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Tellurite-mediated damage to the *Escherichia coli* NDH-dehydrogenases and terminal oxidases in aerobic conditions

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ABSTRACT

Escherichia coli exposed to tellurite shows augmented membrane lipid peroxidation and ROS content. Also, reduced thiols, protein carbonylation, [Fe–S] center dismantling, and accumulation of key metabolites occur in these bacteria. In spite of this, not much is known about tellurite effects on the *E. coli* electron transport chain (ETC).

In this work, tellurite-mediated damage to the *E. coli* ETC's NADH dehydrogenases and terminal oxidases was assessed. Mutant lacking ETC components showed delayed growth, decreased oxygen consumption and increased ROS in the presence of the toxicant.

Membranes from tellurite-exposed *E. coli* exhibited decreased oxygen consumption and dNADH/NADH dehydrogenase activity, showing an impairment of NDH-I but not of NDH-II activity. Regarding terminal oxidases, only the *bo* oxidase complex was affected by tellurite. When assaying NDH-I and NDH-II activity in the presence of superoxide, the NDH-I complex was preferentially damaged. The activity was partly restored in the presence of reducing agents, sulfide and Fe^{2+} under anaerobic conditions, suggesting that damage affects NDH-I [4Fe–4S] centers.

Finally, augmented membrane protein oxidation along with reduced oxidase activity was observed in the presence of the toxicant. Also, the increased expression of genes encoding alternative terminal oxidases probably reflects a cell's change towards anaerobic respiration when facing tellurite.

Introduction

Tellurium is a chalcogen that belongs to the VIA group of the Periodic Table of Elements. While elemental Te does not exhibit apparent toxicity, the oxyanion tellurite (TeO_3^{2-})[2] is extremely toxic for most microorganisms, especially Gram negative bacteria [1].

Once inside the cell, tellurite causes a number of detrimental modifications including a reduced thiol pool [2,3] and increased cytoplasmatic levels of reactive oxygen species (ROS), mainly superoxide, in *Escherichia coli* [4,5], *Pseudomonas pseudoalcaligenes* KF707 [6] and *Rhodobacter capsulatus* [7,8]. In turn, ROS can affect enzyme activity because of protein carbonylation [9,10] and [4Fe–4S] cluster destruction of some dehydratases such as aconitase B and fumarase A [11,12]. This radical can be dismutated to form hydrogen peroxide by superoxide dismutase (SOD), which in turn may provoke macromolecule peroxidation [13]. [Fe–S] center dismantling results in Fe^{2+} release that can react with H_2O_2 to form the noxious hydroxyl radical, which increases membrane lipid peroxidation and also results in the generation of toxic reactive aldehydes such as acrolein and malondialdehyde in *E. coli* [14–16].

Tellurite also affects the activity of some enzymes involved in the central sugar metabolism such as phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, resulting in the accumulation of pyruvate, α-ketoglutarate and a number of phosphorylated sugars [17–19].

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[2] *Abbreviations used:* ETC, electron transport chain; ROS, reactive oxygen species; NDH-I, NADH dehydrogenase I; NDH-II, NADH dehydrogenase II; dNADH, deamino nicotinamide adenine dinucleotide; SQR, quinone oxidoreductase; *bo*, terminal oxidase *bo*; *bd-I*, terminal oxidase *bd-I*; *bd-II*, terminal oxidase *bd-II*; NFMM, non-fermentative minimal medium; H_2DCFDA, 2′,7′-dihydrodichlorofluorescein diacetate; DHE, dihydroethidine; DCPIP, dichlorophenol indophenol; DQH_2, duroquinol; DNPH, 2,4-dinitrophenylhydrazine; XAN, xanthine; XO, xanthine oxidase; WST-1,2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt.

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To face tellurite-induced oxidative stress, a number of microorganisms have evolved toxicant-detoxifying mechanisms such as alkylation [20] and enzymatic or non-enzymatic reduction of tellurite to the less-toxic form elemental tellurium (Te^0) that accumulates intra- or extracellularly [21,22]. General antioxidant activities controlled by SoxRS and OxyR transcriptional factors [23] are also triggered upon tellurite exposure [5]. Enzymes exhibiting the ability of tellurite reduction include nitrate reductases [24], the terminal oxidase *bd-I* from the ETC [25], catalase [26], pyruvate dehydrogenase [17,27], α-ketoglutarate dehydrogenase [18], and the *E. coli* NADH dehydrogenase II [28]. Since this last dehydrogenase forms part of the ETC which could help when facing the toxicant, it was of interest to assess the effects of tellurite on the whole *E. coli* electron transport chain.

Under aerobic conditions, the *E. coli* ETC is composed by the NDH-I complex, NDH-II, SQR complex, ubiquinone and the ubiquinol oxidoreductase complexes *bo*, *bd-I* and *bd-II*. While *bo* is mainly expressed under oxygen-rich conditions and repressed in anaerobiosis, *bd-I* and *bd-II* functions in aerobic and semi-anaerobic conditions [29] and during the stationary phase of aerobic growth, oxygen limitation and inorganic phosphate deprivation, respectively [30]. The ETC utilizes the reduced pyrimidine and flavin cofactors for generating a proton motive force (PMF) through the transfer of protons to the periplasmic space. Electrons are then transferred, via coenzyme Q, to the terminal electron acceptors [31–38] (Scheme 1). Previous studies on the *E. coli* ETC have shown that tellurite exposure results in decreased ATP levels as well as in altered ΔpH [39], suggesting that the ETC would be a potential tellurite target.

Although the NADH dehydrogenase activity of NDH-II is not affected by tellurite, the enzyme generates significant amounts of superoxide while reducing the toxicant [28] which, given the proximity of NDH-I and NDH-II in the supramolecular complex [40], could reach and damage the [Fe–S] centers present in complex I.

The goal of this work was to determine if tellurite exposure results in damage to the ETC's NADH dehydrogenases and/or terminal oxidase *bo* and *bd-I* complexes under aerobic conditions.

