

Proteomic approach of adaptive response to arsenic stress in *Exiguobacterium* sp. S17, an extremophile strain isolated from a high-altitude Andean Lake stromatolite

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Abstract The North-Western part of Argentina is particularly rich in wetlands located in the Puna in an altitude between 3,600 and 4,600 m above sea level. Most of these high-altitude Andean lakes are inhospitable areas due to extreme habitat conditions such as high contents of toxic elements, particularly arsenic. *Exiguobacterium* sp. S17, isolated from stromatolites in Laguna Socompa, exhibited remarkable tolerance to high arsenic concentration, i.e., it tolerated arsenic concentration such as 10 mM of As(III) and 150 mM of As(V). A proteomics approach was conducted to reveal the mechanisms that provide the observed outstanding resistance of *Exiguobacterium* sp. S17 against arsenic. A comparative analysis of S17, exposed and unexposed to arsenic revealed 25 differentially expressed proteins. Identification of these proteins was performed by MALDI-TOF/MS revealing upregulation of proteins involved in energy metabolism, stress, transport, and in protein synthesis being expressed under arsenic stress. To our knowledge, this work represents the first proteomic study of arsenic tolerance in an *Exiguobacterium* strain.

Keywords *Exiguobacterium* · Arsenic stress · Proteomics · Extreme environment

Introduction

Exiguobacterium sp. S17, isolated from a stromatolite from Laguna Socompa, showed a tolerance to high arsenic (As) concentrations. Laguna Socompa is located at the northern part of Salta Province, Argentina (S24°35'34", W68°12'42") at 3,800 m of altitude. L. Socompa is one of the high-altitude Andean lakes (HAAL) in the high altitude desert known as Puna (Farías et al. 2011). It is considered an extreme environment due to conditions like high salinity (125 mS), high UV radiation, wide daily thermic amplitudes ranging from 20 to −10 °C in summer and 10 to −40 °C in winter, and a high As content of 32 mg/l (0.42 mM) (Farías et al. 2011, 2012). Stromatolite communities were found along the southern shore of this lake (Farías et al. 2012). *Exiguobacterium* sp. S17 strain was extracted from one of these stromatolites and belongs to the extremophile culture collection of HAAL described by Ordoñez et al. (unpublished). Several studies have shown that some strains of *Exiguobacterium* possess unique properties of interest for applications in biotechnology, bioremediation, industry and agriculture (Okeke et al. 2007; Lopez et al. 2005; Pattanapitpaisal et al. 2002).

Arsenic (As) is a ubiquitous toxic semi-metal or metalloid released in the environment mainly by volcanic activity (Smedley and Kinniburgh 2002; ATSDR 2000; Ferguson and Gavis 1972). The most common oxidation states for soluble arsenic in nature are the pentavalent, arsenate [As(V)], and the trivalent, arsenite [As(III)], present as (AsO₄^{−3}) and (As(OH)₃), respectively (Rosen 2002; Anderson and Cook 2004), with As(III) being much more toxic than As(V) (Oremland and Stolz 2003; Mukhopadhyay et al. 2002; Rosen 2002). The toxicity to As(V) is due to its similarity to phosphates (Rosen and Lui 2009), thus leading to the disruption of critical cellular

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functions or the impairment of synthesis of essential building blocks. In contrast, As(III) toxicity is believed to be due to its ability to covalently bind protein sulfhydryl groups (Parvatiyar et al. 2005).

To survive in extreme environments, cells trigger programs of specific gene expression, which are manifested as an increase or decrease in the amount of a set of proteins synthesized in response to stress (Duché et al. 2002). In the particular case of deleterious effects of As, it was reported that microorganisms have developed several strategies of detoxification including the reduction of As(V) like arsenite methylation, or its oxidation and further extrusion from the cell by a membrane-associated efflux pump, arsB (Lakshmi Sunita et al. 2012; Silver and Phung 2005; Rosen 2002). The genes encoding the arsenite detoxification machinery (ars genes) are widely distributed in bacteria and archaea and can either be found in plasmids or chromosomes (Achour et al. 2007). In this way, proteomics is an important tool to understand the strategies employed by microorganisms under different stress situations. Proteomics together with bioinformatics constitute complementary approaches that can deal with a huge amount of DNA information from genome sequencing projects and can provide new insights into bacterial functional genomics (Champomier-Vergés et al. 2010). This method is an excellent approach to study changes in the proteins expression patterns to elucidate adaptation mechanisms under different conditions. In several of the As removal processes, bacterial activity may play an important catalytic role (Castro de Esparza 2006). Due to the fact that many bacteria are able to modify or reduce the bioavailability of metals, the use of biological based methods appears to be an attractive tool for arsenic removal. Such change in protein concentrations and enzyme activities are best investigated by proteomics which is chosen here as an approach to understand the mechanisms implicated in the tolerance of *Exiguobacterium* sp. S17 against exposure to As(V) and As(III).

Materials and methods

Arsenic tolerance

The strain *Exiguobacterium* sp. S17 was identified by sequencing of 16S DNA (Ordoñez 2012). The strain was routinely grown in Luria-Bertani broth (LB, Britania) at 30 °C. To determine the tolerance of arsenic [As(V) and As(III)], tubes with 3 ml of LB₅₀ (LB diluted to 50 % final concentration) used as control and LB₅₀ containing different concentrations of arsenic: As(III), 2.5, 5, 10 mM and As(V), 20, 50, 100, 150 mM were inoculated to an initial OD_{600nm} between 0.05 and 0.09. The tubes were incubated

at 30 °C with constant agitation (150 rpm). Samples were taken during 24 h and OD_{600nm} were determined.

