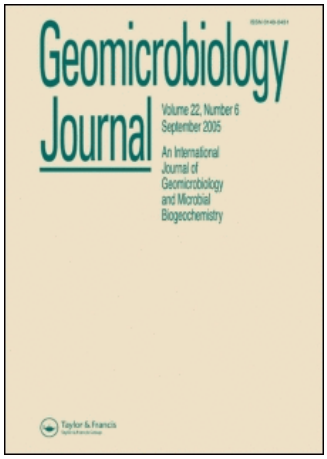

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An abiotic origin has traditionally been assumed for the arsenic minerals realgar and orpiment associated with thermal springs. Microbial precipitation of arsenic, however, has been studied in pure cultures and the isotopic composition of arsenic sulfides associated with some borate deposits suggests a biotic origin for those minerals. The aim of the present study is to demonstrate the role of bacterial arsenic precipitation in the biogeochemical cycle of arsenic in such borate deposits. For this purpose both enrichment and pure cultures were obtained from the natural arsenic minerals and the composition and isotopic signatures of the arsenic sulfide minerals precipitated by the cultures and those associated with boron deposits from an Andean salt flat in northern Chile were compared. Based on the microbiological and chemical evidence gathered, it is concluded that bacteria contributed to the formation of the arsenic minerals. This interpretation is based on the consistent association of a variety of features that strongly indicate microbial involvement in the precipitation process. These include: (1) enrichment and isolation of cultures with arsenic precipitation capacity from arsenic mineral samples, (2) high numbers of arsenic-precipitating bacteria in the Andean minerals and brines, (3) chemical and mineralogical properties of precipitates experimentally formed under biotic and abiotic conditions, (4) similarities in stoichiometry between natural and laboratory obtained minerals, and (5) the consistent depletion in δ^{34}S values for natural versus laboratory obtained sulfides. Thus, microbial precipitation of arsenic sulfides is a geochemically relevant metabolism.

Keywords arsenic respiring microorganisms, sulfate reducing bacteria, borate deposits

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INTRODUCTION

Determining how organisms that utilize or produce minerals may have influenced the chemical and physical features of the planet in the past, and how they continue to do so in the present, is a major challenge for biogeochemistry (Newman 2004). Many types of sedimentary minerals, formerly believed to have an inorganic origin, are now thought to have a complex origin with microbial participation. Progress has been made possible by the combination of two lines of research: Understanding microbial metabolisms involving minerals with pure cultures in the laboratory, and culture-independent analysis of genetic data that allow the description of microbial populations in situ (Macalady and Banfield 2003).

Recently, it has been reported that deep subsurface marine sediments contain a high number of living bacteria with turnover times comparable to those in surface environments (0.25–22 years) (Schippers et al. 2005). On longer time scales, isotopic signatures have evidenced microbial sulfate reduction activity at 3.47 Ga, indicating that this trait evolved early in Earth's history

(Shen et al. 2001). Therefore, microbial activities relevant in mineralization processes are present in appropriate sedimentary environments and have been present almost since the origin of life. In addition, bacterial reduction of Mn and Fe, for example, has been established to account for a significant turnover of organic carbon in many environments (Heidelberg et al. 2002; Nealson et al. 2002). Among the elements recently shown to have a biological cycle are uranium (Lovley 2001), gold (Kashefi et al. 2001; Karthikeyan and Beveridge 2002) and arsenic (Oremland and Stolz 2003).

Arsenic is widely distributed in nature despite its low crustal abundance (0.0001%) and is commonly associated with the ores of metals such as copper, lead and gold, due to its chalcophilic character (Nriagu 2002). These natural arsenic sources are of utmost concern to human health on a global scale (Ball 2005). Anthropogenic point sources like smelter gas emissions and slag also contribute to arsenic found in the environment. Arsenic mobilization from aquatic sediments has been reported to involve a combination of chemical, physical, and microbial factors both in the laboratory and natural environments (Ahmann et al. 1997; Harrington et al. 1998; Ahmann 2002; Redman et al. 2002; Meng et al. 2003).

Bacteria have been identified that can oxidize and/or reduce arsenic compounds for energy conservation. These bacteria are present in many environments, including both aerobic and anaerobic water bodies and sediments (Robertson et al. 2000). Their activities are believed to mobilize and/or precipitate arsenic in the environment and, therefore, their metabolism may be important in detoxification and bioremediation as well as in contamination processes. Thus, interest in the arsenic cycle is clearly justified.

Northern Chile, as a part of the Andean Range, is an excellent place to study microbial implication in the arsenic geochemical cycle. First, ground waters are relatively rich in arsenic due to the volcanic-hydrothermal provenance of this element. As a consequence, arsenic is also present in river sediments, where some of the microbial reactions using arsenic oxianions for energy generation may mobilize arsenic from the solid to aqueous phases, resulting in contaminated drinking water sources. Contamination episodes have taken place in the River Loa with increased arsenic levels (Arroyo et al. 1999). Microbial arsenic mobilization activity has been also found in the arsenic contaminated sediments of the River Loa (Demergasso et al. 2003).

There are extensive salt flats in the Andes due to geomorphology and climate. These conditions include geothermal processes and evaporation of water rich in arsenic and other compounds. These salt flats contain significant borate deposits (mostly in the form of ulexite) that include As-rich red and yellow nodules and lenses, some centimeters thick and a few meters in diameter (Chong et al. 2000). These nodules are rich in the arsenic minerals realgar and orpiment. We have observed these features in Salar de Surire and Salar de Ascotán within the whole ulexite ore profile.

In the Salar de Atacama, ulexite appears in 50-cm-thick layers, related to calcium sulfates, in its eastern and northeastern edges. Such minerals are also common in equivalent environments on the Argentinian side of the Andes. (Alonso 1986). Moreover, major boron ore deposits present similar mineral assemblages and genesis around the world, such as in the U.S.A. (Tanner 2002) or in Turkey (Helvaci 1995; Helvaci and Orti 1998; Helvaci and Orti 2004). For this reason a similar biogeochemical origin would be expected for all of them. The Salar de Ascotán will be used as a model for these systems.

