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Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment

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Abstract

Arsenic is ubiquitous in the biosphere and frequently reported to be an environmental pollutant. Global cycling of arsenic is affected by microorganisms. This paper describes a new bacterial strain which is able to efficiently oxidize arsenite (As[III]) into arsenate (As[V]) in liquid medium. The rate of the transformation depends on the cell density. Arsenic species were separated by high performance liquid chromatography (HPLC) and quantified by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The strain also exhibits high minimum inhibitory concentrations (MICs) for As[III] (6.65 mM (500 mg L^{-1})) and other heavy metals, such as cadmium (1.42 mM (160 mg L^{-1})) or lead (1.20 mM (250 mg L^{-1})). Partial identification of the strain revealed a chemoorganotrophic, Gram-negative and motile rod. The results presented here demonstrate that this strain could represent a good candidate for arsenic remediation in heavily polluted sites.

Introduction

Arsenic is widely distributed in soils and natural waters, released from both natural and anthropogenic sources. Global natural emissions are principally due to igneous activity, and have been estimated to be 8000 tons per year. Whereas human emissions, arising primarily from smelting of metals, combustion of fuels, and use of pesticides, represent about 23 000 tons per year (WH0 1987). The contamination of drinking-water supplies with inorganic arsenic is commonly reported and arsenic has been identified as a major risk for human health (Pontius *et al*. 1994).

The biogeochemical cycle of this element is significantly dependent on microbial transformations which affect the distribution and the mobility of arsenic species in the environment (Tamaki & Frankenberger 1992; Quinn & McMullan 1995). Oxidation states are also correlated to arsenic toxicological properties. As[III] is reported to be on average 100 times more toxic than As[V] (Neff 1997).

A few bacteria catalyzing arsenic oxidation, in *in vitro* conditions, have been previously reported. They were isolated from soil or from cattle dipping fluids; they belong to genera such as *Achromobacter* (Green 1918), *Pseudomonas* (Turner 1949; Turner 1954; Tumer & Legge 1954; Ilyaletdonov & Abdrashitova 1981), *Alcaligenes faecalis* (Osborne & Ehrlich 1976; Phillips & Taylor 1976; Anderson *et al*. 1992), *Thiobacillus ferrooxydans*, and *Thiobacillus acidophilus* (Leblanc *et al*. 1995).

In this report we present a new bacteria, ULPAs1, which shows rapid and extensive oxidation of arsenite into arsenate. This outstanding strain was isolated from arsenic-contaminated water.

Materials and methods

Bacterial strains

The strain ULPAs1 was isolated from an aquatic environment contaminated with arsenic (0.47 mMol kg^{-1}, dry wt). Samples were inoculated at $25 \pm 2\,^{\circ}C$ into a liquid medium (CDM, composition is given below) supplemented with 1.33 mM (100 mg As L^{-1}) as arsenite to develop enriched culture. A pure culture was obtained by successive isolation of colonies at $25 \pm 2\,^{\circ}C$ on As[III]-supplemented medium (CDM), solidified by addition of 20 g L^{-1} of agar-agar (Difco). The strain CCM 999 (*Ochrobactrum antropi*) was kindly supplied by the Czech Collection of Microorganisms (Masaryk University). This strain, first described as *Xanthomonas arsenoxydans* by Turner (1954), was reported to oxidize As[III]. It is the only strain, described by Turner (1954) which is still available in the bacteria collections. The strain S21104 (*Zoogloea ramigera*) was a generous gift from Dr. Hattori, Sendai, Japan. This strain was used as a reference, since ULPAs1 was identified as a member of the same genus.

Media

ULPAs1 strain was cultivated in CDM medium whereas CCM999 and S21104 were cultivated in Luria-Bertani broth (2% w/v, Difco), since they did not grow on CDM medium. The chemically defined medium (CDM) was prepared by mixing the three following solutions.