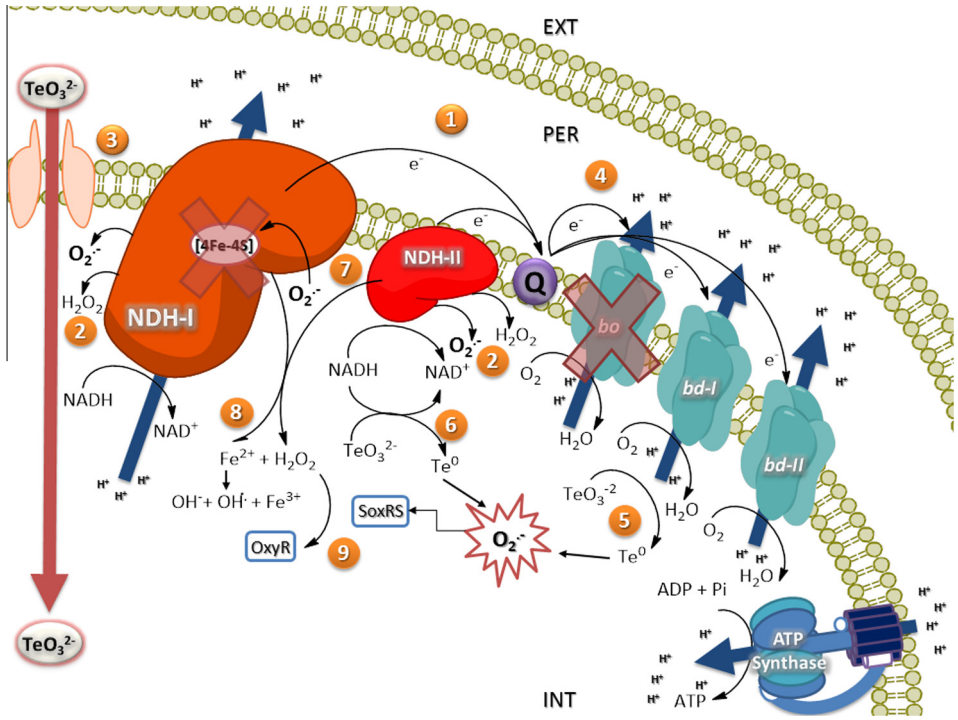
Materials and methods

Bacterial strains and growth conditions

Bacteria used in this work (Table S1) were routinely grown in NFMM that contained M9 1X salts (Na_2HPO_4 34 g, KH_2PO_4 15 g, NH_4Cl 5 g and NaCl 2.5 g in 1 l of a 5X stock solution), casaminoacids (0.1%) and glycerol (1%) [28] at 37 °C with vigorous shaking. This procedure encouraged *bo* and *bd-I* oxidase production while diminishing that of *bd-II* oxidase. When needed, NFMM was amended with the appropriate antibiotic(s). Growth was started with 1:10,000 dilutions of saturated cultures. Unless otherwise stated, in all toxicant treatments exponential cultures (OD_{600} ~0.3) were exposed to sub lethal tellurite concentrations (1.5 μM, corresponding to ½ of the minimal inhibitory concentration, MIC) for 30 min. Solid media contained 2% agar.

E. coli membrane purification

Cells were exposed to TeO_3^{2-} for 30 min and harvested at 8000×*g* for 10 min at 4 °C. After washing, cells were suspended in Tris–HCl 50 mM pH 7.4 buffer (buffer A) containing 20 μM PMSF and disrupted by sonication. The cell debris was eliminated by cen-



Scheme 1. Model showing our current view of tellurite-induced damage to the *E. coli* ETC under aerobic growth conditions. (1) NDH-I and NDH-II oxidizes NADH and electrons are transferred to coenzyme Q (Q) and then to *bo*, *bd-I* and *bd-II* terminal oxidases which reduces oxygen to water. In the process NDH-I, *bo*, *bd-I* and *bd-II* translocate protons to the periplasmic space that are used to synthesize ATP by ATP synthase. Tellurite exposure results in reduced PMF and ATP synthesis [39]. (2) Both NDH-I and NDH-II generate ROS during the normal functioning of the ETC, which can be increased by autoxidation processes. (3) Tellurite enters the cell through phosphate and/or monocarboxylate transporters [65,66]. (4) While *bd-I* oxidase is not affected by the toxicant, *bo* oxidase suffers minor damage. (5) *bd-I* terminal oxidase can use tellurite as alternative electron acceptor [25], which in turn could result in $O_2^{\cdot-}$ generation. (6) NDH-II is a NADH-dependent tellurite reductase that produces superoxide during the reduction process [28]. (7) Superoxide affects NADH dehydrogenase activity of the NDH-I complex in the presence of the NADH substrate, probably by dismantling a [4Fe–4S] cluster. (8) [Fe–S] dismantling results in Fe^{2+} release which could react with H_2O_2 (Fenton reaction) to generate hydroxyl radical ($OH^{\cdot}$) [67]. (9) Superoxide and H_2O_2 activates the *soxRS* and *oxyR* regulons, respectively, which results in antioxidant gene expression [68].

trifugation at 11,000×*g* for 10 min at 4 °C and the supernatant was centrifuged at 120,000×*g* for 1 h at 4 °C. The sediment was considered the membrane fraction. Protein concentration was determined as described previously [41].

Oxygen consumption

To assess the *E. coli* total terminal oxidase activity, oxygen consumption was determined using cells and/or membranes derived from cells previously grown in the absence or presence of the toxicant. Oxygen consumption was assessed using a FIBOX 3 oxygenmeter equipped with a flat oxygen-sensitive probe and an optic fiber detector. Potassium cyanide (2.5 mM) was used to inhibit respiration. After toxicant treatment, 1 ml of the culture was diluted 4-fold with fresh NFMM and oxygen diffusion was avoided by adding 1 ml of sterile mineral oil. Results were expressed as per cent of consumed oxygen in 20 min and normalized by protein concentration.

Assessing ROS generation by flow cytometry

Intracellular ROS levels were determined using H_2DCFDA (λ_{ex} 428 nm, λ_{em} 522 nm). After toxicant exposure, cells were centrifuged at 8000×*g* for 5 min at 4 °C. The pellet was washed with 50 mM Tris–HCl pH 7.4 buffer (buffer A) and diluted 100-fold with the same buffer. The sample was shaken for 30 min with 0.02 mM H_2DCFDA in the dark at 37 °C. After washing with buffer A, the fluorescence intensity was determined in a flow cytometer Becton Dickinson (model Facs Canto II) equipped with an argon laser. The same experimental approach was used to determine superoxide but incubating with DHE (λ_{ex} 520 nm, λ_{em} 610 nm) was for 15 min. Tellurite does not oxidize directly H_2DCFDA or DHE. Fluorescence count assessment was normalized by cell size as described [18].

NADH/dNADH dehydrogenase activity assessment in E. coli membranes

NADH/dNADH dehydrogenase activity was determined in membrane fractions by monitoring NADH oxidation at 340 nm. The reaction mix (500 µl) consisted of buffer A that contained 60 µM NADH or dNADH. To determine NADH/DCPIP dehydrogenase activity, the reaction mixture was supplemented with 0.5 mM of the synthetic substrate DCPIP as described [42].

Duroquinol/ferricyanide activity of cytochrome oxidase complexes in E. coli membranes

Activity of terminal oxidases was determined by measuring oxygen consumption with an oxygenmeter FIBOX 3 or 2 mM ferricyanide [$K_3Fe(CN)_6$] at 500 nm, in the presence of 50 µg of membrane proteins. The reaction mixture (1 ml) contained buffer A, 60 µM NADH or 1.6 mM DQH_2 as electron donor, as required. Results of oxygen consumption were expressed as per cent of oxygen consumed and normalized by the protein concentration.

Assessing membrane protein oxidation

Carbonyl group content in membrane fractions was determined at 370 nm as described previously [13,28]. Membranes were incubated with 4 volumes of 10 mM DNPH for 1 h at room temperature. Proteins were precipitated with 20% trichloro acetic acid and recovered by centrifugation at 10,000×*g* for 5 min. After washing with ethanol:ethyl acetate (1:1) to remove unreacted DNPH, the sediment was dissolved in a solution that contained 6 M guanidine–HCl and 0.1 mM dithiothreitol at 37 °C.

Tellurite reduction assay

Two hundred milligram of membrane protein were tested for tellurite reductase activity at 500 nm in buffer A that contained 0.5 mM NADH and 1 mM 2-mercaptoethanol as described [17]. KCN (2.5 mM) was used to block respiration.

Superoxide generation in E. coli membranes

The superoxide generator system (500 µl) contained buffer B (50 mM MES-NaOH pH 7.4), 0.1 mM XAN and 3.2 mU of XO. Superoxide dismutase (110 U) was included in control assays. Superoxide formation was determined at 438 nm using the water soluble probe WST-1 at 0.1 mM final concentration.

