other *E. coli* antioxidant genes, *btuE* is induced under oxidative stress conditions [19].  

Tellurite toxicity is due at least in part to the generation of oxidative stress that alters different cellular processes [9]; therefore, the role of the *E. coli* *btuE* gene product was examined *in vivo*. The *btuE* gene was cloned and its effects were analyzed in cells exposed to various ROS elicitors. Results were compared to those obtained with mutants lacking *btuE* and to genetically complemented *ΔbtuE* cells. Taken together, the emerging picture is that BtuE is involved in protecting the cell from the deleterious effects caused by exposure to tellurite as well as to other ROS elicitors.

**Results**  
**BtuE mediates resistance to ROS elicitors in *E. coli***  
To assess whether BtuE plays a role in the resistance of *E. coli* to oxidative stress, growth inhibition zones were determined for wild-type, *btuE*-overexpressing (pBAD/*btuE*), *btuE*-deficient (*ΔbtuE*) and genetically complemented *btuE* mutant (*ΔbtuE* pBAD/*btuE*) cells (Table 1). Tested ROS elicitors included the superoxide-generating potassium tellurite [4], the hydroxyl radical elicitor chromate [20,21], and hydrogen peroxide [7]. Cadmium chloride,
whose toxicity seems not to involve ROS generation, was used as control [22].

Cells overexpressing btuE exhibited increased resistance to compounds whose toxicity involves ROS generation. Conversely, the ΔbtuE strain showed increased sensitivity to all these compounds relative to wild type controls. Genetically complemented ΔbtuE cells exhibited resistance levels to K₂TeO₃, K₂CrO₄ and H₂O₂ that were nearly identical to those observed for the btuE-overexpressing wild type strain. In contrast, all tested strains showed similar sensitivity to the non-ROS-producer, thiol oxidizer, CdCl₂ (Table 1).

Interestingly, when minimal inhibitory concentrations (MIC) were determined in liquid medium, the H₂O₂ MIC for pBAD/btuE cells was ten-fold higher than that of the parental, isogenic, control strain (Table S2). This result supports the previous observation that BtuE can function as a glutathione peroxidase in vitro [19].

Table 1. BtuE mediate resistance to ROS elicitors in E. coli.

| Strain              | Growth inhibition zone (cm²) | Control K₂TeO₃ | H₂O₂ | K₂CrO₄ | CdCl₂ |
|---------------------|-----------------------------|----------------|-------|--------|-------|
| BW25113 pBAD        | 6.7±0.3                     | 5.7±0.1        | 6.44  | 4.2±0.2|
| BW25113 pBAD/btuE   | 5.0±0.3                     | 3.3±0.1        | 3.4±0.1| 4.0±0.1|
| ΔbtuE pBAD          | 8.1±1.1                     | 6.7±0.1        | 7.4±0.1| 4.2±0.1|
| ΔbtuE pBAD/btuE     | 4.6±0.2                     | 3.5±0.2        | 4.1±0.1| 3.8±0.2|

Table 2. btuE expression results in decreased intracellular ROS.

| E. coli strain | Control K₂TeO₃ | Paraquat K₂CrO₄ |
|---------------|----------------|----------------|
| BW25113 pBAD  | 14.6±0.8       | 22.0±1.7       |
| BW25113 pBAD/btuE | 10.0±1.2   | 12.7±2.2       |
| ΔbtuE pBAD     | 19.1±2.2       | 23.1±0.9       |
| ΔbtuE pBAD/btuE| 10.1±1.0       | 13.4±2.4       |

Cytoplasmic ROS content was assessed by measuring the activation of 2’,7’-dihydodichlorofluorescein diacetate in wild type, pBAD/btuE, ΔbtuE and ΔbtuE pBAD/btuE cells as described in Methods. Cells were induced with 0.2% arabinose and exposed to K₂TeO₃ (0.5 µg ml⁻¹), paraquat (50 µg ml⁻¹) or K₂CrO₄ (1 mM) for 15 min at 37°C. Fluorescence (AU, arbitrary units) was determined and normalized per mg of protein. Values represent the mean of three independent trials ± SD. doi:10.1371/journal.pone.0015979.t002

