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RESEARCH ARTICLE

ENVIRONMENTAL BIOCHEMISTRY OF ARSENIC SPECIES IN CONTAMINATED AREAS

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ABSTRACT

Arsenic in the Antofagasta Region of Chile is worldwide recognized as highly As contaminated area. Due to its abundance, mainly as As (V) and As (III), certain microorganisms such as several types of bacteria have evolved and developed the necessary genetic components which confer resistance mechanisms. These mechanisms include reactions of reduction, oxidation and methylation. The aim of this work was to study the most relevant arsenic resistant bacteria that exist in highly arsenic contaminated sediments in *El Tatio* geyser field. This place is a suitable habitat to study the adaptation of endemic species subjected to extreme environmental conditions. All bacterial strains isolated were grown with increasing concentration of arsenate, exhibiting high levels of arsenate resistance ranging from to 5 to 30 mM. Inductively coupled plasma mass spectrometry (ICP-MS) was employed to determine the concentration of As within intact cells of each bacterial strain. Results showed a great accumulation of this element. The separation of bacterial cells into cytoplasmic and membrane fractions were carried out by differential centrifugation in order to know the distribution of arsenic in the different cellular compartments. Most of arsenic was mainly located in the cytoplasmic fraction. Indeed, the optimization of chromatographic methods coupled to ICP-MS allowed us to separate and quantify the different arsenic species as a result of bacterial transformations. The results demonstrated that in half of the isolated strains, between 20-70% of arsenate was reduced to arsenite. Only in one case it was observed the presence of methylated species of arsenic such as DMA and MMA.

INTRODUCTION

Arsenic element is highly abundant and worldwide distributed through the Earth crust. Volcanogenic activity, underground waters, thermal springs and mining or industrial activities release this toxic element and left a heritage of arsenic in some well-known contaminated places such as New Zealand, Japan, Argentina, Bangladesh, etc. (Nodrtrom, 2002; Ahsanand Del Valls, 2011; Huang, 2014; Shakoor, 2015; Rahman, 2014). This is also the case of the geothermal springs Tatio Geysers field in Atacama Desert of the II Region of Chile that contain arsenic concentrations in water of 22-60 mg L^{-1} (Cortecci, 2005; Fernandez-Turiel, 2004). This high concentration and high temperature of water, about 60 ºC, together the high UV radiation, produce than only microbial communities able to resist these extreme conditions, could live in this area, probably developing different defence or energy uptake mechanisms in a similar way than reported in other world places (Hsiu-Chuan, 2010; Urbieta, 2014; Landrum, 2009; Langner, 2001; Ashan, 2000; Ashan, 2011). Since the first knowledge about arsenic biotransformation through a series of reductions of pentavalent to trivalent arsenic species and subsequent oxidative methylation with the sulfur atom from S-adenosyl-methionine as redox partner (Challenger, 1945), many studies about the cellular uptake, subcellular distribution, arsenic speciation and toxicity of arsenic compounds in methylating and non methylatingcells has been done (Dopp, 2010). It is believed thatarsenate enters the cell through existing phosphate transporter owing its chemical simility andarsenite in the form of not charged $As(OH)_3$ mainly by aquaporin superfamily of transporters(Rosen, 2002).Arsenate can be used by the cell as an electron acceptor for anaerobic respiration. In fact, in the absence of oxygen, the arsenate reduction to arsenite is a display versatile energy-generating system for respiration mediated by lactate oxidation. This behaviour has been reported in extremophiles bacteria living in hot springs environmental places with respiratory arsenate reductase activity. On the contrary, arsenite can be used as an electron donor to support chemoautotrophic fixation of CO_2 into cell carbon. It is known that arsenite is more noxious to cells than arsenate due to the avidity for thiol groups of biological molecules such as proteins; therefore the oxidation mechanism of arsenite to arsenate could be also considered as a detoxification mechanism (Anderson, 1992; Hamamura, 2009; Donahoe-Christiansen, 2004).

