Full Paper

Diversity of salt-tolerant tellurate-reducing bacteria in a marine environment

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Tellurium (Te) has been increasingly used as a semiconductor material in copious amounts, with a concomitant increase in its discharge from industrial effluents and mining wastewater into the environment. However, soluble Te, such as tellurate (VI) and tellurite (IV), is toxic to organisms. Thus, highly efficient technologies need to be developed for a double-benefit detoxification and recovery of soluble Te from industrial and mining wastewater. Since industrial wastewater contains high concentrations of salt, salt-tolerant microorganisms that metabolize rare metals such as Te have been the subject of focus for the effective detoxification and recovery of Te. In the present study, a total of 52 salt-tolerant tellurate-reducing microorganisms were isolated from marine environmental samples. Of these, 18 strains achieved greater than, or equal to, 50% removal of water-soluble Te from a medium containing 0.4 mM tellurate after 72 h incubation. The 18 isolated strains belonged to 13 species of the following 9 genera: *Sulfitobacter*, *Ruegeria*, *Hoeflea*, *Alteromonas*, *Marinobacter*, *Pseudoalteromonas*, *Shewanella*, *Idiomarina*, and *Vibrio*. No microorganism has been reported to reduce tellurate and tellurite from six of the aforementioned genera, namely, *Sulfitobacter*, *Ruegeria*, *Alteromonas*, *Marinobacter*, *Idiomarina*, and *Vibrio*. Especially, one of the isolates *Sulfitobacter* sp. strain TK39B, removed 82% (w/w) of soluble Te with a 4% NaCl tolerance. These results showed that salt-

tolerant tellurate-reducing bacteria that can be used in the detoxification and recovery of Te are widely present in the marine environment.

Key Words: detoxification; recovery; reducing bacterium; salt tolerance; tellurate; tellurite

Introduction

Tellurium (Te) is a rare metal and is used in various kinds of products, such as in additive agents that improve the resistance and workability of alloys as well as in Cd-Te semiconductors for solar cells (U.S. Geological Survey, 2015). As the industrial demand for Te has increased in recent years, the amount of Te discharged from industrial and mining wastewaters into the environment has also increased concomitantly (Soda et al., 2011; Wray, 1998). In the environment, Te is present in the form of soluble oxyanionic tellurate (VI) and tellurite (IV), or solid elemental Te (0), or highly volatile methylated Te compounds (-I, -II) (Ba et al., 2010; Chasteen et al., 2009). Te in both elemental form and methylated compounds are virtually non-toxic (Ba et al., 2010). However, since Te oxyanions are highly toxic (Ba et al., 2010), there is a concern that its exposure to the environment may have negative consequences on the ecosystem. Industrial wastewater contains a high concentration of salt, while salt-tolerant microorganisms that can metabolize rare metals have been isolated from aquatic environments for the effective recovery of rare metals (Uratani et al., 2014).

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Thus, the development of double-benefit detoxification and recovery techniques that allow purification of soluble Te oxyanions, as well as the recycling and reuse of Te, is highly desirable.

The salt-tolerant tellurite-reducing bacteria, *Pseudoalteromonas telluritireducens* and *Pseudoalteromonas spiralis* (Rathgeber et al., 2006) have been isolated from biofilms on hydrothermal vents located in the deep sea of the Main Endeavor Segment of the Juan de Fuca Ridge in the Pacific Ocean. Other tellurite-reducing bacteria such as *Hoeflea phototrophica* DFL-43 (Biebl et al., 2006), *Psychrobacter immobilis* BNF20 (Arenas et al., 2014), *Shewanella oneidensis* MR-1 (Klonowska et al., 2005), and *Rhodotorula mucilaginosa* 13B (Ollivier et al., 2008) have been isolated from seawater in the North Sea, a salt lake in Antarctica, a freshwater lake in New York, and marine sediments at Rehoboth Beach in the State of Delaware, respectively. Thus, salt-tolerant tellurite-reducing bacteria have been isolated from various aquatic environments. In addition, numerous tellurate-resistant microorganisms are known (Csotonyi et al., 2006), but there is no report of salt-tolerant tellurate-reducing microorganisms, except for *Ochrobactrum anthropi* Ti-3 isolated from the drainage wastewater of a metal refinery plant (Kagami et al., 2012). In order to develop technologies that use microorganisms for the detoxification and recovery of soluble Te, there is a need to isolate not only tellurite-reducing microorganisms but also tellurate-reducing microorganisms, as well as to characterize their reduction ability and deepen our knowledge about the metabolism of Te in a comprehensive manner. In this study, salt-tolerant tellurate-reducing microorganisms were isolated from seawater and ocean floor sediments, and the tellurate-reducing abilities of the isolates were analyzed.