BtuE protects E. coli from intracellular ROS

Cytoplasmic ROS levels were assessed using the probe 2’,7’-dihydodichlorofluorescein diacetate, as described in Methods. All strains exposed to K₂TeO₃, paraquat or K₂CrO₄ exhibited significant probe activation; the slight probe activation observed in untreated cells is presumed to be related to metabolic ROS generation. In the absence of exogenous oxidants, mutants lacking btuE showed higher ROS content than did wild type cells. The E. coli pBAD/btuE strain and the complemented ΔbtuE mutants showed decreased levels of probe activation relative to non-overproducing strains (Table 2).

To further analyze the protective role of BtuE against ROS generated during normal metabolism, we studied the effect of overexpressing btuE in strains lacking superoxide dismutases (ΔsodAB) or catalases/peroxidases (Δhpx). These strains suffer increased levels of O₂⁻ and H₂O₂, respectively [23,24]. Superoxide as well as peroxide levels were assessed by flow cytometry as described in Methods. BtuE production resulted in decreased ROS levels, showing a protective effect both in basal metabolic conditions as well as during oxidative stress caused by ROS elicitors (Fig. S1).

BtuE production results in decreased protein oxidation and damage to membrane lipids

The formation of carbonyl groups in some amino acid side chains is a conventional marker of ROS-mediated protein oxidation [25]. Spectrophotometric determination of derivatized carbonyl groups with 2,4-dinitrophenylhydrazine showed that E. coli ΔbtuE exhibited increased protein oxidation -even in the absence of toxicants- as compared to wild type cells. Genetic complementation of E. coli ΔbtuE, as well as overexpression of btuE, resulted in decreased protein oxidation, regardless of the ROS elicitor (Table 3).

Table 3. btuE expression alleviates oxidation of cytoplasmic proteins.

| E. coli strain | Control K₂TeO₃ | H₂O₂ |
|---------------|----------------|------|
| BW25113 pBAD  | 8.1±1.2        | 16.7±1.2|
| BW25113 pBAD/btuE | 9.6±2.9      | 12.3±2.2|
| ΔbtuE pBAD     | 17.1±6.0       | 39.6±5.0|
| ΔbtuE pBAD/btuE| 8.3±0.9        | 13.2±4.6|

Protein oxidation was determined in wild type, pBAD/btuE, ΔbtuE and ΔbtuE pBAD/btuE cells by the chemical protein carbonyl assay described in Methods. Total protein present in extracts of cells grown in the presence of 0.2% arabinose and exposed for 30 min to K₂TeO₃ (0.5 µg ml⁻¹) or H₂O₂ (100 µM) were reacted with 2,4-dinitrophenylhydrazine, and the specific carbonyl absorbance was read at 370 nm. Values represent the mean of three independent experiments ± SD. doi:10.1371/journal.pone.0015979.t003
Table 4. Elimination of btuE results in increased thiobarbituric acid-reactive substances in E. coli.

| Strain | Control | K2TeO3 | H2O2 |
|--------|---------|--------|-------|
| BW25113 | 24.6±5.7 | 81.20±8.0 | 131.9±18.6 |
| ΔbtuE | 162.0±9.0 | 147.0±27.1 | 144.0±2.5 |

Membrane lipid peroxidation products were determined as thiobarbituric acid-reactive substances (TBARS) in wild type (BW25113) and ΔbtuE strains in the absence (control) or presence of K2TeO3 (0.5 μg ml⁻¹) or H2O2 (100 μM) for 30 min. Values represent the mean of three independent experiments ± SD.