[4Fe–4S] cluster restoration

Fumarase activity was determined as described previously [12]. [4Fe–4S] cluster restoration was determined as reported previously [43] with modifications. Membrane fractions (0.5 mg ml^{-1} protein) were incubated with buffer B which contained 0.1 M NaCl, 0.1 M β-mercaptoethanol and 0.2 mM Na_2S (restoration buffer). Subsequently extra pure nitrogen was bubbled for 45 s to remove any trace of oxygen. Finally $(NH_4)_2Fe$ $(SO_4)_2$ was added up to 0.25 mM and incubated on ice for 5 min.

Relative gene expression

Total RNA was prepared from cells exposed to tellurite for 15 or 30 min using the RNAsy kit (Qiagen) and quantified using the Quant-it Ribogreen Kit (Invitrogen) following the manufacturer's instructions. qRT-PCR was carried out using a Light Cycler apparatus with the RNA Master SYBR Green Kit (Roche Applied Science). 250 ng of purified RNA were used as template. *rpoD* was used as the housekeeping gene. Transcript levels were calculated as described previously [44]. Specific primers to amplify the respective genes are shown in Table S2.

Results

Whole cell studies

Tellurite affects cell respiration

When *E. coli* BW25113, NDH-I$^-$, NDH-II$^-$, *bo*$^-$ or *bd-I*$^-$ strains were independently exposed to tellurite, a diminished growth – which recovered after ~15 h of treatment – was observed (Fig. S1). Particularly, *bd-I*$^-$ cells recovered growth much slower than all other strains when exposed to the toxicant.

Next, oxygen consumption was assessed to determine if tellurite actually affects respiration. In the absence of tellurite, almost all mutants showed the same oxygen consumption as the isogenic parent. The exception was *bd-I*$^-$, which showed decreased respiration (~50%, Fig. 1). In the same line, toxicant-treatment resulted in reduced respiration in *E. coli* BW25113, NDH-I$^-$, *bo*$^-$ or *bd*$^-$ but not in NDH-II$^-$ cells, most probably reflecting a weakened ETC activity (Fig. 1).

Tellurite exposure increases ROS production

Levels of total ROS as well as of superoxide were assessed by flow cytometry using H_2DCFDA (Figs. 2A and S3) and DHE (Figs. 2B and S4), respectively. In the absence of toxicant and excluding the *bd-I*$^-$ strain, ROS levels did not increase in the mutant strains regarding the parental, isogenic control (Fig. 2A). Upon tellurite exposure however and again excluding *bd-I*$^-$

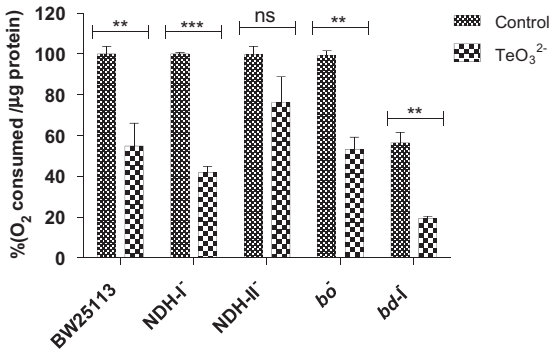


Fig. 1. Oxygen consumption in tellurite-exposed *E. coli*. Kinetics (20 min) of oxygen consumption by untreated and toxicant-exposed *E. coli* BW25113, NDH-I$^-$, NDH-II$^-$, *bo*$^-$ and *bd-I*$^-$ cells. KCN was used to inhibit cytochrome oxidase activity (control). Bars represent the average of 3 independent trials ± SE. $^{**}p < 0.01$; $^{***}p < 0.001$; ns, not significant.

(Fig. S3E), ROS production increased importantly in all strains regarding the respective untreated controls (Figs. 2A and S3A–D).

Studies using membranes

Tellurite damages the E. coli NADH dehydrogenase I

Based on previous observations showing the formation of tellurium crystals along with increased protein carbonylation in membranes from tellurite-exposed cells [28], the NADH dehydrogenase activity present in membrane fractions from NDH-I$^-$ and NDH-II$^-$ cells was determined.

While the absence of a functional NDH-I results in ~60% decrease of the NADH dehydrogenase activity, that of NDH-II increases it ~30% regarding the control (Fig. 3A). Nonetheless, in the presence of tellurite and regarding the respective untreated controls, NADH dehydrogenase activity increased ~90% in NDH-I$^-$ cells while being almost completely abolished in the NDH-II$^-$ strain (Fig. 3A), The same trend was observed when electron transfer to the synthetic substrate DCPIP was assessed (Fig. 3B). To specifically evaluate the activity of the NDH-I complex, dNADH dehydrogenase activity was determined in *E. coli* BW25113 membranes (Fig. 3C).

Tellurite effect on the electron transfer from NADH dehydrogenases to terminal oxidases

Oxygen consumption was evaluated in membrane fractions in the presence of NADH. Under the same experimental conditions, NADH oxidase activity decreased ~50% in the parental strain in the presence of the toxicant (Figs. 4A and S5A) while it increased ~25% in membranes from NDH-I$^-$ cells (Figs. 4A and S5B). When membranes of the NDH-II$^-$ strain were analyzed, activity was almost negligible both in the control situation as well as in the presence of tellurite (Figs. 4A and S5C). To elucidate this issue, membrane fractions were assessed for duroquinol oxidoreductase activity. (Fig. 4B).

Protein carbonylation in membranes of tellurite-exposed E. coli

It was interesting to assess the degree of protein oxidation in membranes from the mutants lacking the terminal oxidases. As expected, carbonyl groups were almost doubled in membrane proteins of the parental and *bo*$^-$ mutant. On the other hand those coming from tellurite-exposed *bd-I*$^-$ cells exhibited a ~25–30% increase in carbonyls groups (Fig. S6).

Tellurite damages bo but not bd-I activity

To determine which terminal oxidase complex is specifically damaged by the toxicant, duroquinol/ferricyanide oxidoreductase activity was determined in membrane fractions from appropriate mutant cells. This was carried out using the synthetic electron donor duroquinol, which bypasses the NADH dehydrogenases allowing electron transfer directly to the ETC's terminal oxidase complexes. While in membranes from tellurite-exposed *bo*$^-$ cells this activity did not change (Fig. 5B), it decreased 2–3-fold in wild type and *bd-I*$^-$ membrane fractions (Fig. 5A and C).

Superoxide damages NDH-I's NADH dehydrogenase activity

It was interesting to assess if this radical damages the NDH-I complex in isolated *E. coli* membranes. For this purpose, the xanthine/xanthine oxidase (XAN-XO) reaction system was used to generate superoxide *in vitro*. NADH dehydrogenase activity was assessed after incubation with in membrane fractions from the parental strain AS454 (*sodC*) did not change upon incubation with the XAN-XO system. However, 50% of the activity was lost when membranes were previously incubated for 10 min with XAN-XO in the presence of the NADH substrate (Fig. 6A).