A number of bacteria are known to process arsenic through different metabolic pathways (Robertson et al. 2000). There are now over two dozen species of prokaryotes that are capable of conserving energy by linking the oxidation of an electron donor (either organic or inorganic) to the reduction of As(V) to As(III) (Oremland and Stolz 2003; Liu et al. 2004; Oremland et al. 2005). *Desulfosporosinus auripigmentum* (Newman et al. 1997; Labeda 2000; Stackebrandt et al. 2003), isolated from lake sediments in USA, and *Desulfovibrio* strain Ben-RB isolated from mud obtained from an arsenic-contaminated reed bed in Bendigo, Australia (Macy et al. 2000), are able to reduce As(V) to As(III) and S(VI) to S(-II).

Desulfosporosinus auripigmentum (Newman et al. 1997; Labeda 2000; Stackebrandt et al. 2003) precipitates arsenic trisulfide (As_2S_3) as a result of the reduction of both As(V) and S(VI). Cultures of *Desulfovibrio* strain Ben-RA (Macy et al. 2000) tended to precipitate As(V) as yellow arsenic sulfide (As_2S_3) even though the As reduction was not associated with energy conservation.

Other arsenic-reducing bacteria cannot precipitate As_2S_3 because they do not reduce S(VI), or because they do not reduce both As(V) and S(VI) to appropriate concentrations of As(III) and S(-II) (Newman et al. 1997; Macy et al. 2000). All these studies have been carried out with pure cultures in the laboratory. However, the isotopic composition of some arsenic sulfides associated to boron deposits in Turkey suggested the involvement of microbial sulfate reduction in the mineral formation (Palmer et al. 2004). This study attempts to demonstrate the involvement of bacteria in the formation of arsenic minerals in the Andean borate deposits.

MATERIALS AND METHODS

Field Locality (Geological Setting)

Salar de Ascotán is part of an evaporitic basin system in the High Andes of northern Chile and is described as an Andean salt flat (Stoertz and Ericksen 1974; Chong 1984). It is located between 22°25′ y 22°45′ South latitude and 68°30′ y 68°10′ West longitude at an average altitude of 3700 m. Salar de Ascotán is at the bottom of a tectonic basin surrounded by volcanic chains to the east and west, including some active volcanoes over 5000 m high, with the highest peaks of about 6000 m. The geological setting is dominated by volcanic structures and includes acidic (rhyolites) and intermediate (andesites) rocks of Tertiary and Quaternary age.

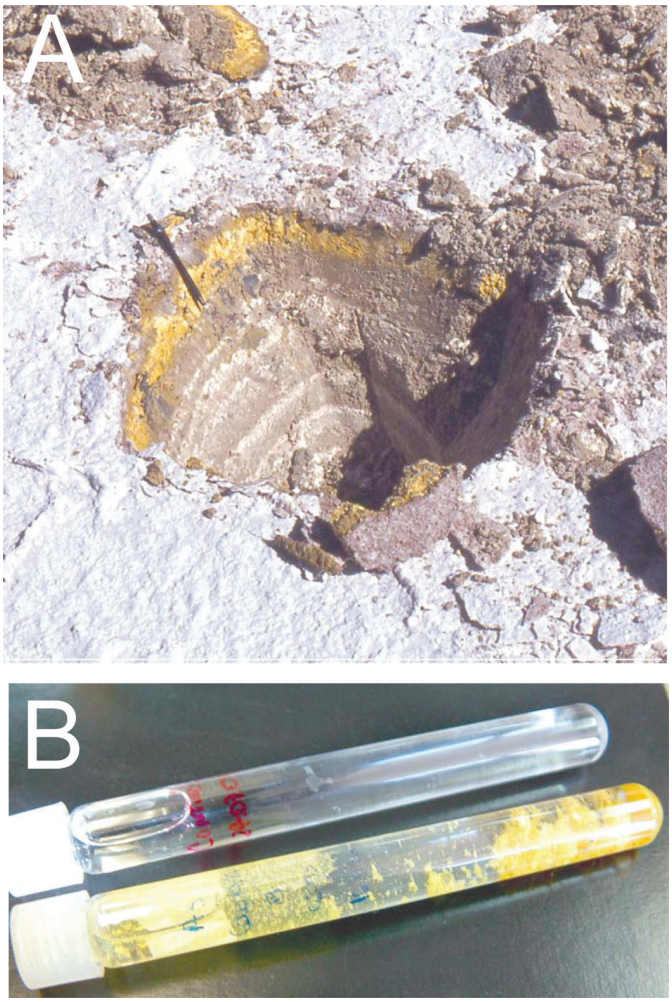

FIG. 1. (A) Position of the yellow material (3 to 5 cm thickness) in the stratigraphic column of Ascotán borate deposits. (B) Yellow precipitate in tubes inoculated with sediments from Ascotán compared to the uninoculated control.

The evaporitic basin contains palaeoshore lines indicating the existence of a former saline lake with deeper bathymetry. Climate is characterized by large daily thermal oscillations. High solar irradiation and strong and variable winds cause intense evaporation (about 4.5 mm/day) while precipitation is about 120 mm/year (Mardones-Pérez 1997). Water input is through surface drainage from the snow fields of volcanoes and underground waters and have a strong geothermal component with spring waters commonly reaching 23 to 25°C. In the eastern border of the basin there are thermal springs (Ojos del Coñapa) with 2700 to 3000 mg L^{-1} of total dissolved solids. The saline crusts are mainly composed of chlorides (halite) and sulfates (gypsum) with important borate ore deposits composed mostly of ulexite with significant amounts of arsenic sulfide minerals (Figure 1A).

Sampling Procedure and Measurements

Large chunks of ulexite with yellow or orange inclusions were extracted from the salt flat with a pull shovel. Samples were selected from these chunks with a sterile spatula and placed in sterile 250 mL polyethylene vials, which were then totally filled with water aseptically collected from the same deposit. Brine samples were also taken in sterilized vials to carry out microbial counts. The vials were stored in an icebox with ice, until further processing, in order to prevent oxidation and changes in microbial composition. Samples for X-ray diffraction (XRD), electron microscope observations, and energy dispersive X-ray microanalysis (SEM/EDS) were freeze-dried in the laboratory in order to preserve the sulfide mineralogy. An Orion model 290 pH meter was used to measure temperature and pH of the brines. Salinity was measured with an Orion model 115 conductivity meter. Two sampling expeditions were conducted, in August 2005 and June 2006.