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BtuE expression results in decreased induction of ibpA, soxS and katG genes

The E. coli reporter strains ADA110 [4,28], SP11 and GS022 were used to assess the protective effect that BtuE confers against ROS elicitors. These strains harbor chromosomal insertions of the lacZ gene under the control of ibpA, soxS and katG promoters, respectively, which are induced under different stress conditions such as misfolding of cytoplasmic proteins and oxidative stress (ibpA), the presence of superoxide-generating compounds (soxS), and peroxides (katG). The effect of btuE overexpression was assessed by transforming them with pBAD/btuE or pBAD (control) plasmids and monitoring β-galactosidase activity after exposure to K2TeO3, menadione or H2O2. As expected, increased β-galactosidase activity was observed after toxicant exposure for all tested strains under control conditions. In turn, btuE overexpression resulted in a considerable decrease of enzyme activity, even in the absence of toxicants (Fig. 1). In fact, by hampering the activation of the ibpA promoter in E. coli ADA110, btuE overexpression resulted in a ~13-fold (control), 2.5-fold (tellurite) and 13-fold (peroxide) diminution of β-galactosidase activity compared to strains harboring the pBAD vector. This result suggests that BtuE might protect the cell by decreasing oxidative stress and cytoplasmic protein misfolding, whether these are generated by basal metabolism or by ROS elicitors (Fig. 1A).

In addition, Figs. 1B and C show that btuE overexpression results in >4-fold (soxS) and ~2-fold (katG) decrease in β-galactosidase activity relative to strains harboring pBAD vector only. By diminishing the response of the ROS defense regulons soxRS and oxyR, these results suggest that BtuE might help to alleviate oxidative stress in the E. coli cytoplasm.

BtuE protects E. coli lacking catalases and peroxidases from oxidative stress

The btuE gene was expressed in different E. coli genetic backgrounds, and growth inhibition zones were determined. Fig. 2A shows that all strains exhibited greater H2O2 tolerance when btuE was overexpressed. Similar results were obtained for potassium tellurite (not shown). The same trend was observed when growth curves of the Hpx- strain overexpressing btuE were analyzed for both H2O2 (Fig. 2B) or K2TeO3 (not shown). These data support the idea that the GPX activity of BtuE protects E. coli from H2O2 exposure.

Given the protective effect of BtuE in E. coli Hpx- against the tested ROS elicitors, it was of interest to analyze the effect of overexpressing other peroxidase genes in this bacterium. Fig. 2C shows that BtuE generates higher H2O2 resistance than KatG and KatE catalases or BCP and Tpx peroxidases.

BtuE production results in increased resistance of E. coli to potassium tellurite and hydrogen peroxide only in aerobic conditions

Since BtuE exhibits peroxidase activity in vitro, it was reasoned that transferring the ΔbtuE mutation to an Hpx- genetic background could help in analyzing the net effect of BtuE when
other H$_2$O$_2$ scavenging enzymes are missing. As seen in Fig. S2, the absence of $\Delta$btuE rendered Hpx$^-$ cells even more sensitive to TeO$_3^{2-}$ in the presence of oxygen. When growth curves were analyzed, the effect of the $\Delta$btuE mutation was more evident in aerobic conditions (Fig. S2A–B). The oxygen requirement was confirmed by determining growth inhibition zones (Fig. S2C).

Finally, the effect of the $\Delta$btuE mutation upon H$_2$O$_2$ tolerance in an Hpx$^-$ genetic background was evaluated. Fig. S2D–E shows that growth of Hpx$^-$ $\Delta$btuE cells is more sensitive to hydrogen peroxide than that of the parental Hpx$^-$ strain only in aerobic conditions. Again, these results were confirmed by determining growth inhibition zones (Fig. S2F).

**Discussion**

Since heavy metal pollution is a serious problem worldwide, there is a growing need to elucidate its toxic effects in sensitive microorganisms. It is also of interest to unveil the resistance mechanisms that protect resistant bacteria [29–32].

The toxicity of some metals is mediated by the generation of oxidative stress, so the cell must invoke a number of antioxidant defences–both enzymatic and non-enzymatic–to cope with this situation. In this regard, tellurite toxicity was initially thought to arise mainly from its ability to oxidize several cellular components [5,6,33]. Later it was recognized that the tellurium oxyanion triggers a series of events that lead to the generation of ROS, particularly superoxide [2,4,8,9,32,34].