Materials and Methods

Sampling of seawater and sediments. Seawater and marine sediments were sampled at 14 locations, namely, Tokyo Bay (TK; 7 stations), the Kanesu-No-Se at Enshu-Nada (EK; 5 stations), and Off-Niigata (NG; 2 stations). At TK, the surface layer water was sampled by using a bucket, and the marine sediments were sampled by using Ekman-Barge sediment samplers (Rigo-sha Co., Ltd., Tokyo, Japan) on *Hiyodori*, a small research and training vessel of the Tokyo University of Marine Science and Technology. At EK, the surface layer water was sampled by using Niskin water samplers (Ocean Test Equipment, FL, USA) with a conductivity, temperature, and depth profiler (CTD; Falmouth Scientific, MA, USA), while the marine sediments were sampled by using the Smith McIntyre sediment sampler (Rigo-sha Co., Ltd.) on *Umitaka-maru*, a large research and training vessel of the Tokyo University of Marine Science and Technology. The samples of NG were harvested by using Niskin water samplers with CTD, while seawater samples were collected from the surface layer as well as from depths of 10, 100, and 300 m, on *Umitaka-maru*. The collected seawater and marine sediments were diluted and mixed with a sterilized physiological saline solution at a proportion of 10% (v/v or w/v). The diluted samples were then used for the isolation of microorganisms. The physical properties and

chemical composition of the surface layer water were estimated by using a multi-sensor (YSI model 556MPS; YSI/Nanotech Inc., Kanagawa, Japan) for TK sample, and by using a CTD and dissolved oxygen sensor (RINKO III, JFE Advantech, Hyogo, Japan) for EK and NG samples.

Media used for the isolation of marine microorganisms.

Marine Broth 2216 (MB; 37.4 g/L) (Becton Dickinson and Company, MD, USA) was utilized as the basal medium. MB culture medium with supplemental sodium telluride or potassium tellurate solution was used as the culture medium for bacterial isolation. Salt-tolerant microbial growth was examined on Luria Broth, Lennox (LB; Nacalai Tesque, Inc., Kyoto, Japan), into which the NaCl concentration was adjusted to 0.5–20% (w/v). To prepare a solid culture medium, 1.5% (w/v) agar was added to the culture medium. The inoculated medium plates were incubated at 30°C, and the liquid culture medium was incubated under rotational shaking at 120 rpm.

Enrichment and isolation of tellurate- and tellurite-reducing bacteria.

Potassium tellurate was added to 50 mL of MB dispensed in 100-mL Erlenmeyer flasks (final concentration of 0.5 mM), and a diluted sample solution was added to obtain a final concentration of 1.0% (v/v). Elemental Te (0), which is produced by the reduction of Te oxyanions, is black in color (Chasteen et al., 2009). After 72 h of incubation, the bacterial culture broth that had turned black was inoculated into new MB medium containing 1.0 mM tellurate at 1.0% (v/v). This procedure was repeated for the same MB culture medium containing tellurate at a final concentration of 2.0 mM. Next, the bacterial culture broth that had turned black was seeded onto the culture medium in MB plates containing tellurate or tellurite at a final concentration of 1.0 mM, and black-colored colonies were isolated from them after the incubation period.