Solution A: 0.0812 M of $MgSO_4$,$7H_2O$ (Sigma), 0.187 M of NH_4Cl (Merck, 99.8%), 0.07 M of Na_2SO_4 (Prolabo, 99%), 0.574 mM of K_2HPO_4 (Prolabo, 97%), 4.57 mM of $CaCl_2$, $2H_2O$ (Merck, 99.5%), 0.446 M Na lactate (Sigma, 98%).
Solution B: 4.8 mM Fe_2SO_4, $7H_2O$ (Prolabo, 99%).
Solution C: 0.95 M $NaHCO_3$ (Prolabo, 99.5%).
100 mL of solution A, 2.5 mL of solution B, 10 mL of solution C were mixed and made up to 1 L with water. The final pH of this complete medium was about 7.2. All the solutions were prepared with doubly deionized water (Milli-Q system, Millipore) previously sterilized by autoclaving (120 °C, 20 mn). Solution A and Luria-Bertani broth are further sterilized by autoclaving (120 °C, 20 mn), and solutions B and C by filtration through a 0.45 μm pore size filter (Millipore). Na lactate at a final concentration of 4.46 mM (5 g L^{-1}) was used as organic carbon source.

Stock solutions

All AsIII solutions were prepared from a 133 mM (10 000 mg As L^{-1}) stock solution of sodium arsenite ($NaAsO_2$), Hg^{2+} solutions from a 50 mM (10 000 mg Hg^{2+} L^{-1}) stock solution of $HgCl_2$, Pb^{2+} solutions from a 48 mM (10 000 mg Pb^{2+} L^{-1}) stock solution of lead acetate ($Pb(CH_3CO_2)_2$, $3H_2O$) and Cd^{2+} solutions from a 89 mM (10 000 mg Cd^{2+} L^{-1}) stock solution of $CdSO_4$. All stock solutions were prepared with doubly deionized water produced with Milli-Q sytem (Millipore).

Growth characteristics

To determine the growth characteristics of the strain ULPAs1, bacteria were grown in 180 ml polystyrene flasks containing 50 mL of arsenite-supplemented (1.33 mM (100 mg L^{-1})) CDM medium inoculated with cells to obtain an initial cellular concentration of about 10^4 cells mL^{-1}. Flasks were incubated in the dark at $25 \pm 2\,^{\circ}C$ and agitated at 250 rpm on a rotary shaker (Biolafitte France; LS Leroy Somer). Numeration of cells was done either by plating 0.1 mL of successive dilutions of the cells suspension on the previously described solid medium, containing 1.33 mM (100 mg L^{-1}) As as arsenite, or by measuring the culture turbidity on aliquots (absorbance at 600 nm. UVIKON 930, Kontron instruments).

Resting cells

Cells of strain ULPAs1 or CCM 999 were grown to late exponential phase (absorbance = 0.4–0.5) in 400 mL CDM medium which was or not supplemented with As[III] or As[V] (1.33 mM (100 mg L^{-1}) in final concentration). In the case of As[V] supplemented medium, no reduction of As[V] to As[III] was detected. Medium was then dispensed into 200 mL centrifuge bottles and cells harvested by centrifugation (KONTRON HERMLE, Centrikon H-401/401B) at 8000 rpm for 10 min at 5 °C. Supernatants were discarded, pellets were suspended in 100 mL Tris HCl buffer (5 mM; pH 7.2) and centrifuged again. This operation was repeated twice. Bacteria were added to 20 mL of buffer (Tris-HCl 5 mM, pH 7.2) to give an adequate absorbance defined for each experiment. As[III] was supplemented to give a final concentration of 1.33 mM (100 mg L^{-1}). Solutions were then incubated with shaking (250 rpm) at $25 \times 2\,^{\circ}C$ and arsenic oxidation was regularly measured over a period of 10 h. Absorbances were monitored in parallel.

Control assays, in which cells had been omitted or boiled (100 °C, 10 mn) before contact with arsenite were monitored in the same way.

Minimum inhibitory concentrations (MICs)

