The tellurite-reducing bacterium *Alteromonas macleodii* from a culture of the toxic dinoflagellate *Prorocentrum foraminosum*

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**A B S T R A C T**

The *Alteromonas macleodii* strain 2328 was isolated from a clonal culture of the toxic dinoflagellate *Prorocentrum foraminosum*. The strain exhibits a resistance to high K2TeO3 concentrations (2500 μg/mL). A study of the growth dynamics of the strain exposed to K2TeO3 has shown a longer lag phase and a reduced stationary phase compared to those during cultivation with no toxicant. The fatty acids profile is dominated by 16:1 (n-7), 16:0, 17:1, 15:0, 18:1 (n-7), and 17:0. The 2328 strain belongs to the Gammaproteobacteria and is related to the genus *Alteromonas* with 99–100% sequence similarity to some intra-genome allele variants (paralogues) of 16S rRNA from *A. macleodii*. A phylogenetic reconstruction (ML and NJ), based on HyHK amino acid sequences, has revealed that the analyzed 2328 strain forms a common cluster with *A. macleodii* strains. In the presented work, the ability of *A. macleodii* to reduce potassium tellurite to elemental tellurium has been recorded for the first time. Bacteria reduce potassium tellurite to Te (0), nanoparticles of which become distributed diffusely and in the form of electron-dense globules in cytoplasm. Large polymorphous metalloid crystals are formed in the extracellular space. Such feature of the *A. macleodii* strain 2328 makes it quite attractive for biotechnological application as an organism concentrating the rare metalloid.

**1. Introduction**

The genus *Alteromonas* (Raumann et al., 1972) belongs to the bacterial class Gammaproteobacteria and currently includes 20 validly published species (http://www.bacterio.net/alteromonas.html). Members of the genus *Alteromonas* are widespread in the marine environment and are isolated from water (Ivanova et al., 2005, 2013; Park et al., 2015), abyssal sediments (Matsuyama et al., 2015), tidal zone sediment (Park et al., 2017), aquatic organisms (Chen et al., 2009), and fouling biofilms (Vandecandelaere et al., 2008). *Alteromonas macleodii*, as well as other members of the genus, has been commonly isolated from many marine samples. The abundance and distribution of *A. macleodii* is not fully understood, however, this bacterium is abundant at middle depths of the photic zone (25–100 m), where it is associated with aggregates of up to 5 mm and actively reproduces during phytoplankton blooms. Two ecotypes have been identified, of which one is found in the upper water layer at temperate latitudes ("surface ecotype"), and the other occurs mostly in the deep waters of the Mediterranean Sea ("deep-sea ecotype") (López-Pérez et al., 2012).

Many of the *Alteromonas* genus members are characterized by resistance to heavy metals. There are mercury-resistant species of *Alteromonas* newly isolated and described from an estuary in Taiwan (Chiu et al., 2007). It is reported that *Alteromonas* produces extracellular polysaccharides capable of binding such metals as zinc, cadmium, lead (Loaec et al., 1998), copper, nickel, and chromium (Zhang et al., 2017). The surface ecotype of *A. macleodii* strain was found to have a plasmid containing genes of multiple resistance to metals, previously identified in the genomes of *A. mediterranea* (deep-sea ecotype), which indicates a genomic exchange existing between related but ecologically different populations (Fadeev et al., 2016). Information on the resistance of *Alteromonas* to tellurite is almost unavailable, and only a statement about such resistance in *A. colwelliana* has been published along with description of its phenotypic traits (Weiner et al., 1988).

Tellurium is a toxic metalloid and one of the rarest elements in the Earth's crust. The mechanisms of toxic effect of Te on bacteria are still poorly understood. One of the hypotheses suggests that the element's...
toxicity can be associated with its soluble form, ion (TeO$_2$), oxidizing thiol and producing reactive oxygen species (Borsetti et al., 2005).

