Potential of tellurite resistance in heterotrophic bacteria from mining environments

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Highlights
Sites with continuous high metal pressure as a source of Te-resistant bacteria diversity
Organism-specific Te (IV) reduction produces unique Te (0) insoluble structures
Unveiled Te resistance genetic determinants and arrangements in Bacillales

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SUMMARY
Untreated mining wastes and improper disposal of high-tech devices generate an environmental increase of bioavailable metalloids, exerting stress on autochthonous microbial populations. Tellurium is a metalloid, an element with raising economic importance; nevertheless, its interaction with living organisms is not yet fully understood. Here we characterized aerobic heterotrophic bacteria, isolated from high metal-content mining residues, able to resist/reduce tellurite into tellurium structures and to determine the presence of confirmed tellurite resistance genetic determinants in resistant strains. We identified over 50 tellurite-resistant strains, among 144 isolates, eight strains reduced tellurite to tellurium at different rates, with the concomitant production of tellurium deposits. Most tellurite resistance genes were found in strains from Bacillales, with the prevalence of genes of the ter operon. This work demonstrated that bacterial isolates, from environments with a persistent selective pressure, are potential candidates for uncovering strategies for tellurite resistance and/or production of valuable Te-containing materials.

INTRODUCTION
Tellurium, although rare in Earth’s crust, is highly sought after in the field of nanotechnology (Mayers and Xia, 2002) owing to the combination of useful properties such as photoconductivity, nonlinear optical response, and relevant thermoelectric capacity. Te exists in nature mostly in five oxidation states, from those Te (IV) or Te (VI) is the most biologically relevant as both are soluble in water and therefore bioavailable. The latter anion is less soluble and less toxic than Te (IV) (Ollivier et al., 2011). Most studies have focused on the most soluble and toxic Te oxyanion, Te (IV), while the toxicity of the elemental form is yet to be determined. The increasing demand for Te will generate the production of components containing this metalloid, which, by reaching their end-of-life, will result in high-tech waste disposal. Improper disposal practices and waste management may create the conditions for Te leaching in the form of Te (IV) and Te (VI), which will seep into the surrounding environment, creating contaminated zones with niches subjected to high selective pressure (Kyle et al., 2011). The bactericidal activity of this metalloid was recognized before the use of antibiotics (Arenas et al., 2014), with a toxicity of the oxyanion tellurite occurring at concentrations as low as $8 \times 10^{-6}$ M in Escherichia coli (Turner et al., 2012).

Biological tellurite resistance (Te’) is not ubiquitous and occurs mainly by the reduction of the anionic form to a less toxic form. To this date, two reduction mechanisms have been described: the generation of the volatile di-methyl tellurite or the direct conversion to Te (0). The resistance to the reduction of Te (IV) to Te (0) can be mediated by several genetic mechanisms. Some are unspecific, such as nitrate-reductases (Sabaté et al., 2001) or other molybdopterin-containing enzymes (Theisen et al., 2013), and others are specific/semi-specific mechanisms.

To this date, although sometimes the mechanisms of resistance are not clear, a few genetic determinants have been linked to Te’. An example of Te’ by methylation is the tehAB genetic system (Dyllick-Brenzinger et al., 2000), which confers moderate levels of resistance in E. coli K12, $1 \times 10^3$ M, this Te’ was also identified in Haemophilus influenzae (Taylor, 1999). In this system, the TehB protein has been identified as a methyltransferase. Te’ by reduction has been thoroughly explored in a Rhodobacter sp. strain possessing the trgAB/cysK determinants (Borghese et al., 2014, 2017). However, the genetic determinant conferring the highest Te’ is the ter operon. The ter operon was initially observed in plasmid pMER610 and R478 and contains seven genes (terZABCDEF). Within the core ter operon three main protein families are present, the
Table 1. Minimal Inhibitory Concentrations in solid and liquid R2A media, in the presence of Te (IV) of identified bacterial isolates from Panasqueira. Mathematical signals indicate growth compared to control situation: + - higher than control, ± - comparable to control and – lesser than control.

