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Arsenic biomineralization and selenium nanoparticles biosynthesis by *Halomonas boliviensis* strain H-10 isolated from the high-altitude Salar de Huasco salt flat (Chile)

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ABSTRACT

High-altitude Andean wetlands of the Atacama Desert (Chile), including the Salar de Huasco salt flat, are unusual environments characterized by a great variability of physical-chemical conditions, often combined with a high concentration of heavy metals, including arsenic (As) and semimetals, such as selenium (Se). Microorganisms present in these environments evolved adaptative strategies to cope with environmental stresses, with biotechnological potentials. The present work investigated the natural bacterial community composition in sediments collected from the Salar de Huasco salt flat, by high-throughput Illumina sequencing, and to isolate bacterial strains able to remove As and biosynthesize Se nanoparticles (SeNPs). Dominant taxonomic groups (abundance ≥1%) were affiliated to *Proteobacteria* (mainly consisting in Delta- and Alfa-Gamma-proteobacteria) *Bacteriodetes*, *Firmicutes*, *Acidobacteria*, *Spirochaetes*, *Deinococcus-Thermus*, *Gemmatimonadetes* and *Verrucomicrobia*. *Halomonas boliviensis* strain H-10 was able to both biomineralize As and biosynthesise SeNPs; therefore, the characteristics of *H. boliviensis* strain H-10 could be used for biotechnological purposes, including As bioremediation from water and bioproduction of SeNPs.

1. Introduction

The Salar de Huasco is a high-altitude (3800 m a.s.l.) salt flat, located in the Andean plateau of the Atacama Desert (northern Chile) with an area of approximately 15,858 ha, which includes a complex system of water sources, such as streams, shallow permanent

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brackish lagoons, ephemeral hypersaline ponds and freshwater springs (Dorador et al., 2010, 2020). Since 1996 it has been classified as a high-altitude wetland according to the Ramsar convention (Eissler et al. 2020). The Salar de Huasco salt flat exhibits the typical environmental conditions of high-altitude Andean Lakes, which are extremely unusual and fragile environments, characterized by important spatial and temporal variability in salinity (from freshwater to salt-saturated water), large daily thermal amplitude (from −10 to +25 °C), whose average annual temperature is <5 °C, low atmospheric pressure, the highest solar radiations registered in the world (over 1000 Wm^{-2}), and high concentrations of heavy metals and metalloids (Albarracín et al., 2016, Hernández et al., 2016; Molina et al., 2016). Arsenic (As), selenium (Se) and other metalloids, originating from the dissolution of minerals in the Andean mountains, are often present in lakes and salt flats of the Atacama Desert, whose toxicity depends on their concentration and speciation. High concentrations of As in surface and groundwater, often exceeding the limits of As in drinking water (10 ug/L) (WHO, 2001; USEPA, 2001), pose serious problems for human health, ranging from skin lesions to cancer of the brain, liver, kidney, and stomach (Sharma and Sohn, 2009). Since 1970, drinking waters supplied to large cities of the Atacama Region (such as Antofagasta) are processed by conventional treatments to remove As, including coagulation, filtration, lime softening, activated alumina adsorption, ion exchange, reverse osmosis, electrodialysis reversal and nanofiltration (Herrera et al., 2021, Nicomel et al., 2015; Nidheesh and Singh, 2017; Luong et al., 2018). However, chronic arsenicism affects nearly 50,000 people, mainly among the rural populations of northern Chile, who drink water from small waterfalls and rivers with As content even higher than 1000 ug/L (Yañez et al., 2005, 2015). The highest concentration of Se(IV), a toxic form of Se, dissolved in the saline waters of the Atacama Desert was reported as 2.65 mg/L (MOP, 2009). When Se combines with other substances in the environment, including drinking water or its accumulation in plants, and if consumed in amounts exceeding acceptable levels it constitutes a risk of Se poisoning (ATSDR, 2023). On the other hand, Se^0, the form in which Se is present in nanoparticles, represents a low-risk form of Se (ATSDR, 2023).

Conventional treatments for water remediation are both expensive and usually generate toxic by-products with negative impacts on ecosystems (Herrera et al., 2021). Therefore, there is growing interest in biology-based approaches to find environmentally safe strategies to remove heavy metals and toxic compounds from waters (Campos et al., 2011).

Microorganisms exhibiting multiple adaptative mechanisms, or poly-extremophiles, are ideal candidates to experimentally addressing questions either to extend our knowledge on the resistance strategies under extreme conditions, and to prospect their use for bioremediation purposes (Zammuto et al., 2018; Merino et al., 2019). Previous investigations reported that Salar de Huasco supports very high microbial diversity, as well as it occurs in many other extreme environments in Atacama Desert (Aguilar et al., 2016; Dorador et al., 2013). Phylogenetic analyses indicated the presence in the Salar de Huasco of dominant bacterial phylotypes referred to *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Firmicutes*, and *Thaumarchaeota* and *Euryarchaeota* as dominant archaea (Dorador et al., 2008a;2008b, 2009, 2013). As other salt flats in the Atacama Desert, Salar de Huasco harbours a rich biodiversity of halophiles, i.e., microorganisms capable to grow in the presence of high salt concentrations, dominated by archaeal Halobacteriales and halotolerant bacteria especially in winter (under scarce water availability) (Dorador et al., 2013).

Most of the halophilic bacteria isolated from Salar de Huasco are able to produce compatible solutes to counteract osmotic pressure and at the same time to protect cells from other stresses, such as dehydration, heat, freezing, UV radiation and the presence of heavy metals at high concentrations (Lebre et al., 2017; Pérez et al. 2017). The adaptations to osmotic stress of the photoheterotrophic *Rhodobacter* sp., including proteins involved in the synthesis and transport of key osmoprotectants (i.e, glycine betaine and inositol) could provide also protection against UV radiation (Pérez et al. 2017;2018). The new halotolerant *Exiguobacterium* sp. strain SH31 was reported to encode a wide repertoire of proteins required to respond to heavy metal stress by Cd, Cu, Hg, Te, Cr and As (Castro-Severyn et al., 2017; Remonsellez et al., 2018). Therefore, the Salar de Huasco can be considered as a source of microorganisms to study the response mechanisms to a wide range of stresses, which could have potential use in bioremediation (Orellana et al., 2018).

Several novel bacterial strains have been isolated from different matrices (superficial waters, sediments, microbial mats and microbialites) of the Salar de Huasco, suggesting that this environment represent a hotspot of diversity with biotechnological potential (Thiel et al., 2010; Farías et al., 2013). Among these bacteria, two novel haloalkaline tolerant *Actinobacteria*, isolated from arid soil samples of the Salar de Huasco, recently classified as *Streptomyces huasconensis* and *Streptomyces altiplanensi*, are able to produce specialized metabolites of valuable pharmaceutical interest (Mitchell and Ferris, 2006; Cortés-Albayay et al., 2019). Among *Alphaproteobacteria*, *Rhodobacter* Rb3 strain, an aerobic anoxigenic phototroph, has been reported to possess multiple-adaptation mechanisms to stress-inducing factors, potentially explaining its success in a poly-extreme ecosystem, such as the Salar de Huasco (Pérez et al. 2018).

As and Se can be transformed by bacteria through dissimilatory processes into less toxic forms for cells and their close environment (Stolz et al., 2002; Dhanjal et al., 2014). As biomineralization, based on microbially induced calcite precipitation (MICP) (Wei et al., 2015) is a process which occurs naturally in almost every environment on Earth (Castro-Alonso et al., 2019). Bacterial-induced production of carbonate minerals have been often reported in *Cyanobacteria* (Jansson and Northern, 2010), sulphate-reducing bacteria (Warthmann et al., 2000), *Myxococcus* (Gonzalez-Muñoz et al., 2010) and *Pseudomonas* (Jha et al., 2009). Concurrently with urea hydrolysis or sulphate reduction, many bacteria can co-precipitate $CaCO_3$ and minerals, resulting in the subsequent deposition of mineral particles in the environment (Muynck et al., 2010). The ability of calcite to trap toxic elements may provide a new in situ remediation method for decontaminating natural systems with high metal concentrations, and therefore, could represent an eco-friendly alternative to traditional techniques to remove As from waters (Achal et al., 2012).

Se in its elemental form (Se^0) is non-toxic, while the selenite oxyanions (SeO_3^{2-}) are soluble in water and toxic to organisms due to their oxidant activity, that can interfere with fundamental cellular functions. Bacteria have evolved different mechanisms to reduce selenite to (Se^0) under different conditions, with the consequent formation of nanoparticles (SeNPs) with different morphologies, both inside and outside the microbial cell. In particular, the formation of extracellular and intracellular metal nanoparticles has been described as a feature of some bacteria belonging to members of *Proteobacteria*, *Firmicutes* and *Deferribacteriales*, and it is considered as

a cellular detoxification mechanism (Eswayah et al., 2019; Xu et al., 2020). The Se bioreduction mechanism could be used for remediating sites and treating wastewaters contaminated with Se oxyanions (Otsuka and Yamashita, 2020; Fadaei and Mohammadian-Hafshejani, 2023). The natural ability to produce nanoparticles has attracted increasing interest in the recent development of nanotechnology. These nanomaterials find potential applications in the electronic field, in procedures for bioremediation of heavy metals, as well as for their antioxidant and antimicrobial properties and also biomedical applications (Natan and Banin, 2017; Baptista et al., 2018 Muzammil et al., 2018; Singh et al., 2018; Yu et al., 2012).

The aims of the present work were to investigate the composition of the natural bacterial community present in sediments collected from the Salar de Huasco salt flat, using the next generation sequencing technique (16 S rRNA Illumina sequencing), and to isolate bacterial strains able to precipitate calcite to immobilize, by co-precipitation, As and concurrently to biosynthesize SeNPs. For these purposes, the strains isolated from the Salar de Huasco salt flat were firstly screened for the production of urease, a key indicator of calcite precipitation, and then their tolerance to heavy metals evaluated. One strain was demonstrated, using atomic absorption spectrometry, to be capable to remove As from the culture medium and, by electron microscopy, to immobilise it in CaCO3 crystals and; furthermore, also using electron microscopy, that the same strain was capable to biosynthesise SeNPs.