The liquid media previously described (CDM or LB), non-amended (controls) or amended with the respective metal elements at different concentrations from stock solutions, were inoculated with cell suspensions from precultures (3 d, 25 °C) to give a final density of approximately 10^6 CFU mL^{-l}. The following concentrations, in mM (or mg L^{-1}) of metal were tested:
Hg^{2+}: 0; 0.01(2); 0.015 (3); 0.025 (5); 0.03 (6); 0.045 (9); 0.06 (12); 0.075 (15); 0.15 (30); 0.225 (45); 0.3 (60); 0.45 (90) and 0.6 (120 mg L^{-1}).
As[III]: 0; 0.025 (2); 0.05 (4); 0.12 (9); 0.17 (13); 0.23 (17); 0.28 (21); 0.57 (43); 0.85 (64); 1.13 (85); 1.73 (130); 2.33 (175); 2.66 (200); 3.35 (250); 4 (300); 4.33 (325); 4.65 (350); 5.3 (400); 5.65 (425); 6 (450); 6.3 (475); 6.65 (500); 7 (525); 7.3 (550); 7.65 (575); 8 (600) and 9.35 (700 mg L^{-1}).
Cd^{2+}: 0; 0.02 (2); 0.035 (4); 0.07 (8); 0.1 (12); 0.14 (16); 0.18 (20); 0.35 (40); 0.53 (60); 0.7 (80); 1 (120); 1.4 2(160); 1.8 (200); 2.2 (250) and 2.7 (300 mg L^{-1}).
Pb^{2+}: 0; 0.01 (2); 0.025 (5); 0.05 (10); 0.065 (14); 0.09 (19); 0.12 (24); 0.23 (48); 0.34 (71); 0.46 (95); 0.69 (143); 0.92 (190); 1.2 (250); 1.45 (300); 1.7 (350); 1.9 (400); 2 (425); 2.2 (450); 2.3 (475) and 2.4 (500 mg L^{-1}).

The MIC is defined as the lowest concentration that causes no visible growth (Courvalin *et al.* 1985): to appreciate the interaction of metals with the strain, colony forming units were determined on samples where no visible cultures were detected.

Arsenic compounds determination

Arsenite transformation was studied on culture supernatants, obtained by filtration through a 0.22 μm pore size sterile Durapore filter (Millipore).

Separation system. The chromatographic system included an isocratic pump (Waters model 501) and a Rheodyne valve fitted with a 100 μl sample loop. Separation was performed on a reversed-phase polymeric resin (Hamilton, PRP-X100, 250 mm $\times$ 4.1 mm i.d., particle size 10 μm) equipped with the corresponding guard column. Arsenic compounds were eluted with a phosphate buffer prepared as further described, degassed and filtered on a 0.65 μm cellulose acetate membrane (Millipore). Link between HPLC column and ICP-AES instrument was carried out with a 20 cm piece of polyethylene tube (i.d. 0.5 mm). Continuous flow was assured with a peristaltic pump (Jobin-Yvon Emission, LabCraft, model hydris 05Mic).

Standard solutions of 0.133 (10 mg L^{-1}), 0.665 (50 mg L^{-1}) and 1.33 mM (100 mg L^{-1}) as arsenite and arsenate were prepared by dissolving sodium arsenite $NaAsO_2$ (Prolabo , 99%), and sodium arsenate Na_2HAsO_4, $7H_2O$ (Prolabo, 98.5%) in doubly deionized water produced with Milli-Q system (Millipore).

Mobile phase. Arsenic compounds were eluted with a phosphate buffer composed of an equal volume of the two following solutions : Solution 1 was prepared by mixing 10 mM sodium monohydrogenphosphate (Na_2HPO_4, 12 H_2O, Prolabo, 99%) and enough solution of sodium dihydrogenphosphate 10 mM (NaH_2PO_4, $2H_2O$, Prolabo, 99%) to reach pH = 6.3. Solution 2 was a Na_2HPO_4,$12H_2O$ (0.1 M) solution (pH = 7.8).

ICP-AES. A sequential Jobin Yvon JY138 ultrace spectrometer operating at a forward power of 1.2 kW (40.68 MHz) and equipped with a Meinhard type nebulizer and a Scott spray chamber was used. Flow-rates: Plasma gas = 16 L min^{-1}, gaining gas = 0.9 L min^{-1}, nebulizer gas = 0.3 L min^{-1} (optimized each day), auxiliary gas = 0.1 L min^{-1}, sample introduction = 1 mL min^{-1}. Wavelength was fixed at 193.7 nm.

Bacteria identification

Bacteria identification was done by biochemical analysis by using the standardized micromethod Api 20 NE (bioMérieux SA, Lyon, France). Partial sequencing of the 16S rDNA was performed as previously described (Heller *et al.* 1997). DNA was extracted from cells harvested from 20 ml stationary phase culture. PCR amplification was done with the two 16S rDNA eubacterial universal primers P8 and Pc1544 (Eurogentec, Seraing, Belgium). Sequencing was performed on the amplified fragments with the Thermosequenase fluorescent labeled primer cycle sequencing kit (Amersham Life Science) on an A.L.F.® DNA Sequencer (Pharmacia Biotech). The sequences obtained were compared with 16S rDNA sequences itemized in the EMBL-GenBank database; they were aligned by using the clustal method (Higgins & Sharp 1988) on DNAStar software.

