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Effects of tellurite on growth of *Saccharomyces cerevisiae*

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Abstract The effects of potassium tellurite on growth and survival of rho$^+$ and rho^0 *Saccharomyces cerevisiae* strains were investigated. Both rho$^+$ and rho^0 strains grew on a fermentable carbon source with up to 1.2 mM K_2TeO_3, while rho$^+$ yeast cells grown on a non-fermentable carbon source were inhibited at tellurite levels as low as 50 μM suggesting that this metalloid specifically inhibited mitochondrial functions. Growth of rho$^+$ yeast cells in the presence of increasing amount of tellurite resulted in dose-dependent blackening of the culture, a phenomenon not observed with rho^0 cultures. Transmission electron microscopy of *S. cerevisiae* rho$^+$ cells grown in the presence of tellurite showed that blackening was likely due to elemental tellurium (Te^0) that formed large deposits along the cell wall and small precipitates in both the cytoplasm and mitochondria.

Keywords Tellurite · *Saccharomyces cerevisiae* · Mitochondria · Transmission electronic microscopy

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Introduction

The tolerance of microorganisms to potentially toxic metals has received considerable attention. Tellurium (Te), a highly toxic metalloid, is a relatively rare element found in nature in its elemental (Te^0), inorganic-(telluride [Te^{2-}], tellurite [TeO_3^{2-}], and tellurate [TeO_4^{2-}]), and organic-(dimethyl telluride [CH_3TeCH_3]) forms. Of these, the oxyanion forms are more common than the non-toxic, elemental state (Cooper 1971).

Microbial resistance to tellurite is poorly understood but is thought to be associated with tellurite reduction and precipitation of metallic tellurium (Lloyd 2003; Zannoni et al. 2008). Tellurite may enter bacterial cell by using the phosphate uptake system and once inside the cell it is reduced by oxidoreductases, including nitrate reductase (Taylor 1999) and terminal oxidases of the respiratory chain (Trutko et al. 2000), glutathione and other thiol-carrying molecules (Turner et al. 2001). Other proposed mechanisms, including cysteine-metabolizing enzymes and methyl

transferases, may be important resistance mechanisms against tellurite toxicity (Taylor 1999; Zannoni et al. 2008).

Growth in the presence of tellurite is often associated with reduction of the oxyanion to Te^0, which leads to blackening of the cells due to either cytoplasmic or periplasmic Te^0 crystalline precipitates (Taylor 1999; Trutko et al. 2000; Zannoni et al. 2008). In some instances the capacity of prokaryotic cells to grow at higher tellurite concentrations depends on the presence of genetic determinants carried on plasmids (Zannoni et al. 2008). Additionally, a number of chromosomal genes have been found to be associated with tellurite resistance or to directly mediate tellurite resistance in a few species, but their role has not been clearly determined (Taylor et al. 1994; Toptchieva et al. 2003; Zannoni et al. 2008).

The capacity to reduce Te is not restricted to prokaryotes, as eukaryotes, including fungi, yeasts and plants, as well as animal tissues may carry out various reactions leading to black Te^0 precipitates (Gharieb and Gadd 1998; Ollivier et al. 2008; Zannoni et al. 2008). However, up to now, the majority of studies with the metalloid Te address its interactions with prokaryotic organisms. In contrast, the study of Te interaction with eukaryotic organisms has received much less attention (Zannoni et al. 2008). To gain insight about the nature of such biological mechanisms, in the present work we analyzed the effects of potassium tellurite on growth, survival and micro-morphology of the model yeast *Saccharomyces cerevisia*.

Materials and methods

Saccharomyces cerevisiae strains Sc57 (YM654 α ura3-52 his3-Δ200 ade2-101 lys2-801 tyr1-501, rho^+, gift from Mark Johnston, Washington University, USA) and its rho^0 derivative Sc57-R3 (Del Giudice et al. 2005) were used. The following media were used: glucose-complete-medium (YEPD): 1% yeast extract, 1% bacto peptone, 2% glucose (dextrose); glucose-complete-medium without bacto peptone (YED); glycerol-complete medium without bacto peptone (YEG): 1% yeast extract, 3% glycerol; minimal medium (YNB): 0.67% yeast Difco nitrogen base without amino acids, 2% glucose, supplementations when necessary. To all solid media 2.5% bacto agar was added. Growth measurements were performed with a Klett–Summerson colorimeter using a glass filter to give a wavelength of 640 nm.