Bacterial strain and culture conditions

A single colony of *Exiguobacterium* sp. S17 was grown in LB₅₀ broth at 30 °C for 24 h and this culture was used for further inoculation of 100 ml of LB (control), 100 ml of LB supplemented with As(V) (100 mM) and 100 ml of LB supplemented with As(III) (7.5 mM) at initial OD_{600nm} 0.1. Erlenmeyer flasks were incubated up to the mid-exponential growth phase (OD_{600nm} 0.55 ± 0.05), the cells were harvested by centrifugation (7,000 rpm, 10 min at 18–20 °C) and washed twice with Tris–HCl buffer (0.1 M) pH 7.5. The pellets were then resuspended in 5 ml of the same buffer and were passed through a French press at 2,000 psi (SLM Instruments, Inc., Haverhill, MA, USA). Unbroken cells and other debris were removed by centrifugation at 14,000 rpm for 10 min at 4 °C (Beckman, USA). The total protein concentration was determined by Bradford method using BioRad reagents (Biorad, Richmond, CA), using bovine serum albumin (Sigma) as the standard. Aliquots of 400 µg of protein were stored at –80 °C for the isoelectrofocusing assay. Three independent assays for each condition were performed.

Two-dimensional electrophoresis (2DE)

Sample preparation and 2DE gels were carried out according to Borja Sánchez et al. (2005) with some modifications. If required, nucleic acids were removed by treatment with 1 µl of benzonase (Novagen®) in the presence of 1 µl of 1 M MgSO₄ for 30 min at 37 °C. Total proteins were precipitated with 3 volumes of cold acetone, and after incubation at –20 °C for 16 h, samples were centrifuged (14,000 rpm, 10 min). The protein pellets were air-dried and solubilized in 40 µl of solubilization mixture. The suspension was centrifugated at 3,500 rpm for 10 min and loaded onto immobilized pH gradient strips (pH 4.0–7.0, 18 cm, GE Health Care, Sweden). Gels were passively rehydrated for 20 h. The isoelectrofocusing assay was performed using IPGphor (GE, HealthCare, Sweden) at 53,500 V/h and the focusing strips were stored at –20 °C until separation in second dimension was performed. The second dimension was performed by SDS-PAGE on gels containing 12.5 % (w/v) polyacrylamide and carried out in a Bio-Rad Protean II xi cell (Biorad, Richmond, CA). Proteins were resolved overnight at a constant current of 11 mA/gel at 4 °C. Gels were stained with Biosafe colloidal Coomassie blue (Biorad, Richmond, CA, USA) according to the manufacturer's instructions, scanned with Image Scanner III and analyzed with Prodigy SameSpots (Nonlinear Dynamic Group, UK).

Protein identification using peptide mass fingerprinting

For each independent assay, the respective 2DE gels were carried out. Briefly, prominent spots were used to manually assign vectors in each gel image and the automatic vectors feature of the software was used to add additional vectors, which were manually verified. These vectors were used to warp the images and align the spot position to a common reference gel. Spot detection performed according to this reference gel was edited and artifacts removed. To correct the variability due to staining and to reflect the quantitative variation in intensity of protein spots, the spot volumes were normalized as a percentage of the total volume in all spots in the gel. A spot was considered significant when its resulting normalized volume showed more than 1.2-fold variation with respect to the control (LB medium) at the level of $p < 0.05$.

Individual spots were excised from the gels and subjected to mass spectrometry analyses that were carried out by CEQUIBIEM (Centro de Estudios Químicos y Biológicos de Espectrometría de Masa), Facultad de Ciencias Exactas y Naturales, UBA, Argentina. Protein identity from peptide mass fingerprints was determined by the MASCOT program (Matrix Science Inc., Boston, MA; <http://www.matrixscience.com/search-form-select.html>). Fragmentation was carried out with more intense MS peaks (MS/MS). When possible, MS and MS/MS information was combined for one or more peptide searches.

