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ORIGINAL PAPER

Enrichment and isolation of *Bacillus beveridgei* sp. nov., a facultative anaerobic haloalkaliphile from Mono Lake, California, that respires oxyanions of tellurium, selenium, and arsenic

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Abstract Mono Lake sediment slurries incubated with lactate and tellurite [Te(IV)] turned progressively black with time because of the precipitation of elemental tellurium [Te(0)]. An enrichment culture was established from these slurries that demonstrated Te(IV)-dependent growth. The enrichment was purified by picking isolated black colonies from lactate/Te(IV) agar plates, followed by repeated streaking and picking. The isolate, strain MLTeJB, grew in aqueous Te(IV)-medium if provided with a small amount of sterile solid phase material (e.g., agar plug; glass beads). Strain MLTeJB grew at high concentrations of Te(IV) (~8 mM) by oxidizing lactate to acetate plus formate, while reducing Te(IV) to Te(0). Other electron acceptors that were found to sustain growth were tellurate, selenate, selenite, arsenate, nitrate, nitrite, fumarate and oxygen. Notably, growth on arsenate, nitrate, nitrite and fumarate did not result in the accumulation of formate, implying that in these cases lactate was oxidized to acetate plus CO_2. Strain MLTeJB is a low G + C Gram positive motile rod with pH, sodium, and temperature growth optima at 8.5–9.0, 0.5–1.5 M, and 40°C, respectively. The epithet *Bacillus beveridgei* strain MLTeJB^T is proposed.

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Introduction

The Group 16 element tellurium is a metalloid that has trace abundance in the Earth's crust (~0.002 ppm). Oxyanions of this element, namely, tellurite [Te(IV)] and tellurate [Te(VI)], are toxic substances and therefore most microbiological studies of Te have focused upon biochemical resistance mechanisms. One such mechanism achieves resistance by reductive precipitation of the soluble oxyanions Te(IV) and Te(VI) to a non-toxic elemental form [Te(0)] (e.g., Averèzi et al. 1997; Moscoso et al. 1998; O'Gara et al. 1997; Ollivier et al. 2008), a strategy that microbes also employ for oxyanions of selenium, a neighboring Group 16 element (Oremland et al. 1994). It was recently discovered that Te-oxyanions can also serve as respiratory electron acceptors to sustain anaerobic growth of certain bacteria (Csotonyi et al. 2006). Both the freshwater ε-Proteobacterium *Sulfurospirillum barnesii* and the haloalkaliphilic Gram positive *Bacillus selenitireducens* carry out the dissimilatory reduction of Te(VI) and Te(IV), respectively, to Te(0) while concurrently showing Te-dependent growth (Baesman et al. 2007), a phenomenon recognized previously for selenium (Stolz and Oremland 1999). When grown with Se-oxyanions as terminal electron acceptors, these microbes produced uniformly sized nanospheres of Se(0) as the endproduct (Oremland et al. 2004). In contrast, the externally accumulated nano-sized particles of Te(0) formed by these two bacteria were drastically

different in shape and appearance. While *S. barnesii* formed small, irregularly shaped nano-granules that clumped together into larger aggregates, *B. selenitireducens* initially formed nano-rods that coalesced into larger shards, which eventually formed rosette-shaped aggregates (Baesman et al. 2007).

Currently, there is research interest in structured nano-scaled minerals composed of Group 15 and 16 elements (e.g., Stefani et al. 2009). These substances have potential photonic applications in the realm of nanotechnology either as "quantum dot" arrays or as "nano-tubes." One advantage of employing biosynthetic routes of nano-mineral formation is that they can circumvent chemical syntheses that usually employ harsh reagents, high temperatures, and/or exposure to high vacuum treatment. Certain materials derived from Te, Se, and in some cases As can be opto-electrically active, and can convert incident light energy into electricity and vice versa. They also can form nano-sized compounds with various metals as well as non-metallic elements (e.g., CdSe; As_2S_3). Some such compounds can be directly generated by the metabolism of anaerobic bacteria like *Veillonella atypica* (Pearce et al. 2008) and *Shewanella* sp. (Lee et al. 2007).

A drawback to working with either *S. barnesii* or *B. selenitireducens* to generate Te(0) particles is that these microbes proved quite sensitive to the concentration of Te-oxyanions added to their growth medium. Tellurium oxyanion concentrations $\geq$1 mM completely inhibited growth of both organisms, which necessitated a continuous pulsing of cultures with $\sim$0.6 mM Te-oxyanion additions over month-long incubation periods in order to achieve adequate Te(0) precipitation and cell densities (Baesman et al. 2007). Clearly these efforts would be facilitated by isolation of a novel anaerobe capable of carrying out dissimilatory Te-reduction at much higher oxyanion concentrations ($\sim$5–10 mM). It is noteworthy that both *S. barnesii* and *B. selenitireducens* were originally isolated from sediments using millimolar levels of selenate and selenite, respectively, as the selective factor (Oremland et al. 1994; Switzer Blum et al. 1998). To date, other than a report of a Te(VI)-respiring bacterium isolated from marine hydrothermal vents (Csotonyi et al. 2006), there are no detailed descriptions of isolations of Te-respiring bacteria using Te-oxyanions as selective factors in the primary enrichment and isolation process.

We chose to employ the sediments of Mono Lake, California as a source for our enrichment because of the considerable past success in isolating a variety of anaerobes that respire oxyanions of selenium and arsenic from this mineral-rich, highly alkaline (pH = 9.8) and hypersaline (salinity = $\sim$75–90 g/L) ecosystem (Oremland et al. 2004). When coupled with the fact that Te(IV) is quite soluble in an alkaline/carbonate matrix as opposed to distilled water, it made this "extreme" environment particularly attractive as a cultivar source from which to obtain novel microbes. We now report the isolation and physiological description of strain MLTeJB from this system and propose the name *Bacillus beveridgei*^{T} to honor the memory and untimely death of our late colleague, Professor T. J. Beveridge of the University of Guelph.

Materials and methods

Sediment slurry incubations

Sediment (100 mL) collected from the shallow littoral region of the northeastern quadrant of Mono Lake were added to 800 mL of sterile, basal salts medium (Switzer Blum et al. 1998; modified as given below) and hand-mixed in a beaker. The resulting dilute slurry was dispensed into serum bottles (70 mL bottle volume; 50 mL slurry volume), plugged with butyl rubber stoppers, crimp-sealed and the headspace flushed with O_2-free N_2. Sodium tellurite (10 mM) was added to all bottles as an electron acceptor, while the electron donor provided was either sodium lactate (30 mM) or molecular hydrogen, the latter achieved by flushing the headspace with a flow of O_2-free H_2. Controls consisted of live slurries incubated without added electron donor, or killed controls with lactate or H_2 that were twice autoclaved (250 kPA, 121°C, 60 min.). The slurries were incubated in the dark at 28°C with constant rotary shaking (150 rpm). Subsamples were periodically withdrawn by syringe (0.5 mL) for analyses of Te(IV) concentrations. The basal salts medium ("AML60") was composed of (g/L): NaCl (60), Na_2CO_3 (10.6), $NaHCO_3$ (4.2), KH_2PO_4 (0.08), K_2HPO_4 (0.15), $(NH_4)_2SO_4$ (0.1), $MgSO_4{\cdot}7H_2O$ (0.025), Na_2WO_4 (8.8 μg/L), and Widdel et al. (1983) trace elements solution (5 mL). The final pH of the medium was adjusted to 9.8.