Tellurate-removal test. Each colony of the isolated strains was inoculated into 50 mL of MB containing 0.5 mM tellurate dispensed in 100-mL Erlenmeyer flasks and incubated for 72 h at 30°C under 120 rpm rotational shaking. The culture broth was inoculated at an amount of 1.0% (v/v) into fresh MB not containing tellurate and incubated for 24 h under the same conditions as described earlier. The bacterial culture broth was inoculated into MB medium to obtain an optical density (OD₆₆₀) of 0.01, and tellurate was also added to the mixture so as to obtain a final concentration of 0.4 mM. The inoculated bacterial culture was incubated for 168 h at 30°C under 120 rpm of rotational shaking. The bacterial culture broths were then harvested as 1.0 mL samples at pre-defined time points and centrifuged at 21,000 × g for 15 min at 4.0°C. The supernatants and the precipitates obtained were used for the quantification of Te concentrations and for the observation of bacteria and solidified products, respectively. MBs without isolates were incubated under the same conditions as mentioned above and were considered as negative controls. The ratio of removal of soluble Te (containing tellurate, tellurite and other dissolved Te compounds, including methylated and unknown Te), from the culture broths was evaluated by using Eq. (1), as follows:

$$\begin{aligned} &\text{Removal ratio of soluble Te (\%)} \\ &= (\text{Te concentration [initial]} - \text{Te concentration [72 h]}) \\ & / \text{Te concentration (initial)} \times 100. \end{aligned} \quad (1)$$

To determine the salt tolerance and capability to remove soluble Te of the isolates, NaCl was added in 1% increments from 0–5.0% (w/v) to MB, and soluble Te-removal tests were performed according to the same procedure as that described earlier. The influence of NaCl concentration on the ability to remove soluble Te was evaluated by calculating the rate of decrease in the levels of soluble Te per unit time.

Analytical procedures. The concentrations of Te in the supernatants and precipitates obtained from the bacterial culture broth were quantified. The supernatants were filtered through 0.2- μm pore size filters (Kurabo, Osaka, Japan); the filtrates were diluted 10- and 100-fold in ultrapure water (Barnstead NANOpureR Diamond; Thermo Fisher Scientific, Rockford, IL, USA), and the resultant concentrations of Te were determined. The precipitates were washed twice with ultrapure water, and completely dissolved in 1.0 mL of 70% nitric acid. The resulting solutions were diluted 10-, 100- and 1000-fold in ultrapure water, and the concentrations of Te were determined. Inductively-coupled plasma-atomic emission spectrometry (ICP-AES, iCAP6300DUO; Thermo Fisher Scientific KK, Yokohama, Japan) was used for the determination of Te concentrations. The instrument was operated and calibrated in accordance with the manufacturer's instructions. An analytical curve was created with reference to a Te standard solution (Wako Pure Chemical Industries, Osaka, Japan). Measurements were performed 3 times per sample, and the mean values with a deviation of $\leq 5.0\%$ were considered.

Identification of isolated bacteria. To determine the growth of the isolates in the presence of NaCl, colony formation of the isolates in the presence of 0.5–20% NaCl was observed. Gram staining of the isolates was performed in accordance with the known method (Cowan and Steel, 1993). The nucleotide sequences of the isolates' 16S rRNA gene were determined as follows. The isolates were cultured for 3 days in MB; the bacterial cells were collected by centrifugation at $21,000 \times g$, 4°C , for 5 min and washed twice with sterilized saline. The genome DNAs of the bacteria were extracted in accordance with the protocol by using ISOPLANT (Nippon Gene, Tokyo, Japan). The isolates' 16S rRNA genes were amplified by using a PCR device (Mastercycler; Eppendorf, Tokyo, Japan; GeneAmp® PCR System 9700; Applied Biosystems, CA, USA), with extracted genome DNAs as templates. A primer set composed of 9F (5'-GAGTTTGATCCTGGCTCAG-3') and either 1510R (5'-GGCTACCTTGTTACGA-3') or 1541R (5'-AAGGAGGTGATCCAGCC-3') was used for the PCR amplification. The nucleotide sequence of the 16S rRNA gene was determined and analyzed with multiple primers [9F, 515F (5'-GTGCCAGCAGCCGCGGT-3'), 785F (5'-GGATTAGATACCCTGGTAGTC-3'), 802R (5'-TACCAGGGTATCTAATCC-3'), and 1115R (5'-AGGGTTGCGCTCGTTG-3'), 1510R, 1541R] (Green and

Table 1. Physical properties of each sampling station and basic information of surface seawater there.