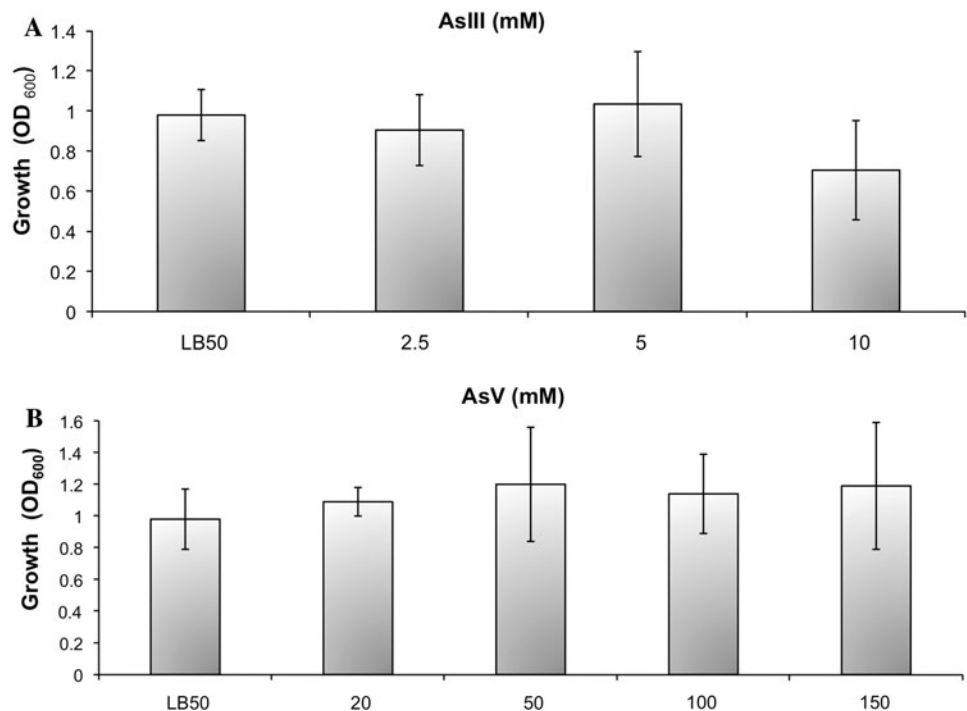
Results and discussion

Growth of *Exiguobacterium* sp. S17 under arsenic stress

To evaluate quantitatively the S17 response to arsenic exposure, we determined the pattern of growth by measuring OD_{600nm} at different times in the presence of increasing concentrations of the arsenic. Figure 1 shows the growth values obtained at 12 h and the tolerance was measured both in arsenate as sodium arsenite. *Exiguobacterium* sp. S17 was able to grow in all concentrations of arsenate assayed, showed similar growth rates, being the concentrations of 150 mM tested in this work (Fig. 1a). With regard to the presence of arsenite, this strain also showed tolerance to all concentrations tested for this metalloid, being 10 mM the highest evaluated concentration (Fig. 1b). The MIC was realized for all conditions. The maximum value was 15 mM of As(III) and 250 mM of As(V). The values selected for proteomic assays [As(V) = 100 mM and As(III) = 7.5 mM] were made to guarantee a good cellular growth.

In contrast with our result, Lakshmi Sunita et al. (2012) reported a strain of *Exiguobacterium*, isolated from estuary of Goa, with a maximum tolerance of 0.5 mM As(III), twenty times lower than the maximum concentration observed in *Exiguobacterium* sp. S17. These results may be due to the fact that *Exiguobacterium* sp. S17 came from

Fig. 1 Growth of *Exiguobacterium* sp. S17 to increasing concentrations of arsenite (2.5, 5, 10 mM) (a) and arsenate (20, 50, 100, 150 mM) (b); LB₅₀ in the absence of arsenic was used as control



Laguna Socompa, which has an arsenic concentration of about 32 mg/l (Farías et al. 2011). The response of a bacterium to a particular stress is related to the environment where it was isolated. Cai et al. (2009) suggest that soils with long-term arsenic contamination may result in the selection of highly diverse arsenic resistance

Table 1 Identification of differentially regulated proteins responding to arsenic stress by *Exiguobacterium* sp. S17

Spot N°	Protein	Accession N°	Mass (Da)	MASCOT Score	Fold change	Comment		
						Control	AsV	AsIII
Transcription and translational components								
28	Sigma 54 modulation protein/ribosomal protein S30EA	gil229916625	21574	128	2.7	ND	E	E
96	Ribosomal protein S2	gil347727010	27729	118	1.3	ND	E	ND
98	Prolyl-tRNA synthetase	gil229918663	63962	129	1.2	ND	E	ND
100	Ribosome recycling factor	gil229918658	20830	82	1.2	ND	E	ND
53	Elongation factor Ts	gil229918656	31767	176	1.7	E	OE	E
Stress proteins, chaperones and anti-oxidants								
9	Cold-shock protein	gil229916626	7176	197	2.4	E	OE	ND
15	Chaperonin GroEL	gil229917843	57572	113	2.1	E	OE	ND
20	Chaperone protein DnaK	gil229916346	65350	273	1.6	E	OE	E
62	Chaperonin Cpn10	gil229917842	10312	63	1.6	E	OE	ND
71	Superoxide dismutase	gil229916297	22931	160	1.5	E	OE	OE
75	UspA domain	gil229916412	16285	128	1.5	ND	E	ND
Metabolic proteins								
48	Phosphopyruvate hydratase	gil229916588	46130	227	1.8	ND	OE	E
37	Fumarate hydratase	gil229915871	50672	167	2.2	ND	E	ND
26	Tranketolase central region	gil229916237	35596	201	2.9	E	OE	ND
27	Transaldolase	gil229917074	23239	206	2.7	ND	E	E
13	Glyceraldehyde-3-phosphate dehydrogenase, type I	gil229916592	36189	83	2.1	E	OE	ND
1	Delta-1-pyrroline-5-carboxylate dehydrogenase	gil229916726	56463	296	5.1	ND	E	ND
8	Glutamine synthetase type I	gil229916142	50258	117	2.4	E	OE	ND
12	3-methyl-2-oxobutanoate dehydrogenase	gil229916238	36832	86	2.1	ND	E	ND
34	Inosine-5'-monophosphate dehydrogenase	gil229917448	53046	163	2.4	E	OE	ND
44	Purine nucleoside phosphorylase I	gil229916181	29464	78	1.9	OE	E	E
67	Serine hydroxymethyltransferase	gil229917051	45287	73	1.5	E	OE	ND
Transport								
97	ABC transporter	gil229918161	41139	113	1.3	ND	E	ND
33	Toxic anion resistance family protein	gil229915810	47343	108	2.5	ND	E	ND
Unknown function								
42	Hypothetical protein ATCR1_03384	gil355538705	10393	85	2.0	ND	E	E

All spots except spot 42, were identified by *Exiguobacterium* sp. ATb1 protein database. The spot 42 was identified by comparison with entries in the *Agrobacterium tumefaciens* CCNWGS0286, database

E expressed, *OE* over-expressed, *ND* not detected

mechanisms that could be probably spread throughout horizontal gene transfer events. Fernández Zenoff et al. (2006) also demonstrated this effect in bacteria isolated from HAAL while investigating UV radiation stress.