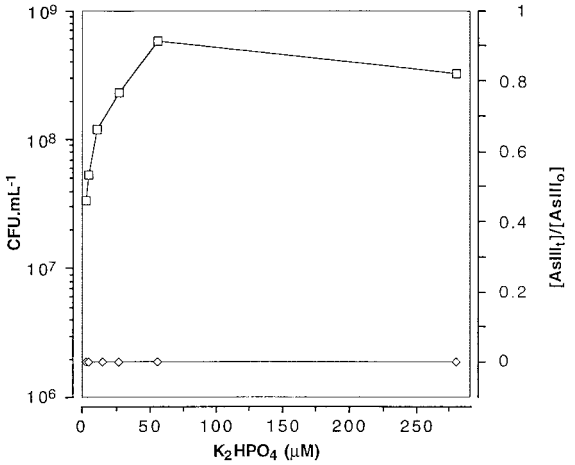

Figure 1. Influence of K_2HPO_4 concentrations (μM) on the grown of ULPAs1 (□) and corresponding As[III] oxidation performed after 3d (◊). Conditions are those described in the materials and methods. Data points represent at least two experiments done in duplicate (SD represent less than 5%).

Reproducibility of results

Data points are average values from two separate experiments, each in duplicate. A maximum variation of 10% was observed between separate experiments.

Results

Oxidation capability of the strain ULPAs1 – Influence of phosphate

Since orthophosphate (PO_4^{3-}) can interfere in transport systems with orthoarsenate (AsO_4^{3-}) because of its chemical similarity (Silver & Walderhaug 1992), we tested different concentrations of KH_2PO_4 (0.00285 (0.5 mg L^{-1})-0.285 mM (50 mg L^{-1})) in lactate supplemented CDM. We determined in each case cell growth and arsenite transformation (Figure 1). The oxidation system was functional at each phosphate concentration tested. After a three days incubation period, As[III] was completely transformed. However, the phosphate concentration had a direct effect on the final cell density. The highest cell density, corresponding to approximately 10^9 CFU mL^{-1} was achieved with a phosphate concentration of 0.057 mM (10 mg L^{-1}). Higher concentrations did not influence the cell density.

We investigated the influence of phosphate concentrations on the arsenite oxidation rate. Figure 2a shows that when the concentration was low (0.00285 mM