Enrichment cultures

An enrichment culture was established with an inoculum (5 mL) from the above live sediments slurries using the AML60 medium supplemented with yeast extract (0.2 g/L), and with lactate (20 mM) as the electron donor. The enrichment was taken through 3 successful growth transfers as indicated by the removal of Te(IV), the darkening of the medium [due to formation of Te(0) particles] and the oxidation of lactate to acetate. The enrichment was streaked onto solidified agar petri-plates which contained the above medium (with 2% agar), but was further supplemented with cysteine–HCl (0.125 g/L) to serve as a reducing agent, and incubated at 35°C. All manipulations and incubations were achieved in an anaerobic glove box. Individual black colonies formed after several days and were re-streaked several

times on fresh agar to obtain purity. Picked isolated colonies, however, were unable to grow in liquid media unless an exogenous, sterilized solid material was included to provide a cell nucleation area. Solid materials that supported growth in aqueous media included (in decreasing order of cell yields) small quantities of sterilized agar, sponge, glass beads, and Phytagel. The isolated culture was subjected to a battery of substrate-affinity and physiological tests (e.g., salinity, temperature, pH) in order to define its phenotype. The pH range and optima for growth were determined by culturing in media with different proportions of three primary buffers (i.e., HEPES, HCO_3^- and CO_3^{2-}) in order to achieve values ranging between 6.5 and 12.0. The final pH of the media was checked after growth in each tube and ranged between 7.0 and 11.5. Respiratory nitrogen metabolism was checked for the presence of either the full denitrification or dissimilatory reduction to ammonia pathways. Nitrate-growing cultures were assayed for N_2O production using the acetylene-block technique of N_2O reductase (Oremland et al. 1984), while subsequently performing ammonia determinations after the end of growth.

Analytical

Total dissolved concentrations of Te and Se in Mono Lake surface water were determined by continuous-flow hydride-generation atomic absorption spectrophotometry (HG-AAS) using a Perkin-Elmer 5000 spectrophotometer equipped with a Varian VGA-76 hydride system. The HG-AAS method requires that Te and Se be present in the +4 oxidation state for accurate measurement. Prior to HG-AAS analysis, filtered (0.2 μm) water samples were subjected to an oxidative nitric/perchloric acid digestion to convert all dissolved Te and Se species to Te(VI) or Se(VI), followed by reduction of these forms to Te(IV) or Se(IV) using concentrated hydrochloric acid as described for Se in Kulp and Pratt (2004). Replicate samples that were spiked with known concentrations of these analytes (added as either the +4 or +6 forms) were used to verify the efficiency of the oxidative and reductive sample pretreatments (average spike recovery was 93%).

Ammonia concentrations were determined by spectrophotometry (Solorzano 1969). Organic acids, Te(VI), and Se(IV) in cultivation experiments were determined by high-performance liquid chromatography, Te(IV) by ion chromatography, and solid phase Te(0) and Se(0) by acidic dissolution and subsequent analysis of the soluble portion by ICP-MS as was described previously (Baesman et al. 2007). Telluride [Te(−II)] and selenide [Se(−II)] were determined by first removing the solid phase by filtration (0.2 μm) followed by precipitation of the aqueous Te(−II) or Se(−II) as cuprous solids by addition of an equal volume of 50 mM $CuCl_2$ solution. After centrifugation and decantation, the solid CuSe or CuTe particles were dissolved in concentrated nitric acid, followed by dilution to 2% HNO_3 and analyzed by ICP-MS (Herbel et al. 2003). Direct counts of living cells were achieved with acridine orange (Hobbie et al. 1977). Methods for obtaining scanning electron microscopic (SEM) images of cells and extracellular Te(0) precipitates have been previously outlined (Oremland et al. 2004; Smith et al. 1985; Baesman et al. 2007). SEM images of Te(0) formed in sediment slurry experiments were obtained by filtering 30 μl of the slurry (0.1 μm) followed by air drying, mounting, Pd/Au-sputter coating and analyzed as outlined previously (Baesman et al. 2007). Thermodynamic calculations were made using the tabulated values of Thauer et al. (1977) and Woods and Garrels (1987). Negative staining and thin sections were prepared following the methods described in Stolz (1990), and observed on a JEOL 100CX transmission electron microscope at 60 kV. Images were captured using a SIA-7C digital camera.

Phylogenetic analysis

The 16S rRNA gene was cloned and sequenced by the methods described in Hoeft et al. (2004) using the 8F and 1492R primers. An amplicon of about 1,500 bp was obtained and cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad CA). The insert was sequenced in both directions using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City CA) using the M13 forward and reverse primers on an ABI 3100 automated DNA sequencer. A sequence of 1,531 unambiguous nucleotides was obtained and assigned the GenBank accession number FJ825145. The sequences of the closest relatives based on a BLAST search were obtained from GenBank. Sequence alignments (1,500 bp) were done using ClustalX (Jeanmougin et al. 1998) and phylogenetic trees (both maximum parsimony and distance matrix) were constructed using PAUP* 4.0b under the default parameters of the program (Swofford 1998). The % G + C analyses were performed at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ).

Results

Lake water Te and Se concentration

Mono Lake surface water collected in Spring 2009 contained 13 nM dissolved Te and 38 nM dissolved Se. We also analyzed an archived sample of Mono Lake surface water that was collected in Summer 1996 as a point of comparison. The archived water sample yielded similar

results (Te concentration, 13 nM; Se concentration, 28 nM).

Sediment slurry incubations

Dilute sediment slurries demonstrated complete removal of 10 mM Te(IV) when incubated with either lactate or H_2 additions, while no such consumption occurred in either the killed controls or in live samples that lacked any added electron donor (Fig. 1). Live slurries with added electron donor turned progressively black with time because of the accumulation of Te(0) precipitates. SEM images from these samples exhibited two primary morphologies of Te(0) nanoparticles, namely, Te-rosettes and Te-granules previously noted after Te-dependent respiratory growth of *B. selenitireducens* and *S. barnesii*, respectively (Baesman et al. 2007). However, both types of Te-morphologies were noted in the SEM examined solid phases of the slurries, regardless of whether they were incubated with lactate (Fig. 2) or hydrogen (not shown).

Growth of strain MLTeJB

Strain MLTeJB demonstrated Te(IV)-dependent growth (Fig. 3). A complete removal of 6 mM Te(IV) occurred concurrent with the oxidation of lactate to acetate and formate, and a tenfold increase in cell abundance (Fig. 3a, b). The sequential removal of Te(IV) was completely balanced by the accumulation of Te(0) (Fig. 3a). Notably, formate accumulated as an end product in quantities equivalent to that of acetate (Fig. 3b). Controls incubated without cells did not demonstrate Te(IV) removal, while live controls incubated without Te(IV) did not demonstrate significant growth or lactate oxidation (Fig. 3c). Strain MLTeJB was also capable of growth on Se(IV) (Fig. 4). A complete reduction of Se(IV) to a mixture of Se(0) plus Se(−II) occurred over 25 days incubation (Fig. 4a), while lactate was concurrently oxidized to acetate plus formate (Fig. 4b). Abiotic controls did not demonstrate Se(IV) reduction, nor did lactate oxidation occur without Se(IV) (Fig. 4c).