Station No.		Latitude (North)	Longitude (East)	Depth to bottom (m)	Water temp. ($^\circ\text{C}$)	Salinity (%)
Tokyo Bay (TK)	St. 1	35.6252	139.8538	3	10.6	3.0
	St. 2	35.6772	139.8490	3	10.1	2.5
	St. 3	35.6510	139.9605	8	9.5	3.1
	St. 4	35.5252	139.9219	22	11.1	3.2
	St. 5	35.4347	139.8697	22	9.6	3.2
	St. 6	35.5269	139.8094	19	11.2	3.1
	St. 7	35.5300	139.7836	4	10.9	2.7
Off-Niigata (NG)	St. 1	37.4368	138.0136	926	25.2	3.4
	St. 2	37.5700	137.9645	1011	26.4	3.4
Kanasu-No-Se, Enshu-Nada (EK)	St. 1	34.4477	138.3178	87	23.1	3.4
	St. 2	34.4135	138.3332	117	23.0	3.4
	St. 3	34.3751	138.3008	340	24.5	3.4
	St. 4	34.3234	138.2921	60	25.0	3.4
	St. 5	34.2749	138.2856	270	25.3	3.4

Sambrook, 2012), as well as by using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) or a 3730xl DNA Analyzer (Applied Biosystems). The use of the Basic Local Alignment Search Tool (BLAST, <http://blast.ncbi.nlm.nih.gov/Bla-st.cgi>) (Altschul et al., 1990) provided by the NCBI-enabled homologous analysis on the 16S rRNA gene sequences. On the basis of the nucleotide sequences of bacterial species showing a high homology, phylogenetic trees were constructed in accordance with the neighbor-joining method (Saitou and Nei, 1987) by using the CLUSTAL W and the phylogenetic tree construction software Molecular Evolutionary Genetics Analysis (MEG5.0). The sequences determined in this study have been deposited in the DNA Data Bank of Japan (DDBJ; <http://www.ddbj.nig.ac.jp>).

Results

Analyses of seawater and sediment samples

The physical properties and chemical compositions of surface water from the sampling points located in TK, EK and NG were analyzed (Table 1). For the environmental samples from TK, the water temperature was approximately 10°C at the time of sampling due to the winter season, while it was approximately 25°C at EK and NG due to the summer season. At the sampling points located near river mouths flowing into TK (Sts. 2, 7), the salinity levels were 2.5% and 2.7%, respectively, which are lower than the normal salt concentration in seawater (3.5%). The seawater samples from TK, EK, and NG did not show any marked difference in the pH range 7.7–8.25 and an electric conductivity in the range 39.88–52.66 mS/cm. All the marine sediments sampled in TK (Sts. 1–7) consisted mainly of black-colored mud, and, in particular, marine sediments collected near estuaries (Sts. 2, 6) contained large numbers of seashells. Marine sediments sampled at TK Sts. 4 and 5 were collected near the center of TK and they smelled like H_2S gas. At all sampling sites in EK (Sts. 1–5), the marine sediments consisted of sand and mud, containing large numbers of gravels and shells. At all sampling sites in NG (Sts. 1, 2), the marine sediments were mud-like and black-colored. Qualitative and quanti-

Table 2. Characterizations of isolated strains.