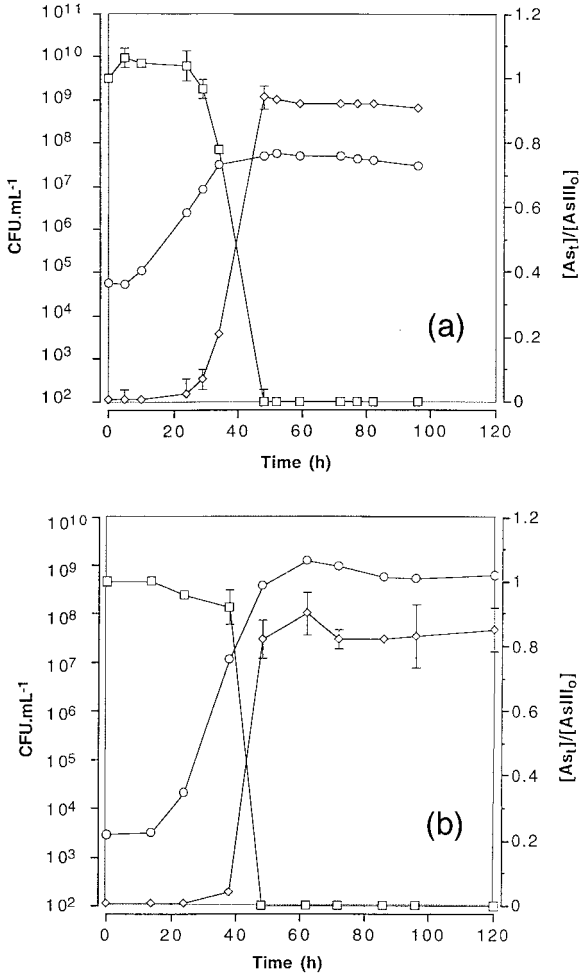

Figure 2. (a) Growth curve of ULPAs1 strain expressed as CFU mL^{-1} in minimum medium containing 0.00285 mM (0.5 mg L^{-1}) of K_2HPO_4 (○) and corresponding kinetics of As[III] oxidation (□) and As[V] formation (◊) in cell-free supernatants. Data points represent at least two experiments done in duplicate. Bars represent the standard error (SD up to ±10%). (b) Growth curve of ULPAs1 strain expressed as CFU mL^{-1} in minimum medium containing 0.057 mM (10 mg L^{-1}) of K_2HPO_4 (○) and corresponding kinetics of As[III] oxidation (□) and As[V] formation (◊) in cell-free supernatants. Data points represent at least two experiments done in duplicate. Bars represent the standard error (SD up to ±13%).

(0.5 mg L^{-1})), the doubling time was approximately 3 h and oxidation of arsenite to arsenate occurred during the exponential growth phase. Complete disappearance of As[III] was achieved in 24 h and was accompanied by appearance of arsenate. No other arsenic species was detected. When the optimum phosphate concentration (0.057 mM (10 mg L^{-1})) was present in the media (Figure 2b), the growth rate was increased (doubling time 1.5 h) as well as the arsenite

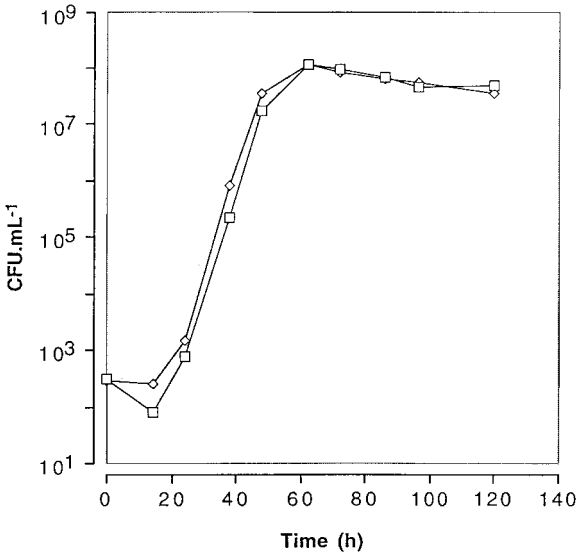

Figure 3. Growth curve of ULPAs1 strain expressed as CFU mL^{-1} in minimum medium containing 0.057 mM (10 mg L^{-1}) of K_2HPO_4 amended (□) or not (◊) with 100 mg L^{-1} As[III]. Data points represent at least two experiments done in duplicate.

oxidation rate (0.11 mM h^{-1}). In similar conditions, uninoculated cultures as well as boiled bacteria suspensions did not show any arsenite transformation even after 14 days (data not shown).

Culture characteristics of strain ULPAs1

In minimum medium (CDM) containing lactate (4.46 mM (5 g L^{-1})) as sole organic carbon source, the growth of the arsenite-oxidizing strain was independent of the presence of arsenic. Results presented in Figure 3 show that the growth was neither inhibited nor stimulated by 1.33 mM (100 mg L^{-1}) As as arsenite. In both experiments (with or without arsenic), the doubling time was 1.5 h. No growth occurred in the absence of an organic carbon source. Acetate or peptone also supported ULPAs1 growth whereas malate, ethanol or starch did not. When incubated in a rich medium, i.e. Luria-Bertani broth (2% w/v, Difco), no growth was observed.

Arsenic and heavy metals resistance

Strain ULPAs1 exhibited a minimum inhibitory concentration (MIC) of 6.65 mM (500 mg L^{-1}) for arsenite. The strain showed lower minimum inhibitory concentration for Pb^{2+} (1.2 mM (250 mg L^{-1})) and Cd^{2+} (1.42 mM (160 mg L^{-1})). The maximum tolerable concentration of Hg^{2+} was 0.01 mM (2 mg L^{-1}).

Table 1. Minimum inhibitory concentrations (MICs) to different heavy metals

Heavy metals	MIC [mM]		
	ULPAs1	CCM999	S21104
Arsenic	6.65	2.33	0.23
Cadmium	1.42	0.07	0.02
Lead	1.20	0.12	0
Mercury	0.01	0	0.01

MICs were also determined for the two other strains and listed for comparison in Table 1.