Proteomic analysis of *Exiguobacterium* sp. S17

The mechanisms of response to the presence of arsenic were investigated by a proteomics approach. Cells were grown in LB (control), LB + As(V) (100 mM) and LB + As(III) (10 mM), and were harvested at the mid log phase of growth ($OD_{600nm} \sim 0.55$). These samples were

subjected to 2DE, which led to successful identification of proteins involved in the arsenic response. Identification of changes is based on a spot-by-spot comparison of gels containing samples extracted under different conditions, always in comparison to a reference (standard gels). Two-dimensional protein patterns revealed a total of 113 spots differentially expressed under the assayed conditions. Twenty-five statistically significant spots, by a factor greater than 1.2, were identified by MALDI-TOF/MS, as shown in Table 1. Among them, eight spots (positions 8, 9, 13, 15, 26, 34, 62 and 67) were over-expressed in the presence of As(V) compared with the control medium and

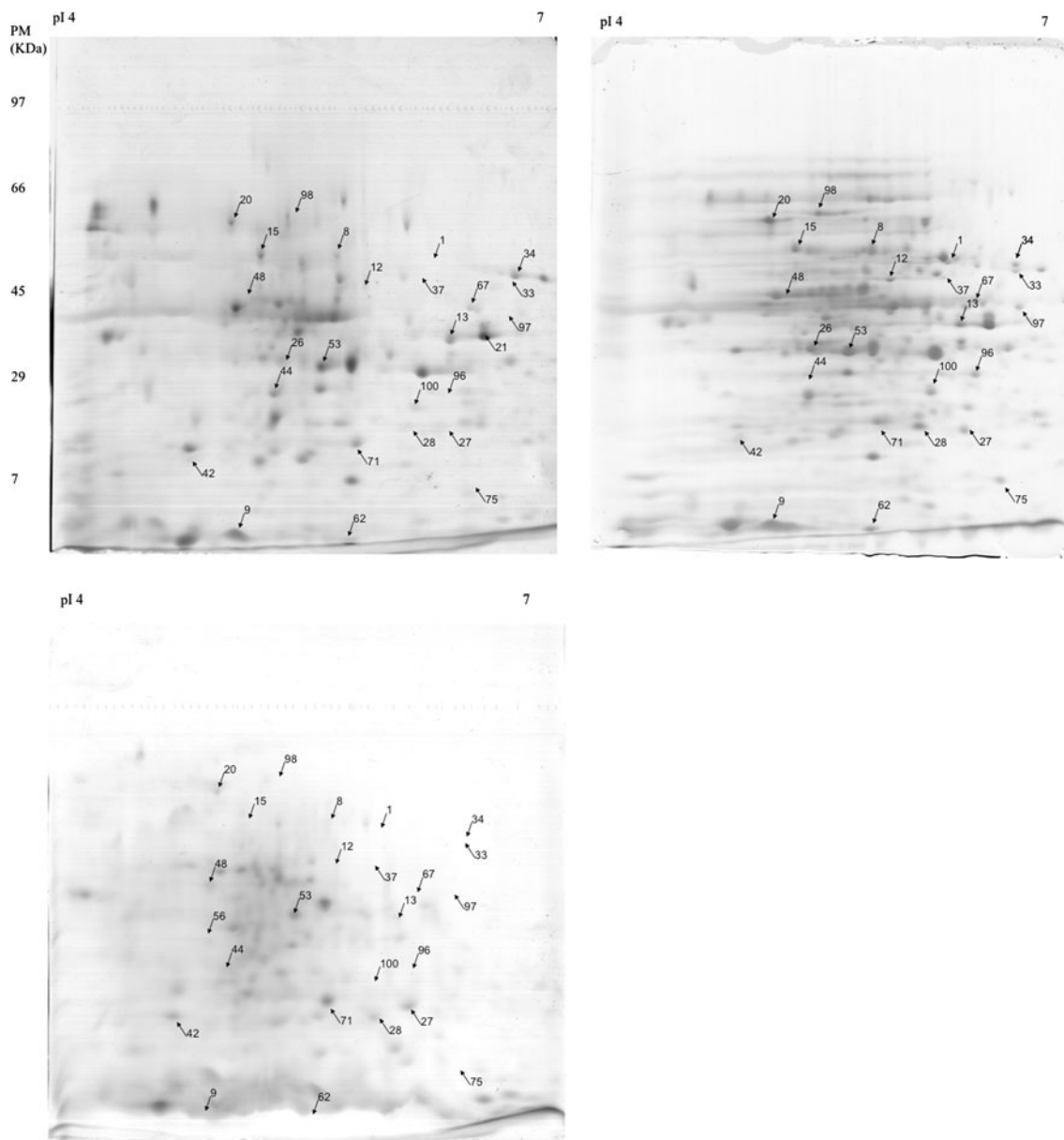


Fig. 2 2DE images of *Exiguobacterium* sp. S17, proteins expressed during growth in LB in the absence of As (*left*), in the presence of AsV (*center*) and AsIII (*right*), the numbers indicate the spots identified by MALDI-TOF/MS. Gels were stained with Coomassie blue BioSafe