Most Probable Numbers, Enrichment Cultures and Isolation

Culturable, arsenic-precipitating cells were detected by most-probable-number (MPN) incubations using fresh minimal medium (Newman et al. 1997) modified by the addition of 0.008% yeast extract, and amended, after autoclaving, with sterile 20 mM sodium lactate, 10 mM sodium sulfate (Na_2SO_4) and 1 mM dibasic sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) under an N_2:CO_2:H_2 atmosphere (80:15:5, v/v). The highest decimal dilution was 10^{-6} and 5 tubes were analyzed for each data point. Cultures were incubated in the dark, at 28°C. The presence of yellow precipitate was considered as a positive result.

Primary enrichment cultures were grown in the fresh, modified, minimal medium described above (Newman et al. 1997). Cultures were incubated in the dark, at 28°C. Primary enrichment cultures were started with sediment samples from Salar de Ascotán. These cultures took several weeks to precipitate arsenic sulfides. Subsequent cultures were maintained by periodic transfer to fresh medium. These cultures repeatedly grew in a few days.

Isolation was carried out by plating on the same medium used for enrichment, containing 2% purified Oxoid agar (Oxoid, Hants, England). Plates were incubated at 28°C, in an anaerobic jar, until the appearance of yellow colonies for further purification. The colonies could then be transferred to liquid medium.

Cell Microscopy

A Leica DMLS microscope was used for phase-contrast observations of morphology. Total cells counts were done by epifluorescence with a DNA-specific dye, 4′, 6-diamidino-2-phenylindole (DAPI) with a Leica DMLS epifluorescence microscope. Cells were fixed with 1.25% glutaraldehyde (final concentration) overnight at 4°C for SEM observation. The concentrated cell suspension from enrichment cultures was placed on polylysine coated glass coverslips (Marchant and Thomas 1983), fixed with osmium tetroxide, dehydrated, critical point dried and coated with gold. Samples were viewed using a Leica Stereoscan S 120 scanning microscope. Pure culture cells were fixed with 1.25% glutaraldehyde (final concentration) overnight

at 4°C, filtered and coated with gold. Samples were examined in a JEOL JSM-6360L scanning electron microscope.

Phylogenetic Analysis

An approximately 600 to 800 bp fragment of 16S rRNA gene was sequenced for strains CC-1, and Asc-3. A similarity matrix was built using those partial sequences with the ARB software package (Technical University of Munich, Munich, Germany; ⟨www.arbome.de⟩). Partial sequences were inserted into the optimized and validated tree available in ARB (derived from complete sequence data), by using the maximum parsimony criterion and a special ARB parsimony tool that did not affect the initial tree topology. Nucleotide sequence accession numbers at EMBL are: EF157293 and EF157294.

Growth Experiments

Strains CC-1 and Asc-3 were grown in the modified minimal medium described above amended with lactate (20 mM) and, as indicated (Fig. 5B), sulphate (10 mM), arsenate (1 mM) or sulphate (10 mM) plus arsenate (1 mM) as electron acceptors. Cysteine (1 mM) was also added. Cultures grown in the complete medium were inoculated (10%) into media with the different growth conditions. Two consecutive subcultures under the same conditions were carried out to avoid the presence of substrates from the initial complete medium. Cell numbers were determined by direct DAPI counts with an epifluorescent microscope, after 2 weeks of incubation. The appearance of an arsenic trisulfide precipitate was considered evidence of both sulfate and arsenate reduction. Sulfate reduction in the tubes without arsenic was observed by adding 1 mL of 5% $Fe(NH_4)_2(SO_4)_2$ after the incubation time. Tubes scoring positive for sulfate reduction turned black.

Arsenic Precipitation by Enrichment Cultures

A few milliliters from each enrichment culture were inoculated into several tubes with fresh medium. Additional tubes were inoculated with strain *D. auripigmentum*, ATCC Orex-4. An abiotic control was carried out in sterile medium without inoculum. Several tubes were sacrificed at each time point and opened in an anoxic glovebox (Coy Laboratory Products, Grass Lake, MI) (N_2:CO_2:H_2, 80:15:5). Medium was centrifuged at 2000 rpm for 5 minutes and filtered through a 0.2 μm cellulose filter; the filtrate was sealed and refrigerated at 4°C to preserve arsenic speciation until analysis. Total soluble arsenic was measured by Hydride Generation-Atomic Absorption Spectroscopy (HG-AAS). The precipitate was analyzed by X-ray diffraction and electron microscopy. Its chemical and isotope composition was also determined.

Analysis of Precipitates in Cultures and Natural Material

X-Ray Diffraction (XRD) . Samples taken from the enrichment and pure cultures 1 week after inoculation were opened in an anaerobic chamber. Precipitated material was recovered by filtration on 0.22 μm pore size Durapore filters and lyophilized. Natural material was directly placed on similar filters. Powdered microsamples of both materials (2 to 5 mg) were placed in 0.5 mm Ø (diameter) Lindemann glass tubes. X-ray diffraction analysis was performed using a Debye-Scherrer diffractometer fitted out with an Inel CPS-120 localization curved counter.

Electron Microscopy. Samples for SEM/EDS were mounted on stubs using a bi-adhesive carbon ribbon, and coated with carbon. Samples for TEM were mounted on a copper grid mesh stage with Mylar, and carbon coated. In addition to morphological observations, these samples were analyzed by SEM and TEM/EDS for determination of elemental composition, and by selected area electron diffraction (TEM/SAED) to establish whether the structures observed were crystalline or amorphous. Observations and SEM/EDS were carried out using a Leica Stereoscan S 120 scanning electron microscope. TEM was carried out with a Hitachi H 600 AB transmission microscope. Molar ratios were calculated from EDS analyses using realgar and orpiment standards.

Chemical Analyses. The relative arsenic and sulfur composition was determined from the average of 2 to 6 SEM-EDS spectra acquired from laboratory and natural materials, and compared to pure realgar and orpiment. Moreover, chemical analyses using Hydride generation atomic absorption spectroscopy (HG-AAS) were performed after acid digestion of the sediment sample.