Similar experiments were carried out with membrane fractions from the *E. coli nuo* strain. As shown in Fig. 6B, NDH-II's NADH dehydrogenase activity was not affected by the XAN-XO system, indicating that this enzyme is not damaged by superoxide. This result is similar to what could occur in the presence of tellurite and NADH. Conversely, exposure of *E. coli ndh* membrane fractions to the superoxide-generating system resulted in a ~50% decrease of NADH dehydrogenase activity which was further reduced in

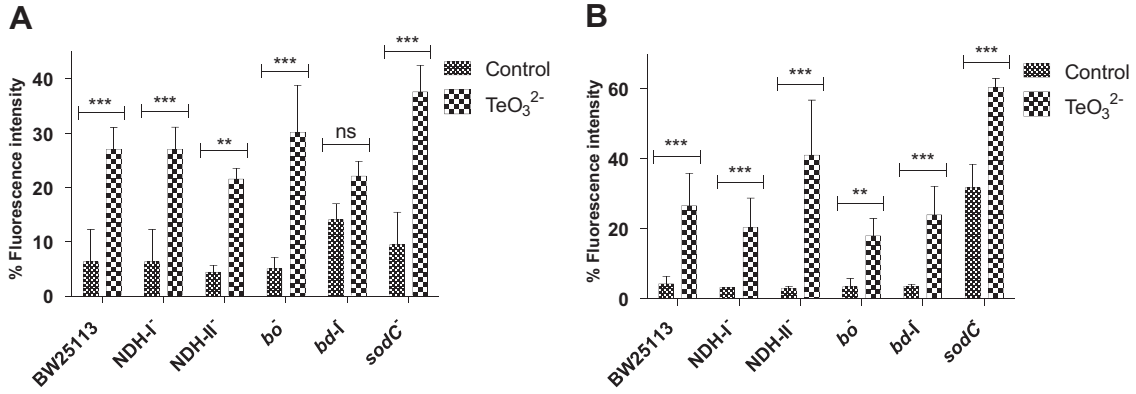


Fig. 2. Total intracellular ROS and superoxide assessed by flow cytometry in tellurite-exposed *E. coli*. Fluorescence intensity of the whole cell population was determined by using H_2DCFDA (total ROS, A) and DHE (superoxide, B). Bars represent the average of 3 independent trials ± SE. $^{**}p < 0.01$; $^{***}p < 0.001$.

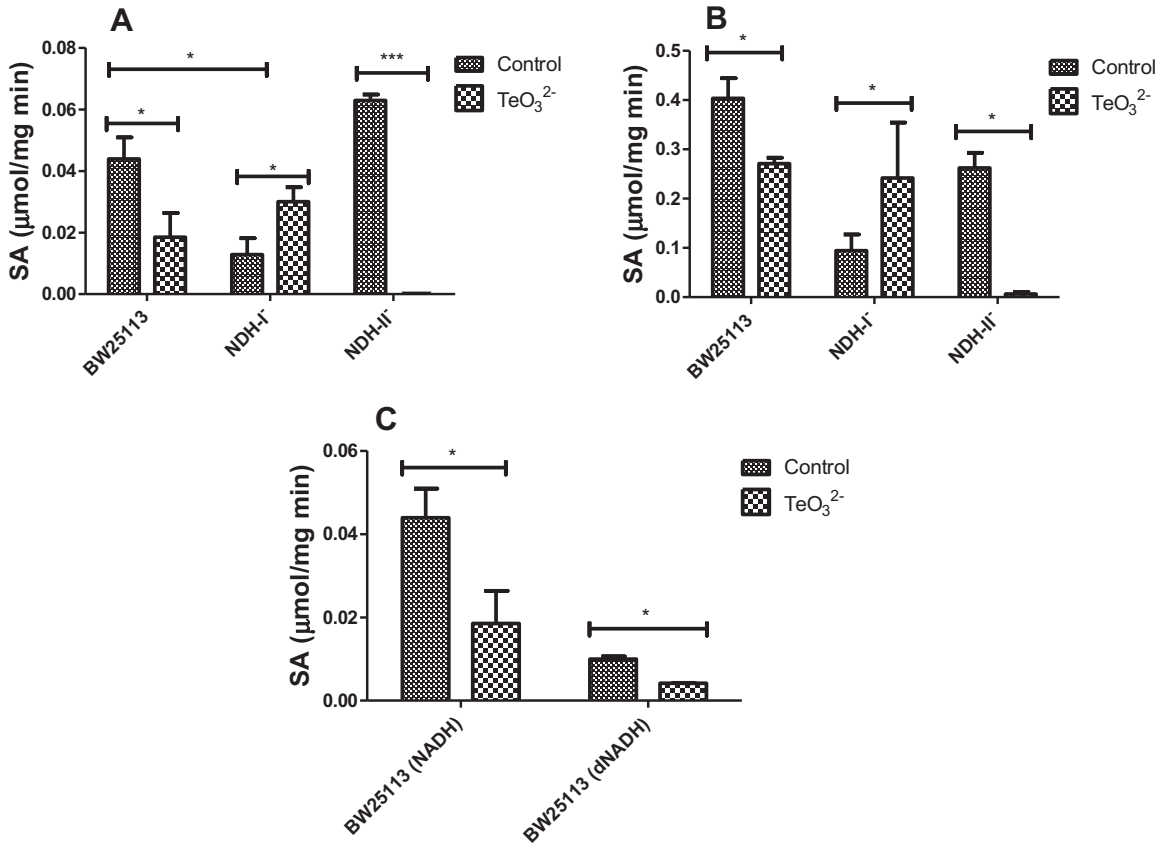


Fig. 3. NADH/dNADH dehydrogenase activity in *E. coli* membrane fractions. NADH (A) and NADH/DCPIP (B) dehydrogenase activity in membranes from the indicated strains in the absence (control) or presence of tellurite. (C) Comparison of NADH and dNADH dehydrogenase activity in the parental strain BW25113. Bars represent the average of 3 independent trials ± SE. $^{*}p < 0.05$; $^{***}p < 0.001$.

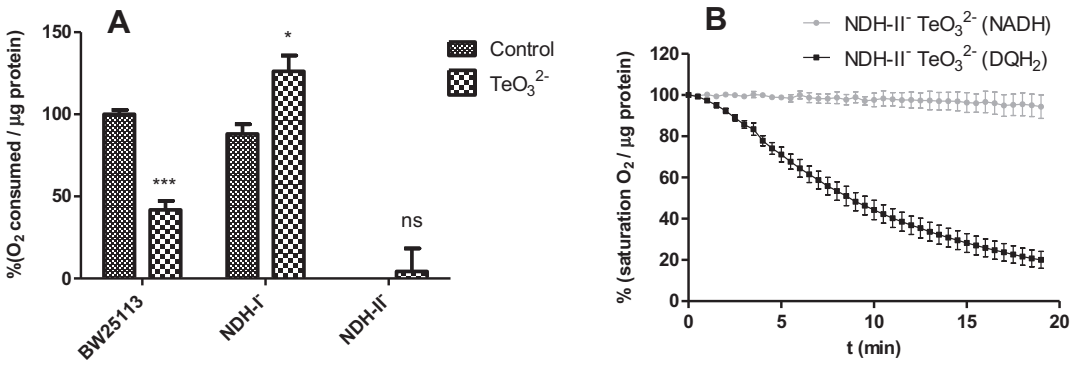


Fig. 4. Cytochrome oxidase activity in membrane fractions of tellurite-exposed *E. coli*. (A) Percent difference of oxygen consumption (NADH oxidase activity) between wild type and the indicated mutant *E. coli* strains. (B) Kinetics of oxygen consumption by membranes of tellurite-exposed NDH-II^{-} cells in the presence of NADH and duroquinol oxidase (DQH_2). KCN was used to inhibit cytochrome oxidase activity (control). Bars represent the average of 3 independent trials ± SE. $^{*}p < 0.05$; $^{***}p < 0.001$; ns, not significant.

the presence of NADH; this last effect is not observed in the presence of SOD (Fig. 6C).

Tellurite damages [Fe–S] centers in NDH-I

Considering that the NDH-I complex contains eight [Fe–S] centers that are well protected from $O_2^{\cdot-}$ [45,46], we tried to determined if tellurite exposure results in a [Fe–S] damage.