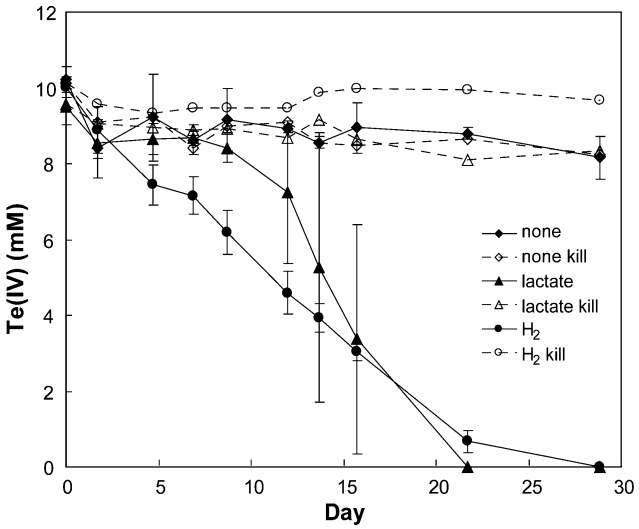

Fig. 1 Consumption of Te(IV) by Mono Lake sediments slurries. Closed symbols each represent the mean of 3 experimental slurries and bars indicate ±1SD. *Open symbols* are killed controls that represent single slurries for each condition

Other electron acceptors that supported lactate-fueled growth of strain MLTeJB included Te(VI), Se(VI), As(V), fumarate, oxygen, nitrate, and nitrite. Respiratory growth on nitrate proceeded via dissimilatory reduction to ammonia because: (1) there was no accumulation of N_2O in the headspace of culture tubes incubated with 10% acetylene to those lacking this inhibitor (data not shown), and (2) live cultures accumulated ammonia in the medium after growth (5.1 mM) compared to uninoculated controls (2.1 mM). The accumulation of formate, however, was noted only for growth with Te(VI), Se(VI), and oxygen (Table 1). We also observed that in the tested cases of Se(IV), nitrate, and fumarate, a small sterile solid agar plug was not needed in the culture medium to initiate growth. Electron donors that supported growth with nitrate included pyruvate, fructose, galactose, glucose, starch, and yeast extract (Table 2). Growth on glucose, fructose, starch or yeast extract, however, did not require the presence of nitrate suggesting a fermentative capacity for strain MLTeJB.

Salinity, temperature, and pH

Strain MLTeJB exhibited a growth optimum for sodium between 0.5 and 1.5 M. Although a steady decline was noted at higher sodium concentrations, significant growth was also recorded at much higher salinities (~4 M) (Fig. 5a). A temperature optimum was observed at 37°C, and growth declined dramatically at temperatures above 45°C (Fig. 5b). Strain MLTeJB exhibited a preference for alkaline conditions, exhibiting growth between pH values of 7 and 10, with an optimum at 8.5 (Fig. 5c).

A negatively stained TEM image of fumarate-grown strain MLTeJB clearly displays a long flagellum, with a subpolar attachment (Fig. 6a). Thin sections reveal that the fumarate-grown cells are rod shaped, have a typical Gram positive cell wall, and divide by binary fission (Fig. 6b). Thin sections of Te(IV)-grown cells are surrounded by dense accumulations of dark precipitates, presumably Te(0) (Fig. 6c). The morphology of the Te(0)-precipitates is not apparent in the TEMs; however, SEM images clearly show they are externally accumulated rosettes (Fig. 6d). Strain MLTeJB also produced nano-spheres composed of elemental selenium when grown on Se(IV) (images not shown) that were comparable in size and appearance to those formed by other anaerobes described previously (Oremland et al. 2004).

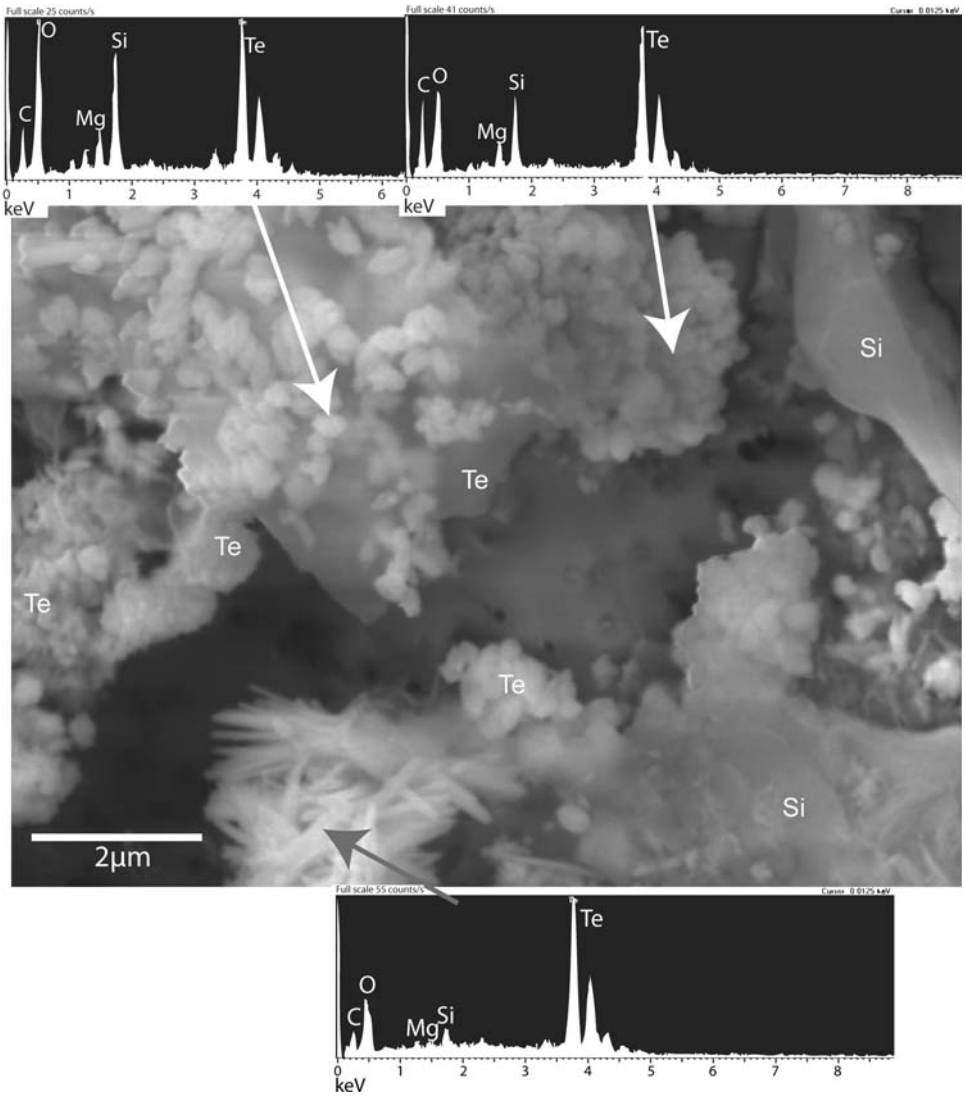

Fig. 2 Scanning electron micrographs of Te(0) nanominerals found precipitated after incubation of Te(IV) amended sediment slurries with lactate. *Arrows* show Te-nanogranules (*top*) and aggregates of Te(0) nanorods (*bottom*) with their dispersive X-ray spectra