|                     | Solid media | Liquid media |
|---------------------|-------------|--------------|
|                     | Te(IV) 10⁻³mM | Control 0.5 1 3 | Te(IV) 10⁻³mM | Control 0.5 3 |
| Panasqueira Water  |             |              |              |
| mine                |             |              |              |
| B2A1ln2             | Rhodanobacter glycinis | +         | ±         | +         | –         |
| B2A2W2              | Diaphorobacter polyhydroxybutyratevarans | +         | +         | –         | –         |
|                     |             |              |              |
|                     |             |              |              |
| Basin 1 core        |             |              |              |
| sediments           |             |              |              |
| B2A1Ga1             | Serratia glossinae | +         | +         | ±         | +         | –         |
| B1S542 5W23         | Bacillus zhanghouensis | +         | +         | +         | +         | –         |
| B1S542 5W30         | Paenibacillus lupini | +         | +         | –         | +         | –         |
| B1S542 10W27        | Bacillus aryabhattachi | +         | +         | –         | +         | –         |
| B1S542 5W4          | Bacillus toyonensis | +         | ±         | –         | +         | –         |
| B1S542 10W15        | Paenibacillus amyloyticus | +         | +         | ±         | +         | –         |
| B1S542 10W28        | Paenibacillus xylanexedens | +         | +         | +         | –         | –         |
| B1S542 5W10         | Cellulomonas fimi | +         | +         | +         | +         | –         |
| B1S422 2As4         | Cellulomonas cellasea | +         | ±         | ±         | ±         | +         |
| B1S422 10W16        | Bacillus simplex | +         | ±         | –         | +         | –         |
| B1S422 5W6          | Fictibacillus enclensis | +         | +         | ±         | –         | –         |
| B1S422 10W32        | Fontibacillus aquaticus | +         | ±         | –         | +         | –         |
| B1S222 10W5         | Paenibacillus xinjiangensis | +         | +         | –         | +         | –         |
| B1S422 3W7          | Paenibacillus etheri | +         | ±         | –         | +         | –         |
| B1S422 5W33         | Paenibacillus sp. | +         | ±         | ±         | ±         | +         | –         |
| B1S542 3W20         | Bacillus altudinins | +         | +         | –         | +         | –         |
| B1S532 10W7         | Paenibacillus sp. | +         | +         | –         | +         | –         |
| B1S532 10W12        | Micrococcus aloeverae | +         | +         | ±         | –         | –         |
| B1S222 3W6          | Paenibacillus xinjiangensis | +         | ±         | –         | +         | –         |
| B1S422 3WS          | Paenibacillus etheri | +         | +         | –         | +         | –         |
| B1S542 10W7         | Bacillus safensis | +         | +         | –         | +         | +         |
| B1S542 10W19        | Bacillus altudinins | +         | +         | –         | +         | +         |
| Panasqueira Basin  |             |              |              |
| 2 core sediments    |             |              |              |
| B2S342 2As5         | Bacillus altudinins | +         | +         | +         | +         | +         |
| B2S322 10W11        | Psychrobacillus psychrodurans | +         | ±         | ±         | –         | –         |
| B2S342 10W7         | Tessaracoccus lapaicaptus | +         | ±         | –         | +         | –         |
| B2S322 2As8         | Flavhumibacter stibioxidans | +         | ±         | –         | +         | –         |
| B2S332 3W8          | Nocardioides paksitanensis | +         | ±         | ±         | –         | –         |
| B2S322 2As7         | Cellulomonas cellasea | +         | ±         | ±         | –         | –         |
| B2S322 10W1         | Bacillus simplex | +         | +         | –         | +         | –         |
| B2S322 3W3          | Bacillus zhanghouensis | +         | +         | –         | +         | –         |
| B2S222 2As2         | Rhizobium selenitreducens | +         | ±         | ±         | –         | –         |
| B2S322 5W2          | Cellulomonas cellasea | +         | ±         | ±         | +         | –         |
| B2S322 3W14         | Cellulomonas cellasea | +         | ±         | ±         | +         | –         |
| B2S222 5W24         | Bacillus zhanghouensis | +         | +         | +         | +         | +         |
| B2S322 10W3         | Actinotalea ferrariae | +         | +         | +         | +         | –         |
| B2S322 10W11        | Cellulomonas cellasea | +         | ±         | ±         | +         | –         |
| B2S222 10W19        | Mesorhizobium qingshengii | +         | ±         | ±         | +         | –         |
| B2S222 2As20        | Cellulomonas cellasea | +         | ±         | ±         | +         | –         |
| B2S222 5W10         | Cellulomonas marina | +         | ±         | –         | +         | +         |

(Continued on next page)
TerB, the TerC, and the TerD family. The three family proteins terB, terC, terD, and terE have been shown to be directly involved in Te' (Turner et al., 1994). Several genetic arrangements of the ter operon have been identified among Actinobacteria, Firmicutes, and Gammaproteobacteria lineages (Anantharaman et al., 2014). In some of these genetic organizations, additional genes for Te’ are present, such as the genes encoding protein TelA and homologs (Franks et al., 2014). The only directly observable effect of Te’ determinants has been the extracellular deposition of Te-containing crystals in the vicinity of the outer membrane, with reduced intracellular Te bioaccumulation (Kormutakova et al., 2000). Early studies also link the ter operon to phage inhibition (Phi) and resistance to pore-forming colicins (PacB) in E. coli (Taylor, 1999).

As verified for other metals, bioreduction leads to the formation of Te-containing intra-/extracellular nanostructures. Diversity of microorganisms has shown the capacity to form these nanostructures, such as Enterobacter cloacae (Contreras et al., 2018), Ochrobactrum sp. (Zonaro et al., 2017), Shewanella sp. (Vai-gankar et al., 2018), and Rhodobacter capsulatus (Borghese et al., 2014, 2017). An increasing interest in understanding the formation of these structures is the result of the growing potential range of applications for bio-produced nanoparticles covering fields such as optical imaging (Plaza et al., 2016) or novel battery technology (Kim et al., 2015). Consequently, the isolation and characterization of organisms from a large number of different environments with potential for Te ions reduction gained importance, namely sea sediments (Csotonyi et al., 2006; Ollivier et al., 2008), fouled waters (Chien and Han, 2009), Antarctic samples (Arenas et al., 2014; Plaza et al., 2016), mine tailings (Maltman et al., 2015) among others. One environment of interest is mines and long-lasting mine tailings where the continuous presence of different mixtures of metals exerts selective pressure on the autochthonous microbial community. The Te (IV) reduction capacity of metabolically active isolates was determined by following Te (IV) depletion and by the visualization of the resulting Te insoluble particles. The presence of genetic determinants conferring resistance to Te (IV) was confirmed by targeted PCR.

RESULTS

Isolation, identification, and screening for tellurite-resistant bacteria

From the three sites sampled (Figure 1) a total of 144 different isolates were collected. From Panasqueira mine, 95 different isolates were recovered, 83 from cores of the tailings basins, 12 from the tailings surface waters. From the entire, identified, collection of Panasqueira microorganisms, 47 were resistant to $1 \times 10^{-3}$ M Te (IV) and of these, 27 were resistant to the concentration of, at least, $3 \times 10^{-3}$ M Te (IV) (Table 1). From
Jales samples, a total of 44 organisms were isolated in R2A agar and 24 were identified, from which 23 isolates were resistant to concentrations of \(1 \times 10^{-3}\) and \(3 \times 10^{-3}\) MT e(I V) and to \(3 \times 10^{-3}\) MT e (IV). From Aljustrel the enrichment yielded five strains, of which three were able to grow in solid media spiked with concentrations of up to \(5 \times 10^{-3}\) MT e(I V) (Table 2).