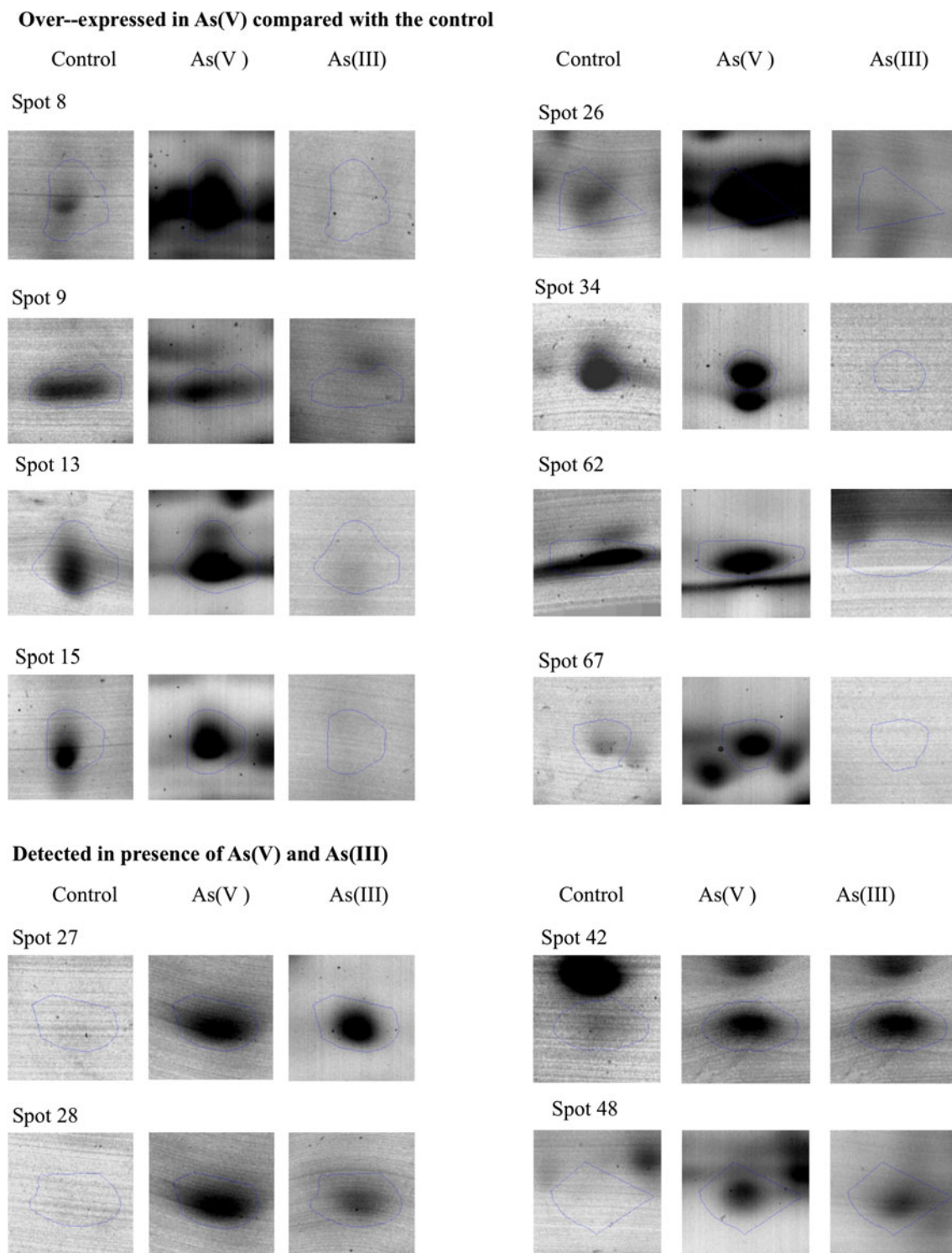


Fig. 3 Portions of 2D gel spots selected for identification; at *left* the spots expressed in control, in the *center* spots expressed in medium with As(V) and at *right* the spots expressed in medium with As(III). *Delimited in blue circle* is the spot considered

were not detected in the presence of As(III), while nine spots (positions 1, 12, 33, 37, 75, 96, 97, 98 and 100) were detected only in the presence of As(V). Four other positions (27, 28, 42 and 48) were detected in the presence of

either As(V) or As(III) but not in the control medium (Figs. 2, 3). On the other hand, under control conditions, one spot (44) corresponding to an over-expressed protein was identified. Finally, three spots (20, 53, 71) were