A stock solution (4 mg/ml) of K_2TeO_3 hydrate minimum 90% (Sigma) was prepared by dissolving 44.4 mg of solute in 10 ml YEG. The solution was adjusted to pH 7–7.5 with 1 M HCl, and sterilized by filtration through a 0.22 micron pore diameter filter (Millipore). The clear stock solution was stored at 4°C. The tellurium precipitation tests were performed utilizing sterile microtiter plate (Falcon), 96 well. 5×10^5 cells per milliliter of fresh stationary phase preculture were inoculated in YE containing the appropriate carbon source. About 0.2 ml of the culture was placed in each of wells of the microtiter plate. Increasing amounts of potassium tellurite were added. Microtiter plate cultures were incubated static at 28°C and precipitation of Te^0 was observed usually after 5–6 days.

The minimum inhibitory concentrations (MIC) for potassium tellurite was determined by using a standard broth macrodilution methods. YED or YEG broth, serial twofold dilution of potassium tellurite and a culture of actively growing yeast cells (about 10^5 colony forming units [CFU] ml^{-1}) were dispensed into polyethylene culture tubes (16 by 125 mm, Corning) to a total volume of 2 ml/tube. Cells were incubated at 28°C for 24–48 h with shaking, and the optical density at a wavelength of 640 nm was monitored.

The survival with potassium tellurite was measured with a preculture of Sc57 strain grown at 28°C with shaking for 24 h. 5×10^5 cells per milliliter of the preculture were inoculated in 5 ml YED tubes containing the appropriate concentration of potassium tellurite. The fresh cultures were incubated at 28°C with shaking for 24 h. Cells at the early stationary phase were washed twice with sterile distilled water by centrifugation at 6,000 rpm for 10 min at 4°C in a Sorvall SS34 rotor. The washed cell pellets were each resuspended, respectively, in 5 ml sterile distilled water. Appropriate dilutions of washed cultures were spread onto YED plates and incubated at 28°C for 2–3 days. Growing cell colonies of each culture sample were counted and the percentage of survivors was calculated comparing to the cell titer of the control culture. Statistical significance was determined by Student's *t* test.

For transmission electron microscopy (TEM) experiments, *S. cerevisiae* strains Sc57 was grown in YED in the presence of 100 μM of potassium tellurite. Samples were collected by centrifugation and fixed with 2% glutaraldehyde and 1% formaldehyde in 0.04 M piperazine-*N*,*N*′-bis (2-ethansulfonic acid) (PIPES) buffer at pH 7.0 for 2 h at room temperature. The samples were rinsed in 0.08 M PIPES buffer and twice in 0.08 M Na-cacodylate buffer and post-fixed in 1% OsO4 in 0.08 M Na-cacodylate buffer, pH 6.7, overnight at 4°C. Following dehydration in a step gradient of ethanol with three changes of anhydrous ethanol and one of propylene oxide incubation step at 4°C, the samples were slowly infiltrated with Epon 912 resin at 4°C, transferred to polypropylene dishes and incubated at 70°C for 24 h. Thin sections were stained with 3% uranyl acetate in 50% methanol for 15 min and in Reynold's lead citrate for 10 min and then examined with a Leo 912AB electron microscope.

Results

Precipitation of tellurium (very likely Te^0) was seen in the presence of increasing potassium tellurite concentrations in the wells of a microtiter plate containing 0.2 ml YED medium inoculated with 5×10^5 cells per milliliter. The amount of Te^0 deposited by Sc57, a respiratory-competent strain, increased as a function of potassium tellurite concentration in the cultures (data not shown). Similar results were obtained by replacing glucose with other fermentable carbon sources (data not shown). In contrast, no Te^0 precipitate was observed when Sc57-R3, a respiratory-deficient derivative of Sc57, was used.

The MIC of potassium tellurite was determined in YE broth containing either glucose (YED) or glycerol as non-fermentable carbon source (YEG). Growth of Sc57 strain was inhibited by 50 μM potassium tellurite in YEG, whilst both Sc57 and Sc57-R3 strains were able to grow in YED up to tested 1.2 mM concentration of the metalloid. MIC of Sc57-R3 could not be determined in YEG due to the inability of this strain to grow on glycerol as a carbon source, while at concentration >1.2 mM MIC measurements were impeded by massive blackening of the cultures (Table 1).

Table 1 MIC of potassium tellurite for *S. cerevisiae* strains Sc57 and Sc57-R3

Yeast strain	MIC of potassium tellurite in YEG medium[a]	MIC of potassium tellurite in YED medium (mM)
Sc57	50 μM	>1.2
Sc57-R3	ND	>1.2

[a] *ND* not determinable

The different behavior of rho^+ and rho^0 strains in the tellurium precipitation test, and the different tolerance to potassium tellurite of rho^+ yeast cells in YED with respect to YEG indicated that this metalloid was inhibiting mitochondrial functions.