Strain	Isolation source (depth)	Removal ratio of soluble Te (%)	Growth on LB in NaCl (%)	Most closely related species (% similarity)	Accession No.
TK11	TK St. 5 Seawater (0 m)	53	0.5–5.0	<i>Pseudoalteromonas elyakovii</i> (99.0%)	LC053426
TK33A	TK St. 4 Seawater (0 m)	51	3.0–5.0	<i>Pseudoalteromonas marina</i> (99.5%)	LC053428
TK33B	TK St. 4 Seawater (0 m)	52	3.0–5.0	<i>Pseudoalteromonas marina</i> (99.5%)	LC053429
TK35	TK St. 6 Seawater (0 m)	65	3.0–5.0	<i>Pseudoalteromonas tetradonis</i> (98.7%)	LC053430
TK36A	TK St. 7 Seawater (0 m)	62	0.5–5.0	<i>Pseudoalteromonas porphyrae</i> (98.7%)	LC053431
TK39B	TK St. 5 Sediment (22 m)	82	0.5–10	<i>Sulfitobacter guttiformis</i> (99.0%)	LC053427
NG47A	NG St. 1 Seawater (300 m)	54	0.5–10	<i>Pseudoalteromonas espejiana</i> (99.1%)	LC053432
NG47B	NG St. 1 Seawater (300 m)	55	0.5–10	<i>Pseudoalteromonas espejiana</i> (98.5%)	LC053433
NG49C	NG St. 2 Seawater (0 m)	55	0.5–5.0	<i>Vibrio penaeicida</i> (98.7%)	LC053434
EK6B	EK St. 1 Sediment (87 m)	55	0.5–10	<i>Marinobacter litoralis</i> (99.3%)	LC053417
EK6C	EK St. 1 Sediment (87 m)	55	no growth	<i>Alteromonas addita</i> (99.5%)	LC053418
EK6D	EK St. 1 Sediment (87 m)	61	0.5–3.0	<i>Marinobacter litoralis</i> (100%)	LC053420
EK6E	EK St. 1 Sediment (87 m)	53	0.5–5.0	<i>Marinobacter litoralis</i> (100%)	LC053421
EK8A	EK St. 1 Sediment (87 m)	55	0.5–3.0	<i>Hoeflea alexandrii</i> (98.5%)	LC053419
EK8B	EK St. 1 Sediment (87 m)	75	0.5–3.0	<i>Shewanella pneumatophori</i> (100%)	LC053422
EK15A	EK St. 2 Sediment (117 m)	79	0.5–15	<i>Idiomarina loihiensis</i> (99.5%)	LC053423
EK15B	EK St. 2 Sediment (117 m)	73	0.5–10	<i>Idiomarina loihiensis</i> (99.5%)	LC053424
EK38B	EK St. 5 Sediment (270 m)	51	3.0–5.0	<i>Ruegeria atlantica</i> (99.6%)	LC053425

tative analysis of the Te content in marine sediments at all sampling sites was performed by using ICP-AES; however, the findings revealed that, at all sites, the content was below the detection level of the analytical instruments (0.1 mg/kg).

Isolation of soluble Te-removal microorganisms

In order to isolate salt-tolerant bacteria capable of removing soluble Te, an attempt was made to isolate microorganisms from the seawater and marine sediments. A total of 52 strains forming black-colored colonies were obtained from MB agar plate containing tellurate. For these 52 strains, the color of the colonies changed to black when inoculated on MB agar containing tellurate and tellurite. The changing color indicated the presence of elemental Te in the colony. These findings suggest that tellurate was reduced into black-colored elemental Te through the formation of tellurite. Classification of the sampling regions of the 52 isolates were as follows: 24 strains were isolated from TK, 6 from NG, and 22 from EK. The above data indicates that salt-tolerant bacteria that remove soluble Te are widely present in the seas near Japan.

Soluble Te-removal tests in isolates

To evaluate the isolate's ability to remove soluble Te, the 52 strains were cultured in MB culture medium containing tellurate. The soluble Te-removal capability was evaluated by calculating the ratio of elimination of Te present in the bacterial culture broths in accordance with Eq. (1). For 18 of the 52 isolates, the percentage removal of soluble Te was greater than or equal to 50% ($\geq 50\%$) after 72 h incubation (Table 2). Particularly, for strain TK39B, the percentage removal of soluble Te was 82%, which was the highest among the isolates. For the remaining 34 strains, the percentage removal of soluble Te was 20–49% after 72 h incubation (data not shown). These findings revealed that all 52 isolates possessed a soluble Te-removal ability and that 18 of these strains possessed a removal percentage of $\geq 50\%$.