Sulfur Isotopic Composition

$\delta^{34}S$ isotope analyses were carried out with natural and laboratory samples. An additional control experiment was carried out to check if there was some isotopic fractionation during chemical precipitation of As_2S_3, without changes in the oxidation state of either element, in the absence of microbial activity. Chemically precipitated As_2S_3 was obtained as previously described (Eary 1992) by adding an excess of $Na_2S \cdot xH_2O$ to a deoxygenated 0.15 *m* $NaAsO_2$ solution, buffered (pH 4 ± 0.2) with 0.1 *m* potassium hydrogen phtalate solution, at 25°C. The yellow precipitate was aged during 1 to 3 days and washed before analysis. Reagents used in the experiments were analytical grade solid Na_2SO_4 (Merck) and $Na_2S \cdot xH_2O$ (x = 7 a 9) (Merck) and were also isotopically analyzed to determine $\delta^{34}S$. The isotopic composition of sulfates was determined on $BaSO_4$ samples precipitated from previously dissolved sulfates. Sulfide samples were directly analyzed.

The sulfur isotopic composition was determined by continuous flow isotope ratio mass spectrometry (IRMS) and is expressed in the standard δ notation given by $\delta^{34}S = ([R_{sample}/R_{standard}]-1) \times 1000$, where $R = {}^{34}S/{}^{32}S$ is the isotopic abundance ratio. Values are expressed on a per mil (‰) basis as deviations from the international standard CDT (Jensen and Nakai 1962). Reproducibility of duplicate analysis of samples and internal standards were all better than 0.1 ‰ (1 σ) for $\delta^{34}S$ values.

Bacterial reduction of sulfate in cultures was allowed to proceed in sealed serum vials without loss of product. In such a closed system the isotopic difference between sulfate and sulfide develops following a Rayleigh distillation model (Canfield 2001). As sulfate depletion proceeds (Canfield 2001), the isotopic composition of the sulfide approaches that of the original sulfate, and after complete sulfate depletion, no fractionation information is preserved. Therefore, the isotopic enrichment factor ε can be calculated using the isotopic differences between dissolved sulfate and precipitated sulfide only when the fraction of sulfate remaining is greater than 95% (Mariotti et al. 1981; Canfield 2001). Then:

$$\varepsilon = [(\delta^{34}S_{\text{sulfide}} + 1000)/(\delta^{34}S_{\text{sulfate}} + 1000) - 1] \cdot 1000$$

where $\delta^{34}S_{\text{sulfide}}$ = isotopic composition of precipitated sulfide, $\delta^{34}S_{\text{sulfate}}$ = isotopic composition of dissolved sulfate.

In order to minimize the Rayleigh effect, the yellow precipitate was harvested as soon as it appeared in the cultures. The mass balance indicated that, at that point, the sulfate remaining fraction was greater than 70% and therefore, it is to be expected that the Rayleigh effect should be insignificant and the fractionation information similar to that observed in an open system. In any case, the Rayleigh effect would decrease the isotopic enrichment factor measured, and fortify the evidence for microbial sulfate reduction.

RESULTS

Bacterial Abundance in Salar de Ascotán

Three sediment samples and three brine samples taken from different ponds on the Salar de Ascotán on 9 August 2005 were analyzed for bacterial counts. Total bacterial numbers, as determined by epifluorescence, are shown in Figure 2A. Values ranged between 1.6 and 6.7 $\times$ 10^5 cells mL^{-1} in the brine samples and between 0.7 and 3.0 $\times$ 10^6 cells g^{-1} of sediment. Most probable numbers (MPN) of arsenic-precipitating bacteria were determined simultaneously (Figure 2A). MPN ranged 5 orders of magnitude between 3.9 $\times$ 10^1 cells mL^{-1} and 1.6 $\times$ 10^6 cells

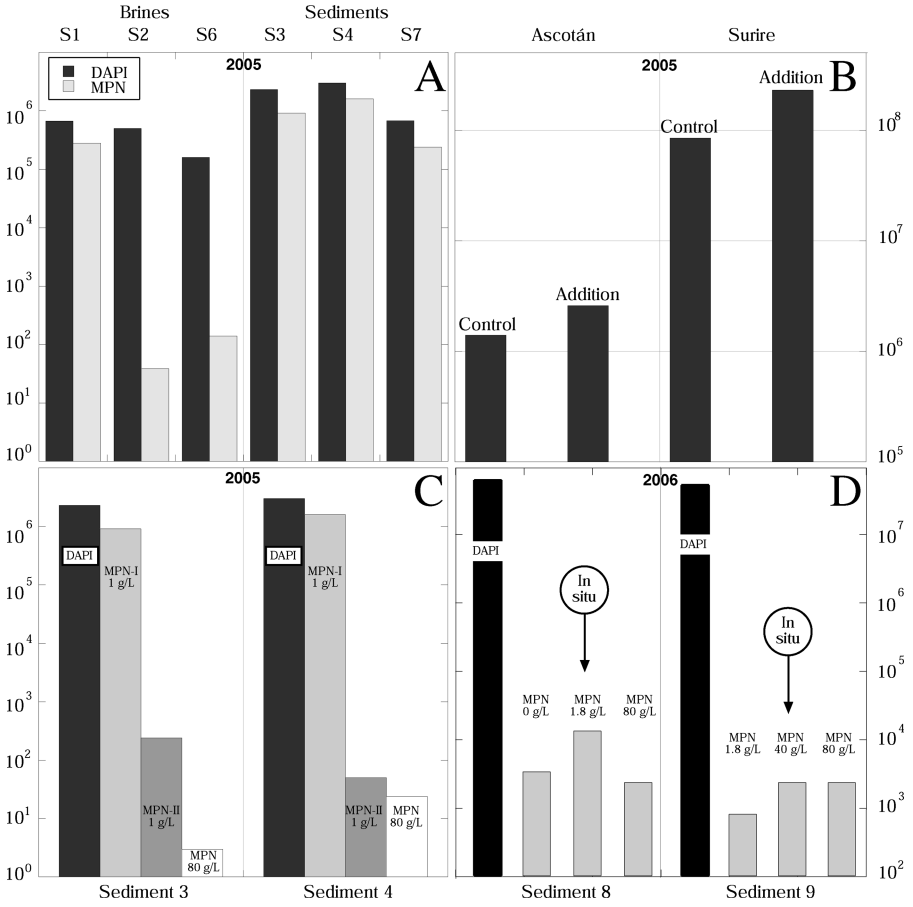

FIG. 2. (A) Total (DAPI) and MPN bacterial cell counts in sediments and brines from the May 2005 samples. (B) Test to control the efficiency of the DAPI method for counting cells in sediment samples from Ascotán and Surire salt flats. The two samples received, respectively, 1 $\times$ 10^6 and 2 $\times$ 10^7 cells g^{-1} from an arsenic-reducing culture obtained from Salar de Ascotán, (C) Effect of salinity on MPN in the May 2005 samples (MPN-I: with 1 g/L of salt, analysis carried out in August 2005; MPN-II: with 1 g/L and MPN 80 g/L salt, analysis carried out in May 2006). (D) Effect of salt concentration in the medium on MPN in two samples taken in 2006. The circles and arrows indicate the in situ salt concentration for each sample.