The percentages of Sc57 and Sc57-R3 colony forming survivors were determined for cells growing in media containing glucose and different potassium tellurite concentrations (Table 2). Viable cells were determined by spreading a measured aliquot of a diluted cell culture onto YED plates and counting the resulting colonies after 2–3 days incubation at 28°C. The percentage of cell survivors was calculated comparing the cell titers of control cultures without potassium tellurite with those of cultures growing in presence of potassium tellurite. The percentage of cell survivors was dependent on tellurite and the presence of Bacto peptone in the media containing a fermentable carbon source. Under these assay conditions, strain Sc57-R3 appeared slightly more sensitive than Sc57, although the differences between two strains were not statistically significant ($P > 0.05$). The number of viable cells of both strains decreased by increasing the potassium tellurite concentration in the media, while the addition of Bacto peptone in the media increased the percentage of viable cells in presence of potassium tellurite suggesting an antagonistic metabolic reaction between Bacto peptone into the media and potassium tellurite. These metabolic reactions may reduce potassium tellurite to its not-toxic Te^0 status encouraging growth of viable cells.

To analyze the tellurium uptake by yeast cells, Sc57 cultures growing in YED containing potassium tellurite were observed microscopically. Transmission electron micrographs (Fig. 1) clearly showed that Te^0 tended to form large deposits along the cell wall (Fig. 1a, asterisks). In addition, small electron-dense precipitates, somewhat round in shape and with

Table 2 Relative CFU on potassium tellurite for *S. cerevisiae* strains Sc57 (rho$^+$) and Sc57-R3 (rho^0) grown in different media

Medium[a]	% Sc57 and Sc57-R3 survivors to different potassium tellurite concentrations (mean ± SD)[b]			
	600 μM		100 μM	
	Sc57	Sc57-R3	Sc57	Sc57-R3
YEPD	30 ± 4	23 ± 4	81 ± 8	60 ± 7
YED	20 ± 4	18 ± 2	53 ± 7	46 ± 5
YNB + aa	17 ± 2	12 ± 2	40 ± 5	29 ± 4
YNB + aa + 0.06% YE	18 ± 3	16 ± 2	47 ± 5	34 ± 4
YNB + aa + 0.06% P	28 ± 4	20 ± 3	68 ± 7	51 ± 6
YNB + aa + 0.06% YE + 0.06% P	27 ± 3	20 ± 3	68 ± 8	50 ± 6

[a] *aa* Amino acid supplement, *YE* yeast extract, *P* bacto peptone

[b] Colony forming units (CFU) from each culture sample were counted and percentages of survivors were calculated with respect to control cultures without potassium tellurite added. Values are means ± SD from at least three culture samples

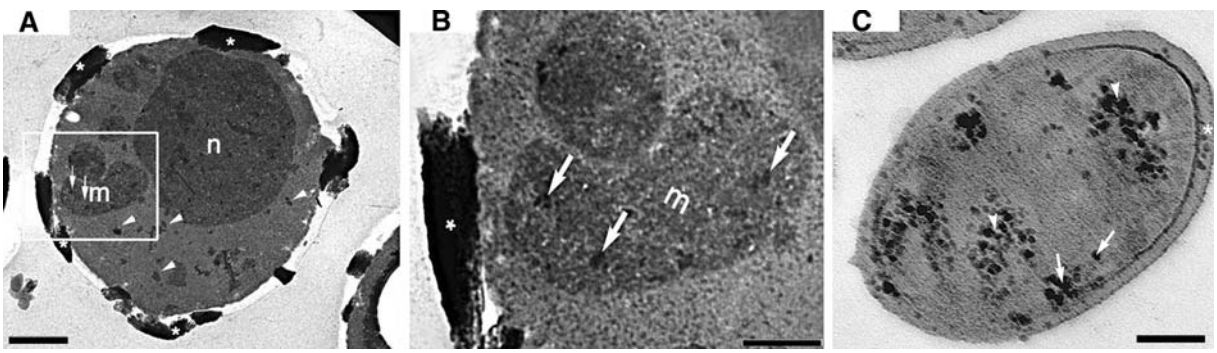

Fig. 1 Transmission electron micrograph sections of *S. cerevisiae* Sc57 yeast cells grown in the presence of potassium tellurite. **a** Elemental tellurium (Te^0) deposits along the cell wall (*asterisks*) and small precipitates in the cytoplasm (*arrowheads*) and mitochondria (*arrows*). **b** Detail of the image in **a** showing Te^0 grains in mitochondria (*arrows*). Mitochondrial ultrastructure appears not completely preserved due to the resistance of the yeast cell wall to the fixative penetration. **c** Te^0 grains (*arrows*) and large deposits (*arrowheads*) in the cytoplasm of dead or severely damaged yeast cells with cell wall deficiency and degeneration of intracellular organelles. Te^0 grains are also visible close to the external side of plasmatic membrane (*asterisk*). *Bars* indicate 1 μm in **a**, 0.3 μm in **b** and 0.8 μm in **c**