The dearth of knowledge about prokaryotic glutathione peroxidases prompted us to analyze the role of the E. coli $\Delta$btuE gene product in cellular resistance to ROS. To assess if BtuE displays a general antioxidant function *in vivo*, the effect of $\Delta$btuE in wild-type, pBAD/$\Delta$btuE, $\Delta$btuE and $\Delta$btuE pBAD/$\Delta$btuE cells exposed to potassium tellurite and other ROS elicitors was evaluated. While in general terms $\Delta$btuE mutants were more sensitive to ROS.
elicitors, btuE overexpression resulted in enhanced cellular resistance to tellurite (4-fold) and hydrogen peroxide (10-fold) as compared to parental, wild type cells. Similar results were observed when cells were exposed to chromium, a generator of hydroxyl radicals [20,21]. In contrast, BtuE did not influence E. coli resistance to CdCl2 (Table 1, Table S2).

To test whether BtuE may affect the level of intracellular ROS, the fluorescent, oxidation-sensitive probe 2',7'-dihydrodichlorofluorescein diacetate was used. Tellurite, paraquat or chromatex exposure resulted in increased ROS levels, above those observed in unexposed cells. E. coli ΔbtuE always exhibited higher basal ROS levels than wild type cells; conversely, E. coli pBAD/btuE showed ROS levels far below those observed in controls (Table 2). Similar results were observed when protein carbonylation was assessed (Table 3), suggesting that BtuE could participate in the response to oxidative stress by lowering cytoplasmic ROS levels.

Since thiohoarbiuric acid responsive substances have been used routinely to assess oxidative stress damage to lipids [4,26,27], the effect of BtuE on membrane lipid damage was studied. A high increase (~6-fold) in the levels of these compounds was observed in E. coli ΔbtuE in the absence of any toxicant, suggesting that BtuE may function in preventing damage to membrane lipids or controlling the level of membrane peroxidation products (Table 4). Given that BtuE exhibits higher peroxidase activity with lipid peroxides in vitro [19], the in vivo situation was analyzed. Table S3 shows that BtuE is involved specifically in lowering lipid peroxide levels in E. coli, again indicating the importance of BtuE in membrane damage. In this context, it is interesting that Se-independent glutathione peroxidases preferentially degrade lipid peroxides [18,35,36].

Since tellurite toxicity is highly dependent on the presence of oxygen [1,37] and Gpxs are involved in oxidative stress, the role of BtuE in E. coli exposed to K2TeO3 both in aerobic and anaerobic conditions was analyzed. It was observed that in aerobic conditions the introduction of the btuE mutation into an Hpx− background resulted in impaired growth and in increased tellurite sensitivity (Fig. S2). Similar results were observed with hydrogen peroxide, except that in anaerobic conditions E. coli wild type strains (BW25113 and MG1655) as well as the ΔbtuE strain showed higher H2O2 sensitivity. This may be due to the fact that in aerobic conditions cells display fully induced antioxidative mechanisms to cope with peroxide [7]. In addition, no difference in peroxide resistance was observed between Hpx− and Hpx− ΔbtuE strains, suggesting that BtuE is important only in aerobic conditions (Fig. S2D-E-F). In support of this, when btuE was expressed in E. coli defective in H2O2-scavenging, increased H2O2 tolerance was observed in all btuE-complemented mutants (Fig. 2A-B).

Given that BtuE also efficiently decomposes lipid peroxides in vitro [19], we speculate that although adding hydrogen peroxide in anaerobic conditions can trigger a number of oxidative events, lipid peroxidation will not occur since it requires molecular oxygen. In this context, the toxic substrates of BtuE will be missing so that the enzyme will have no effect. Further experiments to unveil the global role of BtuE in the E. coli oxidative metabolism are under way in our laboratory.

Materials and Methods

Bacterial strains and culture conditions

Bacterial strains used in this study are listed in Table S1. Cells were grown routinely in LB medium [38] at 37°C with shaking. Growth was initiated by inoculating fresh LB medium with 1:100 dilutions of overnight cultures. Solid media contained 2% (w/v) agar, and plates were incubated overnight at 37°C.