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The methylation cascade of the inorganic As(III) and As(V) species has been also reported as one of the main detoxification mechanism (Cullen, 2010). The contribution of the responses of family of microbes to environmental cycle of arsenic, have been reported in very interesting reviews (Stolz, 2006). The physic-chemical characteristics of the site and the structure and microbial metabolism, determine the arsenic species present the microbial volatilization of arsines in the environment (Paez-Espino, 2009; Stolz, 2006; Wang, 2014).
The oxidation/reduction activity of arsenic inside the cell is mediated by the operons that includes up to five genes. Enzymes arsC operon gene coding for the reduction of As(V) to As(III), and the pump coded by arsB, for As(III) exclusion from the cell, is one of the most common detoxification mechanism(Paez-Espino, 2009). The presence of the arsenic oxidation, reduction and extrusion genes arsC, arrA, aioA and acr3 has been recently explored in a range of natural environments in the Salar de Ascotán, northern of Chile, (Escudero, 2013). Relevant microbial community related to the presence of arsenite-oxidizing and arsenate-reducing bacteria, that exists in rich-arsenic aquifer, soils, sediments, dessert places and geysers in Chile have been characterized (Escalante, 2009; Campos, 2007). Some of these studies have been carried out in arid zones located of the Atacama Desert and infer the functional potential of these communities based on observed phylogenetic association. The analysis revealed high abundances of novel *actinobacteria* and *chloroflexin* and low levels of *acidobacteria* and *proteobacteria* (Neilson, 2012). In the hot spring and geyser sinters of El Tatio, the ferric oxyhydroxides mineral precipitates associated with the microbial matsand accumulate substantial arsenic that was identified as arsenate. This arsenate is easily mobilized by anion exchange or mild dissolution and the ubiquitous microbial mats represent a significant reservoir of arsenic in this system (Landrum, 2009).The fingerprints of organisms observed in a study performed by Fernandez-Turiel, 2005 represent microbes and suggest that the microbial community are mainly *cyanobacteria*, *green bacteria*, and *diatoms* (Fernandez-Turiel, 2005; Jones and Renaut, 1997).

Our objectives about the bioinorganic transformation of As(V) by microorganism present in the Tatio area of Northern of Chile, have been centralized in the bacterial community 4 km down the Geyser El Tatio field. Our studies have been focused to: i) isolate the microorganisms present in sediments and water samples able to live in such extreme arsenic contamination; ii) The semicuantitative and quantitative determination of total arsenic and arsenic species present in the cytoplasm and citoplasmatic membrane of resistant bacteria's.

MATERIALS AND METHODS

Instrumentation and materials

An inductively coupled plasma mass spectrometer (ICP-MS), (HP-4500, Agilent Technologies, Analytical System, Tokio, Japan) operating under normal multi-element tuning conditions was used for total arsenic analysis. The main analytical parameters of the ICP-MS are the following: R.F. power: forward 1350 W; reflected 2.2 W; coolant argon flow rate 14 L min^{-1}; auxiliary argon flow rate 0.9 L min^{-1}; integration time 0.1 s per point, point per peak 3. The solutions were introduced in the plasma through a Babington nebulizer and routed through a double-pass Scott-type spray chamber maintained at 2°C. The signals of arsenic at a mass-to-charge ratio of m/z = 75 (dwell time = 1000 ms), and indium (m/z = 115, dwell time = 1000 ms), were monitored. The signal at m/z 77 was monitored in order to control argon chloride interference. Quantitation was performed by external calibration with an arsenate standard solution and validated by recoveries obtained of different As (V) concentration added to samples. For As speciation, the ICP-MS has been used as the detector system after LC species separation by a gradient HPLC pump Jasco Pu 2089 plus (Tokyo, Japan) and a Hamilton PRP-X100 column (10µm, 250 mm x 4.1 mm) and Phenomenex precolumn (25 x 2.3 mm 12-20µm) (Torrance, CA, USA). The column effluent was directly introduced into a Meinhard-type concentration glass nebulizer and a double-pass Scott-type spray chamber with a surrounding water jacket maintained at 5ºC. Samples of 100 µL were introduced through a 0.45-µm nylon syringe filter into the injection valve Rheodyne 9125 (USA). The connections between the HPLC and the ICP-MS were made of polytetrafluoroethylene tubing (i.d. 0.5 mm).All signal quantification was performed in the peak area mode. The peaks were integrated using either ICP-MS Plasma Lab software or Grams/32 software (Galactic Industries, Salem, NY, USA).

Reagents

The Standard ofAs (III) of 1000 ± 2 mg L^{-1} was prepared in HNO_3 (2%, m/m), TraceCERT™ Ultra (Fluka, Sigma-Aldrich, Steinheim, Germany). The standard of As (V) 1000 ± 2 mg L^{-1}, was prepared in HNO_3 (2% m/m), CertiPUR (Merck, Darmstadt, Germany). Dimethylarsinic acid (DMA) and methylarsonic acid (MMA) standard of 1000 mg L^{-1}, were prepared from methyl disodium arseniate ($Na_2CH_3AsO_3$, 99% Supelco, Bellefonte, PA, USA) and cacodilic sodium trihydro ($(CH_3)_2AsNaO_2.3H_2O$) 98% Fluka) TraceCERT™. Arsenobetaine (AsB) and arsenocholine (AsC) were obtained from Tri Chemical Laboratory Inc. (Japan). These solutions were kept at 4ºC in the dark. Working solutions were daily prepared by dilution with Milli-Q water. All reagents were of analytical grade: HNO_3 60% (Scharlau), HCl 15% (Scharlau), ammonium phosphate 98% (Scharlau). Deionized water (18.2 MΩ cm), obtained with a Milli-Q water system (Millipore, Inc., Spain) was used for all solutions preparation. Plastic and glassware was maintained in 10% HNO_3 for 24 h before use.