TABLE 1
Physicochemical parameters, arsenic-precipitating and total bacterial numbers in samples from Salar de Ascotán

Sample	Sample type	Coordinates	Date	pH	Salinity/per mil	As mg L^{-1}(A) mg Kg^{-1}(B)	DAPI count Cells mL^{-1}(A) Cells g^{-1}(B)
1	brine	7601876N581420E	03-08-2005	7.83	33	4.38 (A)	6.70E+05 (A)
2	brine	7609154N/573967E	03-08-2005	7.98	76	6.5 (A)	5.00E+05 (A)
6	brine	7604253N/577706E	03-08-2005	7.23	309	183 (A)	1.60E+05 (A)
3	sediment	7609154N/574012E	03-08-2005	ND	ND	781 (B)	2.30E+06 (B)
4	sediment	7609035N/573669E	03-08-2005	ND	ND	1210 (B)	3.00E+06 (B)
7	sediment	7609035N/577770E	03-08-2005	ND	ND	6504 (B)	6.80E+05 (B)
8	brine	7609328N/577921E	22-06-2006	5	1.8	3.4	3.9E+06 (A)
8	sediment	7609328N/577921E	22-06-2006	ND	ND	ND	6.03E+07 (B)
9	brine	7610653N/572171E	22-06-2006	6.50	10.5	28	4.25E+07 (A)
9	sediment	7610653N/572171E	22-06-2006	ND	41.4	ND	5.12E+07 (B)

[a]Note that units are different for brine (A) and sediment (B) samples.
ND = Not Determined.

g^{-1}. Both total bacteria and MPN varied considerably between samples.

This is probably due to the different salinities and arsenic concentrations of the samples (Table 1) indicating the heterogeneity of the ponds in the Salar de Ascotán. However, the very high MPN on four of the samples was surprising. Tests were carried out to check the reliability of the counts and additional samples gathered on 22 June 2006. The first test consisted of adding a known number of bacterial cells from a culture isolated from Salar de Ascotán (see later) to two natural samples. As shown in Figure 2B, the total cell counts increased as expected. It is therefore assumed that the total epifluorescence counts are reliable. Since salinity was so variable in this system two additional tests were carried out.

Two samples were selected where the MPN had been highest and this time it was determined at two different salinities without any salt added to the medium and with 8% NaCl added (Figure 2C). In all cases the MPN were several orders of magnitude lower than before. This is to be expected as the samples had been stored in the lab for several months and, thus, lower viability would be presumed. The differences between MPN at the two salinities were not consistent.

In sample Sediment 3, the MPN was one order of magnitude lower at the higher salinity, while in sample Sediment 4, the two values were essentially identical. On 22 June 2006 two additional fresh samples were obtained from the Salar de Ascotán and total counts and MPN were determined in media with different salinities (Figure 2D). At this sampling date, total counts were one order of magnitude higher than in the 2005 sampling, while MPN were about the same as the lower MPN from 2005. The salinity of the medium, again, did not have a marked influence on the results. Notwithstanding, arsenic-reducing bacteria could be detected in all samples, making up between 0.01 and 53% of the total bacterial assemblage. In general, MPN accounted for a larger proportion of the total count in sediments than in brines.

Enrichment Cultures and Isolation

Growth was shown by the appearance of a yellow precipitate (Figure 1B). Growth on Ascotán minerals was observed 12 weeks after inoculation. At this primary enrichment stage several cell morphologies were observed by phase contrast microscopy. Rod-shaped cells (2.5 $\times$ 1 μm) with terminal endospores and spirillum-like, motile microorganisms were the most apparent morphologies. Smaller straight and curved rods and cocci were also abundant. During successive enrichment stages, however, the most abundant and characteristic morphologies were lost, and other morphologies became predominant: straight and slightly curved rods (often paired), rods with pointed ends occurring singly or in pairs, and rod to pear-shaped cells with polar prostheca of varying lengths (Figures 3A–3D).

Some subcultures retained the rods with apparent terminal endospores, but most did not. These subcultures grew much faster and precipitates appeared after a few days of incubation. Soluble arsenic concentration decreased from an average of 77.8 to 33.1 mg/L after three days in the inoculated subcultures with Ascotán enrichment and in a *D. auripigmentum* (Labeda 2000) ATCC 700205™ pure culture, while remaining constant in the abiotic control (Figure 4).

Two strains, CC-1, and Asc-3 were isolated in pure culture. Both of them grew on lactate using sulfate or arsenate as electron acceptors, indicating that arsenate reduction was also associated with energy conservation (Figures 5A and 5B, conditions 1 to 4). Growth on lactate in the absence of an electron acceptor did not occur (conditions 5 and 6). When grown with both arsenate and sulphate, final yield was greater than with sulfate or arsenate alone (Figure 5A, conditions 1 and 2 vs 3 and 4). Cultures were