Earlier, before the discovery of antibiotics, tellurite was used as an antimicrobial agent in treating a range of infections (Taylor, 1999). However, some bacteria remain resistant to high tellurite concentrations. There are reports about the tellurite-resistance of some species such as *Staphylococcus* spp., *Acinetobacter haemolyticus*, *Pseudomonas lini*, *Pseudomonas immobilis* (Arenas et al., 2014), *Baillus* spp. (Sepahei and Rashetnia, 2009), including marine bacilli and yeasts (Ollivier et al., 2008). Moreover, some bacteria of the genera *Rhodococcus* (Presentato et al., 2018), *Erythromicrobiium*, *Erythrobacter*, and *Roseococcus* are resistant to tellurite and able to reduce it (Yurkow et al., 1996). Two *Pseudoalteromonas* species, *P. telluritireducens* and *P. spiralis*, isolated from deep-sea hydrothermal vents have been described as reducing tellurite to tellurium (Rathgeber et al., 2006). There are apparently several mechanisms of tellurite resistance. One of them is the enzymatic or non-enzymatic reduction of tellurite to elemental tellurium which results in the formation of tellurium nanocrystals (Chasteen et al., 2009; Presentato et al., 2018). The unique optic and electronic properties of tellurium has caused a growing demand for this element in the past decade (Bonificio and Clarke, 2014). Industrial production of tellurium is associated with the use of substances harmful for human and environment, and, therefore, alternative ways of extraction of this metalloid are becoming increasingly sought after. For this reason, technologies of microbial concentration of metals from natural ores and tailings with a low level of metal such as, in particular, biosynthesis of nanoparticles of different metals with the use of bacteria, are now more and more popular (Chokriwal et al., 2014; Figueroa et al., 2018; Presentato et al., 2018).

Technological attempts to turn tellurite into metal tellurium by means of bacteria are still sporadic. Studies with the involvement of *Pseudoalteromonas* mandocina as a tellurite reducer are well known (Rajwade and Paknikar, 2003), but active works in this direction are still in progress. The biotechnological potential of marine *Pseudoalteromonas* from deep-sea hydrothermal vents (Rathgeber et al., 2002, 2006), the strict anaerobes *Bacillus senelitireducens* and *Sulfuruspirillum barnesi* (Baensman et al., 2007), marine yeasts of genus *Rhodotorula*, and members of genus *Bacillus* (Ollivier et al., 2008), which are facultative anaerobic bacteria (Sepahei and Rashetnia, 2009), as well as a number of gram-positive and gram-negative bacteria from Antarctica (Arenas et al., 2014) and some of the Latin America countries (Figueroa et al., 2018), seems promising. Thus, the continuous search for new microorganisms with a biotechnological and industrial potential is of great importance.

We could not find information about tellurite reduction by *Alteromonas* in the available literature.

In our research, the 2328 strain resistant to potassium tellurite and reducing it to tellurium was isolated from a clonal culture of the toxic dinoflagellate *Prorocentrum foraminosum* within the framework of the experiment on associative microflora analysis. The goal of the present work was to identify the reducer strain and to analyze the resistance to tellurite ions and reduction to elemental tellurium. For a molecular-genetic study of the 2328 strain, a 16S rRNA analysis was used, which is generally accepted as a “golden standard” in molecular phylogeny, barcoding, and molecular ecology of bacteria. Nevertheless, it is known that the intragenomic heterogeneity of this gene is detected in different groups of bacteria, which in some cases can lead to erroneous species identification. To verify correct identification of species of the 2328 strain, the molecular markers were additionally selected to the genes of nonribosomal peptide synthetase (NRPS)/polyketide synthesis (PKS) in the analysis. These genes are widely represented in all of whole genomes of various strains of *Alteromonas* and closely related *Pseudoalteromonas*.

### 2. Results

The 2328 strain was isolated from a laboratory culture of the microalga *P. foraminosum* on the Plate Count agar containing 100 μg/mL potassium tellurite. At 2 days post-inoculation, black, convex, lustrous colonies of 3 mm in diameter appeared in the dishes; their concentration was 2.3 × 10$^3$ CFU/mL. During further cultivation on media with potassium tellurite (Plate Count agar, Marine agar 2216), the strain colonies always became black in color on day 2. Cultivation on Marine Broth 2216 also caused blackening of the medium (Fig. 1).

On the surface of Marine agar 2216 without potassium tellurite at 25 °C, rounded, smooth, convex, cream-colored colonies of 3 mm in diameter formed within 2 days of cultivation. In the gram-stained smears, the cells had a shape of bacilli of 1.0 μm in width and 2.0–2.5 μm in length, with the gram-negative type of cell wall.

The 16S rRNA sequencing performed in this study revealed that the 2328 strain belongs to Gammaproteobacteria and is related to the genus *Alteromonas*, with a 99–100% sequence similarity to some intragenomic allele variants (paralogues) of 16S rRNA from *A. macleodii* strains and a 98.0% similarity to *A. marina*, a nearest phylogenetic neighbor. A higher degree of variable positions was observed in the 16S rRNA nucleotide sequences of the members of the *A. macleodii* complex.