Sampling places and samples collection

Samples were taken from Platform A1, belonging to the geothermal perforation project of El Tatio, 4 km down the Geyser El Tatio field. Four places very close (22º22´S; 67º 59´O) and above 3700 m of altitude were selected for sampling. Fig.1 shows the places were sediments and water samples were taken. Three water samples were collected in each place in the board of affluent and the water surface and in a column of 10 cm. Three sediments were also taken in the fourth places. All samples were maintained in polyethylene containers at 4ºC in an icebox before transportation to the laboratory and processing.

Bacterial isolates

The 68 arsenate-resistant strains investigated in this study were previously isolated from the sediments or water following the procedure by Simeonova et al. (Simeonova, 2004).

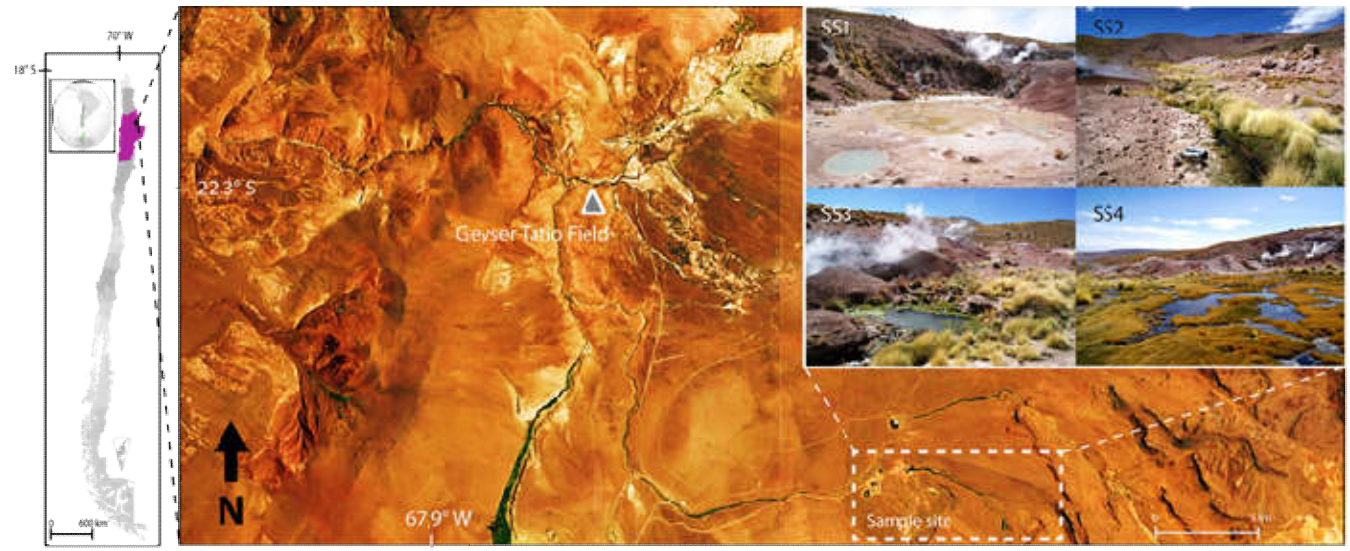

Figure 1. Location and Sampling sites showing the platform of the Project Geothermic *El Tatio*

Sediments or water were placed in sterile flasks containing 12 mL of CDM medium (2% wt/vol) (Muller, 2003) supplemented with 5 Mm of As(V) and incubated at 25±2 ºC for 48 h. Enrichment cultures were centrifuged at 5000 rpm for 15 min, washed twice with sterile deionized water and spread onto a new As(V)-supplemented CDM agar (2%) at 28±2 ºC for 10 days in the dark. The bacterial isolates were submitted to different experiments.

Tolerance level of As and determination of bacterial reduced activity of As(V)

Bacterial isolates resistance to As(V) was evaluated by minimum inhibitory concentrations (MIC) tests under aerobic conditions. MICs of the isolated strains were assessed as previously described (Achour, 2007; Escalante, 2009; Lim and Cooksey, 1993). Aliquots of bacterial cultures (OD600=0.3) were grown on CMD agar plates (2%) containing different concentrations of As(V) (2– 100 mM) and then incubated at 28 °C in oxic or anoxic conditions. The MIC is defined as the lowest concentrations of As(V) that completely inhibited bacterial growth on agar medium after 72 and 120 h of incubation. Control bacteria's were obtained in the same CMD agar without As (V). The abilities of the obtained arsenic-resistant bacteria to reduce As(V) were tested under aerobic conditions. The isolates were first tested using a qualitative $AgNO_3$ screening method as described by Simeonova et al. (Simeonova, 2004). The qualitative method to determine the transformation of original As(V) specie to As(III), is based on the formation of white colored precipitates at pH 6.5–8.5 upon reaction of $AgNO_3$ with Tris–HCl or Tris–maleate.