NADH dehydrogenase activity was completely lost in membrane fractions from *E. coli ndh* cells that had been previously exposed to the toxicant. This enzymatic activity was fully restored after the membranes were incubated in a restoration buffer that contained ferrous ion, sodium sulfide and 2-mercaptoethanol under anaerobic conditions [43] (Fig. 7A and B).

Relative gene expression in tellurite-exposed E. coli

As expected, qPCR showed that *cydB* (encoding the B subunit of the *bd-I* complex) transcription was kept repressed at 15 and 30 min of toxicant exposure (Table 1). On the other hand, and as expected, the expression of *soxS* and *katG* genes was enhanced (5- and 16-fold, respectively), which may represent the activation

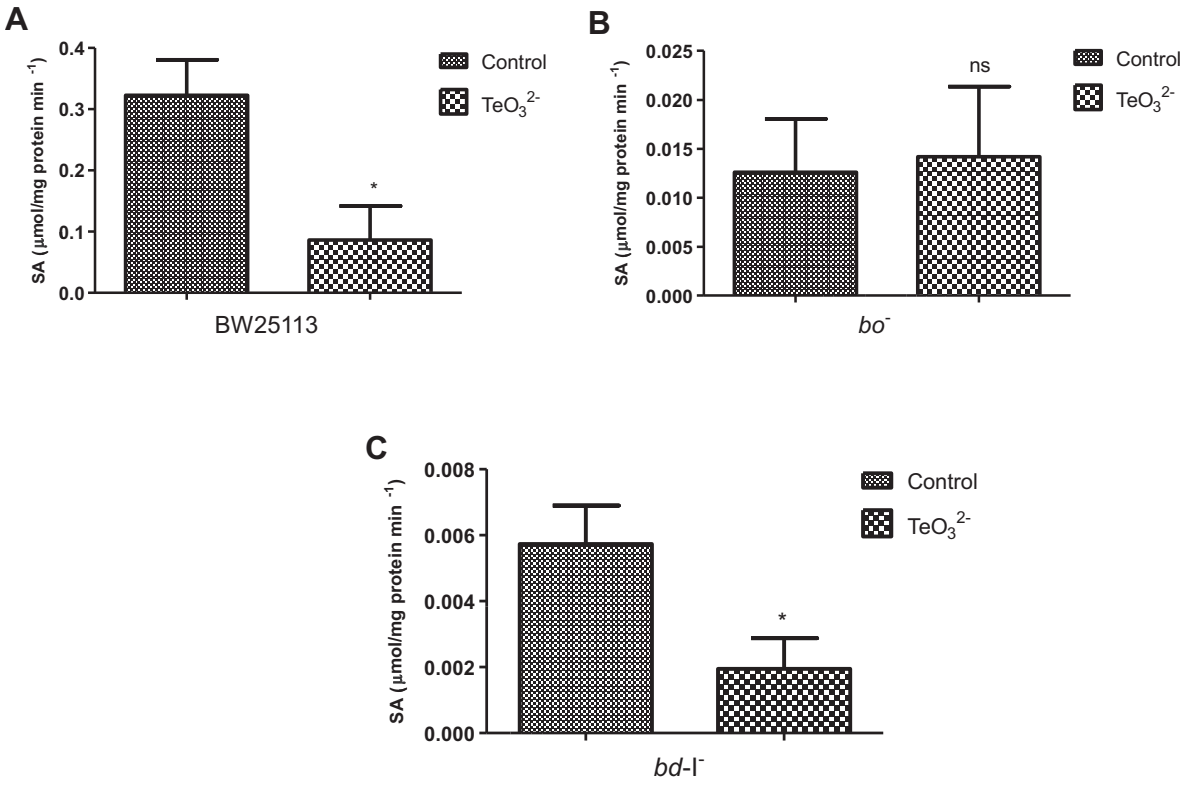


Fig. 5. Duroquinol-ferricyanide reductase activity in *E. coli* membrane fractions. The activity was assessed in *E. coli* BW25113 (A), *bo*$^{-}$ (B) and *bd-I*$^{-}$ membranes (C) as described in Methods. Bars represent the average of 3 independent trials ± SE; $^{*}p < 0.05$; ns, not significant.

of the antioxidant machinery as consequence of toxicant exposure. Regarding terminal oxidases, a strong induction (9.5-fold) of the *frdA* gene (encodes the fumarate reductase A subunit) along with a subtle downregulation of *cyoB* (encoding the B subunit of the *bo* complex) was observed (Table 1).

Discussion

During the last years we and other groups have been interested in deciphering the molecular basis of tellurite toxicity. It has been found that the toxicant affects directly – or indirectly – some pathways that include the loss of activity of a number of key metabolic enzymes [8,19,28,47]. This work focused on the damage that the tellurium oxyanion causes to the *E. coli* ETC, specifically to the NADH dehydrogenases and terminal oxidases.

In the absence of tellurite, terminal oxidase *bd-I* seems to be able to assume completely the respiratory function, showing oxygen consumption levels almost identical to those exhibited by the parental control (Figs. 1 and S2C). Excepting NDH-II^{-}, all tested strains exhibited impaired respiration when exposed to toxicant. Similar observations were communicated by Lohmeier-Vogel et al. [39], who showed that tellurite-sensitive strains exhibited lower PMF that finally resulted in decreased ATP synthesis. Since in normal conditions NDH-II generates substantial amounts of ROS [48,49], and particularly superoxide during tellurite reduction *in vitro* [28], it is likely that in the absence of this enzyme tellurite-exposure results in a less-severe damage to the membrane structure and hence to respiration.

Although *bd-I*$^{-}$ cells did not show changes in ROS generation (Fig. 2A), all other strains showed increased ROS when facing tellurite (Figs. 2A and B, S3 and S4). This suggests that the *bd-I* complex is involved particularly in superoxide generation, which probably results from its tellurite-reducing ability [25] and/or its ROS-scavenging peroxidase activity previously described [38,50]. (Figs. 2B and S4E). This observation agrees with the main oxidative unbalance related to the ETC dehydrogenases [49,51].

Studies using membranes

Tellurite damages the E. coli NADH dehydrogenase I

Total NADH dehydrogenase activity was assessed in membranes from toxicant-exposed *E. coli* to determine which ETC component is the principal tellurite target. Results from NADH dehydrogenase and DCPIP reductase activity indicated that NDH-II is not affected by tellurite. Indeed, both activities were enhanced upon tellurite exposure (Fig. 3A and B), which could result from an increased number of NDH-II molecules to compensate the non-functional NDH-I complex at the membrane. In this context, it was recently shown that the *ndh* gene, encoding NDH-II, is overexpressed in tellurite-exposed *E. coli* [28]. Assays carried out with membranes from the parental strain using dNADH, a NDH-I specific substrate, confirmed that only NDH-I and not NDH-II is damaged by the toxicant (Fig. 3C).