Taxonomic 16S rDNA sequence alignment

Phylogenetic analysis revealed that strain MLTeJB was a low % mole GC Gram positive related to halophilic and haloalkaliphilic species (Fig. 7). It was most closely related (97% identity) to the Mono Lake isolate *B. selenitireducens* (Switzer Blum et al. 1998) and clone, ML-C1, from an enrichment culture capable of sulfide-linked As(V)-reduction (Hollibaugh et al. 2006). Of the other Mono Lake bacilli, the As(III)-oxidizing selenate reducer from Mono Lake, strain ML-SRAO (Fisher and Hollibaugh 2008) and *B. arseniciselenatis* (Switzer Blum et al. 1998) had only 94% identity and 89.5% identity, respectively.

Discussion

Mono Lake contains an abundance of dissolved inorganic arsenic oxyanions within its waters (~200 µM; Oremland et al. 2004). In comparison, the concentrations of dissolved selenium and tellurium are about 4 orders-of-magnitude lower. Nonetheless, previous efforts have readily resulted in the successful cultivation of selenium-respiring bacteria from this unusual environment (Switzer Blum et al. 1998; Fisher and Hollibaugh 2008) implying that a dearth of selenium (or tellurium; Baesman et al. 2007) does not preclude the presence of microbes capable of exploiting oxyanions of these elements to serve as respiratory electron acceptors.

In contrast to the body of earlier work carried out with Te-resistant microbes, very little is known about prokaryotes that have the capacity for energy conservation via dissimilatory reduction of tellurium oxyanions. Although recent work demonstrated the concept that Te(IV) and Te(VI) could serve as electron acceptors for bacterial respiratory growth (Baesman et al. 2007; Csotonyi et al. 2006), these efforts were hampered by the direct toxicity of these compounds when they were included in the medium at

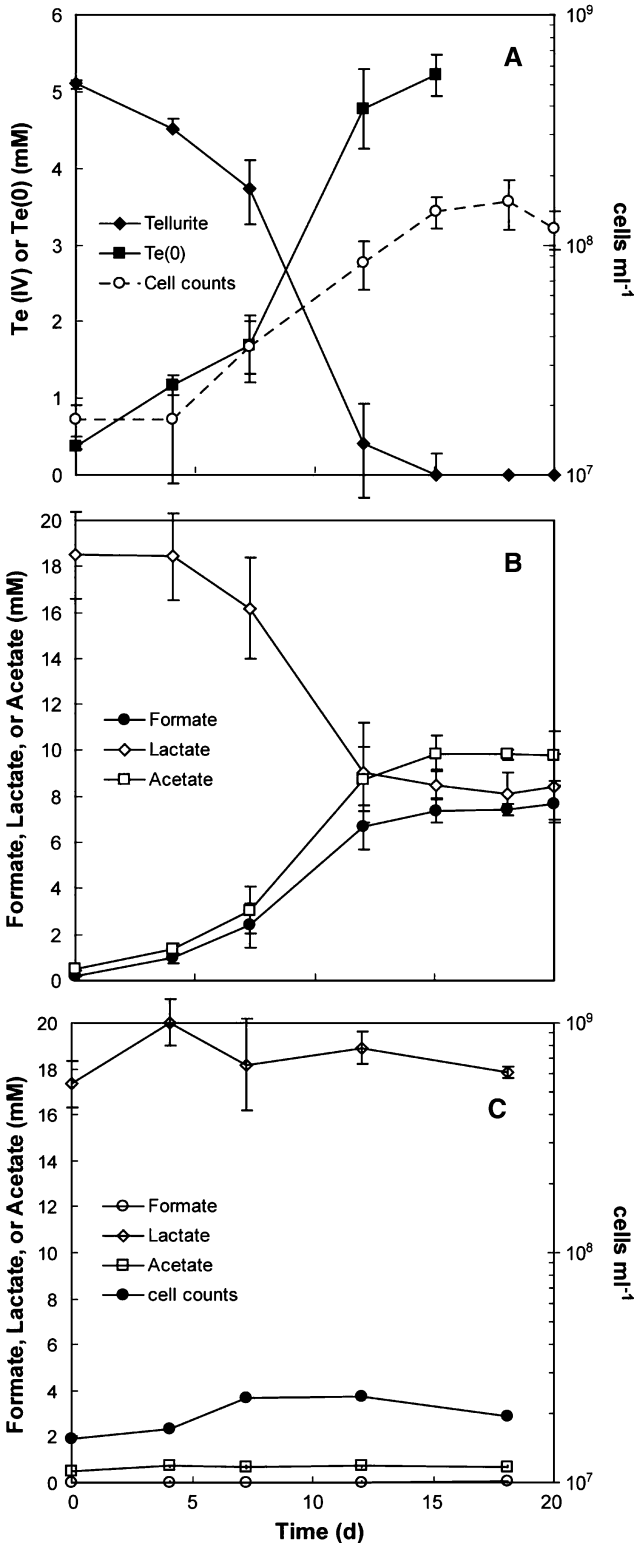

Fig. 3 Growth of strain MLTeJB^T on Te(IV). **a** Te(IV), Te(0), and cells; **b** lactate, acetate, and formate. **c** Controls incubated without Te(IV). *Symbols* represent growth of 3 cultures and bars indicate ±1SD