Precipitates containing arsenic were colored from light yellow of Ag_3AsO_3 (silverorthoarsenite) from the As[III] to light brown-red of Ag_3AsO_4 (silverorthoarsenate) from the As[V](Simonova, 2004).Different concentrations of $AgNO_3$ and Tris–HCl buffer (pH 7.5), supplemented with As[III] or As[V] ions, were investigated to define the correlation between color intensity and the ratio of As[III] and As[V] species. Pictures were taken with a digital camera (Nikon Coolpix 995, Japan), and were processed in PhotoShop 5.0 for Macintosh. This microscreening method will be used in the semiquantitative screening before HPLC-ICP-MS determination.

Membrane and cytoplasm prepared from the bacterial.

Bacterial after sodium arsenate treatment of 5 mM and 20 mM (for resistant's), were submitted to a several centrifugation steps in order to obtain the different parts of the cell. After tissue homogenization, the samples were maintained in tris buffer at pH = 7 to obtain a viscose mass. The centrifugation at 2500 rpm, for 10 minutes, precipitates the cell nucleus. The sobrenadant liquid is centrifuged at 9300 rpm for 10 minutes given a precipitate of the plasmatic membrane. An ultracentrifugation at 15000 rpm for 30 min is used to precipitate chromosome. The supernatant cytoplasm and the plasmatic membrane were analyzed for total arsenic and As speciation.

Total arsenic and As speciation by ICP-MS

Total As determination in the cytoplasm and plasmatic membrane after incubation to 5mM and 20 mM were performed by ICP-MS. Arsenic species were identified by comparison of the LC-ICP-MS chromatograms of the cytoplasm and citoplasmatic membrane with those provided by injection of standards solution of As(III), As(V), MMA, DMA, AsB and AsC. Furthermore, spiking the extracts with a specific As species were also performed to confirm the presence of this species. Since the results obtained by external calibration and standard addition matched well, it was no longer necessary to apply the standard addition method. The chromatographic conditions for HPLC-ICP-MS are given in Table 1.

Table 1. Chromatographic conditions for As speciation

Gradient Mobile phase
$(NH_4)H_2PO_4$ (A: 5 mM, B: 25mM) pH 6
0-15 min (A: 100-0; B: 0-100)
15-25 min (A: 0-100; B: 100-0)
Flow rate: (1 mL min^{-1})
Conditioning: 25-30 min (A:100; B: 0)

Performance parameters of the analytical method were studied, being the detection limits (DL) expresed in µg kg^{-1} of As of 5.0. Relative standard deviation (RSD) was of 5%. Because not certified material for total arsenic or As species are available, the method validation was performed in triplicate by spiking with the As species. In order to check the possible

interference of chloride in the ^{75}As signal due to the formation of $^{40}Ar^{35}Cl^{+}$ during ICP-MS and LC-ICP-MS analysis, the signal at m/z 77 of cytoplasm and membrane samples were monitored. This signal intensity for the all samples was negligible and did not differ from that of a blank solution, indicating that the procedure is free of interference by chloride.

RESULTS

Bacterial isolates and arsenic tolerance

In total, sixty-eight isolates with different colony morphologies were obtained from the El Tatio Geothermal Fieldby enrichment and isolation of single colonies on CMD