A comparison of sequences between the 16S rRNA gene copies of the 2328 strain revealed two heterogenic variants. Differences of 19 nucleotides were found between copies (alleles) of the 16S rRNA gene. Variable positions in alleles of 2328 were strictly located in V1 and V6 regions. Overall, most of the *A. macleodii* strains deposited in GenBank had at least two different 16S rRNA alleles, but a great difference was only observed between alleles of the 2328 strain. The nucleotide sequence of 16S rRNA allele 1 was 100% identical to paralogues within the genome Mex14 strain (CP018023.1–CP018023.2), Balearic Sea AD45 strain (CP003873.1–CP003873.2), English_Channel 673 strain (CP003844.1–CP003844.2), Black_Sea_11 strain (CP003845.1–CP003845.2), Te101 strain (CP018321.1–CP018321.3), MA112 strain (AB491743), and 45Xa1J strain (EU400494) of *A. macleodii*, whereas the nucleotide sequence of 16S rRNA allele 2 was 99% similar to the following strains: HOT1A3 (CP012202.3–CP012202.4) with 8 bp-differences, CP012202.5 (9 bp-differences), Balearic Sea AD45 (CP003873.3–CP003873.5), and Black Sea 11 (CP003845.3–CP003845.5) (10-bp). Intragenomic nucleotide heterogeneity of the 16SrRNA of 2328 strain was 0.007 (0.7%). While 0–0.004 (0.4%) differences were found within the HOT1A3 and ATCC_27126 strains, 0–0.003 (0.3%) differences were found within Te101, 0–0.002 (0.2%) differences were found within Mex14 and Balearic Sea AD45, and 0–0.001 (0.1%) were found within the English_Channel_673 strain. Phylogenetic reconstructions based on 16S rRNA, which were performed with different approaches, show similar topology (Fig. 2). Support values are written on the branches: SH-aLRT
support (%)/aBayes support/ultrafast bootstrap support (%). The sequencing of 16S rRNA, amplified from genomic DNA, classified the allele 1 of the 2328 strain to a cluster of *A. macleodii* and *A. marina* P-2. A phylogenetic analysis, based on site-specific nucleotide substitutions, revealed a recurrent overlap of the allele 2 with genomic alleles and individual gene copies of closely related strains of *A. macleodii* and other species of *Alteromonas* from GenBank. Alleles 1 and 2 in 2328 are paralogous to each other, as are 1 and 2 in genome of *A. macleodii* strains from GenBank. However, sequences of allele 1 from different strains are orthologues, as are alleles “2”. Herewith, the 16S rRNA sequences of subcluster “allele 1” from different strains of *A. macleodii* grouped together with those of the following species: *A. abrolhosensis*,

![Image](image_url)

**Fig. 2.** Phylogenetic position and relationships of the 2328 strain within the *Alteromonas*, as inferred from Bayesian and Maximum likelihood analyses of 16S rRNA gene sequences. Numerals on the branches indicate as follows: SH-aLRT support (%)/aBayes support/ultrafast bootstrap support (%).
A. marina, A. simiduii, A. naphthalenivorans, A. addita, and A. stellipolaris. Another subcluster, “allele 2”, including only sequences of A. macleodii strains, grouped with a subcluster comprising the other Alteromonas species: A. tagae, A. litorea, A. mediterranea, A. genovensis, A. hispanica, A. confluentis, A. oceanica, A. lipolytica, A. halophila, and A. pelagimontana.

When compared against nucleotide sequences in the GenBank database, sequences from mcyE-F2/mcyE-R4 primers were found to most closely align to the sequences of a multi-sensor hybrid histidine kinase (HyHK) of different species of Alteromonadaceae, they shared 99.7–98% nucleotide and amino acid sequence similarity with those of various A. macleodii strains, except for the strain from the Black Sea (77% nt and 92% aa similarity), and 69–68% nt and 82% aa sequence similarity to various of A. mediterranea strains. The HyHK sequences were analyzed from the strains whose HyHK sequences were downloaded as individual nucleotide and amino acid sequences, or extracted from the whole genome sequences of Alteromonadaceae deposited in GenBank. Partial nt sequence of HyHK (1086 bp) from the 2328 strain was translated into amino acid sequence (362 aa). All of amino acid sequences were aligned using the Muscle method implemented in the Geneious R 11.0.4.

A phylogenetic reconstruction (ML and NJ) based on the HyHK amino acid sequences revealed that the analyzed 2328 strain formed common cluster with A. macleodii strains (Fig. 3). The comparison of the HyHK amino acid sequence of the 2328 strain to the other representative strains showed that the 2328 strain and the AD006 strain exhibited 99.7% deduced amino acid identity. The pairwise alignment of 362 amino acid positions of the 2328 and AD006 found only two aa substitutions: Gln/Leu238 and Gln/Glu293. Overall, the number of amino acid substitutions between the HyHK sequences of A. macleodii strains varied from 1 to 18, except for the strain from the Black Sea with 61 aa differences. Recently, the strain Black_Sea_11 was identified as A. macleodii strain through a 16S rRNA sequence analysis (López-Pérez et al., 2012). However, the present phylogenetic analysis of HyHK gene has shown that the strain Black_Sea_11 belongs to a cluster containing A. abrolhosensis with a 100% sequence identity.