Strains with MIC higher than \(5 \times 10^{-4}\) M Te (IV) were identified based on 16S rRNA gene sequence and phylogenetic relatedness with type strains from the NCBI database. The identification results grouped the strains into Actinobacteria (44.7%), Firmicutes (34.2%), and Proteobacteria (21.1%) phyla. The identified bacteria with MIC over \(1 \times 10^{-3}\) M Te (IV) were tested for increasing Te (IV) concentrations in liquid media. The most representative resistant organisms, with a MIC of \(5 \times 10^{-5}\) M Te (IV), belonged to the genera Bacillus, Paenibacillus, and Cellulomonas (Tables 1 and 2).

### Growth kinetics in the presence of tellurite and reduction ability

The strains resistant to \(5 \times 10^{-4}\) M Te (IV) in liquid growth (Table 1) were selected for the evaluation of tellurite reduction. Twelve strains did not show an ability to remove Te (IV) from the media, as the same or
marginally different values of Te (IV), in solution, were detected throughout their growth. The eight remaining strains had their growth affected by Te (IV) (Figure 2). The specific growth rates of cells grown in $5 \times 10^{-4}$ M Te (IV) were lower than the control culture, without Te (IV), for all eight strains (Figure 2). Strains Paenibacillus pabuli ALJ109b, Bacillus mycoides ALJ98a, Paenibacillus taichungensis ALJ98b and Cellulomonas marina strain B2S222 5W10 maintained a growth rate similar to the control condition in concentrations up to $2.5 \times 10^{-4}$ M Te (IV). Bacillus safensis strain B1S542 10W7 (10W7) increased its growth rate to $2.5 \times 10^{-4}$ M Te (IV), decreasing at the highest concentrations.

The strains that performed better at removing Te (IV) from liquid media were Panasqueira isolates Bacillus altitudinis strain B1S542 3W19 (3W19), B. safensis 10W7, and C. marina 5W10 (Figure 3) with Re values ranging from 6.03 to 4.39 Δmg.DO$^{-1}$ (Table 3). The isolates from Aljezur B. mycoides ALJ98a, P. taichungensis ALJ98b and P. pabuli ALJ109b showed moderate depletion efficiencies, with values ranging from 3.35 to 1.08 Δmg.DO$^{-1}$. Finally, the two Jales isolates Mesorhizobium qingshengii Jales Te59 and Mesorhizobium qingshengii Jales Te58 had the lowest depletion efficiencies, with reduction rates in the mid-hundreds of Δmg.DO$^{-1}$. The time required for each different strain to reach the late exponential phase was very distinct, ranging from 8 to 48 h. When considered, B. altitudinis 3W19 remained the highest performer with a reduction rate of 0.75 Δmg.DO$^{-1}$.h$^{-1}$, clearly higher than the second-best performer B. safensis 10W7, with a Re of 0.23 Δmg.DO$^{-1}$.h$^{-1}$ (Table 3).

**Tellurium aggregates production**

Visual identification of Te-containing aggregates was performed by SEM with the identification of Te by coupled EDS for the six strains showing Re over 1 mg.DO$^{-1}$ and Re over 0.05 Re.h$^{-1}$. These were prepared for SEM-EDS imaging as described in the methods section. Cell pellets of all strains revealed the presence of high-density deposits that by EDS were confirmed to have Te in varying abundances (Figure 3). B. safensis 10W7, B. altitudinis 3W19, and C. marina 5W10 formed shard or string-like Te-containing particles, white arrows in Figure 4, all other strains revealed Te-containing nanostructures with unclear geometries (Figure 4).
Screening for tellurite resistance genes

Resistance genes for Te (IV) were found in nine strains mostly belonging to the Bacillus genus. Gene terZ from the TerD family is the most ubiquitous in the strains tested being present in nine strains, Bacillus altitudinis 3W19, Bacillus safensis 10W7, Bacillus mycoides ALJ98a, Mesorhizobium qingshengii Jales Te55, Cellulomonas marina 5W10, Bacillus altitudinis 3W19, Bacillus safensis 10W7, Bacillus mycoides ALJ98a, Paenibacillus tundrae ALJ98b and Paenibacillus pabuli ALJ109b. Comparison of growth in selected concentration of Te (IV), Control (no Te (IV), 1 x 10^{-4} M Te (IV), 2.5 x 10^{-4} M Te (IV), and 5 x 10^{-4} M Te (IV) was conducted in triplicate, with standard deviations indicated in error bars. Statistical significance is represented by symbols *(p \leq 0.05), **(p \leq 0.01) and ****(p \leq 0.001).

Organization of the ter genetic determinants

Bacillus strains possessing more than one element of the ter gene cluster were considered for the determination of the arrangement of the constituting ter genes. From the eight combinations selected for ter genes arrangement only two produced amplicons. All amplicons produced were sequenced and an identification of the amplified region was presented for all the identifiable regions. The four strains tested had amplicons from terZ forward to terC reverse, contrarily only B. subtilis ALJ98a had an amplicon.
from terC forward to telA reverse (Figure 5). Fragment sizes from amplicon terZ-terC ranged from 1.4 kb to 2.9 kb, approximately, with amplicons in each strain varying in number and in size. In *B. mycoides ALJ98a* and *B. safensis 10W7* only one amplicon resulted from the fragment terZ-terC PCR, of approximately 2.9 kb. PSI-Blast identified, in *B. safensis 10W7*, from 5’ to 3’, two TerD family protein/stress response protein (Id = 99.48%/Id = 99.49%) and one TerC family protein (Id = 98.98%). In *B. mycoides ALJ98a*, from 5’ to 3’, one TerD family protein (Id = 99.23%) and one TerC family protein (Id = 99%) (Figure 5 and Table 4). Both *B. altitudinis 3W19* and *B. zhangzhouensis 5W24* produced multiple fragments in terZ-terC PCR. Strain 3W19 produce a fragment of approximately 2.7 kb with, from 5’ to 3’, two TerD family protein (Id = 100%/Id = 99.23%) and one TerC family protein (Id = 99.45%). The smaller amplicon from strain 3W19 that was possible to sequence was 1.4 kb and was identified, from 5’ to 3’, one TerD family protein (Id = 100%) and one TerC (Id = 99.16%). Strain 5W24 produces a fragment of approximately 2.7 kb with, from 5’ to 3’, one VWA domain-containing protein (ID = 99.76%), one tellurium resistance protein TerD (Id = 100%), and one TerC/Alx family metal homeostasis membrane protein (Id = 100). The smaller amplicon from strain 5W24 that was possible to sequence was 2.1 kb and was identified, from 5’ to 3’, one tellurium resistance protein TerD (Id = 100%), one TerD family protein (Id = 99.35%), and one TerC/Alx family metal homeostasis membrane protein (Id = 100%) (Figure 5 and Table 4). The terC-telA amplicon obtained in strain ALJ98a was 2.9 kb in size and was identified, from 5’ to 3’, one TerC family protein (Id = 99.35%) and one toxic anion resistance protein (Id = 99.09) (Figure 5 and Table 4).