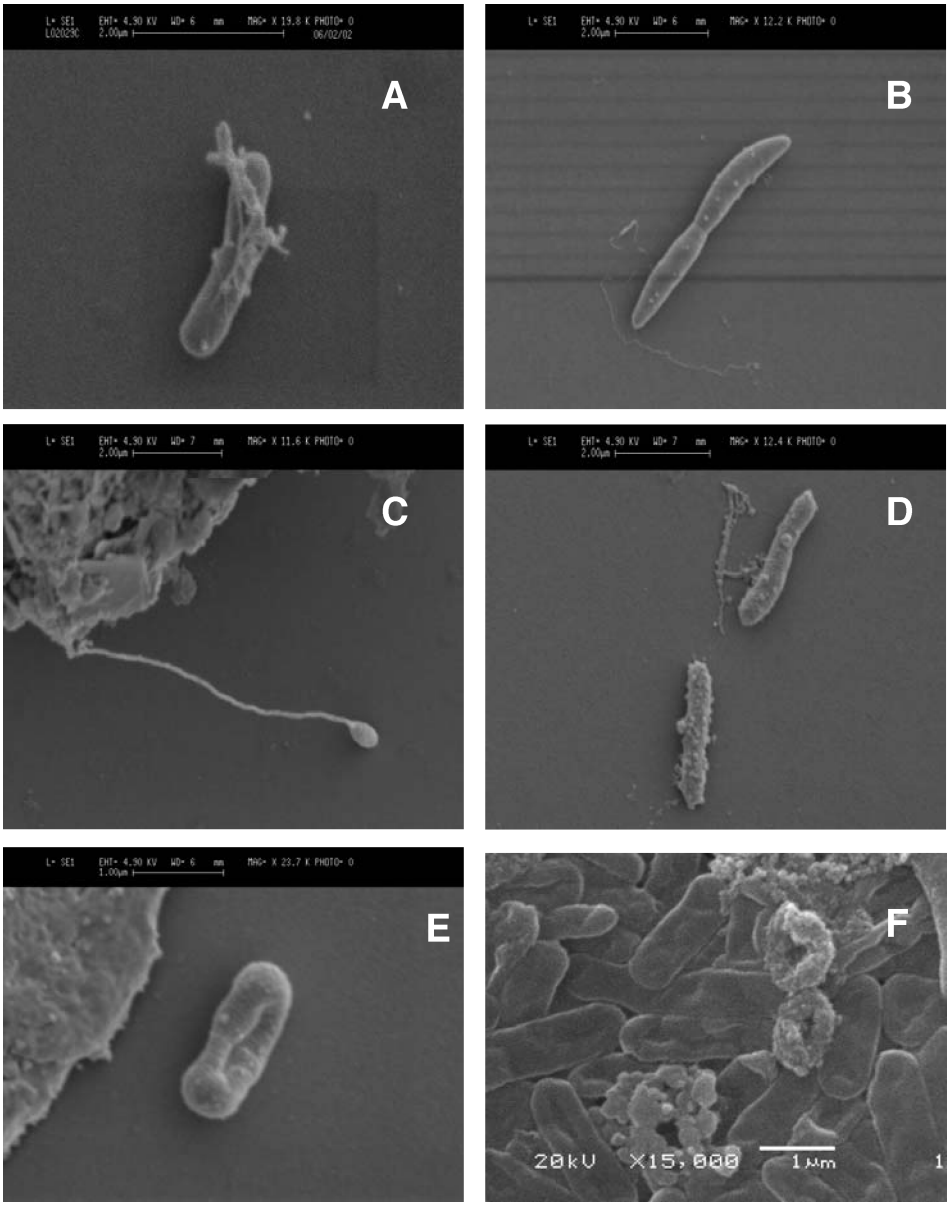

FIG. 3. SEM micrographs of cells observed in enrichment cultures (A–E) after successive steps of reinoculation in fresh medium, and of pure culture Asc-3 (F). Scale bar 2 μm for A to D, and 1 μm for E and F.

grown with and without cystein to check whether this made any difference (conditions 1 vs 2 and 5 vs 6). The two conditions resulted in the same final cell yield. Finally, a control without a sulfur source showed no growth (condition 6). Yellow arsenic sulfides precipitated when cells were grown in 10 mM sulfate and 1 mM arsenate (Figure 5C, conditions 1, 2). Yellow precipitate also appeared in the presence of arsenate and cystein (condition 4) but not in the absence of a source of sulfur (condition 6) nor in the absence of arsenate (condition 3). In order to show that in this culture sulfate had actually been reduced to sulfide, iron was added at the end of the incubation period and a black precipitate formed instantly (Figure 5C, condition 3). Only results for strain Asc-3 are shown in Figure 5, but results for strain CC-1 were identical.

Since salinity in Salar de Ascotán was very variable between sites and at differing times, the range of salinities allowing growth of these two strains was also tested. Both could growth in medium containing up to 3% NaCl after 2 weeks of incubation (0, 3, 5, 7, 10% were tested).

The nearest phylogenetic relative of Asc-3 strain was *Enterobacter* sp. BL-2 (Son et al. 2005) (97% sequence similarity). Strain CC-1, on the other hand, was related to *Pseudomonas* sp. PHLL (Gen Bank description) (99% similarity). Both microorganisms belong to the Gammaproteobacteria class. Both

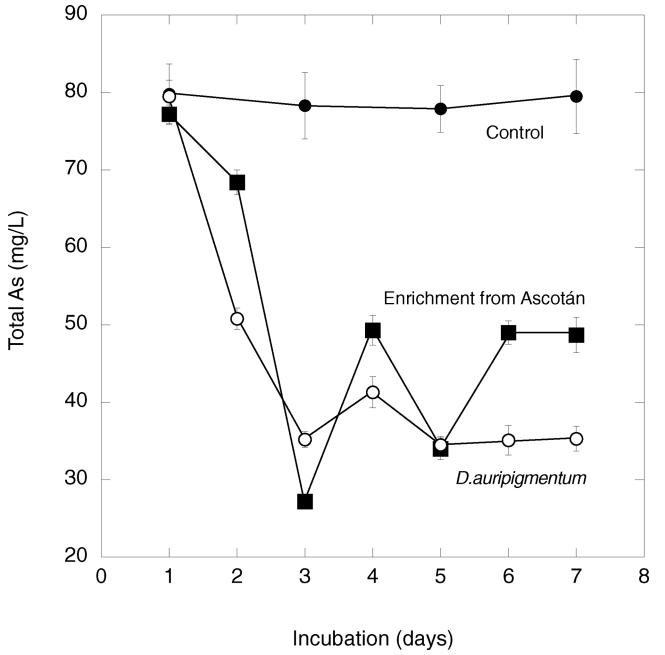

FIG. 4. Changes in the concentration of total soluble arsenic with time in an enrichment culture from Salar de Ascotán.

sequences showed a very low similarity (lower than 90%) to that of strains *D. auripigmentum* Orex-4 (Newman et al. 1997) and to *Desulfovibrio* strain Ben-RB and Ben-RA (Macy et al. 2000).