Identification of isolates

To further study the 18 strains showing $\geq 50\%$ removal of soluble Te, their growths were examined in the presence of NaCl at a concentration of 0.5–20%, except for EK6C. Strains TK33A, TK33B, TK35, and EK38B were found to require NaCl for their growth. These findings revealed that 17 strains, except for EK6C, were salt-tolerant and thus capable of growing in the presence of NaCl in the concentration range of 3.0–15% (Table 2). In addition, all the strains were Gram-negative.

To identify the isolated strains by molecular biological techniques, partial sequencing of the 16S rRNA genes of all the strains was performed, followed by homologous analysis. The results of partial base sequencing from the 16S rRNA gene of all the strains are registered in a DNA data bank (Table 2). Homologous analysis with the BLAST database was performed to reveal the strains with the highest homology with the 16S rRNA genes of the isolates (Table 2). The partial nucleotide sequence homologies of the 16S rRNA gene of some strains were high, for example: 100% homology between strains EK15A and EK15B; 99.4% homology between strains TK33A and TK33B; 100% homology between strains EK6D and EK6E; 99.3% homology between strains EK6B and EK6D (strain EK6E); and 98.6% homology between strains NG47A and NG47B. Homology between strains other than the ones mentioned was $\leq 95\%$. These findings showed that the 18 isolates shared a $\geq 98\%$ homology with the bacteria of the genus *Sulfitobacter*, *Ruegeria*, *Hoeflea*, *Alteromonas*, *Marinobacter*, *Pseudoalteromonas*, *Shewanella*, *Idiomarina*, and *Vibrio* (Table 2). Next, the results of phylogenetic analyses (Fig. 1) revealed that the 18 strains could be classified into 13 species belonging to 9 genera.

Soluble Te-removal tests for strain TK39B

To examine the soluble Te-removal ability of strain TK39B (possessing the highest percentage removal of soluble Te among the tested strains), the Te concentrations in the supernatants and precipitates of strain TK39B were quantified in a timecourse study using MB with 3.0% NaCl (Fig. 2). After 60 h incubation, the concentration of solu-

Fig. 1. Phylogenetic trees inferred from the 16S rRNA gene sequences.

The evolutionary history was inferred by using the neighbor-joining method (Saitou and Nei, 1987). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Numbers at nodes represent bootstrap percentages. Evolutionary analyses were conducted by MEGA5.0 (Tamura et al., 2011).

ble Te decreased from the initial level of 0.4 mM to 0.07 mM, followed by a gradual increase with incubation time. After 168 h incubation, the level of soluble Te increased to 0.17 mM. The Te concentration in the precipitates showed an inverse relation to the decrease in the levels of soluble Te, with a peak of 0.39 mM obtained after 60 h incubation. Later, it decreased to 0.18 mM after 168 h incubation. The total amount of Te contained in the liquid and solid phases was 0.41 mM at 0 h, 0.40 mM at 60 h, and 0.35 mM at 168 h; thus, of the initial soluble Te concentration was decreased by approximately 20%, suggest-

ing volatile Te formation (Bonificio and Clarke, 2014). The bacterial turbidity of the strain TK39B increased from an initial level of $OD_{660} = 0.01$ to approximately 1.6 at 24 h, with a gradual increase thereafter. After 36 h, the color of the culture broth turned black, as a result of which the amount of bacterial cells could not be measured accurately by spectrophotometry. After 72 h, the color of the culture broth changed from black to milky white (data not shown). These findings suggest that strain TK39B may have reduced soluble Te, such as tellurate and tellurite, into elemental Te and eliminated the latter from the culture broth

Fig. 2. Time course of Te concentration in the supernatant and precipitate of cultured strain TK39B.