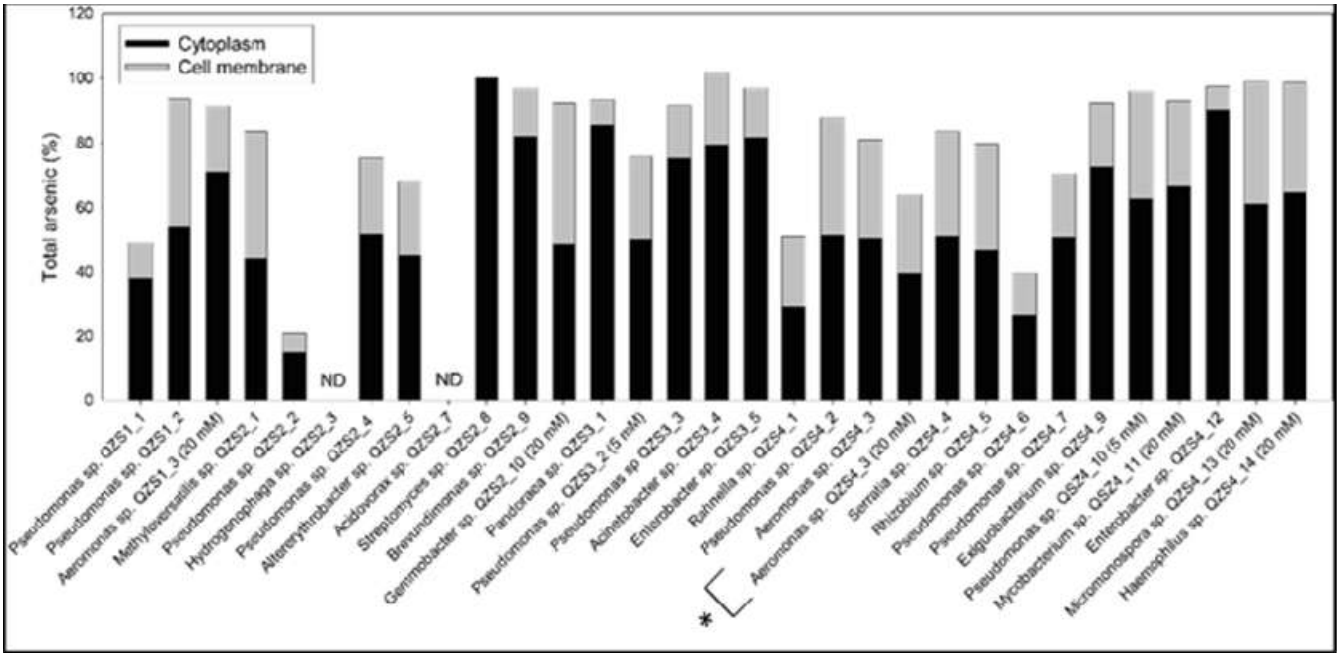

Figure 2. Distribution of total arsenic in the cytoplasm and membrane fractions

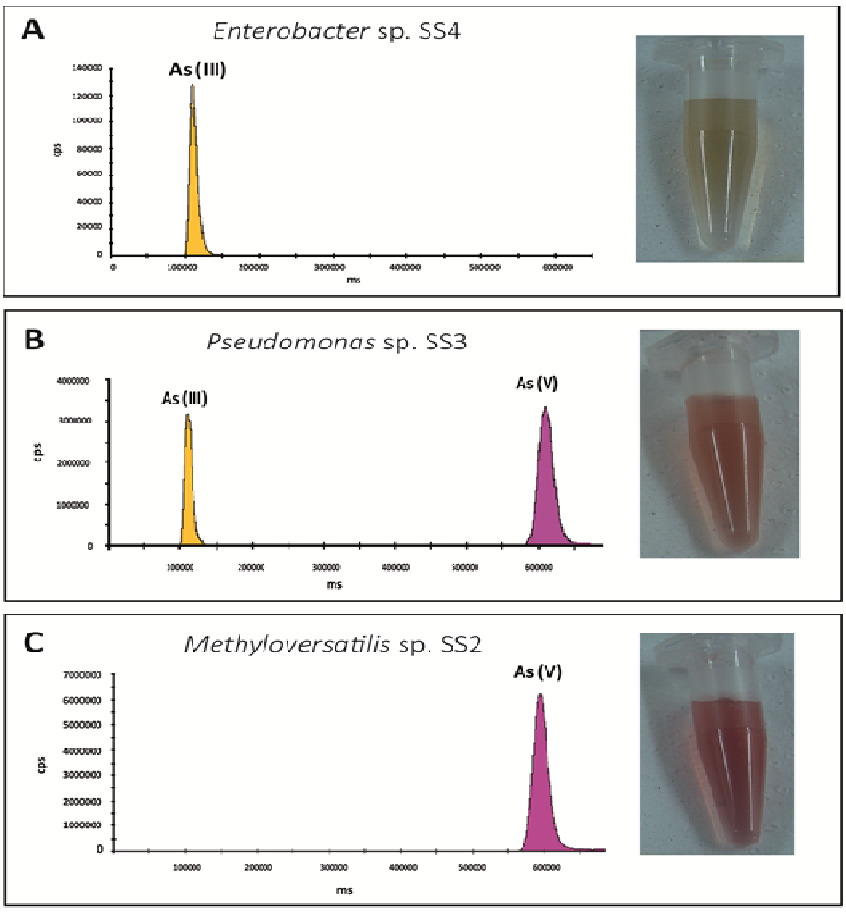

Figure 3. Color precipitates obtained with $AgNO_3$ screening method and corresponding chromatograms obtained by HPLC-ICP-MS in the isolates (A) *Enterobacter* sp SS4, (B) *Pseudomonas* sp. SS3 and (C) *Methyloversatilis* sp. SS2

plates. Isolation of As resistant bacterial strains was possible on the basis of their ability to growing the presence of 5 mM As(V) and according to the criteria of different colony morphologies. Tolerances of the sixty-eight bacterial isolates to As(V) were assessed by MIC tests under aerobic growth conditions. Only Thirty-two isolates were resistant to As(V). Under aerobic growth conditions, all of the bacterial isolates exhibited resistance to As(V) with MICs exceeding 20 mM. Ten isolates (*Hydrogenophaga* sp. SS2, *Brevundimonas* sp. SS2, *Pseudomonas* sp. SS3, *Acinetobacter* sp. SS3, *Rahnella* sp. SS4, *Pseudomonas* sp. SS4, *Pseudomonas* sp. SS4B, *Pseudomonas* sp. SS4C, *Pseudomonas* sp. SS4D, *Enterobacter* sp. SS4), in aerobic growth conditions, showed of 50 mM of As(V). Seven isolates (*Aeromonas* sp. SS1, *Exiguobacterium* sp. SS2, *Streptomyces* sp. SS2, *Rhodobacter* sp. SS2, *Aeromonas* sp. SS4, *Mycobacterium* sp. SS4, *Micromonospora* sp. SS4 and *Haemophilus* sp. SS4) were even resistant to As(V) at 100 mM whereas the remaining isolates showed lower levels (<50 mM) of resistance to As(V).