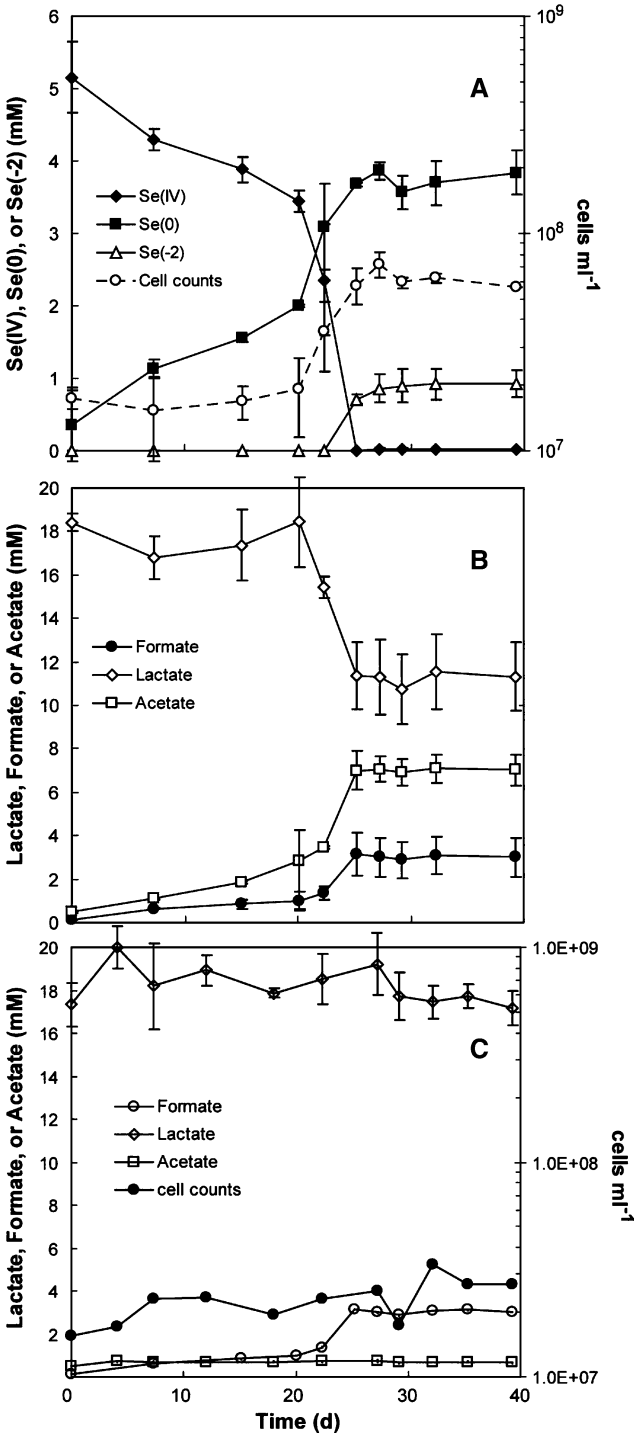

Fig. 4 Growth of strain MLTeJB on Se(IV). **a** Se(IV), Se(0), Se(−II), and cells; **b** lactate, acetate, and formate. **c** Controls incubated without Se(IV). *Symbols* represent growth of 3 cultures and *bars* indicate ±1SD

millimolar concentrations. Indeed, the solubility of these oxyanions also poses a challenge towards isolation, as Te(IV) has limited solubility at acidic to neutral pH values,

Table 1 Growth of MLTeJB on various electron donors and electron acceptors

Electron acceptor[a]	Growth	Electron donor[b]	Growth
Chromate	–	Lactate	+
Tellurite	+	Acetate	–
Tellurate	+	H_2 (1 atm)	–
Selenite	+	H_2 + acetate	–
Selenate	+	Pyruvate	+
Arsenite	–	Galactose	+
Arsenate	+	Glucose	+
Sulfate	–	Glucose w/o NO_3^-	+
Sulfite	–	Succinate	–
Thiosulfate	–	Malate	–
Nitrate	+	Formate	–
Nitrite	+	Propionate	–
Fumarate	+	Citrate	–
Air	+	Glutamate	–
10% O_2	+	Aspartate	–
5% O_2	+	Glycine	–
1% O_2	–	Serine	–
None	–	Ethanol	–
Vanadate	–	Methanol	–
Fe(III)	–	Yeast extract (2 g/L)	+
TMAO[c]	–	Yeast extract w/o NO_3^-	+
		Starch w/o NO_3^-	+
		Benzoate	–
		Sulfide	–

[a] Lactate (10 mM) as the electron donor; all electron acceptors were 5 mM except tellurite (7 mM)

[b] Nitrate (5 mM) as the electron acceptor unless indicated otherwise; all e-donors were 1 mM except benzoate, methanol and ethanol (2 mM)

[c] Trimethylamine oxide

while solubility of Te(VI) is partially constrained at elevated pH. For these reasons we opted to use samples from Mono Lake because its high pH (~9.8) favored the solubility of Te(IV).

The results obtained with the Mono Lake sediment slurries suggest that microbes having the capacity for Te(IV) reduction are relatively easy to culture from this particular environment. Slurries co-incubated with either lactate or H_2 readily demonstrated Te(IV) reduction to Te(0), while those lacking amendment with an exogenous electron donor did not, indicating the diluted nature of the matrix removed endogenous electron donors (Fig. 1). The morphology of the Te(0) precipitates recovered from the sediments were very similar to the "nano-rosettes" and "nano-granules" distinct morphologies formed by *B. selenitireducens* and by *S. barnesii*, respectively, after growth on tellurium oxyanions (Baesman et al. 2007). When taken with the positive results obtained with two electron donors (i.e., lactate and H_2), it suggests that a diversity of Te-respirers may inhabit these sediments. Indeed, we achieved some limited preliminary success in hunting for a H_2-oxidizing Te(IV) reducer as we obtained isolated black colonies on solid AML60 media incubated under a H_2 atmosphere in bottle plates (Hermann et al. 1986). Thus, H_2-oxidizing chemo-autotrophic Te-respiring prokaryotes, analogous to what has been documented for selenate and arsenate respirers (Huber et al. 2000; Liu et al. 2004) may exist in this environment as well.

Culturing strain MLTeJB into aqueous Te(IV)-containing medium after picking colonies from plates impeded progress but this obstacle was empirically resolved by the inclusion of small quantities of sterile, solid surfaces in the medium. The most efficient of these was a small agar plug. However, strain MLTeJB did not require this surface when it was subsequently cultured with Se(IV), fumarate or

Table 2 Comparisons of characteristics of strain MLTeJB with two other low G + C bacilli isolated from Mono Lake, including its closest relative *B. selenitireducens* strain MLS10 and *B. arseniciselenatis* strain E1H

Characteristic	MLTeJB	MLS10	E1H
Morphology	Rods	Rods	Rods
Spores	–	–	+
G + C (moles %)	46.8	49	40
Salinity range	14–261 g/L	20–240 g/L	20–130 g/L
Salinity Optimum	87 g/L	20–60 g/L	60 g/L
pH range	7.0–11.5	7.3–10.5	7.0–10.3
pH Optimum	8.5	9.8	8.5
Mobility	+	–	–
E-acceptors	Te(VI), Te(IV), Se(VI), Se(IV), As(V), Fumarate, O_2, NO_3^-, NO_2^-	Te(IV), Se(IV), As(V), Fumarate, $S_2O_3^{2-}$, S(0), Se(0), O_2, TMAO, NO_3^-, NO_2^-	Se(VI), Te(VI), As(V), Fumarate, NO_3^-, Fe(III)