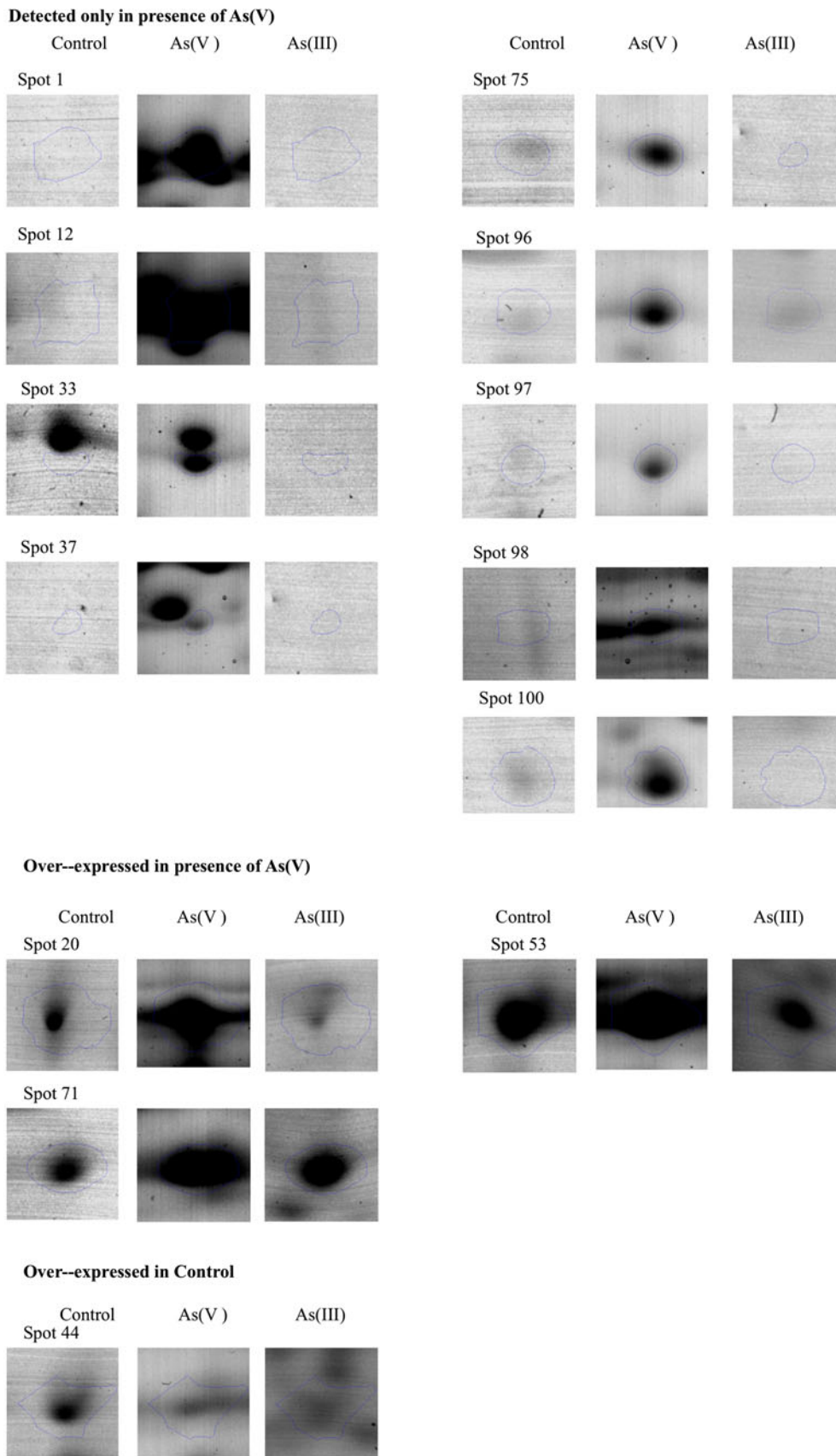


Fig. 3 continued

detected in all tested conditions; nevertheless, the spots' intensity was higher in the presence of arsenate.

Exiguobacterium sp. S17 showed over-expression of a superoxide dismutase "SOD" (spot 71) in the presence of As(V) and As(III) compared to the control. It is known that exposure to arsenic induces response against oxidative stress in bacterial cells (Ciprandi et al. 2012; Rodriguez-Gabriel and Russell 2005; Moore et al. 2005). SOD is an enzyme that catalyzes the dismutation of superoxide ion into oxygen and hydrogen peroxide, which then is reduced to H₂O by peroxidases, constituting an important defense strategy against reactive oxygen species (ROS), responsible for oxidative stress in cells. Ciprandi et al. (2012) described the same effect in *Chromobacterium violaceum* in response to As(III), which increased SOD expression quite comparable to oxidative stress conditions. Furthermore, Kramer and Ames (1988) reported that SOD is an important protein in providing resistance to selenite toxicity in *Salmonella typhimurium*. The over-expression of SOD in *Exiguobacterium* sp. S17 under As exposition is thus considered to be directly involved in the cellular response to reduce the alterations, such as DNA damage and protein oxidation, caused by the presence of this metalloid.

In addition, the differential proteome of *Exiguobacterium* sp. S17 revealed the expression of heat shock proteins (Hsps), e.g., DnaK chaperone (spot 20), GroEL (spot 15) and cpn10 (or GroEs) (spot 62). All these stress proteins are over-expressed in the presence of arsenate. These proteins are highly conserved within species and carry out essential functions such as folding, translocation, and assembly in stressed and non-stressed cells (Hartl 1996; Parsell and Lindquist 1993). The Hsps have an important function against environmental stress conferring tolerance to high temperatures, high salt and heavy metal concentrations (Tkáčová and Angelovičová 2012; Ron 2006; Kiriya et al. 2001; Tedengren et al. 1999; Parsell and Lindquist 1993). With respect to a direct As exposition effect, Zhang et al. (2007) observed an increase of DnaK chaperone in *Comamonas* sp. under arsenate stress. Similar results were reported for *Ferroplasma acidarmanus* Fer1 by Baker-Austin et al. (2007) and for *Pseudomonas aeruginosa* by Parvatiyar et al. (2005) when this bacterium was exposed to As(III). On the other hand, Rodrigues et al. (2008) observed that *Exiguobacterium sibiricum* 255–15 increased the expression of genes *dnaK*, *groES* and *groEL* upon elevated temperature (39 °C). Apparently high temperatures produce denaturation of proteins in *E. sibiricum*, and to overcome this problem, the expression of genes encoding Hsp proteins is induced. Clearly, the finding of an increase of these proteins in *Exiguobacterium* sp. S17 in this study identifies them as an important component of the stress response induced by As exposition apparently requiring the increased