The strain CCM 999 exhibited lower resistance while the growth of strain S21104 was totally inhibited even with low concentrations of As, Cd, Hg or Pb.

Arsenic transformation in resting cells

To test the adaptability of the arsenite oxidation system the ULPAs1 strain was grown with or without arsenite or arsenate. Suspensions of As[III]-grown cells and As[V]-grown cells did completely remove arsenite and transformed it to arsenate. Oxidation of arsenite to arsenate occurred immediately following the contact of the cells with As[III] or with As[V] without any lag phase (Figure 4). The transformation speed was dependent on the cell density. A maximum rate of transformation of 0.4 mM h^{-1} As as As[III] was obtained with approximately 5×10^8 CFU mL^{-1} (Figure 5). No arsenite oxidation was detected with cells grown in the absence of As[III] or As[V], even after 10 h contact. The same result was obtained with the controls, i.e., in the absence of cells or in the presence of cells grown in As[III]-supplemented medium or As[V]-supplemented medium, but which were killed before the contact with arsenite. When As[III]-grown CCM 999 was tested in the same conditions, transformation rates were similar (results not shown).

Identification of the strain ULPAs1

Light microscopy examinations of ULPAs1 revealed motile, Gram-negative rods, slightly curved, approximately 2.5 μm long and 0.5 μm wide. The strain is oxidase-positive and reduces nitrate to nitrite. It does not produce indole from tryptophane, neither does it hydrolyse esculin nor gelatin. It does not harbor arginine dihydrolase, urease and β-galactosidase. The absence of assimilation of either sugars (glucose,

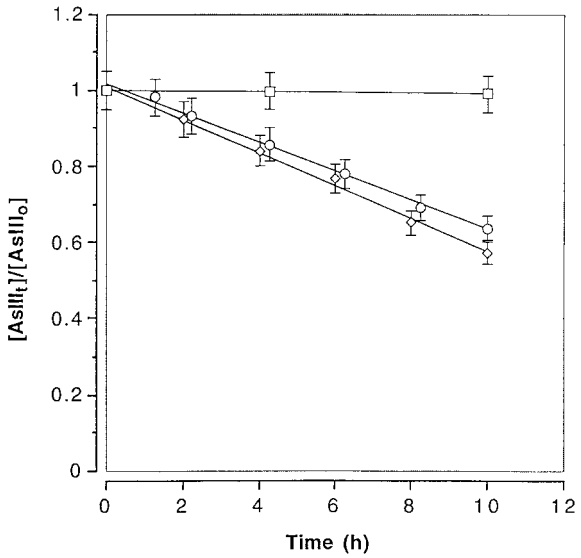

Figure 4. Induction of As[III] oxidation system by ULPAs1. In cell-suspensions of As[III]-grown cells (◊) and As[V]-grown cells (○) compared to non-induced cells (□). Conditions are those described in the materials and methods. Data points represent at least two experiments done in duplicate (SD represent less than 5%).

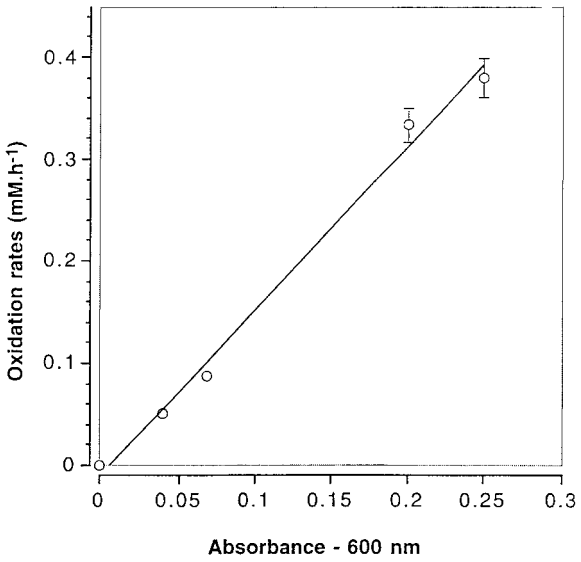

Figure 5. Standard curve showing the correlation between cell concentrations (O.D., absorbance at 600 nm) and As[III] oxidation expressed as mg L^{-1} h^{-1}. Conditions are those described in the materials and methods. Data points represent at least two experiments done in duplicate (SD represent less than 5%).

arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, gluconate, caprate and adipate) or of substrates like citrate, malate and phenyl-acetate excludes the identification as *Pseudomonas* or as *Alcaligenes*. The strain was identified by partial sequencing of 16S rDNA. Comparison with sequences kept in databases showed that the closest genus to ULPAs1 was *Zoogloea*, a group of bacteria found in soils and in activated-sludges.