**Table 3. Description of the reduction efficiency (Re) and reduction rate (Rr) for selected organism from three different mining sites**

| Organism              | Origin    | Re (Δmg/DO) | SD  | Rr (mg/DO/h) | SD  |
|-----------------------|-----------|-------------|-----|--------------|-----|
| *Mesorhizobium qingshengii* Jales TeS8 | Jales     | 0.88        | 0.31| 0.04         | 0.013|
| *Mesorhizobium qingshengii* Jales TeS9 | Panasqueira | 0.52        | 0.04| 0.02         | 0.001|
| *Cellulomonas marina* 5W10 | Panasqueira | 4.39        | 0.51| 0.09         | 0.011|
| *Bacillus altitudinis* 3W19 | 6.03        | 0.12        | 0.75| 0.005        |
| *Bacillus safensis* 10W7 | 5.49        | 0.23        | 0.23| 0.01         |
| *Bacillus mycoides* ALJ98a | Aljezur    | 3.35        | 0.28| 0.17         | 0.014|
| *Paenibacillus tundrace* ALJ98b | 1.08        | 0.21        | 0.05| 0.011        |
| *Paenibacillus pabuli* ALJ109b | 1.26        | 0.05        | 0.06| 0.003        |
DISCUSSION

The mining environments have characteristic elements that shape microbiome including pH, metal concentration/content, temperature, dissolved oxygen, and total organic carbon (Liu et al., 2014; Chung et al., 2019; Sibanda et al., 2019). The continuous exposure to a high concentration of different metals and low carbon selects for distinct microorganisms able to deal with such environments (Liu et al., 2019; Chung et al., 2019). All three sampled mining sites in this study had different features; in none of these Te was a target element for mining (Figure 1). As Te was not the metal extracted, discarded residues were expected to have relatively higher concentrations.

This work presents for the first time strains from the genera *Cellulomonas* and *Mesorhizobium*, as resistant to Te (IV). This opens a new possibility of study in Te’ mechanisms and genes. The overall diversity of Te (IV)-resistant bacteria was large with four different Phyla recovered from all three sampling sites (Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes). The results indicate that, in these specific environments, *Mesorhizobium*, *Cellulomonas*, *Bacillus* and *Paenibacillus*, may be prevalent genera with high tolerance to the presence of Te (IV). In the Panasqueira site, the Te-resistant bacterial isolates recovered belong to some of the most abundant genera of the microbial community, as determined by metagenomic (Chung et al., 2019). As previously illustrated in an earlier microbial diversity study from this site *Bacillus* and *Cellulomonas* are two of the six genera composing the local core microbiome of the mine.
tailings, with Bacillus representing the majority of the community of half the sampling sites (Chung et al., 2019). Most Mesorhizobium isolated, a common genus found in plant rhizobia, originated from samples of the Jales mining site. Unlike the Panasqueira mining site, the Jales gallery is not in operation since 1993 and was, therefore, not impacted by continuous human activity for several years, with the surrounding area being converted into agro/forestry areas. Considering the differences in samples’ characteristics (Figure 1) and human activity on the isolation site it is expected to find variations in isolates recovered.

Figure 5. Genetic organization of ter gene clusters in Bacillus strains
PCR of multi-gene amplicons, identification of amplification products, and putative genetic arrangements.

(A) PCR of fragment terC-telA. 1. Bacillus mycoides ALJ98a, 2. Bacillus zhangshouensis SW24, 3. Bacillus safensis 10W7, 4. Bacillus altitudinis 3W19 and 5. Negative control.
(B and C) PCR of fragment terZ-terC. 1. Negative control, 2. Bacillus altitudinis 3W19, 3. Bacillus zhangouensis SW24, 4. Bacillus mycoides ALJ98a and 5. Bacillus safensis 10W7.
(D) Diagram representation of genetic arrangement based on sequenced and identified amplicons a to g, with abbreviated identification. Size indication based on amplicon positioning on the gel.
Tellurium ions resistance in Bacillus is usually related to their ability to reduce Te (IV), the oxyanion toxic form, to Te (0) (Franks et al., 2014). In Paenibacillus, although there are no publicly available reports concerning Te r mechanisms, considering the phylogenetic proximity of the two taxa, likely resistant strains of Paenibacillus were also able to reduce Te (IV). Our previous study describes in detail the ability of Paenibacillus pabuli ALJ109b, from Aljustrel, to reduce Te (VI) using flagellin (Farias et al., 2021). Most of the recognized Te r genetic determinants were not found in this strain, and those that were found did not display the usual organization of Te (IV) resistance gene clusters or operons.