Tellurite effects on the electron transfer from NADH dehydrogenases to terminal oxidases

In agreement with the previous observation for NADH dehydrogenase activity, membranes from tellurite-treated NDH-I^{-} cells showed increased oxygen consumption, once more supporting the idea that NDH-II is not affected by tellurite (Figs. 4A, S5A and S5B). Curiously, and despite of many attempts, membranes from the NDH-II^{-} strain did not display terminal oxidase activity (Figs. 4A and S5C). These observations could be explained by the ease of the NDH-I complex oxidation [52], which would hamper electron transfer to the terminal oxidases. To discern this point, duroquinol oxidoreductase activity was assessed. This activity

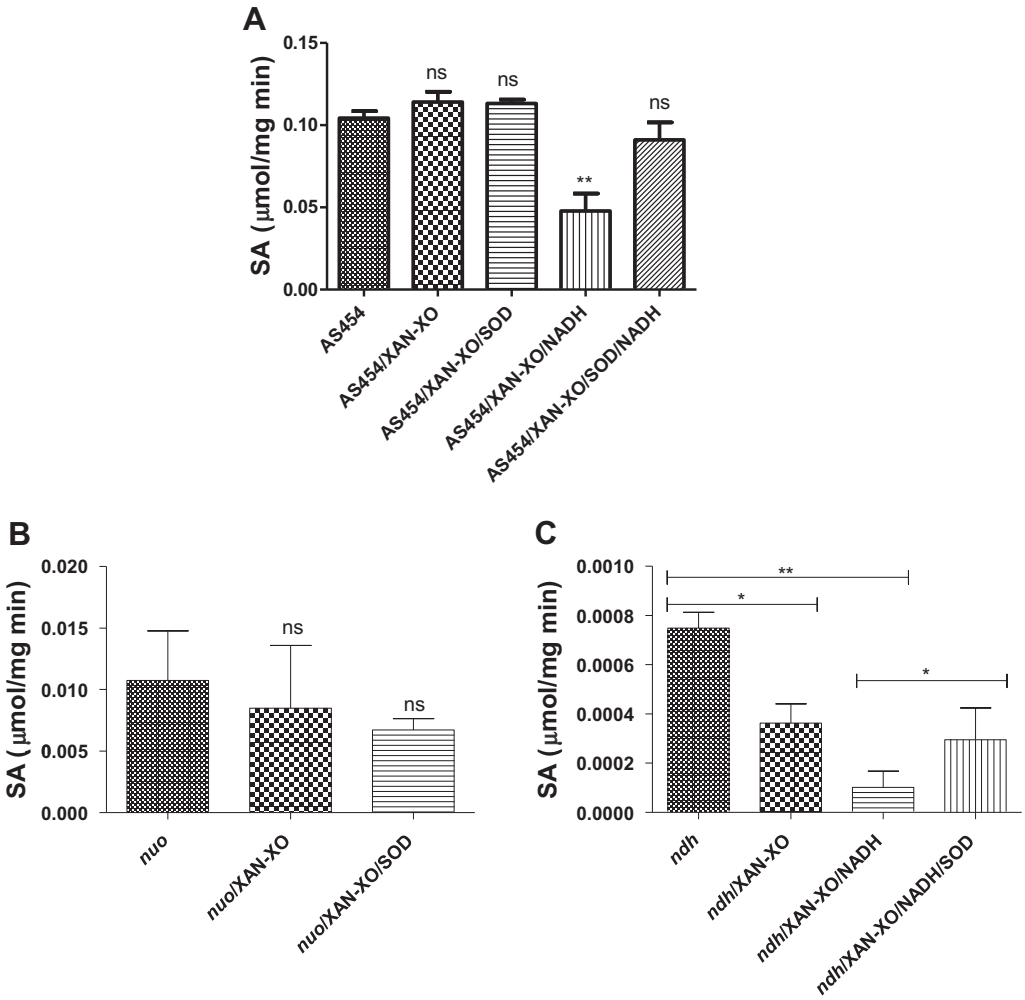


Fig. 6. Effects of superoxide on NADH dehydrogenase activity from *E. coli* membranes. NADH dehydrogenase activity in membrane fractions from *E. coli* AS454 (A), *nuo* (B) and *ndh* (C) was assessed as described in Methods. Membranes were preincubated or not with the NADH substrate as indicated and then assayed in the presence of the XAN-XO system. Bars represent the average of 3 independent trials ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; ns, not significant.

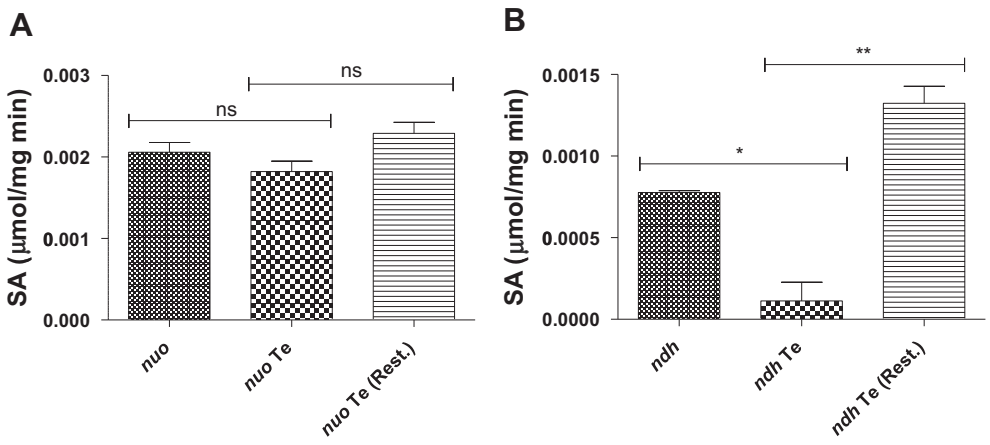


Fig. 7. [Fe–S] center restoration in the *E. coli* NDH-I complex. NADH dehydrogenase activity in membrane fractions from *nuo* (A) or *ndh* (B) strains previously exposed or not to tellurite. Activity was also determined in membranes from toxicant-treated cells after preincubating with restoration buffer (Rest). Bars represent the average of 3 independent trials ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; ns, not significant.

was not affected in membranes from toxicant-exposed NDH-I^{-} cells (Fig. 4B). Thus, these findings suggest that the loss of oxygen consumption does not result from tellurite damage of terminal oxidases and is most probably a consequence of NDH-I oxidation.

Protein carbonylation in membranes of tellurite-exposed E. coli

This kind of protein oxidation comes most probably from tellurite-induced ROS generation, which results in increased reactive aldehydes that ultimately oxidize amino acid side chains [53]. This

Table 1
Relative gene expression in tellurite-exposed *E. coli* as determined by qPCR.

Gene	Relative expression (fold change) Tellurite exposure (min)	
	15	30
cyoB	1.48	0.55
cydB	0.51	0.86
soxS	4.98	2.28
katG	15.89	4.96
frdA	1	9.49

assumption is supported by the fact that tellurite-exposed *E. coli* exhibits augmented lipid peroxidation [15,16]. Results depicted in Fig. S6 and our previous findings [28] indicate that carbonyl groups increase at the membrane level in tellurite-exposed *E. coli*. The main superoxide source at the membrane could be represented by NDH-II's-associated tellurite reductase activity, a process which may occur in a similar way as that described previously for catalase [26]. In addition, the putative superoxide production by terminal oxidase *bd-I* [25] cannot be ruled out.

Tellurite damages bo but not bd-I terminal oxidase activity

Duroquinol/ferricyanide oxidoreductase activity was assessed in membranes to evaluate tellurite sensitivity of these ETC components. The results suggest that the *bo* complex may represent a new intracellular tellurite target and/or that the tellurite-reducing and peroxidase activity displayed by the *bd-I* complex [25,38] confers protection against tellurite-induced oxidative stress.