Discussion

The results presented here demonstrate that the strain ULPAs1, isolated from As-contaminated aquatic environment, has the potential to efficiently transform arsenite into arsenate, when grown in a defined liquid medium, composed of mineral salts and containing an organic source of carbon. The growth of the strain was not affected when the medium was supplemented with As[III] (1.3 mM (100 mg L^{-1})). However it was dependent on the presence of an organic carbon source, indicating that ULPAs1 has a chemoorganothrophic metabolism. Different organic substrates can be used by the strain such as lactate, acetate and peptone. Malate, ethanol and starch did not support growth; arsenic alone cannot support the development of the strain. This first result indicate that the oxydation of As[III] cannot serve as an energy source to support the growth of ULPAs1 strain.

Despite the fact that orthophosphate (PO_4^{3-}) has been shown to interfere with orthoarsenate (AsO_4^{3-}) due to their chemical similarity, a high concentration of phosphate in the medium had no inhibitory effect on the growth of the strain or on the arsenite oxidation efficiency. However, a minimum concentration of 0.057 mM (10 mg L^{-1}) was necessary for an optimum growth.

The arsenite oxidation potential of the strain was detected *in vivo* when cells were grown either with As[III] or As[V]. In both cases resting cells oxidized arsenite to arsenate without any lag time. These results suggest that both As[III] and As[V] may induce the oxidation activity of As[III]. However this hypothesis has to be tested by studying the role of the two arsenic forms on the expression of the gene(s) controlling the arsenite oxidation potential. The literature mentions induction of the oxidation system only with As[III]. Phillips and Taylor (1976) demonstrated that arsenite oxidation of *Alcaligenes faecalis* is brought about by an enzyme and/or an electron acceptor which is

formed only when the cells are grown in the presence of As[III]. Whereas Osborne and Ehrlich (1976) show that a strain of *Alcaligenes* sp. acquired its arsenite-oxidizing enzyme system through growth-dependent induction. However, these two papers do not state whether the oxidation potential is due to the neosynthesis of the enzyme or to the activation of a precursor of the enzyme.

The rate of transformation was directly dependent on the cell yield. A similar result was found by Turner (1954) with 15 different strains in the presence of As[III]. For ULPAs1, when 10^9 CFU mL^{-1} resting cells were used, total oxidation of 1.33 mM (100 mg L^{-1}) As as arsenite was obtained in 2.5 h. In the same conditions the strain CCM 999 (*Ochrobactrum anthropi*) demonstrated the same oxidation rates. This may be due to the occurence of a similar oxidation system in both strains. Up to now, only the arsenite oxidation system of *Alcaligenes faecalis* was studied extensively and was shown to be linked to an oxidase which has been described at a biochemical level (Anderson *et al.* 1992). To our knowledge, no information about the genetic system is available.

The Minimum Inhibitory Concentration of As[III] toward ULPAs1 was 6.65 mM (500 mg L^{-1}). Thus according to Hedges & Baumberg (1973), ULPAs1 can be classified as an highly As-resistant strain. Trevors *et al.* (1985) reported bacteria resistant to 10.95 to 26.60 mM (1500 to 2000 mg L^{-1}) As but unable to oxidize arsenite at such concentrations. For the ULPAs1 strain, on the contrary, growth as well as oxidation efficiency were conserved at a concentration of 6 mM (450 mg L^{-1}) as As[III]. On the other hand it has been shown that in complex media the level of resistance to metals may be higher, due to the reduced availability of metal ions which are chelated by organic ligands, when compared with the values found in minimum medium. In the case of ULPAs1, the MICs tests have been conducted in minimum media with low metal binding capacity; thus the measured levels of metals resistance can be considered without any restriction. In the case of the two strains CCM999 and S21104, since they do not cultivate in the CDM broth, the MICs tests were done in a medium containing organic ligands i.e. Luria-Bertani (diluted 1/20). In consequence, we can suppose that values observed for these two strains could even be lowered.