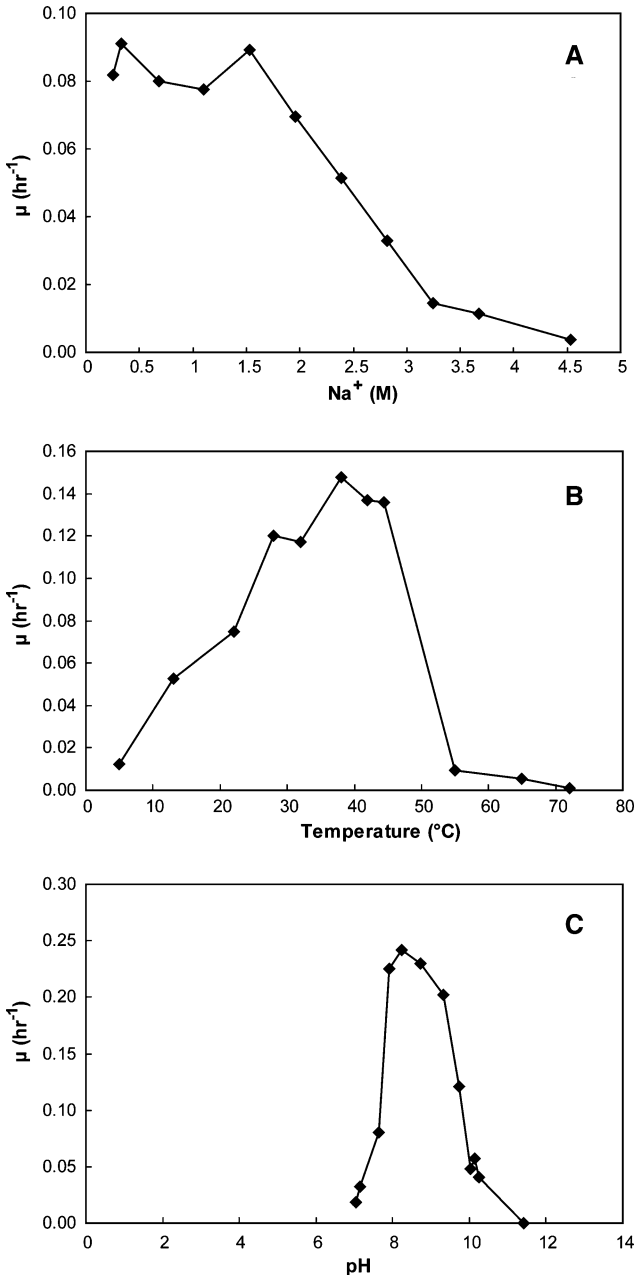

Fig. 5 Specific growth rates of strain MLTeJB over ranges of **a** salinity, **b** temperature, and **c** pH. Cells were grown with lactate (e-donor) and nitrate (e-acceptor)

nitrate as the electron acceptor, but did once again when these cell lines were reintroduced into Te(IV) medium. We conclude that the increased surface area of the agar plug somehow shielded the small quantity of inoculum cells from the initial toxic effects of high concentrations of Te(IV). Application of such a simple technique in the future could aid in the isolation of more Te-respiring microorganisms from other environments.

Growth of strain MLTeJB was evident on Te(IV) and the sole reduction product was Te(0) (Fig. 3a). This occurred despite the fact that there was an excess of lactate in the medium purposefully added to determine if a further reduction to Te(−II) was possible. Clearly this did not occur, a result which contrasts markedly with growth on Se(IV) where reduction proceeded through Se(0) all the way to Se(−II) (Fig. 4a). Hydrogen selenide as an endproduct was noted with *B. selenitireducens* when cells were incubated with an excess supply of lactate and this proceeded by a direct reduction of the accumulated extracellular Se(0) (Herbel et al. 2003). It is not clear why reduction of Se(0) to Se(−II) occurs in these organisms, but not of Te(IV) to Te(−II). It is possible that the morphology of the Se(0) nano-spheres allows for attachment of surface proteins (Pearce et al. 2009) that can act as conduits for electron flow from the cell membrane while these are absent or cannot function in the case of the Te(0) nano-rods.

Although strain MLTeJB is phylogenetically closer to *B. selenitireducens* (Fig. 7) and more distant to *B. arseniciselenatis*, all three Mono Lake bacilli have significant phenotypic commonalities and differences (Table 2). Strain MLTeJB is motile and displays a peritrichous flagella (Fig. 6a) while motility was observed neither in *B. selenitireducens* nor *B. arseniciselenatis* (Switzer Blum et al. 1998). Nonetheless, the annotated genome of *B. selenitireducens* (http://genome.ornl.gov/microbial/bsel/) has a large gene cluster that contains genes encoding flagellum and chemotaxis proteins implying at least the possibility of a latent potential in this regard. Strain MLTeJB can grow using the either +4 and +6 of tellurium and selenium as electron acceptors (Table 1), whereas *B. selenitireducens* can only grow on the +4 oxidation states of these elements and *B arseniciselenatis* only on the +6 oxidation states (Switzer Blum et al. 1998). The standard electrochemical potentials of the Te(VI)/Te(IV) and Te(IV)/Te(0) pairs are +477 mV and −413 mV, respectively. *B. selenitireducens* and *B. arseniciselenatis* oxidize lactate to acetate plus CO_2 when grown with either of their respective selenium or tellurium oxyanions (Switzer Blum et al. 1998; Baesman et al. 2007), whereas formate rather than CO_2 is an endproduct of lactate oxidation in strain MLTeJB. Nonetheless, the bioenergetics of this reaction is still favorable:

$$\text{Lactate}^{-1} + \text{TeO}_3^{2-} + 3\text{H}^+ \rightarrow \text{Acetate}^{-1} + \text{Te(0)} + \text{Formate}^{-1} + 2\text{H}_2\text{O} \quad (1)$$

$$\Delta G_r^{\circ} = -114\,\text{kJ mol lactate}^{-1} \text{or } -71.3\,\text{kJ mole electrons}^{-1}$$

We are at a loss for an explanation for why formate accumulated during growth on Te(IV), as well as on Se(IV) and oxygen but not on other electron acceptors tested that supported growth (Table 1). One possibility is that Te(IV) inhibited formate dehydrogenase. However, we observed

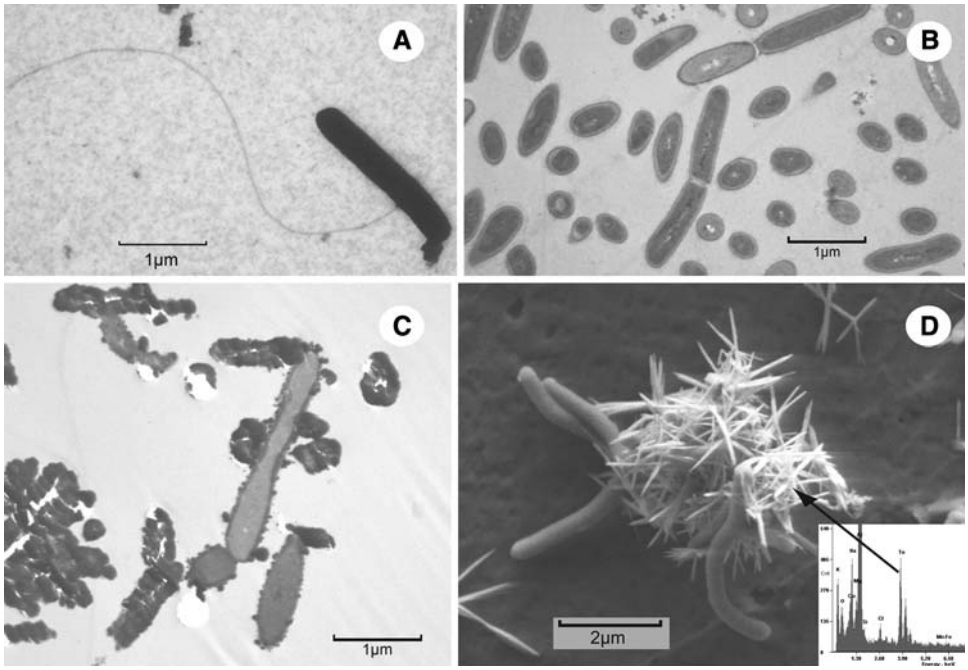

Fig. 6 Transmission electron micrographs of strain MLTeJB. **a** Negatively stained preparation of fumarate-grown cells showing the subpolar insertion of the flagellum. **b** Thin section of fumarate-grown cells revealing typical Gram positive cell wall and division by binary fission. **c** Thin section of Te(IV)-grown cells showing accumulations of elemental tellurium on the external cell wall. **d** SEM image of Te(IV)-grown cells showing extracellular accumulations of Te(0)-rosettes. *Insert* is an EDAX spectral image of a rosette composition (*arrow*)