Characterization of Natural Material and Culture Precipitates

Arsenic-Bearing Natural Material. Bulk chemical analyses of Ascotán ulexite ore, using HG-AAS for As and gravimetry for S, showed concentrations of 0.47% As and 0.57% S (dry weight). The XRD analysis indicated a dominant mixture of calcite, ulexite, halite and quartz minerals. The arsenic-bearing minerals were under the detection limit of the technique. SEM observations showed an ulexite matrix with embedded electrodense grains, 1–3 μm in diameter (Figure 6A) and spicular crystals 0.3 to 0.5 μm wide. EDS analyses of the electrodense grains and crystals revealed sulfur and arsenic as the main components (Figures 6B, 7A). The relative arsenic/sulfur molar ratio in selected As-bearing crystals was 0.68 $\pm$ 0.07 on average (out of five determinations). This value was more closely related to the stoichiometry of orpiment than to that of realgar or pararealgar. The natural arsenic precipitate had an isotopic composition $\delta^{34}S_{CDT}$ = -0.55% as determined by IRMS (Table 2).

Laboratory Bacterially Produced Precipitate. Analysis of the precipitate produced by enrichment culture by XRD showed the presence of pararealgar As_4S_4 (Roberts et al. 1980) (JCPIDS 33-127 and 83-1013) with traces of uzonite (Popova and Polykov 1986) As_4S_5 (ASTM 39-331) (Table 2). SEM observations of the bacterially produced precipitate revealed a matrix of electrodense 0.24 to 0.6 μm thick fibres, 0.3 μm diameter spherical particles, and some framboids 2 μm in diameter (Figure 6C). EDS showed that fibers, spherical particles and framboids were composed of arsenic and sulfur. The As/S molar ratio determined on bacterial surfaces was similar to the stoichiometry of orpiment (Figure 7B). In the bulk, freshly produced precipitate, however, this ratio increased and was closer to that of pararealgar (Figure 7C), matching the XRD results (Table 2).

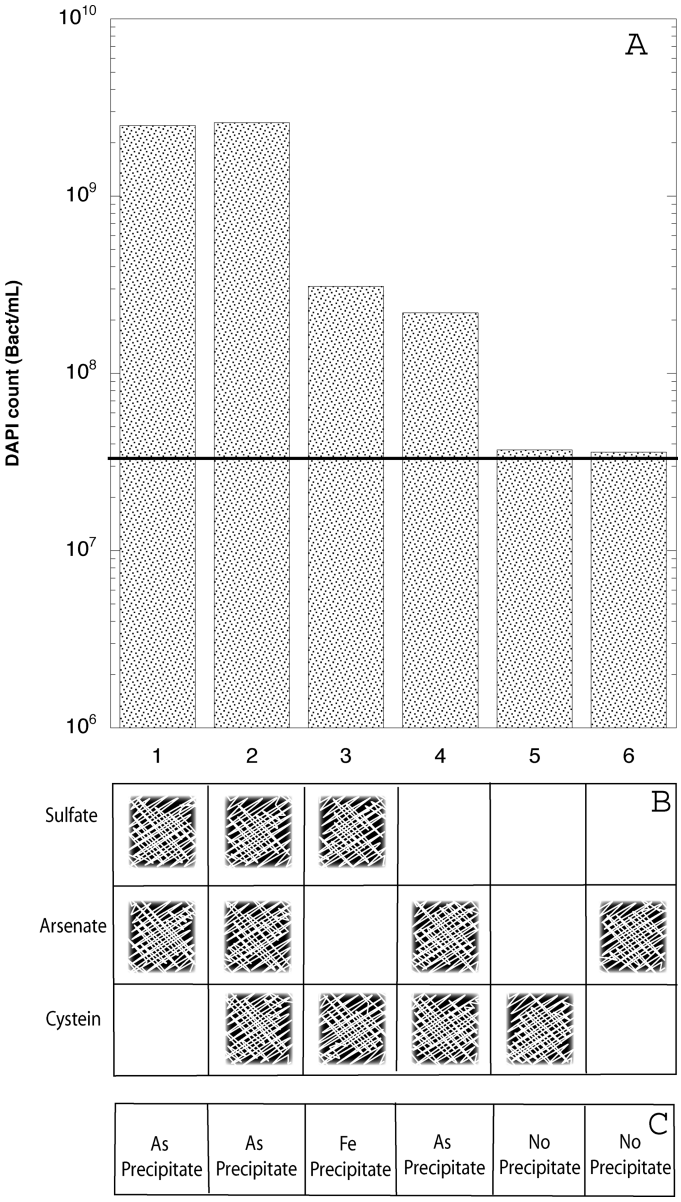

FIG. 5. (A) Growth of strain Asc-3 in modified minimal medium (Newman et al. 1997) with lactate (20 mM), and the addition of sulfate (10 mM), arsenate (1 mM), cysteine (1 mM) as indicated in the lower diagram (B). A culture of the strain grown in the same medium was inoculated (10%) into the experimental tubes at time 0. The black line shows the bacterial number at time 0 (3,5E+07). (B) Experimental conditions for each treatment. (C) Appearance of precipitates after incubation under the different experimental conditions.