2. Materials and methods

2.1. Study site and sample collection

Sediment samples were collected from five fixed stations at the Salar de Huasco salt flat (20° 17' 18'' S / 68° 53' 14'' W), located in the Atacama Desert, Chile, at approximately 4000 m above sea level. Sediment samples (100 g), in triplicate, were collected from the surface up to a depth of 10 cm, using sterile polycarbonate tubes (core). All the samples were refrigerated at 4 °C and then transported to the Environmental Microbiology Laboratory, Department of Microbiology, Faculty of Biological Sciences, Universidad de Concepcion, Concepcion, Chile, for further processing.

The pH and temperature were measured *in situ* using a multi-parameter tester (HI98195, Hanna Instruments, Woonsocket, Rhode Island, USA). Concentrations of As, Cd, Cu, Pb, Zn and Se were quantified using an atomic absorption spectrophotometry AAnalyst 800 equipment (PerkinElmer, Ueberlingen, Germany), according to Leon et al. (2018).

2.2. Bacterial community composition

Total DNA from each homogenized sediment sample was extracted using the E.Z.N.A. DNA/RNA Isolation Kit (Omega Bio-Tek, Inc. Norcross, GA, USA) according to the protocol provided by the manufacturer. DNA extracts were then purified using the PowerClean Pro DNA Clean-up kit (MoBio, Vancouver, Canada) following the manufacturer's instructions. Quality and concentration of DNA were checked by UV/Vis spectroscopy (NanoDrop ND-1000, Peq- lab, Erlangen, Germany).

DNA samples were sequenced at the Greehey Children's Cancer Research Institute (Greehey CCRI) Next Generation Sequencing (NGS) facility, at the University of Texas Health Science Center at San Antonio (San Antonio, Texas, USA). Sequencing was done using the Illumina MiSeq technology with pair end reads, targeting the V1-V2 region of 16 S rRNA gene of Bacteria (primers 27 f 5'- AGA GTT TGA TCM TGG CTC AG-3' and 355r 5'– GCT GCC TCC CGT AGG AGT-3').

Bioinformatic analyses, such as pre-processing of the sequencing data, included quality check, removal of duplicates, alignments to SILVA database, denoising, and chimera removal, were performed using the Mothur software, v.1.33.3 (www.mothur.org). Also, Mothur software was used for distances calculation and clustering of the sequences into OTUs with a distance of 0.03.

For taxonomic assignment, we used the RDP classifier version 2.7 (https://rdp.cme.msu.edu) and the SILVA database (https://www.arb-silva.de/) with a threshold of 0.8. For statistical analysis we used R statistical software version 3.1.0 (https://www.r-project.org).

2.3. Bacterial isolation and screening (urease test) for calcifying ability

Homogenised sediment (1 g) was added to flasks containing 250 mL of Chemically Defined Medium (CDM) Broth at pH 8.0 (Battaglia-Brunet et al., 2002). Flasks were incubated at 25 °C, with shaking (150 rpm), for 48 h in darkness under aerobic conditions. Then, suitable aliquots from the flasks were inoculated onto CDM agar plates and the plates were incubated at 25 °C for 48 h (Campos et al., 2011) in the darkness. Colonies were picked and purified by streaking onto CDM agar plates incubated under the same previous conditions.

To check the production of urease, a key indicator of calcite precipitation, each isolate was transferred to plates containing urea agar base (Christensen Agar, Thermo Scientific, MA, USA) (Achal et al., 2010). After incubation at 25 °C for 48 h, colonies producing urease (colour change of the medium) were selected.

2.4. Tolerance to Arsenic and Selenium

The tolerance levels of the calcifying (urease positive) isolated strains to heavy metals were determined by the agar dilution technique. The strains were seeded on R2A agar (Thermo Scientific, MA, USA) plates supplemented with concentrations ranging from 0.5 to 1000 mM of NaAsO2 (As^{III}), Na2HAsO4 7H2O (As^{V}), Na_2SeO_3 (Se^{IV}), $CuCl_2$ (Cu^{II}), $NiCl_2$ (Ni^{II}), or $CdCl_2$ (Cd^{II}). Each plate was inoculated with cell suspensions from fresh precultures, incubated on CDM agar, adjusted to a final density of approximately 10^7 CFU

mL^{-1} and incubated for 24 h at 25 °C, in the darkness, under aerobic conditions. R2A plates seeded with each bacterial isolate but without heavy metals were used as control.

2.5. Identification of the calcifying strains

The calcifying metal tolerant isolates were identified by PCR amplification of their 16 S rRNA gene. Total DNA of strains was obtained using the UltraClean Microbial DNA Isolation Kit (Thermo Scientific, MA, USA), following the manufacturer's instructions. PCR was performed using the 16 S rDNA bacterial universal primers GM3 (AGAGTTTGATCMTG GC) and GM4 (TACCTTGTTACGACTT) (Herrera et al., 2021). PCR amplification followed the procedure described by Campos et al. (2011). Sequencing was done on the amplified fragments using the Dyenamic ET terminator cycle sequencing kit (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) in a 3100 Avant Genetic Analyzer (Thermo Scientific, MA, USA) according to the manufacturer's instructions. Strains were identified on the basis of sequences stored in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program (Herrera et al., 2021). In addition, for phylogenetic reconstruction, 16S-related sequences were aligned using the MAFFT algorithm (v7.490 version). The parameters used were local-pair, adjust-direction, reorder and maxiterate 100. The alignment obtained was then refined using the Gblocks software, before building the phylogenetic tree by means of the PhyML (v.3.0) algorithm, using a tree search NNIs approach and initial tree as BIONJ, with a nucleotide substitution model set as General Time Reverse (GTR), with a Discrete Gamma Model and Proportion of invariant.

2.6. Bacterial growth kinetic

Bacterial strain was inoculated (~10^4 CFU mL^{-1}) into flasks containing CDM broth supplemented with 2% urea and 25 mM $CaCl_2$ (CDM-Urea broth) plus 0.5 mM As(III) or 0.5 mM As(V) or in the absence of As as control, and incubated at 25 °C under aerobic conditions and agitation (120 RPM). Aliquots were obtained at intervals ranging from 0 to 60 h. The aliquots were transferred to CDM agar supplemented with 2% urea and 25 mM $CaCl_2$ (CDM-Urea agar) and the plates incubated for 24–48 h at 25 °C under aerobic conditions. Growth was determined recording the number of CFU counted for each time of sampling (Herrera et al., 2021). The growth curves were analysed in accordance with the mathematical modelling of Gompertz. Graphs and models were made using GraphPad Prism version 5.0 (GraphPad software, San Diego, California, USA).

2.7. Arsenic removal by MICP

A batch system was performed in flasks containing 100 mL of CDM-Urea broth, 200 μL of an overnight culture (equivalent to 10^7 CFU mL^{-1}) of selected strain, supplemented with 0.5 mM As(V) or without As, or without bacterial cells, as control, incubated at 25 °C for 48 h under aerobic conditions (Herrera et al., 2021). The total As removal was evaluated in the cell-free supernatant, as described by Yañez at al. (2015), using atomic absorption spectrometry (AAS). In addition, $CaCO_3$ crystals were visualized and analysed by Scanning Electronic Microscopy (SEM), according to Campos et al. (2011). In addition, $CaCO_3$ crystals were visualized by Transmission Electron Microscopy (TEM), according to Torres et al. (2012).

2.8. Biosynthesis of Selenium nanoparticles (SeNPs)

To verify the capacity of the selected calcifying strain to produce SeNPs, it was firstly necessary to verify the ability of the strain to convert selenite into $Se(^0)$. The selected strain was grown in R2A broth supplemented with 1 mM sodium selenite (Sigma-Aldrich. St. Louis, MO, USA). The selenite supplemented R2A broth medium (9 mL) was inoculated with 20 μL of an overnight culture of the strain and incubated at 25 °C under aerobic conditions. The appearance of red colour in the culture indicated the presence of $Se(^0)$ (Klonowska et al., 2005). As negative control, the strain was cultured in the absence of selenite. Samples from bacterial culture were aseptically withdrawn and processed for SEM-EDS and TEM.

2.9. Electron microscopy and energy dispersive spectroscopy

For SEM-EDS analyses, the samples were harvested and washed with distilled water. Washed cells were collected by centrifugation, at 3000 g for 10 min, and further processed as described by Campos et al. (2011). The samples were visualized under a JEOL SEM, Model JSM 6380LV (JEOL, Tokyo, Japan), equipped for Energy Dispersive Spectroscopy (EDS). TEM samples were prepared centrifuging the samples at 3000 g for 10 min, harvested cells were washed with isotonic saline solution and then with distilled water. The pellet was re-suspended in distilled water to prepare a suspension. One drop of this suspension was placed on a copper electron microscope grid pre-coated with a carbon film, air-dried overnight and observed using a JEOL JSM 1200EX-II TEM (JEOL, Tokyo, Japan), according to Torres et al. (2012).

2.10. Statistical analysis

Data was evaluated by means of one-way ANOVA using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). p values equal to or less than 0.05 were considered as significant. Diversity indices was carried out using the R software version 3.1.0. using the 'diverse' packages.

3. Results

3.1. Characteristics of the sediment samples

The temperature and pH, measured on the superficial sediments of the Salar de Huasco salt flat, were in average 12 °C and 7.0, respectively. The concentrations of the chemical elements were: As: 440 mg/kg, Se: 110 mg/kg, Cd: 67 mg/kg, Cu: 350 mg/kg, Pb: 90 mg/kg, and Zn: 120 mg/kg of sediment.