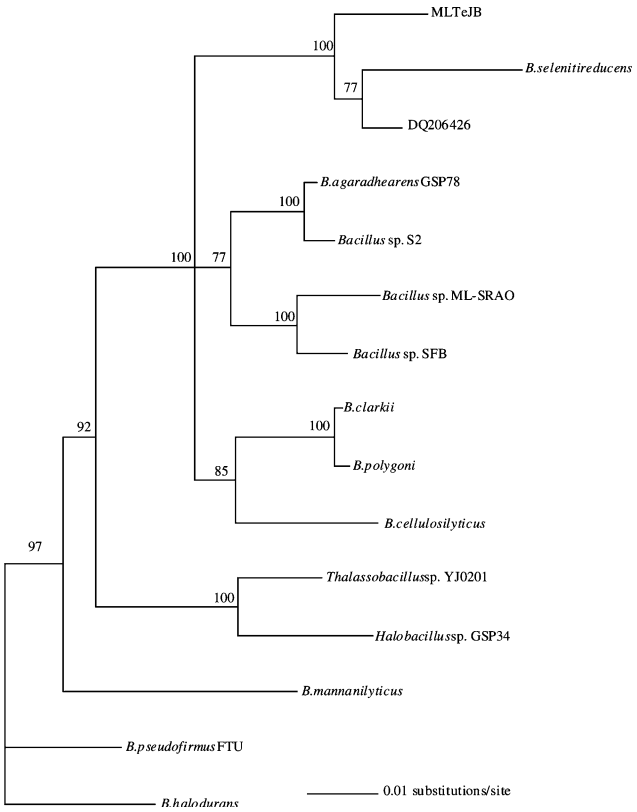

Fig. 7 Neighbor joining tree of the 16S rRNA gene showing the phylogenetic relatedness of strain MLTeJB

that Te(IV)-grown cell suspensions consumed added formate whether co-incubated with either Te(IV) or nitrate, thereby indicating the presence of a functioning formate dehydrogenase (data not shown).

The Te(0) produced by strain MLTeJB was in the form of "nano-rods" and their associated aggregations whether grown on Te(IV) or Te(VI) (Fig. 6) thereby making them very similar in appearance to those formed by *B. selenitireducens* (Baesman et al. 2007). The fact that we also observed Te(0) "nano-granules" in the initial sediment slurry experiment (Fig. 2) that were similar to those formed by *S. barnesii* (Baesman et al. 2007) also suggests a wider diversity of Te-respirers are present in this anoxic mud than is revealed by this simple microbiological investigation. Relatively little is known about the geochemistry of this element, although it is extracted as a byproduct for commercial purposes in association with the refining of copper sulfide ore deposits (Cooper 1972). Such ores are thought to arise from sulfide-rich magmatic processes, but potential commercial-level enrichment in marine ferro-manganese crusts has been noted as well (Hein et al. 2003). It is unknown if microorganisms are involved in either process, but the recovery of Te-metabolizing bacteria from marine hydrothermal vent sites suggests such a possibility (Ratheber et al. 2002; Csotonyi et al. 2006). There is a growing research interest in devising bench-top physical/chemical means of producing Te-nano materials because of the potential use of this element in the realm of nano-photonics (e.g., Ujjall and Rao 2004; Zhu et al. 2006a, b; Song et al. 2008; Yuan et al. 2008). Consideration of microbiological mechanisms for producing diverse Te-nano-sized minerals should also be evaluated with practical economy in mind as well as with an eye towards finding any unique properties for potential commercial exploitation.

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Appendix 1: Description of *Bacillus beveridgei*, sp. nov.

Bacillus beveridgei (bev.er.rig'.ei.N.L. gen.n. *beveridgei* of Beveridge, named in honor of the memory of Professor Terry J. Beveridge for his broad contributions and teaching in the realm of Geomicrobiology, with specific reference to his research on the formation of mineral phases by microorganisms). Cells are Gram positive, motile rods with a peritrichous flagellum (0.25 × 1.3–2.6 μm) and have a DNA G + C content of 46.8%. Colonies on agar are round, smooth, 4–28 mm in diameter and appear dark black when grown on Te-oxyanions due to precipitation of Te(0). Haloalkaliphilic, with growth optimal at pH 9.0 and 0.5–1.5 M sodium chloride. Meso-thermophilic with a temperature optimum at 40°C. Facultative anaerobe, able to grow in air and with 5% v/v headspace O_2, but cells are not microaerophilic. Anaerobic growth demonstrated with Te(VI), Te(IV), Se(VI), Se(IV), As(V), nitrate, nitrite, trimethylamine oxide, and fumarate as electron acceptors. Growth on nitrate is via dissimilatory reduction to ammonia. Electron donors include lactate, pyruvate, glucose and galactose, starch and complex substances like yeast extract. Growth on glucose, starch and yeast extract does not require the presence of an electron acceptor and can be fermentative. The type strain, MLTeJB^T (DSMZ = DSM 22320; ATCC = BAA-1786) was isolated from Mono Lake, an alkaline hypersaline soda lake in California, USA.

References

Averèzi C, Truner RJ, Pommier J, Weiner JH, Giordano C, Vermégio (1997) Tellurite reductase activity of nitrate reductase is responsible for basal resistance of *Escherichia coli* to tellurite. Microbiology 143:1181–1189

Baesman SN, Bullen TD, Dewald J, Zhang D, Curran S, Islam FS, Beveridge TJ, Oremland RS (2007) Formation of tellurium nanocrystals during anaerobic growth of bacteria that use Te oxyanions as respiratory electron acceptors. Appl Environ Microbiol 73:2135–2143

Cooper CW (1972) Tellurium: element and geochemistry. In: Fairbridge RW (ed) The encyclopedia of geochemistry and environmental sciences, van Nostrand Reinhold Co., New York, pp 1164–1165

Csotonyi JT, Stackebrandt E, Yurkov V (2006) Anaerobic respiration on tellurate and other metalloids in bacteria from hydrothermal vent fields in the eastern Pacific Ocean. Appl Environ Microbiol 72:4950–4956

Fisher JC, Hollibaugh JT (2008) Selenate-dependent anaerobic arsenite oxidation by a bacterium from Mono Lake, California. Appl Environ Microbiol 74:2588–2594

Hein JR, Koschinsky A, Halliday AN (2003) Global occurrence of tellurium-rich ferromanganese crusts and a model for the enrichment of tellurium. Geochim Cosmochim Acta 67:1117–1127