recovery of misfolded proteins caused by the presence of this metalloid (Nguyen et al. 2009; Mogk et al. 2003). Interestingly, one universal stress protein, UspA (spot 75) was expressed only in the presence of As(V). In contrast, Cleiss-Arnold et al. (2010) reported UspA induction by As(III). Multiple members of the UspA family of proteins are found in the genomes of bacteria, archaea and fungi, and it has been reported that this protein is expressed in response to a large variety of stress conditions (Kvint et al. 2003). However, the biological and biochemical functions of the UspA protein family are still not known (Siegele 2005). Curiously, the Hsps proteins (cold-shock protein, GroEL, Cpn10 and UspA), were not detected in the presence of arsenite, probably due to the fact that the cell removes the arsenite and the defense machinery in the presence of this metalloid may not be activated. Ordoñez (2012) reported that the genome of *Exiguobacterium* sp. S17 carries a copy of an *ArsB* gene encoding a protein that acts as an efflux pump of cytoplasmic arsenite. Additionally, in the same strain, an As(III) resistance protein (ARC3) has been reported that also acts as an efflux pump. The presence of this gene in the *Exiguobacterium* genus has not been reported before. The combined work of both efflux pumps (*arsB* and *ARC3*) might explain the enhanced tolerance of this strain to As(III) (Ordoñez et al. unpublished). Proteomic analysis did not allow detecting differential expression of the protein responsible for the transport of arsenic. This is probably due to the fact that these transport proteins are expressed under all the studied conditions as constitutive proteins, and thus are not detected as differential spots. It should also be kept in mind that *ArsB*, the protein responsible to the arsenite resistance, is a membrane protein which often is highly insoluble and, therefore, is not readily resolved by 2D-PAGE (Baker-Austin et al. 2007). However, two proteins involved in transport were detected only in the presence of As(V), and ABC transporter (spot 97) and a protein member of the toxic anion resistance family protein (spot 33). It has been reported that the inward transport of arsenate is carried out by phosphate transport membrane systems as Pit (phosphate transport) and Pst (phosphate specific transporter) (Paez-Espino et al. 2009). This latter protein is an ABC transporter that binds and uses the energy of ATP to transport substances across membranes (Cough Uicab et al. 2010). In *Exiguobacterium* sp. S17, this protein is probably involved in the transport of As(V) into the cell, since its presence under As(III) expositions and in the control medium was not observed. In relation with the other identified protein (spot 33), a toxic anion resistance family protein, it might be assumed that the cells promote the elimination of toxic anion arsenite as mechanism of arsenic resistance.

Additionally, as shown in Table 1, sigma 54 modulation protein/ribosomal protein S30EA (spot 28), ribosomal

protein S2 (spot 96), ribosome recycling factor (spot 100), prolyl-tRNA synthetase and elongation factor TS (spot 53) are all overexpressed in the presence of As(V). All of these proteins are involved in transcriptional and translational processes. With respect to the EF-Ts, it is responsible for mediating the regeneration of EF-Tu–GDP complex, catalyzing the addition of aminoacyl tRNA into the ribosome enabling protein synthesis. According to Caldas et al. (1998), the EF-Tu has a dual function in protein synthesis and stress response. The results obtained here are in agreement with this finding as spot corresponding to EF-Ts were observed in the three study conditions, and in fact, it was the most intense spot under arsenate presence. Similar results were also described by Pandey et al. (2011), who observed an over-expression of EF-Tu in *Anabaena* sp. PCC7120 under arsenate stress. Baker-Austin et al. (2007) suggested that the amount of ribosomal sub-units increases due to the requirement of increased protein biosynthesis to combat the cellular stress caused by the presence of metalloids. Based on these constraints, the results presented here reflect the ability of *Exiguobacterium* sp. S17 to adapt and survive in the presence of arsenic.

Screening of response against arsenic stress by 2DE yielded also proteins that belong to the general metabolism pathways (carbohydrate and amino acid metabolism) and identified their differential expression in *Exiguobacterium* sp. S17. In the presence of As(V), a differential up-regulation, glyceraldehyde-3-phosphate dehydrogenase (spot 13) and phosphopyruvate hydratase (spot 48), enzymes involved in the glycolytic pathway were detected. Both proteins are involved in the second stage of glycolysis, and thus in energy (ATP-) generation. In addition, the results showed that, in the presence of arsenate, proteins involved in amino acid metabolism were expressed. Up-regulation was found for: 3-methyl-2-oxobutanoate dehydrogenase (spot 12), involved in the metabolism of leucine, valine and isoleucine, delta-1-pyrroline-5-carboxylate dehydrogenase (spot 1), implicated in arginine and proline metabolism, and finally serine hydroxymethyl transferase (spot 67), performing conversion of serine to glycine. Identification of enzyme involved in the amino acid metabolism is again indicative of an increased energy generation, as deaminated amino acids (after conversion into alpha-keto acids) are fed into the tricarboxylic acid cycle (TCA). TCA cycle is the final pathway for the oxidation of molecules such as amino acids, fatty acids and sugars, and also provides intermediates for biosynthesis. In accordance to this finding, a change was also detected in fumarate hydratase concentration (spot 37), another enzyme of the TCA cycle. These results concur with reports by TCA cycle is the final pathway for the oxidation of molecules such as amino acids, fatty acids and sugars, and also provides intermediates for biosynthesis. Baker-