3.2. Bacterial community diversity

Sequencing data of bacterial 16 S rRNA universal V1-V2 region and diversity indices (Shannon diversity index and non-parametric Chao1 and ACE richness estimators) are reported in Table S1. A total of 450,220 sequences were retrieved and after a quality check, using the SILVA database and removing chimeras, 447,928 (99.5%) high quality sequences remained. The accession number to the metagenomic sequences is SUB14162480.

3.3. Composition of the bacterial community

The metagenomic analysis of OTUs retrieved from the superficial sediment of the Salar de Huasco salt flat showed the presence of 11 different bacterial phyla. The dominant taxonomic groups (abundance ≥1%) were affiliated to *Proteobacteria* (38.5%), *Bacteriodetes* (34.6%), *Firmicutes* (5.8%), *Acidobacteria* (5.8%), *Spirochaetes* (3.8%), *Deinococcus-Thermus* (1.9%), *Gemmatimonadetes* (1.9%) and *Verrucomicrobia* (1.9%) (Fig. 1). Within *Proteobacteria*, the most abundant class was *Deltaproteobacteria* (17.33%), followed by *Alphaproteobacteria* (11.5%), *Epsilonproteobacteria* (3.8%), *Gammaproteobacteria* (3.8%) and *Betaproteobacteria* (1.9%) (Table S2).

3.4. Identification of calcifying and non-calcifying bacterial strains recovered from the sediment and their tolerance to heavy metals and selenium

Isolates from sediments of the Salar de Huasco salt flat and their phylogenetic affiliation, tolerance to heavy metals and Se, and urease production are reported in Table 1. Isolates were affiliated with three different genera and four species, in particular: Dietzia maris (H-3R), Marinobacter excellens (H-8, HF-15, HF-16B, HF-10), Halomonas boliviensis (H-3, H-6, H-10 and H-10R) and Halomonas neptunia (H-9R) (Table 1). Three from the 10 strains were resistant to 128 mM As(III) (Dietzia maris H3R, Halomonas boliviensis H10 and Halomonas boliviensis H10R) and Se tolerance, the highest level was shown by H. boliviensis H3 strain (Table 1). Three strains produced urease during 48 h at 25 °C under aerobic conditions (Table 1). Moreover, all bacterial isolates were able to grow at 25 °C on R2A medium plus urea supplemented with As(III), As(V), Cu (II) or Se (IV) (tested in the presence of 0.5–1000 mM of

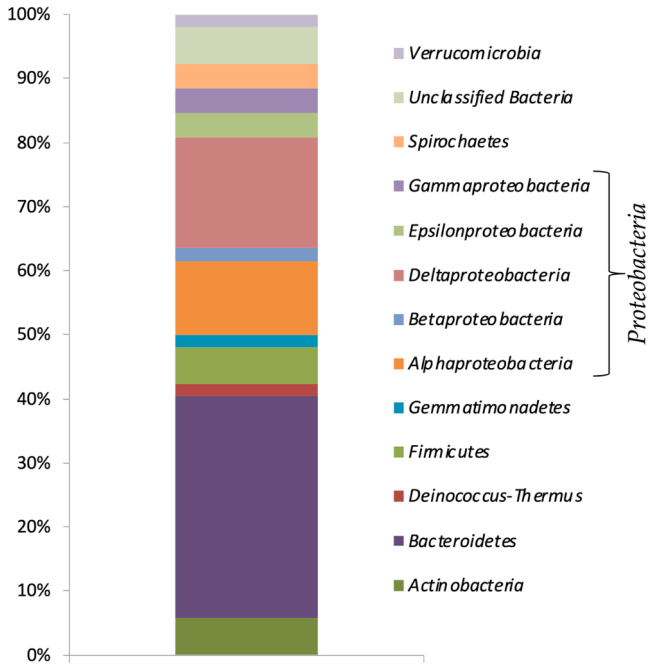

Fig. 1. Relative abundance of sequences (expressed as percentage) assigned to bacterial phylogenetic groups present in the superficial sediment of the Salar de Huasco salt flat, Atacama Desert, Chile.

each heavy metal), to evaluate the tolerance of the isolated bacterial strains to these chemical elements. In addition, all the strains were able to grow in the presence of f 0.5–20% (W/V) NaCl (data not shown).

Strain H-10 was phylogenetically affiliated, with a 99% similarity, to H. boliviensis (Figure S1). This strain was able to grow at the highest concentrations of As(III), and As(V) and Se(IV) (Table 1) tolerated among the strains capable to hydrolyse urea (as shown when cultured in urea agar base medium). Therefore, given these characteristics, the strain H. boliviensis H-10 was selected for the following assays, including biomineralization of As and biosynthesis of Se nanoparticles.

3.5. Growth kinetics of the H. boliviensis H-10 in the presence of As(III) and As(V)

In order to determine the effect of As(III) and (As(V) on the growth kinetics of the *H. boliviensis* H-10, its growth was studied in the presence of 0.5 mM As(III) or As(V) or in the absence of As, and the results were analysed using the Gompertz growth model (Fig. 2). The model demonstrated that after 60 h of incubation, the *H. boliviensis* H-10 cultured in the presence of As(V) showed a similar growth than the control, exhibiting growth rates (μm) of 0.068 and 0.061 h^{-1}, respectively ($p>0.05$). The Gompertz model also showed that in the presence of As(III) the growth rate (μm) was 0.055 h^{-1}, showing less growth than the control ($p>0.05$).

3.6. Arsenic biomineralization by microbially induced calcite precipitation (MICP)

The removal of As, by means of microbiologically induced biomineralization, using H. boliviensis H-10 was evaluated by atomic absorption spectrometry (AAS). H. boliviensis H-10 removed 94% of the total As present in the CDM-Urea medium, measured in a cell-free supernatant sample, after 48 h of incubation at 25 °C (Fig. 3).

SEM observations of H. boliviensis H-10 cultured in CDM-Urea broth either in the presence or absence of As for 48 h at 25 °C under aerobic condition allowed to detect the presence of a precipitate (crystals) around bacterial cells (Fig. 4A and 4B). The SEM-EDS analyses of the precipitate when the strain was cultured in the absence of As allowed to detect the presence of $CaCO_3$ (40.36%), SiO_2 (51.97%), Albite (0.83%), Al_2O_3 (1.70%) and SiO_2 (4.73%). No peak for As was detected under this culture conditions (Fig. 4C). The SEM-EDS analysis of the precipitate when the strain was cultured in the presence of As showed the presence mainly of $CaCO_3$ (41.9%), SiO_2 (44.50%) and As (10.23%). In lesser percentages, Albite (1.04%), Al_2O_3 (2.03%) and Ag (0.35%) were also detected. SEM-EDS detected the presence of As peaks only in the sample from the culture incubated in the presence of As (Fig. 4D), indicating that the biomineralization of As was mediated by the bacterial strain subject of the study.

In addition, TEM and TEM-SAED analyses of the H. boliviensis H-10 strain cultured for 48 h at 25 °C in CDM-Urea medium supplemented with 0.5 mM As(V) were done (Fig. 5). Fig. 5 shows TEM and TEM-SAED images confirming the bioproduction of $CaCO_3$ sub-micrometric crystals (≤1 μ) nucleation obtained, having a poli-rhombohedral morphology. Particularly, Fig. 5C shows, by TEM-SAED, the structure of the calcite crystal after biomineralization by H. boliviensis H-10 strain.

3.7. Biosynthesis of Se nanoparticles by H. boliviensis H-10 strain

After culturing H. boliviensis H-10 strain for 24 h at 25 °C under aerobic conditions in R2A medium supplemented with 0.5 mM Se (IV), the reduction of Se(IV) to Se(^{0}) by the bacterial strain was detected by the red-coloured precipitate in the culture resulting from the reduction of selenite and/or selenate into red Se(^{0}) precipitate, the form having biological activity. Red precipitate was not observed in the cultures of E. coli K-12, used as negative control (data not shown).

The production of nanoparticles by *H. boliviensis* H-10 was observed by TEM after culturing the bacterium in the presence of Se(IV) but not when cultured in the absence of Se (negative control, Fig. 6A). TEM images of the bacterium cultured in medium plus 0.5 mM selenite showed the accumulation of intracellular and extracellular spherical nanoparticles (Fig. 6B and 6C). The size of the nanoparticles varied from nearly 80 to nearly 300 nm. SEM analysis showed the presence of bacillary bacteria in the negative control (absence of Se in the culture medium) and experimental cultures (presence of Se in the culture medium), but nanoparticles were only present in the experimental cultures (Fig. 7A and 7B). SEM-EDS confirmed the absence of nanoparticles and of Se peaks in the negative control (Fig. 7C) but their presence in the culture supplemented with 0.5 mM selenite. The detection of Se peaks is indicative that

Table 1
Identification, production of urease and tolerance to Arsenic and Selenium (mM) of the bacterial strains isolated from sediments of the Salar de Huasco salt flat, Atacama Desert, Chile.