To confirm the taxonomic status of the isolate, the fatty acid (FA) composition of cell-wall lipids was analyzed; the bacteria were grown with and without K$_2$TeO$_3$. The dominant FAs in both cases were 16:1 (n-7), 16:0, 17:1, 15:0, 18:1 (n-7), and 17:0 (Table 1).
The analysis of resistance to potassium tellurite did not reveal any inhibition of growth of Alteromonas macleodii strain 2328 at all the tested K₂TeO₃ concentrations (from 10 to 2500 μg/mL) on Marine agar 2216. On day 2 of growth, black colonies were visible on the agar surface, which indicated the reduction of potassium tellurite to tellurium and accumulation of the latter in the cell culture. The cells did not lose their viability on the marine agar with 2500 μg/mL K₂TeO₃, as shown by their further cultivation on the same medium with no potassium tellurite. The visible growth appeared after a day of cultivation, and after 2 days the morphology of colonies completely matched the described one for the growth conditions without tellurite ions. Minimal inhibitory concentration (MIC) was not determined, because no inhibition of growth was observed at all the tellurite concentrations.

Bacterial growth curves (Fig. 4) showed an elongated lag phase and a slightly shorter stationary phase in the case of exposure to K₂TeO₃ at a concentration of 1000 μg/mL as compared to normal conditions (growth with no tellurite ions).

An energy-dispersive X-ray spectroscopy analysis of the elemental composition of bacteria, carried out using an X-MAX Silicon Drift detector (Oxford Instruments, GB) mounted on an EVO40 scanning electron microscope (Carl Zeiss), showed the presence of Te atoms in specimens of Alteromonas macleodii 2328, as was shown by their further cultivation on the same medium with no potassium tellurite. The visible growth appeared after a day of cultivation, and after 2 days the morphology of colonies completely matched the described one for the growth conditions without tellurite ions. Minimal inhibitory concentration (MIC) was not determined, because no inhibition of growth was observed at all the tellurite concentrations.

The ultrastructural study of bacteria of the 2328 strain, examined using the TEM method, showed that these are typical gram-negative rods of 1.4 μm in width and 1.4 μm in length, surrounded by the double-membrane cell wall (Fig. 6A–F).

In the control, the culture grown on Marine agar 2216 consists of cells with different degrees of maturity, from long thin bacilli with the electron-dense cytoplasm and extensive nucleoid zone to oval ones, with the comparatively small nucleoid zone and cytoplasm of medium electron density. In the cytoplasm, electron-light vacuoles can be present also in the extracellular space, where they can have a shape of globules and create large polymorphous crystals (Fig. 6F).

### Table 1

| Fatty acids | P 2328 without K₂TeO₃ | P 2328 with K₂TeO₃ | A. macleodii† |
|------------|-----------------------|--------------------|---------------|
| 12:0       | 0.42                  | 0.27               | n/d           |
| 13:0       | 0.47                  | 0.21               | 0.4           |
| 14:0       | 0.52                  | 0.61               | 0.2           |
| 14:0       | 3.10                  | 1.09               | 3.0           |
| 15:0       | 4.06                  | 3.3                | 3.7           |
| 15:1       | 1.16                  | 0.59               | 1.7           |
| 16:0       | 3.72                  | 2.79               | 2.0           |
| 16:1 (n-7) | 28.35                 | 14.40              | 34.6          |
| 16:0       | 21.86                 | 28.15              | 25.0          |
| 17:0       | 0.79                  | 0.70               | 0.4           |
| 17:1 (n-8) | 11.77                 | 11.58              | 8.0           |
| 17:0       | 5.71                  | 12.41              | 5.6           |
| 18:1       | 0.84                  | 0.30               | 0.9           |
| 18:1 (n-7) | 12.76                 | 17.39              | 9.4           |
| 18:1       | 2.16                  | 2.96               | 1.0           |
| 19:1       | 0.82                  | 1.56               | 0.2           |

Note: *Data from Mikhailov et al. (2006).

### 3. Discussion & conclusion

Members of the species Alteromonas macleodii were first isolated from sea water off the Hawaiian Islands (Baumann et al., 1972). In our research, the 2328 strain has been isolated from the enrichment culture of the microalgae P. foraminosum, maintained for 8 months without subculturing. According to our observations, Alteromonas macleodii 2328 is a component of associative microflora of microalgae which has been cultivated under laboratory conditions during 5 years. It was previously noted that phytoplankton stimulates proliferation of Alteromonas macleodii, being a supply of available organic matter (López-Pérez et al., 2012). There is also an inverse relationship: thus, bacteria associated with dinoflagellates play a significant role in the production of various metabolites by microalgae, including toxins (Pérez-Guzmán et al., 2008).