Herbel MJ, Switzer Blum J, Borglin S, Oremland RS (2003) Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se-oxyanions. Geomicrobiol J 20:587–602

Hermann M, Noll KM, Wolfe RS (1986) Improved agar bottle plate for isolation of methanogens or other anaerobes in a defined gas atmosphere. Appl Environ Microbiol 51:1124–1126

Hobbie JE, Daley RL, Jaspar S (1977) Use of nuclepore filters for counting bacteria for fluorescent microscopy. Appl Environ Microbiol 33:1225–1228

Hoeft SE, Kulp TR, Stolz JF, Hollibaugh JT, Oremland RS (2004) Dissimilatory arsenate reduction with sulfide as the electron donor: experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate-respirer. Appl Environ Microbiol 70:2741–2747

Hollibaugh JT, Budinoff C, Hollibaugh RA, Ransom B, Bano N (2006) Sulfide oxidation coupled to arsenate reduction by a diverse microbial community in a soda lake. Appl Environ Microbiol 72:2043–2049

Huber R, Sacher M, Vollmann A, Huber H, Rose D (2000) Respiration of arsenate and selenate by hyperthermophilic archaea. Sys Appl Microbiol 23:305–314

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23:403–405

Kulp TR, Pratt LM (2004) Speciation and weathering of selenium in Upper Cretaceous chalk and shale from South Dakota and Wyoming, USA. Geochim Cosmochim Acta 68:3678–3701

Lee J-H, Kim M-G, Yoo B, Myung NV, Maeng I, Lee T, Dohnalkova AC, Fredericksson JK, Sadowsky MJ, Hur H-G (2007) Biogenic formation of photoactive arsenic-sulfide nanotubes by *Shewanella* sp. strain HN-41. Proc Natl Acad Sci USA 104:20410–20415

Liu A, Garcia-Dominguez E, Rhine E, Young LY (2004) A novel arsenate respiring isolate that can utilize aromatic substrates. FEMS Microbiol Ecol 48:323–332

Moscoso H, Saavedra C, Loyola C, Pichuantes S, Vásquez C (1998) Biochemical characterization of tellurite-reducing activities of *Bacillus stearothermophilus* V. Res Microbiol 149:389–397

O'Gara JP, Gomelsky M, Kaplan S (1997) Identification and molecular genetic analysis of multiple loci contributing to high-level tellurite resistance in *Rhodobacter sphaeroides* 2.4.1. Appl Environ Microbiol 63:4713–4720

Ollivier PRL, Bahrou AS, Marcus S, Cox T, Church TM, Hanson TE (2008) Volatilization and precipitation of tellurium by aerobic, tellurite resistant marine microbes. Appl Environ Microbiol 74:7163–7173

Oremland RS, Umberger C, Culbertson CW, Smith RL (1984) Denitrification in San Francisco Bay intertidal sediments. Appl Environ Microbiol 47:1106–1112

Oremland RS, Switzer Blum J, Culbertson CW, Visscher PT, Miller LG, Dowdle P, Strohmaier FE (1994) Isolation, growth and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl Environ Microbiol 60:3011–3019

Oremland RS, Stolz JF, Hollibaugh JT (2004) The microbial arsenic cycle in Mono Lake, California. FEMS Microbiol Ecol 48:15–27

Pearce CI, Coker VS, Charnock JM, Pattrick RAD, Mosselmans JFW, Law N, Beveridge TJ, Lloyd JR (2008) Microbial manufacture of chalcogenide-based nanoparticles via the reduction of selenite using *Veillonella atypica*: an in situ EXAFS study. Nanotechnology 19:155603–155616

Pearce CI, Pattrick RAD, Law N, Charnock JM, CokerVS, Fellowes JW, Oremland RS, Lloyd JR (2009) Investigating different mechanisms for biogenic selenite transformations: *Geobacter sulfurreducens, Shewanella oneidensis* and *Veillonella atypical.* Environ Technol (in press)

Ratheber C, Yurkova N, Stackebrandt E, Beatty JT, Yurkov V (2002) Isolation of tellurite- and selenite-resistant bacteria from hydrothermal vents of the Juan de Fuca Ridge in the Pacific Ocean. Appl Environ Microbiol 68:4613–4622

Sen S, Bhatta UM, Kumar V, Muthe KP, Bhattacharya S, Gupta SK, Shashwati JV, Yakhmi JV (2008) Synthesis of tellurium nanostructures by physical vapor deposition and their growth mechanism. Crystal Growth Des 8:238–242

Smith RL, Strohmaier FE, Oremland RS (1985) Isolation of anaerobic oxalate degrading bacteria from freshwater lake sediments. Arch Microbiol 14:8–13

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Song J-M, Lin Y-Z, Zhan Y-J, Tian Y-C, Gand L, Yu S-H (2008) Superlong high-quality tellurium nanotubes: synthesis, characterization, and optical property. Crystal Growth Des 8:1902–1908

Stefani FD, Hoogenboom JP, Barkai E (2009) Beyond quantum jumps: blinking nano scale light emitters. Physics Today 62:34–39

Stolz JF (1990) Introduction to the phototrophic prokaryotes and ultrastructural techniques used in their study. In: Stolz JF (ed) Structure of phototrophic prokaryotes. CRC Press, Boca Raton, pp 1–14

Stolz JF, Oremland RS (1999) Bacterial respiration of selenium and arsenic. FEMS Microbiol Rev 23:615–627

Switzer Blum J, Burns Bind A, Buzzelli J, Stolz JF, Oremland RS (1998) *Bacillus arsenicoselenatis* sp nov., and *Bacillus selenitireducens* sp. nov.: two haloalkaliphiles from Mono Lake, California which respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods) version 4, Sinauer Associates, Sunderland, MA

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Microbiol Rev 4:100–180

Ujjall KG, Rao CNR (2004) Controlled synthesis of crystalline tellurium nanorods, nanowires, nanobelts and related structures by a self-seeding solution process. J Mat Chem 14:2530–2535

Widdel F, Kohring G-W, Mayer F (1983) Studies on the dissimilatory sulfur-reducing bacteria that decompose fatty acids. 3. Characterization of the filamentous gliding *Desulfonema limicola*, gen. nov., sp. nov. and *Desulfonema magnum*, sp. nov. Arch Microbiol 134:286–294

Woods TL, Garrels RM (1987) Thermodynamic values at low temperature for inorganic materials: an uncritical summary. Oxford University Press, New York

Yuan L, Schmalz H, Xu Y, Miyajima N, Drechsler M, Möller MW, Schacher F, Müller AHE (2008) Room-temperature growth of uniform tellurium nanorods and the assembly of tellurium or Fe_3O_4 nanoparticles on the nanorods. Adv Mat 20:947–952

Zhu W, Wang W, Xu H, Zhou L, Zhang L, Shi JJ (2006a) Ultrasonic-induced growth of crystalline tellurium nanorods and related branched structures. J Crystal Growth 295:69–74

Zhu W, Wang W, Xu H, Zhou L, Zhang L, Shi J (2006b) Controllable, surfactant-free growth of 2D, scroll-like tellurium nanocrystals via a modified polyol process. Crystal Grow Des 6:2804–2808