The impact of Te (IV) on the growth of tested strains was mainly negative, decreasing growth with increasing Te (IV) concentrations, with few exceptions. Highest significant variations in the specific growth rate were mainly observed comparing the highest concentration of Te (IV), as expected. Low concentrations of Te (IV) significantly increased specific growth rates in C. marina 5W10 and this result is unexpected and not yet found in previous studies with other bacterial strains. Nevertheless, this variation was small in comparison to the other strains tested. The Te (IV) reduction capacity of metabolically active isolates was determined by following Te (IV) depletion and by the visualization of the resulting Te insoluble particles. In this work, bacterial strains demonstrated varied Te (IV) reducing performance, either calculating by cell mass or time, between strains. Isolates from Panasqueira mine Bacillus altitudinis 3W19, Bacillus safensis 10W7, and Cellulomonas marina 5W10 performed better than isolates from Aljustrel and Jales in reduction efficiency, mostly for the highest depletion values of Te (IV) in liquid media, no records were found in the literature of Te (IV) reduction by Cellulomonas strains. The reduction rate was also higher for strains Bacillus altitudinis 3W19 and B. safensis 10W7 as well as for Bacillus mycoides ALJ98a, all these strains have high specific growth rates with small variation in growth, with $5 \times 10^{-4}$ M Te (IV), compared to most other strains. Previous works demonstrated that Bacillus sp. strain STG-83 was able to reduce 50% of 1 $\times 10^{-3}$ M of Te (IV) in 104 h (Soudi et al., 2009), and a Bacillus thermoamylolavorans strain SKC1 was able to completely reduce the $5 \times 10^{-4}$ M of soluble Te (IV) in 10 days (Slobodkina et al., 2007). In this study, Bacillus strains are, from an application perspective, the best reducers, having highest Te (IV) depletion with less cell mass in shorter times. Despite the lack of comparative measurements in the literature, for the Te (IV)-Te(0) biologic conversion, the determination, in this study, of reduction efficiencies and reduction rates allows an efficient strategy to select strains able to manufacture Te structures. Absolute variations in Te (IV) depletion, seen in

### Table 4. Identification obtained by PSI-Blast of fragments indicated on Figure 5/bottom image, left to right

| Amplicon | Blastx Identification | Accession number | Identity (%) |
|----------|-----------------------|-----------------|--------------|
| a        | TerC family protein   | WP_153252914.1  | 99.35        |
|          | toxic anion resistance protein | WP_098333049.1 | 99.09        |
| b        | TerD family protein   | WP_007498343.1  | 100          |
|          | TerD family protein   | WP_008342133.1  | 99.23        |
|          | TerC family protein   | WP_007498342.1  | 99.45        |
| c        | TerD family protein   | WP_039166394.1  | 100          |
|          | TerC family protein   | WP_144556175.1  | 99.16        |
| d        | VWA domain-containing protein | WP_202671625.1 | 99.76        |
|          | Tellurium resistance protein TerD | WP_071681669.1 | 100          |
|          | TerC/Alx family metal homeostasis membrane protein | WP_024485071.1 | 100          |
| e        | Tellurium resistance protein TerD | WP_071681669.1 | 100          |
|          | TerD family protein   | WP_141130909.1  | 99.35        |
|          | TerC/Alx family metal homeostasis membrane protein | WP_024485071.1 | 100          |
| f        | TerD family protein   | WP_007498343.1  | 99.23        |
|          | TerC family protein   | WP_144556175.1  | 99           |
| g        | Stress response protein SCP2 | SDD49373.1 | 99.49        |
|          | TerD family protein   | WP_025751616.1  | 99.48        |
|          | TerC family protein   | WP_153252914.1  | 98.98        |

All identified fragments with coverage E $\leq 0.01$. 

Tellurium ions resistance in Bacillus is usually related to their ability to reduce Te (IV), the oxyanion toxic form, to Te (0) (Franks et al., 2014). In Paenibacillus, although there are no publicly available reports concerning Te r mechanisms, considering the phylogenetic proximity of the two taxa, likely resistant strains of Paenibacillus were also able to reduce Te (IV). Our previous study describes in detail the ability of Paenibacillus pabuli ALJ109b, from Aljustrel, to reduce Te (VI) using flagellin (Farias et al., 2021). Most of the recognized Te r genetic determinants were not found in this strain, and those that were found did not display the usual organization of Te (IV) resistance gene clusters or operons.

The impact of Te (IV) on the growth of tested strains was mainly negative, decreasing growth with increasing Te (IV) concentrations, with few exceptions. Highest significant variations in the specific growth rate were mainly observed comparing the highest concentration of Te (IV), as expected. Low concentrations of Te (IV) significantly increased specific growth rates in C. marina 5W10 and this result is unexpected and not yet found in previous studies with other bacterial strains. Nevertheless, this variation was small in comparison to the other strains tested. The Te (IV) reduction capacity of metabolically active isolates was determined by following Te (IV) depletion and by the visualization of the resulting Te insoluble particles. In this work, bacterial strains demonstrated varied Te (IV) reducing performance, either calculating by cell mass or time, between strains. Isolates from Panasqueira mine Bacillus altitudinis 3W19, Bacillus safensis 10W7, and Cellulomonas marina 5W10 performed better than isolates from Aljustrel and Jales in reduction efficiency, mostly for the highest depletion values of Te (IV) in liquid media, no records were found in the literature of Te (IV) reduction by Cellulomonas strains. The reduction rate was also higher for strains Bacillus altitudinis 3W19 and B. safensis 10W7 as well as for Bacillus mycoides ALJ98a, all these strains have high specific growth rates with small variation in growth, with $5 \times 10^{-4}$ M Te (IV), compared to most other strains. Previous works demonstrated that Bacillus sp. strain STG-83 was able to reduce 50% of 1 $\times 10^{-3}$ M of Te (IV) in 104 h (Soudi et al., 2009), and a Bacillus thermoamylolavorans strain SKC1 was able to completely reduce the $5 \times 10^{-4}$ M of soluble Te (IV) in 10 days (Slobodkina et al., 2007). In this study, Bacillus strains are, from an application perspective, the best reducers, having highest Te (IV) depletion with less cell mass in shorter times. Despite the lack of comparative measurements in the literature, for the Te (IV)-Te(0) biologic conversion, the determination, in this study, of reduction efficiencies and reduction rates allows an efficient strategy to select strains able to manufacture Te structures. Absolute variations in Te (IV) depletion, seen in
Figure 3, points to B. *safensis* 10W7 as the best Te (IV) reducer by a two-fold difference compared to most strains tested. Moreover, the formation of Te precipitates was observed for selected strains. Most show a clear indication of the formation of Te (0) aggregates with different levels of structural organization. The formation of different structures of aggregates is indicative of different metabolic mechanisms involved in the formation of said structures, as is observed in previous works (Wang et al., 2018). The strain *B. altitudinis* 3W19 appears to produce two morphologically distinct Te-containing structures. The formation of string-like deposits of Te in the case of *B. safensis* 10W7 and *B. altitudinis* 3W19 is indicative of template base formation of nanoparticles on cell structures, i.e. the flagellum, like what is suggested for *P. pabuli* ALJ109b (Farias et al., 2021). Shard/rod-like structures, observed in *B. altitudinis* 3W19 and *C. marina* 5W10, were previously observed in *Pseudomonas putida* strain BS228 (Suzina et al., 1995), in *Pseudomonas pseuodocaligenes* strain Te (Forootanfar et al., 2015) or in *Bacillus selenitireducens* and *Sulfurospirillum barnesii* (Baesman et al., 2007), indicating that these geometries are common in Te-containing nanostructures.