This study is the first report on the molecular phylogeny of Alteromonas based on the multi-sensor hybrid histidine kinase gene. Furthermore, we conducted a comparative analysis of 16S rRNA, including all available intragenomic copies from all the Alteromonas strains and other species of Alteromonas from the NCBI database.

Allele variants of 16S rRNA of Alteromonas macleodii 2328 are distinct at 19 nucleotide positions. One of them corresponds to the several intragenomic alleles of Alteromonas strains from GenBank, while the other copy corresponds to another allele from the genome of other strains and species of Alteromonas. Alleles 1 and 2 in 2328 are paralogous to each other, as are 1 and 2 in genome of Alteromonas strains from GenBank. However, sequences of allele 1 from different strains are orthogonal, as are alleles 2.

The genetic heterogeneity of Alteromonas macleodii at the intra-species level is low with a 99% sequence similarity, and a high level of genetic differentiation of Alteromonas was detected at the inter-species level. The obtained results revealed that the HyHK gene is a suitable molecular marker to be used in species identification and phylogenetic analysis of Alteromonadaeae, because it is more variable than 16S rRNA and it does not have multiple copies. This region may also be used to develop new molecular markers for species-specific identification of Alteromonas and discrimination of Alteromonas spp.

The results indicate the importance of combined use of 16S rRNA and HyHK markers for correct species identification in Alteromonas. The use of two genetic markers made it possible to determine, with a high degree of reliability, the belonging of the 2328 strain to the species Alteromonas macleodii, which was confirmed by data of the chemotaxonomic analysis.

The fatty acid (FA) composition of cell-wall lipids in the investigated strain characterizes it as a member of the genus Alteromonas (Svetashev et al., 1995). The two variants of the 2328 strain, grown with and without potassium tellurite, had some differences in the FA composition (Table 1). The strain exposed to potassium tellurite showed an amount of 16:1 (n-7) decreased two-fold compared to that grown without tellurite, and the amount of 17:0, on the contrary, increased two-fold. It is likely that tellurite ions affect the biosynthesis of these FAs. This fact seems to be worth consideration and requires further research.

An analysis of the growth dynamics of the 2328 strain showed that potassium tellurite ions exert a toxic effect, which was manifested as a decrease in the rate of growth and variations in the duration of its phases (Fig. 4). El-Moselhy et al. (2013) suggested that a reduction in the stationary phase duration is a result of the metal’s toxic effect on the physiological processes in bacteria during the exponential growth phase. Elongation of the lag phase can obviously be explained by bacteria’s adaptation. The stationary phase of growth of the 2328 strain exposed to metalloid ions was somewhat shorter compared to normal. A similar pattern was observed in the case of cultivation of Alteromonas macleodii with cadmium (El-Moselhy et al., 2013). It should be noted, however, that the effect of high potassium tellurite concentrations on the 2328 strain was negligible. Thus, when exposed to the toxicant, the 2328 strain had a high level of cell density in the stationary phase, which was only slightly
The lag phase was longer compared to the control, but then the cells resumed growth curves in the presence of \( \text{K}_2\text{TeO}_3 \), and the presence of the toxicant significantly influenced the dynamics of their growth (Arenas et al., 2014). There was a slight toxic effect of \( \text{TeO}_3^{2-} \) on the growth of a number of gram-positive and gram-negative bacteria isolated from Latin America (Figueroa et al., 2018). In strains of \( \text{Staphylococcus sciuri} \) and \( \text{Exiguobacterium acetylicum} \) exposed to the toxicant in the culture medium, the lag phase was longer compared to the control, but then the cells resumed their growth. In two strains of \( \text{Acinetobacter schindleri} \) and \( \text{E. acetylicum} \), the growth curves in the presence of \( \text{K}_2\text{TeO}_3 \) and without it almost coincided.

The characteristic feature of the \( \text{A. macleodii} \) 2338 is its ability to reduce tellurium to elemental metal. The presence of Te in the specimens of bacteria grown on a medium containing \( \text{K}_2\text{TeO}_3 \) is confirmed by data of X-ray spectroscopy. As for other members of the genus, this information is almost unavailable in the literature. Nevertheless, some traits are noted which indirectly indicate the ability to reduce metals. In particular, the isolate from the surface sea water, which produced unusual exopolysaccharides (EPS) and was able to absorb such metals as copper, nickel, and chromium, was preliminarily attributed to \( \text{A. marina} \) (Zhang et al., 2017). Earlier, it was reported about the production of EPS binding heavy metals by a strain of \( \text{Alteromonas} \) sp. isolated from deep-sea polychaetes (Vincent et al., 1994). The presence of EPS is characteristic for tellurite-and selenite-reducing \( \text{Pseudoalteromonas} \) from deep-sea hydrothermal vents (Rathgeber et al., 2002, 2006).