only twenty-seven showed significant decrease of arsenic concentration in the culture broth (Figure 2). Percentage of arsenic removal at the end of the experiment (after all the cultures reached a stationary phase) was determined by the precipitation of arsenite with a particular color depending of the arsenic species. As it can be seen in the Figure 3,the yellow precipitates obtained show a arseniate reduction, meanwhile a reddish coloration indicate a low or null arsenite presence. Arsenic transformation by isolates was further confirmed by data from HPLC-ICP-MS analysis. The profilechromatograms obtained for them show a complete reduction of As (V) to As(III) has been produced in the isolate *Enterobacter* sp. SS4 (Fig. 2A). Figure 2B show a partial reduction of original As(V) in *Pseudomonas* sp. SS3 and the Figure 2C show null reduction of sodium arseniate in *Methyloversatilis* sp. SS2. This screening methodology is very rapid and sensitive and very useful to investigate genes involved in oxidation or reduction of arsenic previous its quantification by HPLC-ICP-

Table 2. Arsenic species in the cytoplasm and membrane from bacterial isolates

ISOLATE	CYTOPLASM (mgL-1)		MEMBRANE (mgL-1)		RECOVERY (%)
	As (III)	As (V)	As (III)	As (V)	
Pseudomonas sp. SS1	0.15 ± 0.01	37.87 ± 0.06	1.10 ± 0.20	9.73 ± 0.15	49
Pseudomonas sp. SS1B	51.67 ± 0.49	1.87 ± 0.15	30.43 ± 0.90	9.37 ± 0.45	93
Methyloversatilis sp. SS2	ND ±	43.93 ± 1.10	3.50 ± 0.20	35.93 ± 0.91	83
Pseudomonas sp. SS2	0.48 ± 0.01	14.57 ± 0.31	3.50 ± 0.20	2.23 ± 0.23	21
Pseudomonas sp. SS2B	2.17 ± 0.57	49.30 ± 0.80	21.67 ± 0.71	1.87 ± 0.25	75
Altererythrobacter sp. SS2	23.63 ± 0.93	21.23 ± 0.31	20.13 ± 0.65	2.87 ± 0.35	68
Exiguobacterium sp. SS2	ND	78.87 ± 3.50	ND	12.57 ± 0.31	91
Acidovorax sp. SS2	ND	ND	ND	ND	ND
Streptomyces sp. SS2	97.27 ± 0.21	2.60 ± 0.10	ND	ND	100
Brevundimonas sp. SS2	0.24 ± 0.01	81.73 ± 0.21	1.23 ± 0.25	13.50 ± 0.40	97
Pandoraea sp. SS3	1.07 ± 0.20	84.30 ± 0.72	5.93 ± 0.55	1.87 ± 0.21	93
Pseudomonas sp SS3	24.70 ± 0.36	50.27 ± 1.62	12.87 ± 0.12	3.53 ± 0.45	91
Acinetobacter sp. SS3	31.13 ± 1.31	48.20 ± 1.14	19.53 ± 0.35	2.80 ± 0.30	102
Enterobacter sp. SS3	52.00 ± 1.00	29.50 ± 0.26	13.53 ± 0.45	1.70 ± 0.20	97
Rahnella sp. SS4	4.60 ± 0.10	24.30 ± 0.56	3.27 ± 0.21	18.50 ± 0.10	51
Pseudomonas sp. SS4	11.73 ± 0.21	39.27 ± 1.80	9.40 ± 0.10	27.30 ± 0.75	88
Aeromonas sp. SS4	5.17 ± 1.16	44.87 ± 0.15	3.03 ± 0.25	27.77 ± 0.15	81
Serratia sp. SS4	0.22 ± 0.02	50.43 ± 0.86	2.20 ± 0.26	30.67 ± 0.51	84
Rhizobium sp. SS4	2.15 ± 0.15	44.27 ± 1.16	24.53 ± 0.35	8.67 ± 0.35	80
Pseudomonas sp. SS4B	0.87 ± 0.10	25.53 ± 0.55	7.57 ± 0.60	5.57 ± 0.35	40
Pseudomonas sp. SS4C	34.93 ± 0.15	15.47 ± 0.51	16.80 ± 0.70	2.87 ± 0.25	70
Exiguobacterium sp. SS4	0.74 ± 0.01	71.47 ± 1.46	15.40 ± 0.30	4.50 ± 0.20	92
Exiguobacterium sp. SS4B	61.23 ± 1.55	0.44 ± 0.01	18.47 ± 1.05	9.23 ± 0.25	89
Enterobacter sp. SS4	90.07 ± 2.90	ND	ND	7.10 ± 0.20	97
Hydrogenophaga sp. SS2	ND	ND	ND	ND	ND
Pseudomonas sp. SS3	48.53 ± 0.55	138.50 ± 1.18	72.77 ± 2.15	23.90 ± 1.20	76
Aeromonas sp. SS4	63.33 ± 0.42	94.80 ± 0.30	60.80 ± 1.15	26.37 ± 0.60	65
Pseudomonas sp. SS4D	44.80 ± 0.20	190.50 ± 0.89	89.27 ± 1.35	35.50 ± 2.55	96
Aeromonas sp. SS1	701.50 ± 3.44	360.27 ± 8.46	300.83 ± 1.10	10.17 ± 0.45	92
Exiguobacterium sp. SS2	ND	737.17 ± 2.75	12.90 ± 0.10	495.43 ± 0.67	83
Rhodobacter sp. SS2	292.27 ± 5.18	434.17 ± 3.58	198.57 ± 0.90	459.63 ± 9.20	92
Aeromonas sp. SS4	6.07 ± 0.60	586.87 ± 2.07	158.27 ± 1.85	210.77 ± 1.85	64
Mycobacterium sp. SS4	27.53 ± 0.55	971.33 ± 4.66	218.43 ± 0.61	176.87 ± 2.37	93
Micromonospora sp. SS4	45.67 ± 0.68	873.30 ± 3.04	480.23 ± 12.79	85.03 ± 0.42	99
Haemophilus sp. SS4	24.40 ± 0.79	946.90 ± 14.37	403.10 ± 6.61	106.07 ± 4.11	99