Most of the Te’ genes identified in the strains tested belonged to the TerD family, nine terZ and three terD according to our identification, despite PSI-Blast identification available only identified most genes as belonging to TerD family. Some genes from this family, terD paralogs, terE, and terZ are highly conserved among Firmicutes lineage (Anantharaman et al., 2014), hence the higher abundance in the strains tested, mostly from the *Bacillus* and *Paenibacillus* genus. The TerC family is also represented in the strains tested but the TerB family was not found. Considering that, in the strains tested, Te (IV) resistance involve the ter operon, this result is contrary to what is seen in the literature for several strains, namely *Escherichia coli* O 157:H17, where it was demonstrated that for Te’, the genes terB, terC, terD, and terE were required (Taylor et al., 2002). This result is a variation on the known genetic organization of Te’ that may translate into a different resistance mechanism, albeit undefined as the ter Te’ mechanism is still unknown.

The study of the arrangement of genes from the ter operon in the strains possessing more than one gene revealed two possible organizations. *B. mycoides* ALJ98a indicated a structure similar to the yceC operon in *Bacillus subtilis* strain trpC2 attSPβ, with two terD family genes, a terC and a telA, only lacking the yceG upstream to telA. Contrarily, *B. altitudinis* 3W19, *B. safensis* 10W7, and *B. zahngouensis* 5W24 only share the initial structure with two/three terD family genes and a terC. Considering that telA was also present in these three strains, the genetic arrangement present seems more related to that of *Chitinophaga pinnenisi* DSM 2588 where the terC-telA region is interrupted by a biosynthetic module (Anantharaman et al., 2014). Only one amplicon of the gene terB was found, in *B. altitudinis* 3W19. TerB coding gene is more often found in ter arrangements that incorporate biosynthetic modules (Anantharaman et al., 2014). According to this author, the function attributed to TerB, is to link TerD and TerC either to the enzymes encoded by the biosynthetic module or to the diffusible product synthesized by them. The absence of terB in *B. safensis* 10W7 and *B. zhangzhouensis* 5W24 strains suggests an alternative ter system organization. In these strains unidentified protein could be involved in TerD-TerC binding to biosynthetic module enzymes or its products, or the organisms does not require TerB owing to the absence of biosynthetic module.

In this work, we demonstrated that an organized cluster of ter determinants was present in strains with resistance to Te (IV) and demonstrated Te (IV) high reducing ability, except for the slow reducer *C. marina* 5W10. The relation between Te (IV) reduction and the existence of Te’ genetic determinants is visible in the *Bacillus* genera in mining isolates. The organization, as well as the components present in the ter gene cluster, is worthy of study to understand better why many variations of this gene cluster exist and how it affects Te’.

**Limitations of the study**

This study uses exploratory PCR techniques to determine the presence of previously described Te (IV) resistance genes. Owing to a large number of bacterial strains and broad coverage of genera, families and orders the authors decided to use degenerate oligonucleotides that guaranteed amplification of desired genes from the maximum number of strains. This may have produced some false negatives for strains containing genes with sequences not contemplated in the oligonucleotide design.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104566.