The ability to reduce tellurium to elemental state is reported for bacteria of different taxa isolated from extreme deep-sea vents with high metalloid concentrations such as, in particular, deep-ocean vents (Rathgeber et al., 2006). Other active reducers are found mostly at sites enriched in or polluted by metalloids (Sepahei and Rashetnia, 2009; Amoozegar et al., 2008). However, tellurite-reducing bacteria are also isolated from usual, not extreme, habitats (Ollivier et al., 2008; Soda et al., 2018). Obviously, bacteria have different types of specialized mechanisms providing them with a resistance to heavy metals and metalloids.

A substantial part of determinants of gram-negative bacteria's resistance to tellurite is localized on plasmids (Turner et al., 1999). Moreover, the membrane-associated nitrate reductases NarG and NarZ play a key role in reduction of potassium tellurite in \( \text{E. coli} \) (Avazeri et al., 1997). Calderon et al. (2006) report isolating a protein that reduces tellurite to tellurium via NADPH oxidation. Evidence has been obtained that anaerobic bacterial growth can be achieved by using tellurium oxyanions as electron acceptors (Sepahei and Rashetnia, 2009).

The mechanisms of resistance to Te oxyanions are associated with their physical removal from cell. This can be achieved by either volatilization to form dimethyl telluride or reductive precipitation to form insoluble Te (0). The precipitated Te (0) can occur outside or, more commonly, inside cells (Sepahei and Rashetnia, 2009), and bacterial colonies of tellurite-reducing strains become black in color because of the elemental tellurium accumulated in them.

In this study, TEM was used for the visualization of particles of reduced Te in cells of the 2338 strain bacteria, which is traditionally applied for solving such problems. According to the literature data, the reduced Te (0) may be deposited in the form of globules or nanorod crystals (Vurkov et al., 1996; Sepahei and Rashetnia, 2009; Presentato et al., 2018), which depends on the cultivation conditions. We have shown the presence of electron-dense globules in the 2328 strain cultivated on a medium containing \( \text{TeO}_3^{2-} \); at the same time, the control bacteria grown on the medium without \( \text{TeO}_3^{2-} \) lacked such structures. Consequently, the electron-dense globules are particles of reduced Te (0). The globules are localized mostly in the cytoplasm of cells, which indicates the cytoplasmic localization of the apparatus of tellurite reduction to Te (0). Nevertheless, bacteria of the 2338 strain lack any extracellular capsuloid-like components which, according to the literature data, play the protective role by providing the metal-resistance (Vincent et al., 1994; Wrangstadh et al., 1986).

Thus, the mechanism of Te resistance in the 2338 strain bacteria is associated with its transfer into the insoluble form. Initially, metal particles are accumulated in the cytoplasm of bacterial cells. According to the literature data, a similar pattern is observed in different species of bacteria such as photosynthetic \( \text{Rhodopseudomonas palustris} \) (Xie et al., 2018), \( \text{Shewanella oneidensis} \) (Klonowska et al., 2005), and two new species \( \text{Pseudoalteromonas telluritireducens} \) and \( \text{P. spiralis} \) (Rathgeber et al., 2002, 2006).

The presence of bacteria at the final life-cycle stage, having the degraded cytoplasm and containing electron-dense globules of the reduced metal, in the studied culture allows assuming that elemental Te enters the culture medium as a result of lysis of these cells. The subsequent, already abiological processes of growth and formation of large Te (0) crystals take place in the extracellular environment.

It is worth mentioning the high resistance of the 2328 strain to tellurite ions. We could not increase the potassium tellurite concentration in the medium higher than 2500 \( \mu \text{g/mL} \) due to the formation of white...
opaque precipitate hampering the further analysis; other researchers faced the same problem (Rathgeber et al., 2002). At all the tested concentrations, the 2328 strain did not manifest visible signs of growth inhibition. An analysis of growth curves showed some features associated with the presence of high $K_2TeO_3$ concentrations in the medium, but they did not prevent reaching a significant cell density by the stationary phase of growth. Such feature of the strain makes it quite attractive for biotechnological applications as a biological object extracting the metalloid, which is extremely rare in the lithosphere, and concentrating it in cells.