Strain	Closest relative	% similarity	Accession Number	Urease Test	As (III)^T	As (V)^T	Se (IV)^T	Ni (II)^T	Cd (II)^T	Cu (II)^T
H3	*Halomonas boliviensis*	99%	KC178891	+	16	724	128	0.5	0	2
H6	*Halomonas boliviensis*	99%	NR029080	-	8	362	128	1	16	4
H8	*Marinobacter excellens*	98%	KJ004408	-	4	362	128	0	0	1
H10	*Halomonas boliviensis*	99%	MZ604313	+	128	256	128	0.5	0	2
H3R	*Dietzia maris*	99%	MK318606	-	128	256	32	0	16	4
H9R	*Halomonas neptunia*	97%	MK070060	-	16	128	128	1	4	1
H10R	*Halomonas boliviensis*	99%	KC178891	+	128	256	32	0	1	1
HF15	*Marinobacter excellens*	93%	KJ004408	-	64	128	128	0	32	2
HF16B	*Marinobacter excellens*	98%	KJ004408	-	16	128	128	2	4	8
HF10	*Marinobacter excellens*	97%	KJ004408	-	32	32	128	4	1	0.5

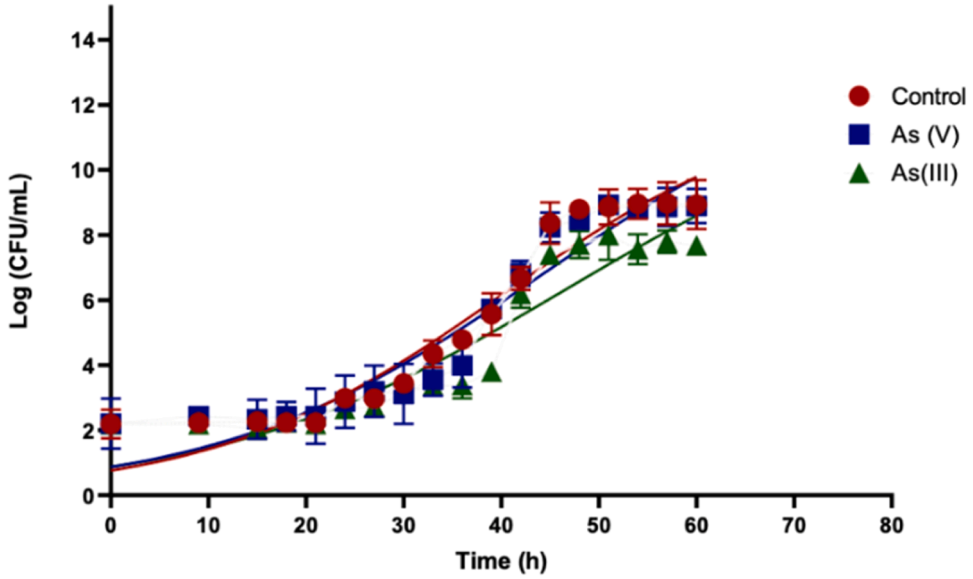

Fig. 2. Growth kinetics of *H. boliviensis* H-10 strain as evaluated in the presence of 0.5 mM As(III) or As(V) or in the absence of As, assessed by the plate count technique. Kinetics was adjusted using the Gompertz growth model.

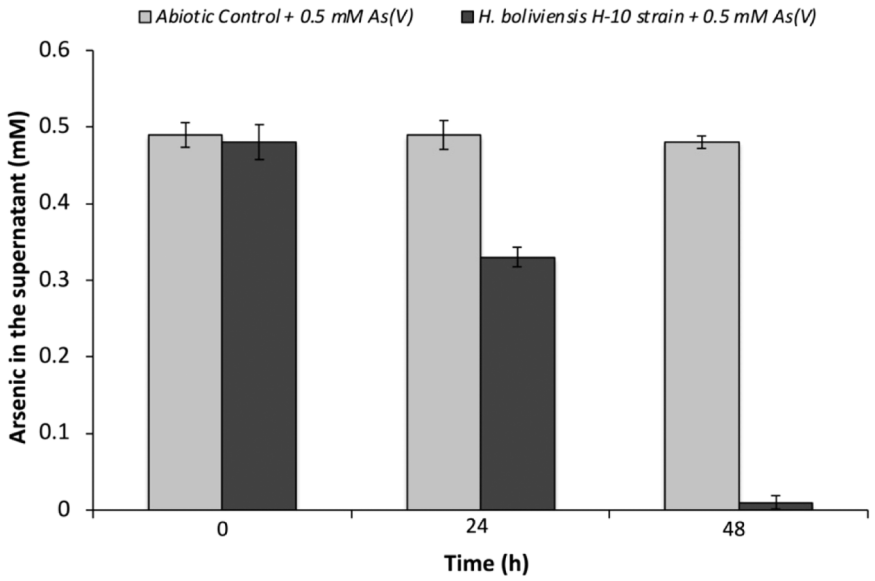

Fig. 3. Arsenic removal by *H. boliviensis* H-10 strain in CDM-Urea broth supplemented with 0.5 mM As(V). An abiotic control (lacking the bacterial strain) was also included. Incubation was carried out at 25 °C.

nanoparticles were composed of Se (Fig. 7D). Other peaks revealed by SEM-EDS corresponded to Na and Cl, components of the culture medium or saline solution with which the samples were washed. Also, C and O peaks associated with the cellular exudate were detected.

4. Discussion

In natural poly-extreme environments, such as the high-altitude Andean Salar de Huasco (northern Chile) salt flat, the average abundance of heavy metals is generally higher in sediments and soil than in the water column. Microorganisms have the ability to adapt and have evolved, developing a wide variety of mechanisms to counteract environmental stresses and heavy metals. In natural habitats, heavy metals concentration depends on the physico-chemical properties and the microbial community of the environment (Coker, 2016). As revealed in our study using Illumina high-throughput sequencing, the bacterial community of the sediment from the

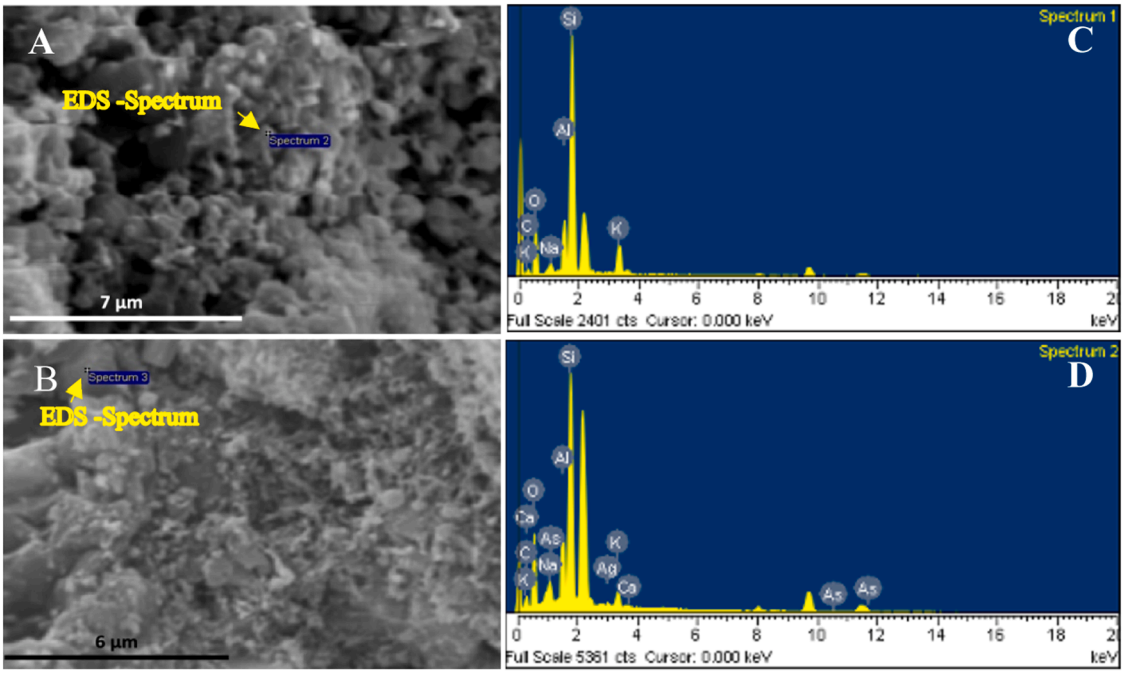

Fig. 4. Scanning electron microscopy (SEM) (micrographs at left) and SEM-energy dispersive spectroscopy (SEM-EDS) (images at right) of *H. boliviensis* H-10 strain cultured for 48 h at 25 °C under aerobic conditions in CDM-Urea broth supplemented with 0.5 mM As(V) or in the absence of As showing the $CaCO_3$ crystals obtained. A) *H. boliviensis* H-10 strain cultured in the absence of As (control); B) *H. boliviensis* H-10 strain cultured in the presence of 0.5 mM As(V); C) SEM-EDS of a culture incubated in the absence of As (control) D) SEM-EDS of a culture incubated in the presence of 0.5 mM As showing the presence of As peaks. Yellow arrows show the EDS analysed spot.

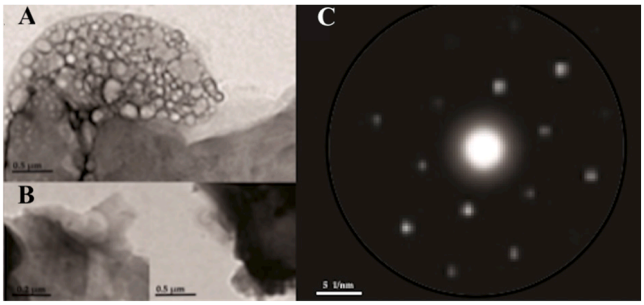

Fig. 5. Transmission electron microscopy (TEM) and TEM selected area electron diffraction pattern (TEM-SAED) showing the presence of $CaCO_3$ crystals obtained after culturing *H. boliviensis* H-10 strain for 48 h at 25 °C under aerobiotic conditions in CDM-Urea broth supplemented with 0.5 mM As(V). A) TEM micrograph showing $CaCO_3$ nucleation and the shape of calcite crystals; B) TEM micrograph showing poli-rhombohedral calcite crystals; C) TEM-SAED of calcite crystals.

Salar de Huasco salt-flat was composed of few dominant phyla, referred to *Proteobacteria* (mainly represented by Delta- and Alpha-proteobacterial subclasses) and *Bacteroidetes*. These findings confirmed previous investigations performed in water samples of the high Andean plateau (Aguilar et al., 2016; Eissler et al., 2020).