Superoxide damages NDH-I's NADH dehydrogenase activity

Given the proximity between NDH-I and NDH-II at the membrane, superoxide generated by NDH-II during tellurite reduction [28] could dismantle [Fe–S] clusters of the NDH-I complex. In this line, *in vitro* damage to NADH dehydrogenases was assessed using the xanthine-xanthine oxidase (XAN-XO) superoxide-generating system. Membranes purified from the parental strain and previously incubated with the XAN-XO system did not lose NADH dehydrogenase activity, except if the NADH substrate is present (Fig. 6A). A putative interpretation of these findings is that the supramolecular organization of the NDH-I/NDH-II complex [54] is responsible for avoiding excessive damage by superoxide, a situation that changes radically in the presence of NADH. This would allow a significant protection against tellurite-mediated oxidative damage and also a better electron transfer to terminal oxidases. The XAN-XO system did not cause damage to NDH-II in *nuo* membranes, most probably because the enzyme lacks [Fe–S] clusters (Fig. 6B).

On the other hand, the initial reduction of NADH dehydrogenase activity observed in *ndh* membranes preincubated with the XAN-XO system was more evident in the presence of NADH (Fig. 6C). Taken together, these findings could reflect that a number of [4Fe–4S] centers may be rendered susceptible to superoxide damage most probably because of NADH-induced conformational changes of the NDH-I complex.

Tellurite damages [Fe–S] centers in NDH-I

The NDH-I complex contains eight [Fe–S] centers that are well protected from $O_2^{\cdot-}$ [45,46]. However and as mentioned above, it is likely that some of them turn superoxide sensitive because of NADH-induced conformational changes of NDH-I dehydrogenase [55,56].

Interestingly, damaged NADH dehydrogenase activity in membranes from toxicant-exposed *ndh* cells could be restored upon preincubation in anaerobic conditions in the presence of ferrous ion as was previously reported for fumarase A [12] and pyruvate formate-lyase-activating enzyme [43]; this suggests that the NDH-I complex is in fact undergoing [4Fe–4S] center dismantling in the presence of tellurite. Since [Fe–S] cluster disassembling implies that it should be exposed to the solvent, in our opinion the best candidate to suffer superoxide-mediated dismantling in the presence of the NADH substrate is NDH-I's N2 [4Fe–4S] center, which is involved in proton translocation and electron transfer to ubiquinone 8 [57–61]. In this line, Berrisford and Sazanov [55] have suggested that in the presence of NADH, Complex I from *Thermus thermophilus* undergoes oxygen-dependent chemical modifications that occur near of the N2 [Fe–S] center. Nevertheless, further experimental evidence is required to identify the ultimate [Fe–S] target within the *E. coli* NDH-I complex. (Fig. 7A and B)

Next, changes in the expression of respiration-related genes were assessed by qPCR. The results (Table 1) suggest that cells are most probably switching to anaerobic metabolism, a situation that was also observed by microarray analysis [62].

Summarizing, under aerobic conditions tellurite exposure provokes an oxidative stress status which damages significantly the functioning of the *E. coli* electron transport chain. Particularly damaged results the NDH-I complex, most probably by [Fe–S] cluster dismantling. In this scenario, it is probable that the SQR complex, a [Fe–S] cluster-containing complex, undergoes similar modifications. In the same line, tellurite-mediated damage to the *bo* terminal oxidase cannot be ignored. A direct effect of the damaged ETC components could result in decreased electron flux through coenzyme Q thus impairing oxygen reduction by terminal oxidases. Thus, under aerobic conditions the ETC could still function thanks to the rather tellurite-insensitive enzymes NDH-II and the terminal oxidase *bd-I* in a similar way to what occurs when oxygen becomes limiting.

Finally and since that (i) NDH-I's NADH dehydrogenase activity is sharply reduced while that of NDH-II is not affected in the presence of tellurite, (ii) NDH-II is the most important dehydrogenase during aerobic growth [54], (iii) the *bo* oxidase suffers minor damage in the presence of tellurite while *bd* oxidase is not affected by tellurite, (iv) qPCR experiments showed that *ndh* as well as other anaerobic respiration-related genes such as *napA* and *narG* are overexpressed in the presence of tellurite [28], (v) [4Fe–4S] center dismantling of NDH-I could result in Fe^{2+} freeing that in turn can react with H_2O_2 coming from tellurite-mediated oxidative damage and/or auto-oxidation processes at the NDH-I complex [48] or NDH-II [49,52,63], (vi) peroxide can result in a substantial increase of membrane lipid peroxidation [15,16] and protein carbonylation [28,64], the model depicted in Scheme 1 is proposed to shed light to the molecular basis underlying tellurite-induced membrane damage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.abb.2014.10.011.