4. Materials and methods

4.1. Origin of A. macleodii strain 2328 and analysis of phenotypic traits

The 2328 strain was isolated from a culture of the dinoflagellate *P. foraminosum* (clone MBRU_Pr07) maintained in the collection of marine microalgae “Marine Biobank” (National Scientific Center of Marine Biology (NSCMB), FEB RAS) http://marbank.dvo.ru. The clone MBRU_Pr07 is a producer of dinophysis toxin-1 (DTX-1) (Kameneva et al., 2015). Supernatant from the batch culture, preliminarily maintained during 8 months without subculturing and adding a fresh growth medium, was used in the amount of 0.1 mL, according to the standard dilution procedure, to inoculate the solid medium Plate Count agar (Merck) containing 5 mL/L of 2% potassium tellurite solution (Sigma-Aldrich), according to the standard cultivation procedure. The MBRU2328 strain of *A. macleodii* is stored at −80 °C in the LiCONiC STC Compact ULT automated biobanking system of Marine Biobank, NSCMB FEB RAS under accession number MBRU_B-AM-001 (see http://marbank.dvo.ru/index.php/en/). Besides, the bacterial strain is stored at −85 °C in cryotubes with seawater containing 1% peptone, 30% glycerol, and MgSO4 at a concentration of 35 g/L in the Museum of Marine Heterotrophic Bacteria, NSCMB FEB RAS.

Dishes with inoculations were kept in an incubation chamber at 23 °C.

![Fig. 5. X-ray spectra of the 2328 strain bacteria grown on a standard medium (A) and a medium with the addition of $K_2TeO_3$ (B). The presence of Te atoms confirms the ability of the studied bacteria to reduce tellurite to Te (0). X-axis is the spectral range, keV; Y-axis is the intensity of the signal, imp/s/keV.](image-url)
Some colonies, after 3 passages on the Marine agar 2216, were taken for the further study.

The strain was cultivated on Marine agar 2216 and Marine Broth 2216. The morphology of cells was studied in gram-stained smears under a Carl Zeiss Primo Star light microscope; to characterize the ultrastructure of cells grown with and without potassium tellurite, transmission electron microscopy (TEM) was applied.

The analysis of FA lipid content of cell walls in two variants of the 2328 strain, grown on the Marine agar 2216 with 100 μg/mL of K₂TeO₃ and without it, was conducted as described earlier (Svetashev et al., 1995). For this, the culture had been grown during 3 days at 23°C.

4.2. DNA extraction, PCR amplification, and DNA sequencing

Bacterial genomic DNA was extracted with a NucleoSpin® Tissue Kit (MACHEREY-NAGEL GmbH & Co.). Amplifications were carried out using the DreamTaq DNA polymerase (Thermo Fisher Science, USA) under aseptic conditions. The nuclear 16S rRNA and nonribosomal peptide synthetase (NRPS)/polyketide synthase (PKS) genes were selected as targets for PCR. The amplification was carried out in a thermal cycler for 40 cycles using the following reaction protocol: denaturation of DNA at 95°C for 30 s, primer annealing at 56°C for 1 min, and primer elongation at 72°C for 1 min 15 s, with the initial period of 3 min at 94°C and the final period of 7 min at 72°C. The PCR product (~1412 bp) of 16S rRNA was generated with 8F (Zhou et al., 1996) and 1492 primers (Lane, 1991). Randomly selected primer sets were used to screen for PKS-NRPS-encoding genes. The PKS-NRPS-specific primer pairs that were tested for the analysis of the 2328 strain, isolated from the toxic marine dinoflagellate P. foraminosum, included as follows: M4/M5 and M13/M14 (Schembri et al., 2001), mcyA-Cd/FR (Hisbergues et al., 2003), mcyDF/mcyDR, and mcyE-F2/mcyE-R4 (Rantalä et al., 2004). Only the mcyE-F2/mcyE-R4 primer pair successfully amplified the fragment, which was a candidate for PKS-NRPS-encoding genes with PCR-product at ~1086 bp. Enzymatic PCR cleanup was treated with Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (rSAP) (New England Biolabs). Purified products were cloned using the InstAclone PCR Cloning Kit (Fermentas, Lithuania) into the pTZ57 R/T DNA cloning vector. Bacterial clones were blue-white screened for inserts and colony-PCR using the universal primers M13 and then sequenced with a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing reaction products were cleaned by gel filtration through Sephadex G-50 Superfine (GE Healthcare Bio-Sinces) and sequenced on the ABI3500 automated sequencer (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). The sequences were deposited in GenBank under the accession nos. MH569097–MH569098 and MH568796.

Alignments were performed using Clustal W and MAFFT implemented in Geneious R11 software (v11.0.3, Biomatters Limited, Auckland, New Zealand) and checked visually to exclude ambiguous regions which are difficult to align. The support values on the branches were as follows: SH-aLRT support (%)/aBayes support/ultrafast bootstrap support (%) performed in the programs implemented in Geneious R11.0.4 and IQ-TREE.