Microorganisms can metabolize metal ions and obtain energy through oxidation and reduction process by dissolving or precipitating them. In the present study, strains able to tolerate heavy metals (As, Cu, Ni, Cr, Cd) and Se were mainly ascribed to halotolerant members of the *Gammaproteobacteria* group, affiliated to *Halomonas* and *Marinobacter* genera, and *Actinobacteria*. Castro-Severyn et al. (2020) isolated, from the Salar de Huasco, *Exiguobacterium* strains, with different As tolerance levels. In addition, *Pseudomonas arsenicoxydans* and *Pseudomonas migulae* were isolated from As-rich sediments of the Camarones river, Atacama Desert and they showed high tolerance to As (Campos et al., 2011; Herrera et al., 2021). *H. boliviensis* was described as a new species in 2004 by Quillaguamán et al. (2004), on the basis of two isolates obtained from the soil around Laguna Colorada (Bolivia) lagoon. This lagoon is a hypersaline shallow lake located at 4300 m a.s.l. in the southwest of the Bolivian altiplano, close to the border with Chile. The original description of *H. boliviensis* reports it as an alkalitolerant and moderate halophile bacterium (Quillaguamán et al., 2004). García (2017) evaluated the production of bioplastic, particularly PHA, from cereal mash by *H. boliviensis*.

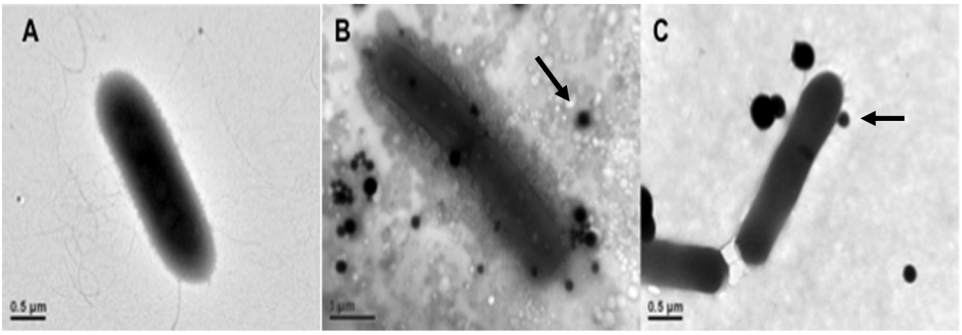

Fig. 6. TEM images of *H. boliviensis* H-10 strain cultured in CDM medium for 24 h at 28 °C. A) Negative control, cultured in the absence of Se; B) and C) Biogenic nanoparticles produced by a culture in the presence of 0.5 mM selenite. Arrows indicate selenium nanoparticles. (Scale bar: A) 0.5 µm; B) 1 µm; C) 0.5 µm).

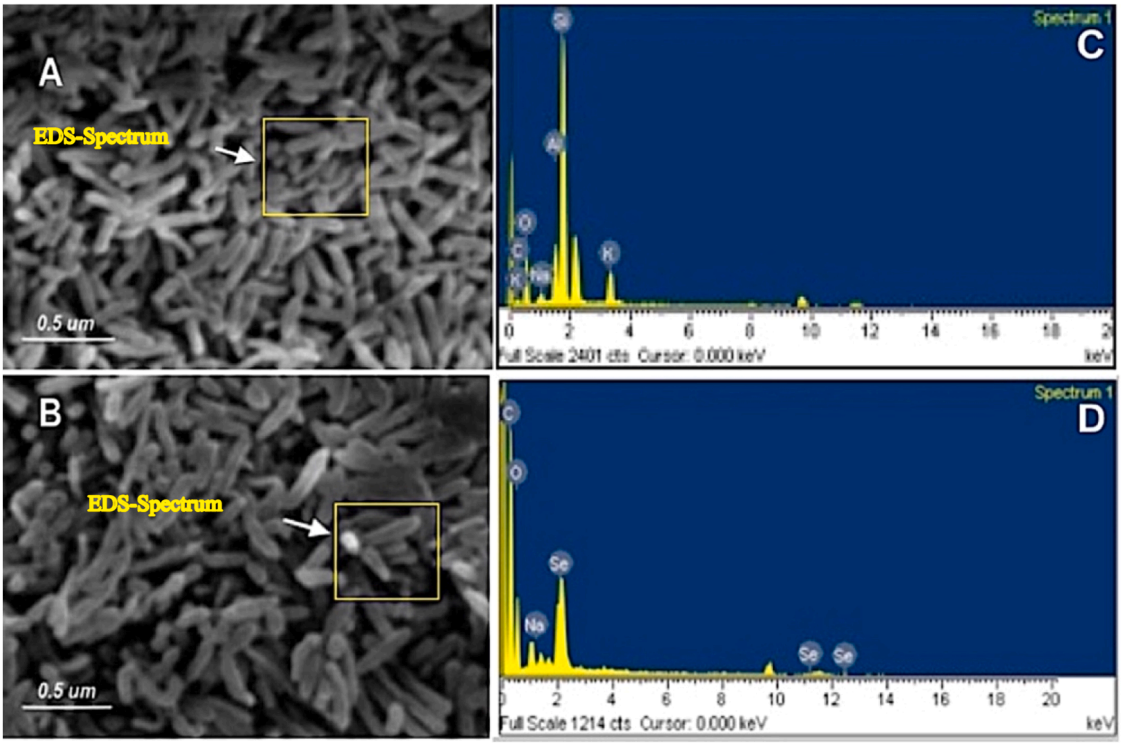

Fig. 7. *H. boliviensis* H-10 cultured for 24 h at 25 °C under aerobic conditions in CDM medium supplemented with 0.0 mM or 0.5 mM selenite. Scanning electron microscopy (SEM) images at left and SEM-energy dispersive spectroscopy (SEM-EDS) analysis images at right. A) SEM micrograph of *H. boliviensis* H-10 cultured in CDM medium in the absence of Se (negative control); B) SEM micrograph of a similar culture but supplemented with 0.5 mM Se. C) SEM-EDS of the indicated area of micrograph A. D) SEM-EDS of the indicated area of micrograph B.

H. boliviensis strain H-10 was able to grow in the presence of high concentrations of As and Se. Due to its ability to meditate the precipitation of calcite, entrapping As and sequestering this heavy metal from the environment, strain H-10 could be considered as a promising candidate in bioremediation of As. Moreover, this strain is also able to biosynthesize SeNPs, which have biotechnological potential. It is reasonable to assume that strain *H. boliviensis* H-10, being able to hydrolyse urea, increase the alkalinity of the culture medium, and consequently favours the co-precipitation of As together with $CaCO_3$, the heavy metal substituting some Ca ions. The negative charge and the functional groups of the cell-wall could facilitate the adsorption of As (Mitchell and Ferris, 2006).

The incorporation of heavy metals into calcite is an essentially competitive co-precipitation reaction, in which suitable divalent cations, such as As, are incorporated into the calcite lattice, forming carbonate minerals with very low solubility (Hammes et al., 2003a; Colwell et al., 2005). Hammes et al. (2003b) used bacterially mediated $CaCO_3$ precipitation to remediate Ca(II)-rich industrial wastewater. Calcium rich wastewater often results from the processing of bones, production of citric acid, paper recycling, and landfill leachates. The addition of urea into a reactor system containing a sludge, allowed to remove over 80% of the Ca(II) present in the

system, bioprecipitating $CaCO_3$. Fujita et al. (2004) used *Bacillus pasteurii* for the precipitation of $CaCO_3$ in a chemistry based simulated groundwater of the Snake River Aquifer (southern Idaho, USA). The study showed that the $CaCO_3$ produced by *B. pasteurii* incorporated ^{90}Sr into the calcite crystal matrix due to the high $CaCO_3$ precipitation rates, consequence of bacterial urea hydrolysis. Altaş et al. (2011) described the use of a biocatalytic calcification reactor containing a waste ureolytic mixed culture as a Cr(VI) sorbent from an aqueous solution. Achal et al. (2012) described an environmental As resistant bacterial strain, identified as *S. ginsengisoli*, able to co-precipitate calcite and As from contaminated soil. In addition, Li et al. (2013) isolated *Bacillus* spp and *Sporosarcina* spp strains, from nursery garden soil from Tsinghua University, Beijing, China, able to transform heavy metal ions by depositing as mineral crystals, which may play a role in removal of heavy metals in wastewater and soils.

Over the last two decades there has been considerable interest in the microbiological transformation of Se oxyanions, as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), into volatile forms of Se, such as dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe), or insoluble forms, such as elemental Se (Maltman and Yurkov, 2018). In particular, the reduction of Se oxyanions to SeNPs has been regarded as a detoxification mechanism, with potential to be considered as a bioremediation because, due to its insoluble nature, elemental Se is less toxic and biologically unavailable (Torres et al., 2012). This mechanism has been reported in bacteria such as *Pantoea agglomerans*, *Sulfurospirillum barnesii*, *Bacillus selenitireducens, Rhodococcus aetherivorans*, *Bacillus* spp., *Klebsiella pneumoniae*, *Selenihalanaerobacter shriftii* and *Rhodospirillum rubrum,* among others (Escobar-Ramírez et al., 2021). Many of these bacteria are known to possess excellent Se resistance and to be capable to biosynthesize SeNPs under anaerobic or aerobic conditions; therefore, they have been considered as a biological resource to produce SeNPs (Kessi et al., 1999; Oremland et al., 2004; Fesharaki et al., 2010; Li et al., 2014; Shakibaie et al., 2015; Presentato et al., 2018). The transformation of selenite to insoluble Se(^{0}) is catalysed by nonspecific selenite reductases and the extracellular and intracellular formation of SeNPs has been discussed by various authors (Kessi et al., 1999; Kessi and Hanselmann, 2004; Ridley et al., 2006). Some authors suggested that the SeNPs found outside cells are released by cell lysis (Xu et al., 2018b; Wang et al., 2020), whereas others suggested that a membrane efflux pump rapidly expels the nanoparticles (Dhanjal and Cameotra, 2010; Pang et al., 2019). Kessi et al. (1999) speculated that a vesicular mechanism of Se excretion occurs in *R. rubrum*. However, there is not enough experimental evidence in support of either vesicular secretion or cell lysis as the mechanisms responsible for the release of intracellularly produced SeNPs.