Usually the investigation of the occurrence of metals in the environment is only based on the determination of the total metal content. However since the toxicological effects of metals depend on their forms, the speciation of the different forms is of essential importance (Cullen & Reimer 1989). In previous studies both quantification and speciation of arsenate and arsenite anions were done with the iodometric titration. The two forms of arsenic were distinguished by separation *via* thin layer chromatography and quantification of each form was estimated by total arsenic assay after elution of the visualized spots corresponding either to As[III] or to As[V] (Turner 1954; Osborne & Ehrlich 1976; Philips & Taylor 1976; Ilyaletdonov & Abdrashitova 1981). Such a technique is rather time-consuming and the reproducibility of the results is function of the skill of the manipulator.

Recently, metal speciation studies have developed new attention on the potential of separation techniques such as liquid chromatography coupled to atomic spectrometric detection systems. Several methods for As[III] and As[V] speciation have been described (Rauret *et al.* 1991; Rubio *et al.* 1992; Sarzanini & Mentasti 1997). The technique used in the present work is based on the coupling of HPLC and ICP-AES (El Moll *et al.* 1996; Amran *et al.* 1997). Its detection limit is 3.2 μM (240 μg L^{-1}) for arsenite. In our range of concentration, the linearity of the method is good ($r^2 = 0.999$), and the quantification of arsenite and arsenate is rapid, taking only a few minutes and reproducibility is reliable. Moreover this technique allows the detection of the eventual presence of other species such as dimethylarsinic and monomethylarsonic acids as well as their quantification, which was not the case with the previously used techniques.

This new strain can be favorably compared with the most efficient arsenite-oxidizing strains which have been described previously: *Pseudomonas arsenoxidans-quinque* (Turner 1954) and *Alcaligenes faecalis* (Osborne & Ehrlich 1976; Phillips & Taylor 1976). ULPAs1 is efficient in an arsenite concentration range corresponding to the highest concentrations which have been described in soil or in aquatic environments (Seiler 1996). Thus this strain could be a good candidate to be used for bioremediation of arsenic in environmental compartments. The presence of arsenite, highly soluble and toxic, in acidic freshwaters from volcanic areas is frequently reported (Leblanc *et al.* 1995). The best approach to remove arsenite is to oxidize it into arsenate; this last form is less soluble and much more easily removed, i.e, by alkaline precipitation technologies on various solids formed during softening including $CaCO_3$, $Mg(OH)_2$, $Mn(OH)_2$ or $Fe(OH)_3$ (McNeill & Edwards 1997). Oxidation can be achieved chemically with various

oxidation treatments, i.e., by adding potent oxidants. The major drawbacks of these processes are they can generate additional pollution and moreover are generally highly expensive. An alternative method could be a biological oxidation. An efficient strain like ULPAs1 could be used and would allow a much more economical way to perform this oxidation. Nevertheless, the corresponding innovative industrial process remains to be developed. Its importance is also emphazised in a future context of more stringent regulation. The current maximum concentration limit for arsenic in drinking water is 0.66 μM (50 μg L^{-1}) which will be lowered to a range of 0.0266-0.266 μM (2–2 μg L^{-1}) (Pontius 1995).

Biochemical tests as well as partial sequencing of the 16S RNA gene indicated that ULPAs1 belongs to the genus *Zoogloea*. This strain does not correspond to the previously described arsenite-oxidizing microorganisms: *Bacillus arsenoxidans* (Green 1918), *Pseudomonas arsenoxidans*, *Xanthomonas arsenoxidans*, *Achromobacter arsenoxidans* (Turner 1954), *Alcaligenes faecalis* (Osborne & Ehrlich 1976; Phillips & Taylor 1976) *Pseudomonas arsenitoxidans* (Ilyaletdonov & Abdrashitova 1981). Our results extend the known diversity of organisms involved in As[III] oxidation. A detailed characterization of the strain, based on sequencing of the total 16S rDNA, on fatty acid analysis and on DNA/DNA hybridization, is in progress.

Further investigations still remain to be carried out to complete taxonomic identification and strain oxidation efficiency in aquatic and soil microcosms.

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