To study the overall 16S rRNA diversity within the strains and between the species, the mean pairwise genetic distances between sequences were calculated in MEGA 7 (Kumar et al., 2016), using the
LogDet (Tamura-Kumar) model (Tamura and Kumar, 2002), with 500 bootstrap replicates and the “pairwise deletion” option of missing data. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The matrix of pairwise genetic distances is provided in Supplementary Table S1.

Bayesian (BI) and Maximum-likelihood (ML) trees for 16S rRNA were constructed using Geneious R11.0.4 and IQ-TREE (Nguyen et al., 2014) under the best-fitting substitution-model. The GTR + F + R2 according to Akaike Information Criterion and TPM2u + F + R2 according to Bayesian Information Criterion were used for the 16S rRNA dataset. Model of rate heterogeneity: Invar + Gamma with 4 categories; proportion of invariant sites: 0.7628; Gamma shape alpha: 0.6823. Branch supports was assessed using the following options: bootstrap method based on 1000 ultra-fast bootstrap (UFBoot2) replicates (Hoang et al., 2017) with a 0.99 minimum correlation as convergence criterion, 1000 replicates of the with SH-aLRT (approximate likelihood ratio test [aLRT] and Shimodaira–Hasegawa [SH]-aLRT) branch test [-alrt] (Guindon et al., 2010) and Approximate Bayes test [-abays] (Anisimova et al., 2011). Five alleles of 16S rRNA gene of Pseudoalteromonas atlantica T6c CP000388 were extracted from the whole genome and used as an outgroup. The phylogenetic trees were subsequently viewed and graphically edited in Inkscape 0.92.3 (https://inkscape.org/).

The LG model (an improved general amino acid replacement matrix) (Le and Gascuel, 2008) of amino acids substitution according to Akaike Information Criterion was used for the HyHK dataset. Phylogenetic trees were inferred from amino acid sequence alignments of the histidine kinase region starting at the Cache domain and ending with PAS domain-containing protein. ML and Neighbor-Joining (NJ) trees for HyHK dataset were constructed using PhyML 3.0 (Guindon et al., 2010) and Geneious Tree Builder implemented in the Geneious R 11.0.4.

4.3. Tellurite resistance and bacterial growth curves

The analysis of resistance to potassium tellurite and its reduction was carried out according to (Rathgeber et al., 2002, 2006). The strains were grown in aerobic conditions in dishes with Marine agar 2216; then the over-night culture was transferred to Marine Broth 2216 (Difco) to obtain a dense cell suspension. A loop of bacterial suspension was transferred over-night culture was transferred to Marine Broth 2216 (Difco) to obtain a dense cell suspension. A loop of bacterial suspension was transferred onto a marine agar containing 0, 10, 100, 500, 1000, 1500, 2000, and 2500 μg/mL K2TeO3, and the dishes were incubated during 5 days at 30°C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which the growth of the strain was inhibited. To clarify the issue of the loss of cell viability after a long-term (7 days) cultivation in a K2TeO3-rich medium (2500 μg/mL), the strain was subcultured on a marine agar with no potassium tellurite.

For the growth test at different conditions (with 1000 μg/mL of K2TeO3 and without it), cells of the 2328 strain grown over-night at 23°C were inoculated (1%, v/v) into Marine Broth 2216 (Difco) and cultivated in a shaking flask on Exella E5 (New Brunswick, USA) at 130 rpm (Math et al., 2012). Growth was monitored in specified time intervals by measuring the OD 600 of the cultures using a spectrophotometer Apel pd 303 (Japan) in cuvettes with 1-cm light path.

4.4. Transmission electron microscopy

Samples for TEM were fixed with 2.5% glutaraldehyde in a 0.1 M cacodylate buffer solution, post-fixed with 1% solution of OsO4 in 0.1 M cacodylate buffer, dehydrated in an acetone series, and embedded in Epon-Araldite. Ultrathin sections were cut on a Leica EM UC6 ultramicrotome (Leica Microsystems, Germany). The sections were stained with uranyl acetate and lead citrate and examined under a Libra 120 (Carl Zeiss, Germany) transmission electron microscope.

4.5. Analysis of the elemental composition of bacteria

Bacterial specimens were dried at 45°C and placed on an aluminum sample holder; the elemental composition was analyzed using an X-Max Silicon Drift Detector (Oxford Instruments, GB) mounted on an EVO40 scanning electron microscope (Carl Zeiss).

Declarations

Author contribution statement

Irina A. Beleneva: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kseniya V. Efimova, Marina G. Eliseikina: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Vasilii I. Svetashev: Performed the experiments; Analyzed and interpreted the data.

Tatiana Yu. Orlova: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at MBRRU - Marbank NSCMB FEB RAS under the accession number MBRRU Pr07.

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