SeNPs are important not only for their technological applications, anticancer, antioxidant, antimicrobial, and anti-biofilm properties which include taking advantage of their semiconductor and photoelectric properties and their X-ray-sensing properties (Dhanjal and Cameotra, 2010; Hosnedlova et al., 2018) but also for their biological activity. In this respect, SeNPs interact with functional groups of proteins (C–N, COO–, C═O, NH) favouring their adsorption (Zhang et al., 2004) and SeNPs have been shown to increase the activity of enzymes such as glutathione peroxidase and thioredoxin reductase, both reducing agents of oxidative stress (Zhang et al., 2005), reason to include SeNPs in the diet of several production animals. SeNPs have a better performance than Na_2SeO_3 when used as a diet supplement in rainbow trout (Yanez-Lemus et al., 2022). On the other hand, Xu et al. (2018a) also demonstrated that SeNPs showed a better performance than Na_2SeO_3 when they evaluated several physiological parameters in human cell lines. Additionally, hollow spherical SeNPs have been demonstrated to reduce the risk of Se toxicity (Gao et al., 2002; Wang et al., 2007).

5. Conclusion

The ability of the bacterium *H. boliviensis* strain H-10 to concurrently biomineralize As, co-precipitating it with calcite, and to biosynthesise SeNPs, is here reported for the first time when cultured under aerobic conditions at 25 °C. Our results provide the evidence that *H. boliviensis* strain H-10 can be used to bioremediate both toxic As and Se in one step. Moreover, Se can be recovered in the form of Se^0 nanoparticles, which can be used for other purposes.

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CRediT authorship contribution statement

Ruben Moraga: Investigation, Methodology, Validation. **Florencia Bravo:** Formal analysis, Methodology, Visualization. **Victor Campos:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Concetta Gugliandolo:** Conceptualization, Methodology, Writing – original draft. **Vincenzo zammuto:** Writing – original draft. **Felipe Contreras:** Writing – original draft. **Carlos Smith:** Conceptualization, Writing – original draft, Writing – review & editing. **Cristian Valenzuela:** Formal analysis, Methodology, Visualization. **Paulina Aguayo:** Formal analysis, Methodology, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103575.

References

Achal, V., Pan, X., Fu, Q., Zhang, D., 2012. Biomineralization based remediation of As(III) contaminated soil by *Sporosarcina ginsengisoli*. J. Hazard. Mater. 201-202, 178–184. https://doi.org/10.1016/j.jhazmat.2011.11.067.

Aguilar, P., Acosta, E., Dorador, C., Sommaruga, R., 2016. Large differences in bacterial community composition among three nearby extreme waterbodies of the high Andean plateau. Front. Microbiol 7, 1–8. https://doi.org/10.3389/fmicb.2016.00976.

Albarracín, V.H., Gärtner, W., Farías, M.E., 2016. Forged Under the Sun: life and Art of Extremophiles from Andean Lakes. Photochem. Photobiol. 92 (1), 14–28.

Altaş, L., Kiliç, A., Koçyiğit, H., Işik, M., 2011. Adsorption of Cr(VI) on ureolytic mixed culture from biocatalytic calcification reactor. Colloids Surf. B Biointerfaces 86 (2), 404–408. https://doi.org/10.1016/j.colsurfb.2011.04.031.

ATSDR (Agency for Toxic Substances and Disease Registry). 2003. Public health statement Selenium. https://www.atsdr.cdc.gov/ToxProfiles/tp92-c1-b.pdf, accessed January 20, 2023.

Baptista, P.V., McCusker, M.P., Carvalho, A., Ferreira, D.A., Mohan, N.M., Martins, M., Fernandes, A.R., 2018. Nano-strategies to fight multidrug resistant bacteria -"a battle of the titans. Front. Microbiol. 9, 1441. https://doi.org/10.3389/fmicb.2018.01441.

Battaglia-Brunet, F., Dictor, M.C., Garrido, F., Crouze, C., Morin, D., Dekeyser, K., Clarens, M., Baranger, P., 2002. An arsenic(III)-oxidizing bacterial population: selection, characterization, and performance in reactors. J. Appl. Microbiol. 93, 656–667.

Campos, V.L., León, C., Mondaca, M.A., Yañez, J., Zaror, C., 2011. Arsenic mobilization by epilithic bacterial communities associated with volcanic rocks from Camarones River, Atacama Desert, northern Chile. Arch. Environ. Contam. Toxicol. 61, 185–192.

Castro-Alonso, M.J., Montanez-Hernandez, L.E., Sanchez-Munoz, M.A., Macias-Franco, M.R., Narayanasamy, R., Balagurusamy, N., 2019. Microbially induced calcium carbonate precipitation (MICP) and its potential in bio-concrete: microbiological and molecular concepts. Front. Mater. 6, 126. https://doi.org/10.3389/fmats.2019.00126.

Castro-Severyn, J., Remonsellez, F., Valenzuela, S.L., Salinas, C., Fortt, J., Aguilar, P., Pardo-Esté, C., Dorador, C., Quatrini, R., Molina, F., Aguayo, D., Castro-Nallar, E., Saavedra, C.P., 2017. Comparative genomics analysis of a New *Exiguobacterium* strain from Salar de Huasco reveals a repertoire of stress-related genes and arsenic resistance. Front. Microbiol. 8, 456. https://doi.org/10.3389/fmicb.2017.00456.

Castro-Severyn, J., Pardo-Esté, C., Mendez, K.N., Morales, N., Marquez, S.L., Molina, F., Remonsellez, F., Castro-Nallar, E., Saavedra, C.P., 2020. Genomic variation and arsenic tolerance emerged as niche specific adaptations by different *Exiguobacterium* strains isolated from the extreme Salar de Huasco environment in Chilean Altiplano. Front. Microbiol. 11, 1632.

Coker, J.A., 2016. Extremophiles and biotechnology: current uses and prospects. F1000Res 5, 396. https://doi.org/10.12688/f1000research.7432.1.

Colwell, F.S., Smith, R.W., Ferris, G.F., Reysenbach, A.L., Fujita, Y., Tyler, T.L., Taylor, J.L., Banta, A., Delwiche, M.E., McLing, T.L., Watwood, M.E., 2005. Microbially mediated subsurface calcite precipitation for removal of hazardous divalent cations. Microbial activity, molecular biology, and modeling, Subsurface Contamination Remediation: Accomplishments of the Environmental Management Science Program. ACS Symposium Series, Vol. 904. American Chemical Society, Washington, D.C., pp. 117–137.

Cortés-Albayay, C., Silber, J., Imhoff, J., Asenjo, J., Andrews, B., Nouioui, I., Dorador, C., 2019. The polyextreme ecosystem, Salar de Huasco at the Chilean Altiplano of the Atacama desert houses diverse *Streptomyces* spp. with promising pharmaceutical potentials. Diversity 11 (5), 69. https://doi.org/10.3390/d11050069.

Dhanjal, S., Cameotra, S.S., 2010. Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil. Microb. Cell Fact. 9, 52.

Dhanjal, S., Singh, A., Cameotra, S., 2014. Global gene expression analysis of bacterial stress response to elevated concentrations of toxic metalloids-Selenium and Arsenic. Geomicrobiol. J. 31 (6), 480–492. https://doi.org/10.1080/01490451.2013.822613.

Dorador, C., Busekow, A., Vila, I., Imhoff, J., Witzel, K.P., 2008a. Molecular analysis of enrichment cultures of ammonia oxidizers from the Salar de Huasco, a high altitude saline wetland in northern Chile. Extremophiles 12, 405–414. https://doi.org/10.1007/s00792-008-0146-x.

Dorador, C., Vila, I., Imhoff, J.F., Witzel, K.P., 2008b. Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion? FEMS Microbiol. Ecol. 64 (3), 419–432. https://doi.org/10.1111/j.1574-6941.2008.00483.x.

Dorador, C., Meneses, D., Urtuvia, V., Demergasso, C., Vila, I., Witzel, K.-P., Imhoff, J.F., 2009. Diversity of bacteroidetes in high altitude saline evaporitic basins in northern Chile. J. Geophys. Res. 114, G00D05. https://doi.org/10.1029/2008JG000837.

Dorador, C., Vila, I., Remonsellez, F., Imhoff, J.F., Witzel, K.-P., 2010. Unique clusters of Archaea in Salar de Huasco, an athalassohaline evaporitic basin of the Chilean Altiplano. FEMS Microbiol. Ecol. 73 (2), 291–302. https://doi.org/10.1111/j.1574-6941.2010.00891.x.

Dorador, C., Vila, I., Witzel, K.-P., Imhoff, J., 2013. Bacterial and archaeal diversity in high altitude wetlands of the Chilean Altiplano. Fundam. Appl. Limnol. /Arch. FüR. Hydrobiol. 182, 135–159. https://doi.org/10.1127/1863-9135/2013/0393.

Dorador, C., Molina, V., Hengst, M., Eissler, Y., Cornejo, M., Fernández, C., et al., 2020. Microbial communities composition, activity, and dynamics at Salar De Huasco: a polyextreme environment in the Chilean Altiplano. In: Farías, M.E. (Ed.), Microbial Ecosystems in Central Andes Extreme Environments. Springer International Publishing, New York,NY, pp. 123–139.

Eissler, Y., Dorador, C., Kieft, B., Molina, V., Hengst, M., 2020. Virus and potential host microbes from viral-enriched metagenomic characterization in the high-altitude wetland, Salar de Huasco, Chile. Microorganisms 8, 1077. https://doi.org/10.3390/microorganisms8071077.

Escobar-Ramírez, M., Castañeda-Ovando, A., Pérez-Escalante, E., Rodríguez-Serrano, G., Ramírez-Moreno, E., Quintero-Lira, A., Contreras-López, E., Añorve-Morga, J., Jaimez-Ordaz, J., González-Olivares, L., 2021. Antimicrobial activity of se-nanoparticles from bacterial biotransformation. Fermentation 7 (3), 130. https://doi.org/10.3390/fermentation7030130.