Austin et al. (2007) reported an up-regulation of enzymes that feed into or are part of TCA cycle in *Ferroplasma acidarmanus* Fer1 under As(III). Taking together reports from the literature and findings from this study, the results could suggest that in *Exiguobacterium* sp. S17 upregulates significantly the increment of energy-providing metabolic pathways to overcome the cellular stress caused by the exposition may be necessary to manage the cellular stress caused by the addition of arsenic.

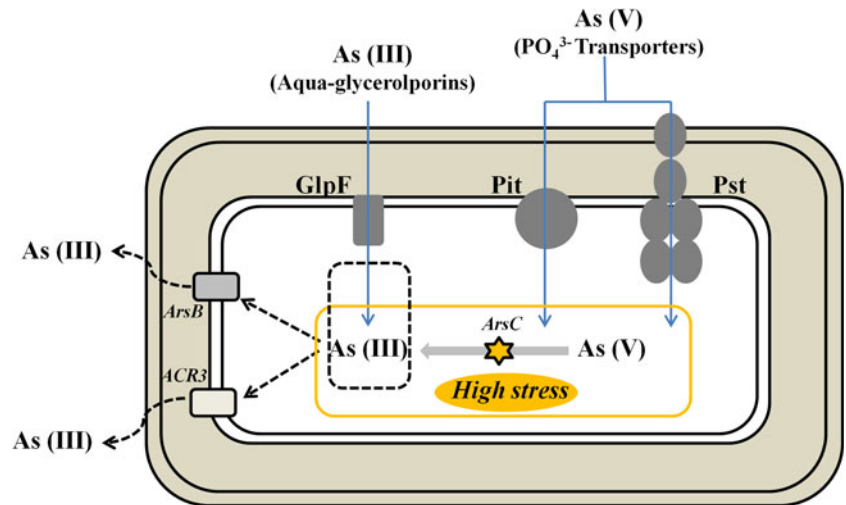
Again directing in the energy-providing pathways in the identification of two enzymes involved in purine metabolism was also detected in 2DE gels. Inosine 5-monophosphate dehydrogenase (spot 34) becomes over-expressed in the presence of As(V), as compared with the control, but it is not detected in the presence of arsenite. Purine nucleoside phosphorylase (spot 44) was found over-expressed in control conditions and downregulated in cells under stress. These enzymes have an essential role in providing two precursors (adenine and guanine) for RNA and DNA synthesis. Overexpression is thus understandable as it is known that bacteria have evolved salvage pathways of purine and pyrimidine, which can be used as a source of energy, and for providing carbon and nitrogen (Middelhoven et al. 1984). On the other hand, the down-regulation under arsenic presence suggests that the cells by strictly regulating the purine synthesis, thus insuring bacterial survival under harmful conditions.

Finally, one other down-regulated protein, transketolase (spot 26), was detected under arsenic stress compared with the control. This enzyme participates in the formation of xylulose 5-phosphate in the pentose phosphate pathway for NADPH production. The same effect was observed in *Anabaena* under arsenic stress by Pandey et al. (2011). According to Kaiser (1979), this down-regulation may be due to high sensitivity toward accumulated H_2O_2 in the cells after As treatment. Alternatively, one might speculate that this diminution is due directly to the arsenate presence, which acts as competitive inhibitor of the reaction catalyzed by transketolase (Gorbach et al. 1981). These results constitute evidence of the adaptive response of *Exiguobacterium* sp. 17 against arsenic stress conditions, which thereby guarantee its survival.

Concluding remarks

In conclusion, 25 proteins were described that are differentially expressed under As(V) or As(III) exposition; all (100 %) of these proteins were expressed or over-expressed in the presence of As(V), while 32 and 48 % of these were detected in the As(III)-supplemented medium with As(III) and or in the control, respectively. Probably, most of them are synthesized by the cells but in lower

Fig. 4 Schematic representation of the proteins involved in the transport of arsenic (adapted from Paez-Espino et al. 2009)



concentrations than in medium with As(V) and were undetected. The lower expression of proteins detected in the presence of As(III) could be explained by the finding that *Exiguobacterium* sp. S17 is able to remove the As(III) through ACR3 and ArsB pumps, while the As(V) has to be reduced and recently expelled prior excretion. In fact, the genes responsible for the reduction and transport of arsenic were found in the strain under study (unpublished). It is highly probable that the greater permanence and higher concentration of metalloid inside within the cells could be responsible for triggering the defense machinery to insure adaptation and survival, exactly as reflected in the increased expression of stress-related proteins, described in this work (Fig. 3). The current findings are the basis for future studies, e.g., as the construct generation of deletion mutants for of the *arc3* gene, one of the most prominent proteins identified in this survey might help understand if this gene in *Exiguobacterium* sp. S17 is responsible for the ability to adapt and survive under arsenate stress conditions (Fig. 4).

This report, representing the first proteomic study of arsenic tolerance in an *Exiguobacterium* strain isolated from the HAAL, constitutes an important contribution to future studies about the potential application of this strain in bioremediation processes.

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