Open circle, concentration of soluble Te; close circle, concentration of Te in precipitate.

through solidification. Furthermore, after 72 h incubation, elemental Te (solid) may have changed into soluble Te (liquid), and thus dissolved as methylated, or chemically unknown, Te again (Bonificio and Clarke, 2014; Kagami et al., 2012).

Effect of NaCl on soluble Te-removal in strain TK39B

To determine the effects of NaCl on the ability of strain TK39B to remove soluble Te, 0.0–5.0% (w/v) of NaCl was added to MB in increments of 1.0%, and the rate of decrease in the amounts of soluble Te per unit time in the presence of strain TK39B was calculated (Fig. 3). Strain TK39B showed the highest removal rate of soluble Te (0.011 mM/h) at 1.0% NaCl. Even at 5.0% NaCl, the rate of removal of soluble Te was found to be approximately 30% (0.003 mM/h) of that at 1.0% NaCl. These findings suggest that the strain TK39B was a salt-tolerant microorganism capable of removing soluble Te extensively at added NaCl concentrations of 0.0–5.0% (w/v).

Discussion

Sampling of seawater and marine sediments at 3 locations (TK, EK, and NG) resulted in the isolation of 52 bacterial strains possessing the capability of removing soluble Te. The 52 isolates included 24 strains from TK (21 strains from seawater and 3 strains from marine sediments), 6 from seawater of NG, and 22 from marine sediments of EK.

Eighteen of the 52 isolates reduced the concentration of soluble Te by $\geq 50\%$. The 18 strains were Gram-negative bacteria and could grow on LB in the presence of NaCl at concentrations of 0.5–15%, except strain EK6C (Table 2). Molecular biological analyses of the 18 strains showed that they could be classified in the following 9 genera: the genus *Pseudoalteromonas* (7 strains), *Marinobacter* (3 strains), *Idiomarina* (2 strains), *Sulfitobacter* (1 strain), *Ruegeria* (1 strain), *Hoeflea* (1 strain), *Alteromonas* (1 strain), *Shewanella* (1 strain), and *Vibrio* (1 strain). All of the most closely related strains within the 9 genera (Table 2) were members of the class *Proteobacteria* and belonged to Gram-negative bacteria (Donachie et al., 2003; Hirota et al., 2005; Ishimaru et al., 1995; Ivanova et al., 2005;

Fig. 3. Effect of NaCl on soluble Te removal rate in strain TK39B.

Strain TK39B was cultivated in MB with added NaCl at 0.0–5.0% (w/v) with 0.4 mM tellurate, and the rate of decrease in the amounts of soluble Te in the culture per unit time was calculated.

Labrenz et al., 2000; Palacios et al., 2006; Sawabe et al., 2000; Uchino et al., 1998; Yoon et al., 2003). In consideration of the findings pertaining to colony morphology (data not shown), 16S rRNA gene sequence homology, salt tolerance, and the rate of removal of soluble Te, strains TK33A and TK33B, as well as strains NG47A and NG47B, seemed to be similar. The differences between the other isolated bacterial strains will need to be further examined through detailed biochemical characterization. However, according to the molecular biological and biochemical properties of the isolates, not many strains seemed similar. Thus, surprisingly, aerobic tellurate-reducing bacteria were successfully isolated from seawater and marine sediments collected from 3 different marine areas; this finding showed that tellurate-reducing bacteria are present over large marine areas.

Classification according to marine areas showed that the bacteria collected from TK belonged to the genus *Pseudoalteromonas* and *Sulfitobacter*, those from NG belonged to *Pseudoalteromonas* and *Vibrio*, and those from EK belonged to *Marinobacter*, *Alteromonas*, *Hoeflea*, *Shewanella*, *Idiomarina*, and *Ruegeria*. The only bacteria to be present in multiple marine areas belonged to the genus *Pseudoalteromonas*, whereas other bacteria were present only in one marine region at a time. To our knowledge, no report is yet available concerning the aerobic tellurate- and tellurite-reducing bacteria in these 6 genera *Sulfitobacter*, *Ruegeria*, *Alteromonas*, *Marinobacter*, *Idiomarina*, and *Vibrio*. Bacteria belonging to 4 of the 6 genera were isolated from EK. The area surrounding EK is known to have cold seeps, which give spurts of underground water from fissures developed in the Earth's crust at temperatures similar to those of seawater (Kobayashi, 2002). Cold seeps are inhabited by chemosynthetic organisms (chemoautotrophs) that use methane and hydrogen sulfide contained in the underground waters from cold seeps as substrates (Li et al., 1999). Various microorganisms capable of metabolizing metals have been found in environments composed of chemolithoautotrophic ecosys-