Eswayah, A.S., Hondow, N., Scheinost, A.C., Merroun, M., Romero-González, M., Smith, T.J., Gardiner, P.H.E., 2019. Methyl Selenol as a precursor in selenite reduction to Se/S Species by methane-oxidizing bacteria. Appl. Environ. Microbiol. 85 (22) https://doi.org/10.1128/AEM.01379-19 e01379-19.

Fadaei, A., Mohammadian-Hafshejani, A., 2023. Selenium removal from water and wastewater by different technologies: a systematic review. Iran. J. Public Health 52 (1), 64–77. https://doi.org/10.18502/ijph.v52i1.11667. PMID: 36824240; PMCID: PMC9941446.

Farías, M.E., Rascovan, N., Toneatti, D.M., Albarracín, V.H., Flores, M.R., Poiré, D.G., Collavino, M.M., Aguilar, O.M., Vazquez, M.P., Polerecky, L., 2013. The discovery of stromatolites developing at 3570 m above sea level in a high-altitude volcanic lake Socompa, Argentinean Andes. PLoS ONE 8, e53497. https://doi.org/10.1371/journal.pone.0053497.

Fesharaki, P.J., Nazari, P., Shakibaie, M., Rezaie, S., Banoee, M., Abdollahi, M., Shah-Verdi, A.R., 2010. Biosynthesis of selenium nanoparticles using *Klebsiella pneumoniae* and their recovery by a simple sterilization process. Braz. J. Microbiol. 41, 461–466.

Fujita, Y., Redden, G.D., Ingram, J.C., Cortez, M.M., Ferris, F.G., Smith, R.W., 2004. Strontium incorporation into calcite generated by bacterial ureolysis. Geochim. Cosmochim. Acta 68, 3261–3327.

Gao, X., Zhang, J., Zhang, L., 2002. Hollow sphere selenium nanoparticles. Their in vitro anti-hydroxyl radical effect. Adv. Mater. 14, 290–293.
García Torreiro, M., 2017. Biopolymers Production by *Halomonas boliviensis* in an Integrated Biorefinery. Doctoral Thesis, Universidad de Santiago de Compostela, Spain.
Gonzalez-Muñoz, M.T., Rodriguez-Navarro, C., Martinez-Ruiz, F., Arias, J., Mohamed, M., Rodriguez-Gallego, M., 2010. Bacterial Biomineralization: New Insights From *Myxococcus*-Induced Mineral Precipitation. In: Geological Society, 336. London Special Publications, pp. 31–50.
Hammes, F., Seka, A., Van Hege, K., Van de Wiele, T., Vanderdeelen, J., Siciliano, S.D., Verstraete, W., 2003b. Calcium removal from industrial wastewater by bio-catalytic CaCO3 precipitation. J. Chem. Technol. Biotechnol. 78, 670–677. https://doi.org/10.1002/jctb.840.
Hammes, F., Boon, N., Villiers, J., Verstraete, W., Siciliano, S., 2003a. Strain-specific ureolytic microbial calcium carbonate precipitation. Appl. Environ. Microbiol. 69 (8), 4901–4909.
Hernández, K.L., Yannicelli, B., Olsen, L.M., Dorador, C., Menschel, E.J., Molina, V., Remonsellez, F., Hengst, M.B., Jeffrey, W.H., 2016. Microbial activity response to solar radiation across contrasting environmental conditions in Salar de Huasco, northern Chilean Altiplano. Front. Microbiol. 7, 1857. https://doi.org/10.3389/fmicb.2016.01857.
Herrera, C., Moraga, R., Bustamante, B., Vilo, C., Aguayo, P., Valenzuela, C., Smith, C.T., Yáñez, J., Guzmán-Fierro, V., Roeckel, M., Campos, V.L., 2021. Characterization of arsenite-oxidizing bacteria isolated from arsenic-rich sediments, Atacama Desert, Chile. Microorganisms 9 (3), 483. https://doi.org/10.3390/microorganisms9030483.
Hosnedlova, B., Kepinska, M., Skalickova, S., Fernandez, C., Ruttkay-Nedecky, B., Peng, Q., Baron, M., Melcova, M., Opatrilova, R., Zidkova, J., Bjørklund, G., Sochor, J., Kizek, R., 2018. Nano-selenium and its nanomedicine applications: a critical review. Int. J. Nanomed. 13, 2107–2128. https://doi.org/10.2147/IJN.S157541.
Jansson, C., Northern, T., 2010. Calcifying cyanobacteria-the potential of biomineralization for carbon capture and storage. Curr. Opin. Biotechnol. 21, 1–7.
Jha, B.K., Gandhi Pragash, M.G., Cletus, J., Gandhi Pragash, M., Cletus, J., Raman, G., Sakthivel, N., 2009. Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent *Pseudomonas* strains, *Pseudomonas aeruginosa*, *P. plecoglossicida* and *P. mosselii*. World J. Microbiol. Biotechnol. 25 (4), 573–581.
Kessi, J., Hanselmann, K.W., 2004. Similarities between the abiotic reduction of selenite with glutathione and the dissimilatory reaction mediated by *Rhodospirillum rubrum* and *Escherichia coli*. J. Biol. Chem. 279, 50662–50669.
Kessi, J., Ramuz, M., Wehrli, E., Spycher, M., Bachofen, R., 1999. Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium *Rhodospirillum rubrum*. Appl. Environ. Microbiol. 65 (11), 4734–4740. https://doi.org/10.1128/AEM.65.11.4734-4740.1999.
Lebre, P.H., De Maayer, P., Cowan, D.A., 2017. Xerotolerant bacteria: surviving through a dry spell. Nat. Rev. Microbiol. 15, 285–296. https://doi.org/10.1038/nrmicro.2017.16.
Leon, C.G., Moraga, R., Valenzuela, C., Gugliandolo, C., Lo Giudice, A., Papale, M., Vilo, C., Dong, Q., Smith, C.T., Rossello-Mora, R., Yañez, J., Campos, V.L., 2018. Effect of the natural arsenic gradient on the diversity and arsenic resistance of bacterial communities of the sediments of Camarones River (Atacama Desert, Chile). PLoS ONE 13 (5), e0195080. https://doi.org/10.1371/journal.pone.0195080.
Li, B., Liu, N., Li, Y., Jing, W., Fan, J., Li, D., Zhang, L., Zhang, X., Zhang, Z., Wang, L., 2014. Reduction of selenite to red elemental selenium by *Rhodopseudomonas palustris* strain N. 2014. PLoS ONE 9 (4), e95955. https://doi.org/10.1371/journal.pone.0095955.
Li, M., Cheng, X., Guo, H., 2013. mHeavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int. Biodeterior. Biodegrad. 76, 81–85. https://doi.org/10.1016/j.ibiod.2012.06.016.
Luong, V.T., Cañas Kurz, E.E., Hellriegel, U., Luu, T.L., Hoinkis, J., Bundschuh, J., 2018. Iron-based subsurface arsenic removal technologies by aeration: a review of the current state and future prospects. Water Res. 133, 110–122. https://doi.org/10.1016/j.watres.2018.01.007.
Maltman, C., Yurkov, V., 2018. Bioremediation potential of bacteria able to reduce high levels of selenium and tellurium oxyanions. Arch. Microbiol. 200 (10), 1411–1417. https://doi.org/10.1007/s00203-018-1555-1556.
Merino, N., Aronson, H.S., Bojanova, D., Feyhl-Buska, J., Wong, M.L., Zhang, S., Giovannelli, D., 2019. Living at the extremes: extremophiles and the limits of life in a planetary context. Front. Microbiol. Sec. Extrem. Microbiol. 10, 780. https://doi.org/10.3389/fmicb.2019.00780.
Mitchell, A., Ferris, F., 2006. The Influence of *Bacillus pasteurii* on the nucleation and growth of calcium carbonate. Geomicrobiol. J. 23 (3-4), 213–226. https://doi.org/10.1080/01490450600724233.
Molina, V., Hernández, K., Dorador, C., Eissler, Y., Hengst, M., Pérez, V., Harrod, C., 2016. Bacterial active community cycling in response to solar radiation and their influence on nutrient changes in a high-altitude wetland. Front. Microbiol. 7, 1823. https://doi.org/10.3389/fmicb.2016.01823.
MOP., 2009. Levantamiento hidrogeológico para el desarrollo de nuevas fuentes de agua en áreas prioritarias de la zona norte de chile, regiones XV, I, II Y III. https://snia.mop.gob.cl/sad/REH5161v4.pdf, accessed January 230, 2023.
Muynck, W.D., Belie, N.D., Verstraete, W., 2010. Microbial carbonate precipitation in construction materials: a review. Ecol. Eng. 36, 118–136.
Muzammil, S., Hayat, S., Fakhar-E-Alam, M., Aslam, B., Siddique, M.H., Nisar, M.A., Saqalein, M., Atif, M., Sarwar, A., Khurshid, A., Amin, N., Wang, Z., 2018. Nanoantibiotics: future nanotechnologies to combat antibiotic resistance. Front. Biosci. (Elite Ed.) 10 (2), 352–374. https://doi.org/10.2741/e827.
Natan, M., Banin, E., 2017. From nano to micro: using nanotechnology to combat microorganisms and their multidrug resistance. FEMS Microbiol. Rev. 41, 302–322. https://doi.org/10.1093/femsre/fux003.
Nicomel, N.R., Leus, K., Folens, K., Van Der Voort, P., Du Laing, G., 2015. Technologies for arsenic removal from water: current status and future perspectives. Int. J. Environ. Res. Public Health 13 (1), 0062. https://doi.org/10.3390/ijerph13010062.
Nidheesh, P.V., Singh, T.S.A., 2017. Arsenic removal by electrocoagulation process: recent trends and removal mechanism. Chemosphere 181, 418–432. https://doi.org/10.1016/j.chemosphere.2017.04.082.
Orellana, R., Macaya, C., Bravo, G., Dorochesi, F., Cumsille, A., Valencia, R., Rojas, C., Seeger, M., 2018. Living at the frontiers of life: extremophiles in Chile and their potential for bioremediation. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.02309. Their Potential for Bioremediation. Front. Microbiol. 9, 2309. 10.3389/fmicb.2018.02309.
Oremland, R.S., Herbel, M.J., Switzer-Blum, J., Langley, S., Beveridge, T.J., Ajayan, P.M., Sutto, T., Ellis, A.V., Curran, S., 2004. Structural and spectral features of selenium nanospheres produced by Se-respiring bacteria. Appl. Environ. Microbiol. 70, 52–60.
Otsuka, O., Yamashita, M., 2020. Selenium recovery from wastewater using the selenate-reducing bacterium *Pseudomonas stutzeri* NT-I. Hydrometallurgy 197, 105470. https://doi.org/10.1016/j.hydromet.2020.105470.
Pang, Z., Raudonis, R., Glick, B.R., Lin, T.-J., Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol. Adv. 37 (1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013.
Pérez, V., Hengst, M., Kurte, L., Dorador, C., Jeffrey, W.H., Wattiez, R., Molina, V., Matallana-Surget, S., 2017. Bacterial survival under extreme UV radiation: a comparative proteomics study of *Rhodobacter* sp., isolated from high altitude wetlands in Chile. Front. Microbiol. 8, 1173. https://doi.org/10.3389/fmicb.2017.01173.
Pérez, V., Dorador, C., Molina, V., Yáñez, C., Hengst, M., 2018. *Rhodobacter* sp. Rb3, an aerobic anoxygenic phototroph which thrives in the polyextreme ecosystem of the Salar de Huasco, in the Chilean Altiplano. Antonie Van. Leeuwenhoek 111 (8), 1449–1465. https://doi.org/10.1007/s10482-018-1067-z. Epub 2018 Mar 22. PMID: 29569108.
Presentato, A., Piacenza, E., Anikovskiy, M., Cappelletti, M., Zannoni, D., Turner, R.J., 2018. Biosynthesis of selenium-nanoparticles and nanorods as a product of selenite bioconversion by the aerobic bacterium *Rhodococcus aetherivorans* BCP1. N. Biotechnol. 41, 1–8. https://doi.org/10.1016/j.nbt.2017.11.002.
Quillaguamán, J., Hatti-Kaul, R., Mattiasson, B., Alvarez, M.T., Delgado, O., 2004. *Halomonas boliviensis* sp. nov., an alkalitolerant, moderate halophile isolated from soil around a Bolivian hypersaline lake. Int. J. Syst. Evol. Microbiol. 54 (3), 721–725. https://doi.org/10.1099/ijs.0.02800-0.
Remonsellez, F., Castro-Severyn, J., Pardo-Esté, C., Aguilar, P., Fortt, J., Salinas, C., Barahona, S., León, J., Fuentes, B., Areche, C., Hernández, K.L., Aguayo, D., Saavedra, C.P., 2018. Characterization and salt response in recurrent *Halotolerant Exiguobacterium* sp. SH31 isolated from sediments of Salar de Huasco, Chilean Altiplano. Front. Microbiol 20 (9), 2228. https://doi.org/10.3389/fmicb.2018.02228.