Anaerobic growth liquid (liquid and solid media) was carried out in a Coy chamber (Coy Laboratory Products, Inc.) under 85% N2, 10% H2, and 5% CO2. Anaerobic buffers and media were moved into the chamber immediately after being autoclaved and allowed to equilibrate with the anaerobic atmosphere for at least 24 h prior to use.

E. coli harboring pBAD or pBAD/btuE plasmids (see below) were grown in LB containing ampicillin (100 µg ml−1) at 37°C with continuous agitation. When the cultures reached an OD600 ~0.4, L-arabinose (0.2% final concentration) was added. Induction was for 4 h at 37°C with shaking. Strains lacking btuE (ΔbtuE) and all other mutants were grown in LB medium containing kanamycin (100 µg ml−1).

Growth curves

To ensure that all studies were being conducted with exponentially growing cells, aerobic or anaerobic overnight cultures were diluted in fresh LB medium to an OD600 ~0.005 and grown at 37°C until they achieved an OD600 of ~0.1–0.2. Cultures were then diluted 10-fold into fresh medium containing K2TeO3 or H2O2, and they were grown at 37°C. Absorbance at 600 nm was monitored at 30 min intervals. Cell blackening due to tellurite reduction was negligible at tellurite concentrations up to 0.1 µg ml−1. In determining anaerobic growth, absorbance measurements were carried out at 1 h intervals.

Cloning the E. coli btuE gene and strain construction

In order to amplify the btuE gene from the E. coli genome, specific primers (Table S1) were designed using the VECTOR 9 NTI (Invitrogen®) software. The PCR product was inserted into pBAD/TOPO (Invitrogen®) vector, according to manufacturer’s instructions, resulting in plasmid pBAD/btuE. Identity/integrity of btuE was checked by DNA sequencing.

Strain Hpx− ΔbtuE was constructed by P1 transduction [39] between JEM216 x ΔbtuE (Table S1), selecting for kanamycin resistance. The btuE mutation in the resulting strain was confirmed by PCR using primers listed in Table S1.

Determination of growth inhibition zones

Growth inhibition zones were determined in LB-agar plates as described [40]. In brief, overnight cultures were diluted with LB and grown at 37°C for 4 h. After dilution to an OD600 ~0.1, 100 µl of each culture was evenly spread on the plates. Plates were air dried, and toxins to be tested (10 µl) were deposited on sterile 6 mm filter disks placed on the centres of the plates. Growth inhibition areas were determined after overnight incubation at 37°C. Determination of growth inhibition zones in anaerobic conditions followed an identical protocol, but all manipulations were carried out inside a Coy anaerobic chamber.

Determination of the minimal inhibitory concentration

Sterile stock solutions of appropriate concentrations of K2TeO3, K2CrO4, CdCl2 or H2O2 were serially diluted in a 96-well ELISA plate containing 200 µl of LB medium (plus the appropriate antibiotic) per well. Five µl of cultures grown at 37°C in LB medium supplemented with the required antibiotic(s) to an OD600 ~0.4 were added to each well, and the plate was incubated at 37°C. Turbidity was observed visually after 24 h. MIC determinations in anaerobic conditions followed the same protocol in a Coy chamber.

Determination of intracellular reactive oxygen species

In general, cellular oxidants, including ROS, were assessed using the oxidation-sensitive probe 2',7'-dichlorofluorescein diacetate. As
demonstrated by Royall and Ischiropoulos [41], once inside the cell this esterified probe is deacylated by intracellular esterases and the resulting compound, dichlorofluorescein, is susceptible to oxidation by ROS. Briefly, cells grown aerobically in LB medium to an OD₆₀₀ ~0.4 were exposed for 30 min to K₂TeO₃ (0.5 μg ml⁻¹), paraquat (50 μg ml⁻¹) or K₂CrO₇ (1 mM). They were then centrifuged, washed with 10 mM potassium phosphate buffer, pH 7.0, and incubated for 30 min in the same buffer containing the probe (10 mM final concentration). Cells were subsequently washed and disrupted by sonication. One hundred μl of the resulting cell extracts were mixed with 1 μl of the same buffer, and fluorescence intensity was determined using an Applied Biosystems Citifluor 4000 Fluorescence Multi-well plate reader (excitation 490 nm, emission 519 nm). Emission values were standardized by protein concentration [4,42].