a tendency to cluster into bigger spots, were observed in both the cytoplasm (Fig. 1a, b, arrowheads) and mitochondria (Fig. 1a, b, arrows). In these organelles, Te^0 grains were presumably associated either to the internal membrane of mitochondrial crests or to the interspace between the internal and external mitochondrial membranes supporting the hypothesis that mitochondria were involved in tellurite toxicity. The effects of tellurite toxicity were demonstrated by massive accumulation of Te^0 grains in the cytoplasm of dead or severely damaged yeast cells (about 50% in the examined sample) with cell wall deficiency and degeneration of intracellular organelles (Fig. 1c).

Discussion

The nature of tellurite toxicity and tellurite reduction to Te^0 has to be determined in yeast. The results of MIC experiments with respiratory-competent strain Sc57 are consistent with previous data on the influence of the complex media ingredients with special reference to the nature of the carbon source on the potassium tellurite resistance during bacterial growth (Borghese et al. 2004, 2008). The strong growth inhibition of Sc57 (rho$^+$) in glycerol medium with low concentrations of potassium tellurite, and very limited inhibition of both Sc57 and Sc57-R3 (rho^0) in glucose

medium containing up to 1.2 mM of potassium (Table 1) clearly suggest that tellurite has an effect on mitochondrial functions. This hypothesis was supported by the evidence that growth of rho^+ yeast cells in the presence of increasing amount of tellurite resulted in dose-dependent blackening of the culture, a phenomenon that was not observed with rho^0 cultures.

Indeed, the direct involvement of the respiratory electron transport chain, particularly the cytochrome c oxidase (COX), in tellurite reduction has been reported in Gram-negative bacteria (Trutko et al. 2000), although this interpretation has been recently questioned (Zannoni et al. 2008). Utilization of tellurite as a terminal electron acceptor has been documented in the strict anaerobes *Bacillus selenitireducens* and *Sulfurospirillum barnesii* (Baesman et al. 2007) and in hydrothermal vent isolates (Csotonyi et al. 2006). It is reasonable to speculate that the respiratory electron transport chain is involved in potassium tellurite reduction to elemental tellurium (Te^0) and that in *S. cerevisiae* tellurite itself or, more likely, Te^0 deposits may be, at least in part, responsible for toxicity. Indeed, tellurite toxicity was greatly attenuated in media containing high levels of fermentable carbon sources, which are expected to down-regulate strongly the respiratory activity (the so called "Crabtree effect").

In addition to the nature of the carbon source, another factor affecting the level of resistance to potassium tellurite was the presence of Bacto peptone in the media. The addition of Bacto peptone in the media increased the percentage of viable Sc57 and Sc57-R3 cells in presence of potassium tellurite (Table 2). This finding was taken as an indication that this component of the rich medium may exert a protective, anti-oxidative action on the cells in the presence of potassium tellurite, which is believed to have strong oxidant properties.

In the present study it was not possible to determine the specific site(s) of tellurite reduction in the yeast cell. In a previous study with vacuole-lacking and -defective mutants of *S. cerevisiae* it was reported that accumulation of Te^0 occurs mainly in the cytosolic compartment of the cell, although no electron microscopic evidence was provided to support this conclusion. Furthermore, it was suggested that tellurite detoxification is influenced by the presence of a functionally active vacuole, which may play a role in compartmentation as well as regulation of the cytosolic compartment for optimal expression of a detoxification mechanism, e.g., reduction (Gharieb and Gadd 1998). In this study, by using TEM we demonstrate that in rho^+ yeast cells Te^0 form large deposits along the cell wall and small electrondense precipitates in both the cytoplasm and mitochondria (Fig. 1). The exact location of Te^0 grains in these organelles (i.e., the internal membrane of mitochondrial crests or the interspace between the internal and external mitochondrial membranes) remains to be established. Nevertheless, the TEM data support the above-mentioned hypothesis that mitochondria may be involved in both tellurite reduction to Te^0 and, very likely, tellurite toxicity in *S. cerevisiae*.

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