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AUTHOR CONTRIBUTIONS
PF: Data curation, performed all benchwork, analyzed all data using bioinformatic and statistical analyses. Wrote the original draft. RF: Heading field sampling and processing of some analyses in the laboratory, assisting in strain isolations and growth and reduction assays. Reviewed the statistical analyses. Reviewed and edited article. PVM: Conceptualized whole experiment and secured funding. Supervised the laboratory, bioinformatics, and statistical analyses. Contribute to the original draft and revised article. All authors read and approved the article.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCE TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial strains** | | |
| Bacillus safensis strain B1S542 10W7 | This study | UCCCB 133 |
| Bacillus mycoides strain AL98a | This study | UCCCB 134 |
| Mesorhizobium qingshengii strain Jales Te58 | This study | UCCCB 135 |
| Mesorhizobium qingshengii strain Jales Te59 | This study | UCCCB 136 |
| Bacillus zhangouensis strain B2S222 SW24 | This study | UCCCB 137 |
| Paenibacillus tundrae strain AL98b | This study | UCCCB 138 |
| Bacillus altitudinis strain B1S542 3W19 | This study | UCCCB 87 |
| Cellulomonas marina strain B2S222 SW10 | This study | UCCCB 86 |
| Paenibacillus pabuli strain AL109b | Farias et al 2021 | UCCCB 89 |
| **Chemicals, peptides, and recombinant proteins** | | |
| Reasoners 2A agar (R2A) | VWR | 84671.0500 |
| Luria Bertani (LB) | NZYTech | MB14501 |
| Sodium tellurite | Sigma | 400688-10G |
| Diethyl-di-thiocarbamate | Sigma | 228680-5G |
| **Critical commercial assays** | | |
| Gel purification kit | EZNA, VWR | D2500-02 |
| **Oligonucleotides** | | |
| Primers for "Screening for tellurite resistance genes" | See Supplementary Material S1 | |
| Primers for "Organization of the ter genetic determinants" | See Supplementary Material S1 | |
| This paper | N/A | |
| **Deposited data** | | |
| 16S rRNA sequencing data from all samples | This study; Genbank | accession numbers from OK644207 to OK644280 |
| ter determinants sequencing data | This study; Genbank | accession numbers from OL344531-OL344548 |
| **Software and algorithms** | | |
| MegaX | MEGA software | https://www.megasoftware.net/ |
| EzBioCloud 16S rRNA gene sequence database v. PKSSU4.0 | ChunLab; Kim et al., 2015; Yoon et al. (2017) | https://help.ezbiocloud.net/mtp-pipeline/; https://doi.org/10.1099/jjs.0.038075-0; https://doi.org/10.1099/ijs.0.001755 |
| IDFix | SAMx | http://www.samx.com/microanalysis/products/idfix_us.html |
| Prism | GraphPad software | https://www.graphpad.com/scientific-software/prism/ |
| **Other** | | |
| Infinite® 200 PRO Fluorimeter | Tecan Instruments | https://lifesciences.tecan.com/plate_readers/infinite_200_pro |
| FEI Quanta 400FEG | ThermoFisher Scientific | N/A |
| INCA Energy 350 | Oxford Instruments | N/A |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Paula V Morais (pvmorais@ci.uc.pt).
Materials availability
Strains of interest, used in this study, were deposited in the UCCCB culture collection under the identifiers:
- *Cellulomonas marina* B2S222 5W10 (UCCCB 86)
- *Bacillus altitudinis* B1S542 3W19 (UCCCB 87)
- *Paenibacillus pabuli* ALJ109b (UCCCB 89)
- *Bacillus safensis* B1S542 10W7 (UCCCB 133)
- *Bacillus mycoides* ALJ98a (UCCCB 134)
- *Mesorhizobium qingshengii* Jales Te58 (UCCCB 135)
- *Mesorhizobium qingshengii* Jales Te59 (UCCCB 136)
- *Bacillus zhangouensis* B2S222 5W24 (UCCCB 137)
- *Paenibacillus tundrae* ALJ98b (UCCCB 138).

Data and code availability
All data supporting findings of this study are provided within the manuscript and its supplemental information section. Whole 16S rRNA sequencing data of strains has been deposited on the Genbank repository, under accession numbers from OK644207 to OK644280. Partial and whole sequencing data of ter determinants has been deposited on the Genbank repository, under accession numbers from OL344531-OL344548.

EXPERIMENTAL MODEL AND SUBJECT DETAILS
Sample collection
Sediment samples were collected from two mining sites the Aljustrel copper mine (37°52′07.3″N 8°09′24.7″W), in southern Portugal, and the Panasqueira tungsten mine (40°07′32.7″N, 7°42′51.1″W), in central Portugal. Additionally, a test gallery for gold mining, in Jales north Portugal (41°28′13.1″N 7°34′38.1″W). All sites have unique characteristics (see Figure 1 for sample/site description). Aljustrel samples were recovered by digging 1 m deep, into the discarded material of the sedimentary basin. Samples from Panasqueira were collected at 2 and 4 m from boreholes, using a dynamic geotool probe and a 50 mm poly-vinyl carbonate sampler with a diameter of 50 mm. Between each core sampling (B2S2, B2S3, B1S4 and B1S5) the sampler was decontaminated using 70% ethanol. Jales disturbed samples were collected from the walls of the gallery. All samples were collected and transported in sterile containers. Sediment samples were broken apart, homogenized and partitioned for further studies.

METHOD DETAILS
Bacterial strains isolation and growth
The bacterial isolation protocol targeted aerobic heterotrophic bacterial strains from sediments from all isolation sites. Aliquots from sediment samples, Panasqueira and Jales mining sites, were collected and serial dilutions were prepared, with saline solution (NaCl 0.85%) for inoculation of Reasoner 2A (R2A) agar (NZYTech, Portugal). Plates were incubated at 25°C for up to one month. Pure isolates were obtained from repeated streaking of a selection of colonies with different morphology and preserved at −80°C in growth media containing 15% glycerol.

Sediment samples from Aljustrel mining site were suspended in 50% diluted Luria Bertani (LB) medium (Sigma). The samples were incubated at 25°C for 7 days in an orbital shaker. Timely increments of sodium tellurite (Sigma) were added to the cultures, increasing from $5 \times 10^{-4}$ M, $1 \times 10^{-3}$ M, $3 \times 10^{-3}$ M, $5 \times 10^{-3}$ M up to $1 \times 10^{-2}$ M. Prior to each Te (IV) enrichment, an aliquot of the suspension was plated in 50% diluted LB agar for selection of isolates, as described above.

DNA extraction, 16S rRNA gene amplification and identification
DNA from each isolate was obtained using the standard freeze-thaw method (Nielsen et al., 1995). Amplification of the nearly full-length 16S rRNA gene sequence was performed by PCR with universal primers (Rainey et al., 1996), (see Supplementary Table S1 for PCR conditions). The resulting amplicons, sequenced by Sanger method (Stabvida) were matched with the existing sequences using a nBlast search against the reference type strain database on the EzTaxon portal (Rutherford et al., 2000; Kanz et al., 2005).