Ridley, H., Watts, C.A., Richardson, D.J., Butler, C.S., 2006. Resolution of distinct membrane-bound enzymes from *Enterobacter cloacae* SLD1a-1 that are responsible for selective reduction of nitrate and selenate oxyanions. Appl. Environ. Microbiol. 72, 5173–5180.

Shakibaie, M., Forootanfar, H., Golkari, Y., Mohammadi-Khorsand, T., Shakibaie, M.R., 2015. Anti-biofilm activity of biogenic selenium nanoparticles and selenium dioxide against clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. J. Trace Elem. Med. Biol. 29, 235–241. https://doi.org/10.1016/j.jtemb.2014.07.020.

Sharma, K., Sohn, M., 2009. Aquatic arsenic: toxicity, speciation, transformations, and remediation. Environ. Int. 35 (4), 743–759. https://doi.org/10.1016/j.envint.2009.01.005.

Singh, P., Garg, A., Pandit, S., Mokkapati, V., Mijakovic, I., 2018. Antimicrobial effects of biogenic nanoparticles. Nanomaterials 8 (12), 1009. https://doi.org/10.3390/nano8121009.

Stolz, J., Basu, P., Oremland, R., 2002. Microbial transformation of elements: the case of arsenic and selenium. Int. Microbiol. 5, 201–207. https://doi.org/10.1007/s10123-002-0091-y.

Thiel, V., Tank, M., Neulinger, S.C., Gehrmann, L., Dorador, C., Imhoff, J.F., 2010. Unique communities of anoxygenic phototrophic bacteria in saline lakes of Salar de Atacama (Chile): evidence for a new phylogenetic lineage of phototrophic Gammaproteobacteria from pufLM gene analyses. FEMS Microbiol. Ecol. 74, 510–522. https://doi.org/10.1111/j.1574-6941.2010.00966.x.

Torres, S.K., Campos, V.L., León, C.G., Rodríguez-Llamazares, S.M., Rojas, S.M., González, M., Smith, C.T., Mondaca, M.A., 2012. Biosynthesis of selenium nanoparticles by *Pantoea agglomerans* and their antioxidant activity. J. Nanopart. Res. 14, 1236. https://doi.org/10.1007/s11051-012-1236-3.

USEPA, 2001. Implementation Guidance for Arsenic. Draft report, Cincinnati, EPA 816-D-01-002.

Wang, H., Zhang, J., Yu, H., 2007. Elemental selenium at nano size possesses lower toxicity without compromising the fun-damental effect on selenoenzymes: comparison with selenomethionine in mice. Free Radic. Biol. Med. 42, 1524. –153.

Wang, Y., Shu, X., Zhou, Q., Fan, T., Wang, T., Chen, X., Li, M., Ma, Y., Ni, J., Hou, J., Zhao, W., Li, R., Huang, S., Wu, L., 2020. Selenite reduction and the biogenesis of selenium nanoparticles by *Alcaligenes faecalis* Se03 isolated from the gut of *Monochamus alternatus* (Coleoptera: Cerambycidae). Int. J. Mol. Sci. 21 (4), 1294.

Warthmann, R., Van Lith, Y., Vasconcelos, C., McKenzie, J.A., Karpoff, A.M., 2000. Bacterially induced dolomite precipitation in anoxic culture experiments. Geology 28, 1091–1094.

Wei, S., Cui, H., Jiang, Z., Liu, H., He, H., Fang, N., 2015. Biomineralization processes of calcite induced by bacteria isolated from marine sediments. Braz. J. Microbiol. 46 (2), 455–464. https://doi.org/10.1590/S1517-838246220140533.

WHO, 2001. Arsenic compounds: Environmental health criteria, 2nd ed., 224. World Health Organization, Geneva.

Xu, C., Qiao, L., Guo, Y., Ma, L., Cheng, Y., 2018a. Preparation, characteristics and antioxidant activity of polysaccharides and proteins-capped selenium nanoparticles synthesized by *Lactobacillus casei* ATCC 393. Carbohydr. Polym. 195, 576–585.

Xu, D., Yang, L., Wang, Y., Wang, G., Rensing, C., Zheng, S., 2018b. Proteins enriched in charged amino acids control the formation and stabilization of selenium nanoparticles in *Comamonas testosteroni* S44. Sci. Rep. 8, 4766. https://doi.org/10.1038/s41598-018-23295-5.

Xu, Q., Song, Y., Lin, Z., Bañuelos, G., Zhu, Y., Guo, Y., 2020. The small RNA chaperone Hfq is a critical regulator for bacterial biosynthesis of selenium nanoparticles and motility in *Rahnella aquatilis*. Appl. Microbiol. Biotechnol. 104, 1721–1735. https://doi.org/10.1007/s00253-019-10231-4.

Yañez, J., Fierro, V., Mansilla, H., Figueroa, L., Cornejo, L., Barnes, R., 2005. Arsenic speciation in human hair: a new perspective for epidemiological assessment in chronic arsenicism. J. Environ. Monit. 7, 1335–1341. https://doi.org/10.1039/b506313b. PMID: 16307093.

Yañez, J., Mansilla, H.D., Santander, I.P., Fierro, V., Cornejo, L., Barnes, R.M., et al., 2015. Urinary arsenic speciation profile in ethnic group of the Atacama Desert (Chile) exposed to variable arsenic levels in drinking water. J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng. 50, 1–8. https://doi.org/10.1080/10934529.2015.964594.

Yanez-Lemus, F., Moraga, R., Mercado, L., Jara-Gutierrez, C., Smith, C.T., Aguayo, P., Sanchez-Alonzo, K., García-Cancino, A., Valenzuela, A., Campos, V.L., 2022. Selenium nanoparticles biosynthesized by *Pantoea agglomerans* and their effects on cellular and physiological parameters in the rainbow trout *Oncorhynchus mykiss*. Biology 11, 463. https://doi.org/10.3390/biology11030463.

Yu, B., Zhang, Y., Zheng, W., Fan, C., Chen, T., 2012. Positive surface charge enhances selective cellular uptake and anticancer efficacy of selenium nanoparticles. Inorg. Chem. 51, 8956–8963.

Zammuto, V., Fuchs, F.M., Fiebrandt, M., Stapelmann, K., Ulrich, N.J., Maugeri, T.L., Pukall, R., Gugliandolo, C., Moeller, R., 2018. Comparing spore resistance of Bacillus strains isolated from hydrothermal vents and spacecraft assembly facilities to environmental stressors and decontamination treatments. Astrobiology 18, 1425–1434.

Zhang, J., Xu, J.X., Kong, Y.Z., et al., 2005. Generation of chemical-inducible activation tagging T-DNA insertion lines of Arabidopsis thaliana. Acta Genet. Sin. 32, 1082–1088.

Zhang, S., Zhang, J., Wang, H., Chen, H., 2004. Synthesis of selenium nanoparticles in the presence of polysaccharides. Mater. Lett. 58 (21), 2590–2594.