References

[1] D.E. Taylor, Trends Microbiol. 7 (1999) 111–115.
[2] R.J. Turner, J.H. Weiner, D.E. Taylor, Microbiology 145 (1999) 2549–2557.
[3] R.J. Turner, Y. Aharonowitz, J.H. Weiner, D.E. Taylor, Can. J. Microbiol. 47 (2001) 33–40.
[4] J.C. Tantaleán, M.A. Araya, C.P. Saavedra, D.E. Fuentes, J.M. Pérez, I.L. Calderón, P. Youderian, C.C. Vásquez, J. Bacteriol. 185 (2003) 5831–5837.
[5] J.M. Pérez, I.L. Calderón, F.A. Arenas, D.E. Fuentes, G.A. Pradenas, E.L. Fuentes, J.M. Sandoval, M.E. Castro, A.O. Elías, C.C. Vásquez, PLoS One 2 (2007) e211.
[6] F. Borsetti, V. Tremaroli, F. Michelacci, R. Borghese, C. Winterstein, F. Daldal, D. Zannoni, Res. Microbiol. 156 (2005) 807–813.
[7] V. Tremaroli, S. Fedi, D. Zannoni, Arch. Microbiol. 187 (2007) 127–135.
[8] V. Tremaroli, M.L. Workentine, A.M. Weljie, H.J. Vogel, H. Ceri, C. Viti, E. Tatti, P. Zhang, A.P. Hynes, R.J. Turner, D. Zannoni, Appl. Environ. Microbiol. 75 (2009) 719–728.
[9] E. Cabiscol, J. Tamarit, J. Ros, Int. Microbiol. 3 (2000) 3–8.
[10] N. Contreras, P. del, C.C. Vásquez, Arch. Microbiol. 192 (2010) 969–973.
[11] J.A. Imlay, Mol. Microbiol. 59 (2006) 1073–1082.
[12] I.L. Calderón, A.O. Elías, E.L. Fuentes, G.A. Pradenas, M.E. Castro, F.A. Arenas, J.M. Pérez, C.C. Vásquez, Microbiology 155 (2009) 1840–1846.
[13] H. Semchyshyn, T. Bagnyukova, K. Storey, V. Lushchak, Cell Biol. Int. 29 (2005) 898–902.
[14] J.M. Pérez, F.A. Arenas, G.A. Pradenas, J.M. Sandoval, C.C. Vásquez, J. Biol. Chem. 283 (2008) 7346–7353.
[15] G.A. Pradenas, B.A. Paillavil, S. Reyes-Cerpa, J.M. Pérez-Donoso, C.C. Vásquez, Microbiology 158 (2012) 1279–1283.
[16] G.A. Pradenas, W.A. Díaz-Vásquez, J.M. Pérez-Donoso, C.C. Vásquez, BioMed. Res. Intern. 2013 (2013) 563756.
[17] M.E. Castro, R. Molina, W. Díaz, S.E. Pichuantes, C.C. Vásquez, Biochem. Biophys. Res. Commun. 375 (2008) 91–94.
[18] C.A. Reinoso, C. Auger, V.D. Appanna, C.C. Vásquez, Biochem. Biophys. Res. Commun. 421 (2012) 721–726.
[19] M. Valdivia-González, J.M. Pérez-Donoso, C.C. Vásquez, Biometals 25 (2012) 451–458.
[20] T.G. Chasteen, R. Bentley, Chem. Rev. 103 (2003) 1–25.
[21] D.E. Taylor, E.G. Walter, R. Sherburne, D.P. Bazett-Jones, J. Ultrastruct. Mol. Struct. Res. 99 (1988) 18–26.
[22] G. Lloyd-Jones, A.M. Osborn, D.A. Ritchie, P. Strike, J.L. Hobman, N.L. Brown, D.A. Rouch, FEMS Microbiol. Lett. 118 (1994) 113–119.
[23] J.A. Imlay, Annu. Rev. Microbiol. 57 (2003) 395–418.
[24] C. Avazeri, R.J. Turner, J. Pommier, J.H. Weiner, G. Giordano, A. Vermeglio, Microbiology 143 (1997) 1181–1189.
[25] S.M. Trutko, N.E. Suzina, V.I. Duda, V.K. Akimenko, A.M. Boronin, Dokl. Akad. Nauk 358 (1998) 836–883.
[26] I.L. Calderón, F.A. Arenas, J.M. Pérez, D.E. Fuentes, M.A. Araya, C.P. Saavedra, J.C. Tantaleán, S.E. Pichuantes, P.A. Youderian, C.C. Vásquez, PLoS One 1 (2006) e70.
[27] M.E. Castro, R.C. Molina, W.A. Díaz, G.A. Pradenas, C.C. Vásquez, Biochem. Biophys. Res. Commun. 380 (2009) 148–152.
[28] W.A. Díaz-Vásquez, M.J. Abarca-Lagunas, F.A. Arenas, C.A. Pinto, F.A. Cornejo, P.T. Wansapura, G.A. Appuhamillage, T.G. Chasteen, C.C. Vásquez, Biometals 27 (2014) 237–246.
[29] R.B. Gennis, FEMS Microbiol. Lett. 46 (1987) 387–399.
[30] J. Dassa, H. Fsihi, C. Marck, M. Dion, M. Kieffer-Bontemps, P.L. Boquet, Mol. Gen. Genet. 229 (1991) 341–352.
[31] B.A. Haddock, C.W. Jones, Bacteriol. Rev. 41 (1977) 47–99.
[32] W.J. Ingledew, R.K. Poole, Microbiol. Mol. Biol. Rev. 48 (1984) 222–271.
[33] K. Matsushita, L. Patel, H.R. Kaback, Biochemistry 23 (1984) 4703–4714.
[34] J.A. Garcia-Horsman, B. Barquera, J. Rumbley, J. Ma, R.B. Gennis, J. Bacteriol. 176 (1994) 5587–5600.
[35] T. Friedrich, Biochim. Biophys. Acta 1364 (1998) 134–146.
[36] G. Cecchini, I. Schroder, R.P. Gunsalus, E. Maklashina, Biochim. Biophys. Acta 1553 (2002) 140–157.
[37] A.M.P. Melo, T.M. Bandeiras, M. Teixeira, Microbiol. Mol. Biol. Rev. 68 (2004) 603–616.
[38] V.B. Borisov, R.B. Gennis, J. Hemp, M.I. Verkhovsky, Biochim. Biophys. Acta 1807 (2011) 1398–1413.
[39] E.M. Lohmeier-Vogel, S. Ung, R.J. Turner, Appl. Environ. Microbiol. 70 (2004) 7342–7347.
[40] P.M. Sousa, S.T. Silva, B.L. Hood, N. Charro, J.N. Carita, F. Vaz, D. Penque, T.P. Conrads, A.M. Melo, Biochimie 93 (2011) 418–425.
[41] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[42] A.S. Fernandes, M.M. Pereira, M. Teixeira, J. Bioenerg. Biomembr. 34 (2002) 413–421.
[43] R. Kulzer, T. Pils, R. Kappl, J. Huttermann, J. Knappe, J. Biol. Chem. 273 (1998) 4897–4903.
[44] M.W. Pfaffl, G.W. Horgan, L. Dempfle, Nucleic Acids Res. 30 (2002) e36.
[45] T. Ohnishi, Biochim. Biophys. Acta 1364 (1998) 186–206.
[46] G. Yakovlev, T. Reda, J. Hirst, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 12720–12725.
[47] T.G. Chasteen, D.E. Fuentes, J.C. Tantaleán, C.C. Vásquez, FEMS Microbiol. Rev. 33 (2009) 820–832.
[48] D. Esterhazy, M.S. King, G. Yakovlev, J. Hirst, Biochemistry 47 (2008) 3964–3971.
[49] K.R. Messner, J.A. Imlay, J. Biol. Chem. 274 (1999) 10119–10128.
[50] D. Wall, J.M. Delaney, O. Fayet, B. Lipinska, T. Yamamoto, C. Georgopoulos, J. Bacteriol. 174 (1992) 6554–6562.
[51] A. Lindqvist, J. Membrillo-Hernández, R.K. Poole, G.M. Cook, Antonie Van Leeuwenhoek 78 (2000) 23–31.
[52] L.C. Seaver, J.A. Imlay, J. Biol. Chem. 279 (2004) 48742–48750.
[53] J.R. Requena, R.L. Levine, E.R. Stadtman, Amino Acids 25 (2003) 221–226.
[54] G. Unden, J. Bongaerts, Biochim. Biophys. Acta 1320 (1997) 217–234.
[55] J.M. Berrisford, L.A. Sazanov, J. Biol. Chem. 284 (2009) 29773–29783.
[56] R.G. Efremov, R. Baradaran, L.A. Sazanov, Nature 465 (2010) 441–445.
[57] U. Weidner, S. Geier, A. Ptock, T. Friedrich, H. Leif, H. Weiss, J. Mol. Biol. 233 (1993) 109–122.
[58] P. Hellwig, D. Scheide, S. Bungert, W. Mantele, T. Friedrich, Biochemistry 39 (2000) 10884–10891.
[59] S. Magnitsky, L. Toulokhonova, T. Yano, V.D. Sled, C. Hagerhall, V.G. Grivennikova, et al., J. Bioenerg. Biomembr. 34 (2002) 193–208.
[60] D. Flemming, A. Schlitt, V. Spehr, T. Bischof, T. Friedrich, J. Biol. Chem. 278 (2003) 47602–47609.
[61] D. Flemming, P. Hellwig, S. Lepper, D.P. Kloer, T. Friedrich, J. Biol. Chem. 281 (2006) 24781–24789.
[62] R.C. Molina-Quiroz, D.E. Loyola, W.A. Díaz-Vásquez, F.A. Arenas, U. Urzúa, J.M. Pérez-Donoso, C.C. Vásquez, Res. Microbiol. 165 (2014) 566–570.
[63] K.R. Messner, J.A. Imlay, J. Biol. Chem. 277 (2002) 42563–42571.
[64] H.H. Refsgaard, L. Tsai, E.R. Stadtman, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 611–616.
[65] F. Borsetti, A. Toninello, D. Zannoni, FEBS Lett. 554 (2003) 315–318.
[66] A.O. Elías, M.J. Abarca, R.A. Montes, T.G. Chasteen, J.M. Pérez-Donoso, C.C. Vásquez, MicrobiologyOpen 1 (2012) 259–267.
[67] J.A. Imlay, Annu. Rev. Biochem. 77 (2008) 755–776.
[68] G. Storz, J.A. Imlay, Curr. Opin. Microbiol. 2 (1999) 188–194.