tems such as cold seeps and hydrothermal vents (Jeanthon and Prieur, 1990). In fact, various microorganisms that reduce tellurite under aerobic or anaerobic conditions have been isolated in hydrothermal vents (Csotonyi et al., 2006; Rathgeber et al., 2006). The fact that various species of tellurate-reducing microorganisms were isolated from EK may be associated with the fact that the latter is a sea area with cold seeps. Our finding revealed that 13 species of salt-tolerant aerobic tellurate-reducing bacteria belonging to 9 genera, that can be used for environmental cleanup and recovery of resources, are widely present in marine environments.

Te could not be detected in seawater and marine sediments used in the present study; however, the presence of trace amounts of Te previously reported in seawater and marine sediments (Schirmer et al., 2014). It is probable that the isolates obtained in this study may be involved in the circulation of Te in the marine environment.

As for strain TK39B, which showed as high as 82% removal of soluble Te, genetic analysis revealed a homology of 99.0% with *Sulfitobacter guttiformis* PM-3 and *Sulfitobacter pseudonitzschiae* H3. *S. guttiformis* EL38 (a type strain of *S. guttiformis*) was previously isolated from Lake Ekho, a high-salt concentration lake in Antarctica, and could grow in the presence of 1.0–4.0% NaCl concentration (Labrenz et al., 2000). On the other hand, *S. pseudonitzschiae* H3, which was previously isolated from the toxic diatom *Pseudo-nitzschia multiseries*, could grow in the presence of 1.0–9.0% NaCl concentration (Hong et al., 2015). Strain TK 39B could grow on LB in a wider range of NaCl concentrations (0.5–10%) as compared with the type strains EL-38 and H3 (Table 2). As far as we know, there exists no previous report about the reduction of tellurate and tellurite in association with the genus *Sulfitobacter*. Thus, the metabolism of Te by bacterial strains of the genus *Sulfitobacter* is a new discovery that is reported for the first time in this paper.

Strain TK39B reduced the concentrations of soluble Te from an initial level of 0.4 mM to 0.07 mM within 60 h incubation, and synthesized elemental Te by the same amount as soluble Te was decreased (Fig. 2). *Ochrobactrum anthropi* Ti-3, which is an aerobic tellurate-reducing bacterium, reduced the concentrations of tellurate from an initial level of 1.0 mM to ≤ 0.2 mM after 72–100 h of culturing (Kagami et al., 2012). Although the experimental conditions were different, strain TK39B appeared to possess a Te-removal ability that was at least equal to, or greater than, that of *O. anthropi* Ti-3. In addition, observations under SEM-EDX of the precipitate after 72 h of culture of strain TK39B showed the presence of extracellular elemental Te (data not shown). The soluble Te-removal ability in strain TK39B was greatest in MB with 1.0% NaCl added, and $\geq 50\%$ of the highest level of ability to remove soluble Te (0.011 mM/h) was achieved even with 4.0% NaCl; this finding shows that strain TK39B possessed a salt-tolerant removal ability of soluble Te with 4.0% NaCl. From the results, we identified the salt-tolerant tellurate-reducing bacterial strain as *Sulfitobacter guttiformis* TK39B. Finally, we showed that salt-tolerant tellurate-reducing bacteria are widely present even in the marine environment at very low concentrations of tellu-

rate, and one of them, strain TK39, could be expected to be used in the process of detoxification and recovery of Te.

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