ND: No Detected

Total As concentration in cytoplasm and citoplasmatic membrane and bacterial reduction capacity of As(V)

The abilities of the isolated bacteria to reduce As(V) under aerobic growth conditions were further tested. We used two approaches to determine the As reduction, a qualitative and quantitative method. Of sixty-four bacterial strains examined, MS. To test the isolates for the ability to grow in arsenate, total arsenic concentration in cytoplasm and cell citoplasmatic membrane was conducted (Fig. 3). Also the analysis performed in DNA and mitochondrial fraction of all bacteria's showed also a slight. As concentration in the range of $\mu g\ kg^{-1}$, that could be attributed to contamination of these fractions (data not shown). All of the isolates grew with 5 mM sodium

lactate as the electron donor and 1.33-20 mM sodium arsenate as the electron acceptor. In all the cultures arsenic was observed, mainly found in the cytoplasm and in with a less content in the cell membrane, in which the arsenic rate not exceed the 40% in the last case. The recovery is, in the most cases up to 80%, but not in all cases. In *Pseudomonas* sp. SS2 isolate, the recovery respect incubation is only 21% and the arsenic is mainly content in the cytoplasm. Other strains had a slightly lower total arsenic presence, even two of them, showed the lack of total arsenic presence (*Acidovorax* sp. SS2 and *Hydrogenophaga* sp. SS2) (Table 2).

The HPLC-ICP-MS analysis shows the presence of specific arsenic form in two different part of the cell by ultracentrifugation. No methylated forms of arsenic such as monomethyl arsonic acid, dimethyl arsinic acid and trimethylarsine oxide known to be produced by bacteria (Bentley and Chasteen, 2002) were detected. In the cytoplasm (Table 2), both As(III) and As(V) species were be detected. From all the 28strain tested, in the 15% of the bacterial from sediments and water, practically only As(III) is present. This is the case of *Streptomyces* sp. SS2, *Exiguobacterium* sp. SS4B, *Pseudomonas* sp. SS1Band *Enterobacter* sp. SS4. 20% of the bacteria's were able to reduce the 25-50% of As (V) to As(III), 15% of them were able to reduce the 75-100% the As(V) initial. The 40% of other bacterial showed practically only As (V) It is remarkable than different branches of the phylogenetic tree show different behavior about tolerance and reduction capability. For example, in *Pseudomonas spSS1* the As(V) remained in altered but the *Pseudomonas spSS1B*, reduce practically all the As(V) to As(III). At 20mM incubation concentrations the concentration of As(III) in the cytoplasm is very low. On the other hand, the distribution of arsenic species in the cell membrane (Table 2) were almost equally distributed, but with different concentration of As(III) and As(V) in most of the bacterial isolates, even when the species is not found in the cytoplasm. For example, in the case of *Methyloversatilis sp.SS2*, As(III) is not detected in the cytoplasm, but about a 10% of the total As in the membrane is As(III). For *Streptomyces sp.SS2*, and *Enterobacter sp.SS4*, no arsenic or very low amount can be detected in the membrane, however the recovery in both bacterial are very high as As(III) in the cytoplasm. In the case of incubation at 20 mM of As(V), on the contrary that in most cases happens in the cytoplasm, the As(III) concentration is really high and in some case as in the *Aeromonas sp.SS1* practically is the unique species present.