Isolates metal resistance determination and growth
Metal resistance of the isolates was determined by minimal inhibitory concentration (MIC). Strains were grown in R2A agar (Jales and Panasqueira isolates) and LB agar (Aljustrel isolates), with increasing concentrations of Te (IV), $5 \times 10^{-4}$ M, $1 \times 10^{-3}$ M and $3 \times 10^{-3}$ M. All bacterial growth was performed with incubation at 25°C for 24 to 48 h.
For the determination of specific growth rates, each isolate was grown in increasing concentrations of Te (IV), 1 × 10⁻⁴ M, 2.5 × 10⁻⁴ M and 5 × 10⁻⁴ M in LB and R2A broth, while comparing against growth in the absence of Te (IV). Growth kinetics were determined by evaluation of optical density (OD) variation (Abs 600 nm) of bacterial strains grown at 25°C, 100 rpm. Specific growth rates were determined for each strain at each concentration considering all values obtained at timepoints between early exponential and late exponential/early stationary phases (growth curves not shown).

**Tellurite bio-reduction from liquid media**

Tellurite reduction was evaluated in R2A for Jales and Panasqueira isolates and in LB for Aljustrel isolates, at the concentrations of 1 × 10⁻⁴ M, 2.5 × 10⁻⁴ M, 5 × 10⁻⁴ M. Aliquots for Te (IV) reduction testing were recovered at four points, as lag/early exponential, mid-exponential, late exponential and late stationary growth phase. After growth, cells were centrifuged for 20 min at 4000 g, the pellets were preserved for further tests and the supernatant was stored for evaluation of Te (IV) reduction. Quantitative depletion of Te (IV) was evaluated using a chromophore Diethyl-di-thiocarbamate (DDTC) method adapted from Turner and colleagues (Turner et al., 1992). The reagent mixture was prepared with 1 mM DDTC, Tris-HCl pH7 buffer and each sample was incubated for no more than 15 min prior to absorbance reading at 340 nm in an Infinite 200 PRO fluorimeter. Quantitative data was obtained from a minimum of three experimental replicates.

The efficiency of Te (IV) depletion, Reduction efficiency (Re), was determined as the ratio of the absolute variation of Te (IV) in grams, from time 0 (T0) to late exponential growth (Tf), per growth, expressed as a variation on optical density, Tf – T0. Reduction rate (Rr) was determined as reason of the Re per time at Tf, as demonstrated in the equation.

\[
Re = \frac{|\Delta Te|}{\Delta OD(Tf - T0)} \quad Rr = \frac{Re}{t(Tf)}
\]

**Tellurium aggregates production**

Demonstration of Te precipitation was performed by scanning electron microscopy with coupled energy-dispersive X-ray spectroscopy (SEM-EDS) on samples of cells recovered from a late exponential phase in the presence of 5 × 10⁻⁴ M of Te (IV). Cell pellets from cultures were collected by centrifugation at 4000 g, washed twice in cold saline phosphate buffer (PBS), and resuspended in 0.1 mL of the same buffer. Droplets of cell concentrate = 30 μL were dried in a 5 × 5 mm stainless steel plate, at room temperature, followed by two-step fixation with 2.5% glutaraldehyde followed by desiccation with increasing ethanol concentration, 70/80/90/95%. SEM micrographs were obtained on a FEI Quanta 400FEG ESEM and EDS analysis was accomplished using an Oxford INCA Energy 350 equipped with the SAMX IDIFIX software, with an accelerating voltage of 15 kV and a beam current of 20 nA.

**Screening for tellurite resistance genes**

DNA from each isolate was obtained as described in section “DNA extraction, 16S rRNA gene amplification and identification”. Targeted genes for screening were selected based on proven demonstration of conferring Te (IV) resistance (BacMet used for reference) with preference given to genes conferring resistance with absolute or narrow specificity to Te ions. Oligonucleotide design was conducted considering, at least, inter-genus genetic homology, between selected strains to reduce degeneracy (see Supplementary Table S1 for oligonucleotide details and PCR conditions). Amplification of terB gene using primers dterB_F and dterB_R with annealing 45 s, 55°C; elongation 30 s, 72°C; terD gene using primers terD_F and terD_R with annealing 45 s, 50°C; elongation 1 min, 72°C; terZ gene using primers yceD_F and yceD_R with annealing 1 min, 58°C; elongation 1 min, 72°C; terC gene using primers yceF_F and yceF_R with annealing 1 min, 54°C; elongation 1 min, 72°C and telA/yceH gene using primers yceH_F and yceH_R with annealing 1 min, 57°C; elongation 1 min, 72°C and tehA gene using primers tehA_F and tehA_R with annealing 1 min, 50°C; elongation 1 min, 72°C. PCR amplicons were observed on a 1% agarose electrophoresis and purified using a gel purification kit (EZNA, VWR). The resulting amplicons, sequenced by Sanger method (Stabvida) were matched with the existing sequences using a PSI-Blast search.

**Genetic organization of ter gene cluster**

Organization of the ter operon was determined by PCR for the *Bacillus* strains SW24, 3W19, ALJ98a and 10W7. Sets of primers for genes terZ and terC were designed based on the known sequences of terZ...
and terC genes from *B. altitudinis* 3W19. Combinations of forward-reverse primers were designed to generate four sets of amplicons with primers from terZ and terC: terZ_F + terC_R, terZ_F + terC_F, terZ_R + terC_F and terZ_R + terC_R. The same combinations were performed with primers from terC and telA: telA_F + terC_R, telA_F + terC_F, telA_R + terC_F and telA_R + terC_R. Full list of PCR conditions can be seen in Supplementary Table S1.

**Phylogenetic reconstruction and statistical analysis**

Alignment of PCR amplified ter genes and closely related reference genes, obtained by PSI-Blast, was performed with ClustalW, and the phylogenetic reconstruction was performed using MegaX software package. All data presented in graphs is calculated with statistic based on three biological replicates, with standard deviations represented in error bars, and statistical significance is calculated, when applicable, using one-way ANOVA and Dunner’s post-hoc test, using Prism, GraphPad10 software. Significant difference between experimental groups and control is represented as *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.