E. coli ΔoddB and Hpx⁻ strains transformed with the indicated plasmids (Table S1) were used to determine intracellular ROS by flow cytometry. Cells were grown to an OD₆₀₀ ~0.5 in the presence of arabinose at 37°C, and they were then exposed to K₂TeO₃ (0.5 μg ml⁻¹) for 30 min. After centrifugation at 5,000 g for 10 min, cells were washed with saline phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.3) and diluted 1:10 with the same buffer. Cells were incubated with 10 mM 2',7'-dihydodichlorofluorescein diacetate (or 127 μM dihydrodichlorofluorescein diacetate or 127 μM dihydrodichlorofluorescein diacetate and dissolved in 1 ml of ethanol:chloroform (2:1 v/v). After vigorous shaking for 1 h, FOXII reagent (ferrous oxidation in the presence of xylenol orange) was added, and the mixture was shaken again for 1 h at room temperature. After centrifuging at 13,000 g for 10 min, the clear supernatant was used to determine the content of membrane lipid peroxides at 360 nm [16].

Supporting Information

Figure S1 Effect of BtuE in the generation of intracellular ROS. Cytoplasmic superoxide (A) or ROS (B) were determined by flow cytometry using dihydroethidine or 2',7'-dihydorochlorofluorescein diacetate in E. coli ΔoddB or Hpx⁻ strains, respectively, exposed or not to K₂TeO₃ (0.5 μg/ml) for 30 min in the presence of 0.2% L-arabinose. Representative profiles of fluorescence intensity regarding the cell number (above) for the analyzed strains and histograms representing % of fluorescence intensity of control (pBAD) and pBAD/btuE cells (below) are shown. 100% of fluorescence intensity corresponds to the strain carrying pBAD only. Bars represent the average of three independent experiments ± SD. Numbers above each condition represent the pBAD/pBAD/btuE ratio. (TIF)

Figure S2 BtuE protects E. coli from potassium tellurite and hydrogen peroxide in aerobic conditions. E. coli Hpx⁻ and Hpx⁻ ΔbtuE strains were grown aerobically (A) or anaerobically (B) in LB medium to an OD₆₀₀ ~0.01, and K₂TeO₃ was added to a final concentration of 0 (control, C), 0.001 (D), 0.005 μg ml⁻¹ (E, A). Data are representative of three independent experiments. (C), Growth inhibition zones were assessed for Hpx⁻ and Hpx⁻ ΔbtuE cells grown aerobically (+O₂) or anaerobically (−O₂) and exposed to K₂TeO₃ (10 μl, 1 μg/ml). Values represent the mean of three independent experiments ± SD. E. coli Hpx⁻ and Hpx⁻ ΔbtuE cells grown aerobically (+O₂) or anaerobically (−O₂) and exposed to H₂O₂ (10 μl, 1 M). Values represent the mean of three independent experiments ± SD. (TIF)

Table S1 Bacterial strains, plasmids and primers used in this study. (DOCX)

Table S2 BtuE mediates resistance to potassium tellurite and other ROS elicitors in E. coli. (DOCX)

Table S3 Elimination of btuE results in decreased lipid peroxide levels in E. coli. (DOCX)

Author Contributions

Conceived and designed the experiments: FAA PCC JAI CCV. Performed the experiments: FAA PCC. Analyzed the data: FAA JMS JMP JAI CCV. Contributed reagents/materials/analysis tools: JAI CCV. Wrote the paper: FAA CCV. Allowed a stay of FAA at his lab: JAI.
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24. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
25. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
26. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
27. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
28. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
29. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
30. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
31. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
32. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
33. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
34. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
35. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
36. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
37. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
38. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
39. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
40. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
41. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
42. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.
43. Miller JR (1972) Experiments in Molecular Genetics, 201–205, 352–355, and 730–750.