RESULTS AND DISCUSSION

Large variations in the levels of temperature and acidity determine the colonization by different extremophiles, with communities of meso- thermo- hyper thermophiles, and acidophilus thermophiles. The hot springs and geysers of El Tatio, that is the world´s highest field ringed by high volcanoes, are a natural laboratory of extraordinary interest because water temperature and salinity, determine the microbial communities. In the Tatio Geyser field, the different color on the surface depends on the different microbial communities that are consequence of the gradient of water temperature (Barbieri and Calavazzi, 2014). At the Tatio area, several unusually high elements concentration such as arsenic, possibly related to the leaching of metal sulfides of volcanic origin. The microbial community under study shifted with very different phylogenetic composition and behavior under As(V) stress, therefore, it is difficult to established a preliminary approach to part of the geo-microbial model that might be operating in the Tatio area. The naturally high As concentration, ranging up to 67 mg L^{-1} and 80 mg L^{-1}, of the place and the capacity to develop defence mechanisms determine the different communities and the different behaviour of the bacterial community towards As. One of the characteristics of all these bacteria's is the lack of capacity to developed methylation mechanisms. Only As(III) and As(V) have been found as As species present in the cytoplasm and citoplasmatic membrane for all of them. In a precedent study about the characterization of bacterial microorganisms in the hot spring and geyser sinters of Tatio, biota comprises mainly non-photosynthetic hyperthermophilic bacteria such as *cyanobacterias, green bacterias and diatoms* (Fernandez-Turiel, 2005). A study about isolation of arsenic resistance bacteria from volcanic rocks of QuebradaCamarones, Parinacota Region of Chile, with environmental soil and water As conditions similar to Tatio zone, showed that Gram negative no fermentative bacillus, identified as *Pseudomonas alcaligenes* bacteria's were able to tolerate concentrations of As above 2 mM and *Acinetobacter calcoaceticus, Enterobacter cloacae Burkordeliacepacea* up to 8 Mm. All these bacterias showed arsenate-reductase activity determined through the ars ABC operons (Campos, 2007). The microbial reduction of Arsenic determined through the ars ABC operons have been reported in a number of genes including *Desulfovibrio, Alcaligenes, Bacillus, Pseudomonas, Shewanella, Emnterobacter, Escherichia, Thauera* and in some *Cyanobacterias* and bacterias with capacity to reduce sulphates (Yamamura, 2003; Niggemyer, 2001). It is difficult to explain resistance between microbial species to As(V) and in non-methylatedbacteria's, the transformation to As(III), one possible explanation as can be found in the literature is that arsenate can be used by the cell as an electron acceptor for anaerobic respiration in the absence of oxygen, and therefore, the arsenate reduction to arsenite is a display versatile energy-generating system for respiration. ArsC gene coding for the reduction of As (V) to As(III), is one of the most common detoxification mechanisms. The arrA gene, involved in anaerobic respiration using As (V) as electron acceptor was found in bacterial obtained in natural environments in northern of Chile such as *Firmicutes*-like genes (Escudero, 2013). In our case the presence of As[III] in cytoplasm in isolates *Pseudomonas* sp. QZS1_1, *Methyloversatilis sp.* QZS2_1 and *Brevundimonas sp.* QZS2_9at either 1.33 or 20 Mm. On the contrary, a total reduction to As[III] was seen for *Streptomyces sp. QZS2_8* and *Enterobacter sp. QZS4_12* bacteria. Arsenic (mainly As[III]) was present in the cell membrane of both *Aeromonas sp. QZS4_3* and *Pseudomonas sp. QZS1_2.*

Other possibility is that As(III) could be removed from the cell easier than As(V) through the arsB pumps. In fact, in some bacterial species the As(III) concentration can be seen in the citoplasmatic membrane but not in the cell cytoplasm. Finally, another explanation could be the synthesis by the cell the specific peptides such as glutathione than bind As(III) as a detoxificant mechanism previous to the expulsion. The reducible capability is found mainly in the Actinobacter family. Acidovorax and Hydrogenophagabacterias do not accumulate any As in the cytoplasm and citoplasmatic membrane even at 5mM incubation. It is known that Acidophile bacteria havean intrinsic tolerance to cations and most of them can live in very high concentration of metals.

They have an inside positive cytoplasmic trans membrane potential (Dopson, 2014). Arsenic is present in bio mining environments as the metalloids arsenate (AsO_4^{3-}) or arsenite (AsO_3^{3-}). At neutral pH, that is the incubation media, AsO_4H^{2-} and $As(OH)_3$ are the species present, therefore, the intrinsic acidophil cation tolerance system would not aid to explain the arsenic tolerance or lack of capacity to penetrate into the cell. Therefore other metalloid resistancesystems could be developed to permit arsenic does not enter in the cell in very high As concentration incubation. In some bacterial, the relative uptake of the arsenic compounds was higher at lower concentration. This fact could indicate an inhibition of uptake or an increased afflux at higher concentration. This is the case of *Streptomyces sp. QZS2_8*. However, no always happens as in the case of *Pseudomonas* sp. QZS1_1 bacteria. This fact has been also observed in cell from urothelial and fibroblasts human cells and as in our case, the highest amount of arsenic was found in the cytoplasmic fractions (Dopp, 2010).

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