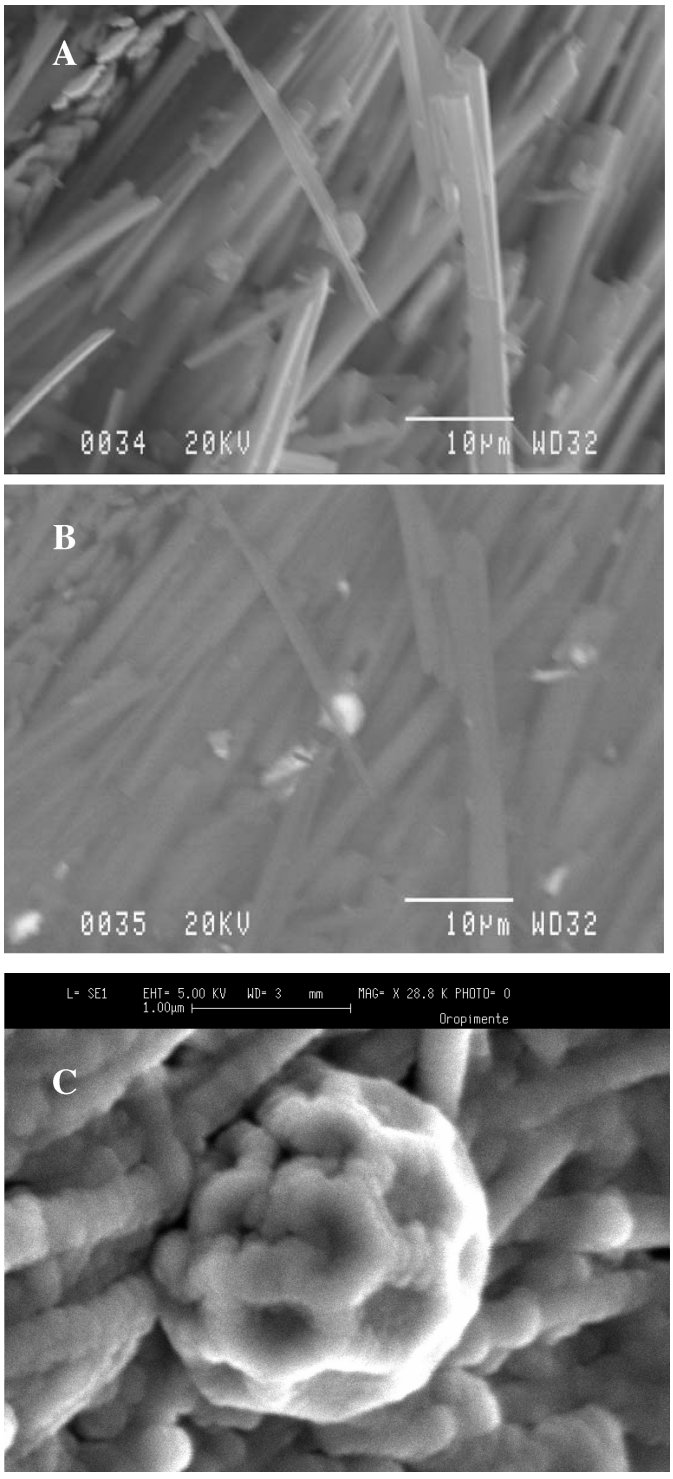

FIG. 6. (A) SEM micrograph of a natural sample from Salar de Ascotán. Note the fibrillar matrix of ulexite and the grain in the center. (B) SEM image of the same field taken with back-scattered scanning electrons. Note the lighter shade of the grain indicating the presence of heavy atoms. (C) SEM micrograph of laboratory-produced microbial precipitate. Note the framboid structure and the fibrillar structures surrounding it.

TEM observations revealed microfibers from 0.05 to 0.6μm thick and rectangular crystals from 0.05 to 0.3 μm in size that appeared to be fragments of the fibers. TEM-SAED confirmed the crystalline nature of these fragments. Likewise, TEM-EDS analyses confirmed that these crystals were formed by arsenic and sulfur. IRMS of the laboratory arsenic precipitate showed isotopic compositions of $\delta^{34}S_{CDT}$= –0.55% and –0.79 in two separate determinations (Table 2). The As/S molar ratio of the precipitate produced by pure cultures was closer to orpiment stoichiometry.

DISCUSSION

Bacterial Abundance in the Salar de Ascotán. Total bacterial numbers in the brine samples were within the normal range of values found in planktonic environments (Whitman et al. 1998). Abundance in the sediment samples with high As content (over 700 mg g-^{1}), however, was two to three orders of magnitude lower than the normal values usually found in sediments (Whitman et al. 1998). It is even more surprising that a very high percent of these were retrieved as arsenic-precipitating bacteria in MPN incubations. It is unusual to find MPN of a specific physiological group to make up more than 1% of the total count (Simu et al. 2005).

In the Ascotán samples collected in 2005, however, arsenic-precipitating bacteria accounted for approximately half of the total count in four out of six samples. This suggests that arsenic-based metabolisms must be very significant processes in this ecosystem with high arsenic content. The methodological tests carried out indicated that both the total and MPN counts were reliable despite the different salinities used in the MPN medium and the original in situ salinity. When counts were carried out again in June 2006, both total counts were higher and MPN lower than in 2005. It should be concluded that the rather variable conditions found in the Salar de Ascotán, both in space and time generate large differences in bacterial numbers.

Thus, neither the high nor the low numbers can be considered as representative. However, the high percent of MPN found in the 2005 sampling demonstrates that arsenic reducing bacteria may account, at times, for a very significant part of the bacterial assemblage. MPN results from Mono Lake showed that arsenate-respiring bacteria (AsRB) were much lower than total bacterial counts, accounting only 0.001% of the total population (Oremland et al. 2000). However the radioassay results in the same study measuring the reduction of ^{73}As (V) to ^{73}As (III) suggested that the population size of AsRB by MPN might have been underestimated by as much as two to three orders of magnitude (Oremland et al. 2000). If this were the case, Mono Lake would also show a significant percent of the total bacterial assemblage as bacteria able to reduce As.

Enrichment Cultures. Bacteria able to precipitate arsenic sulfide were successfully enriched from the natural arsenic-rich

TABLE 2
Isotopic signatures from natural and laboratory sulfur compounds and enrichments of different reduction processes

Substrate	Product	$\delta^{34}S‰$	Process	ε
Na_2SO_4		2.67 to 3.35		
	As_2S_3, As_4S_4,As_4S_5	−0.56 to −0.79	Precipitation in enrichment cultures	−3.5 to −3.9
	As_2S_3	ND	Precipitation in pure culture	ND
$Na_2S \cdot xH_2O$		1.73		
	As_2S_3	4.21 to 4.24	Chemical precipitation[c]	2.5
Sulfate in Ascotán brines		2.95 to 4.29 (3 samples)		
	Ascotán mineral As_2S_3	−0.55	Biogeochemical precipitation	−3.5 to −4.8
Gypsum		20.9		
	As_2S_3	−30.4 to −35.3	Biogeochemical precipitation[a]	−50.2 to −55.0
Sulfate				
	Metal sulfides in sediments		Biogeochemical precipitation[b]	<−4 to −46%
Sulfate				
	Sulfide		Reduction in pure cultures[b]	−4 to −46%

[a](Palmer et al. 2004).
[b](Canfield 2001).
[c](Eary 1992).

material. Soluble arsenic concentration decreased as a yellow precipitate appeared. Analysis of this precipitate showed it to be an arsenic sulfide with molar composition ranging between those of orpiment and realgar. The enriched bacteria showed a metabolic potential similar to that of these two isolated strains. However, they differed in the molar composition of the precipitate. This could be due to different culture conditions, which are known to alter the stoichiometry of the products. Alternatively,

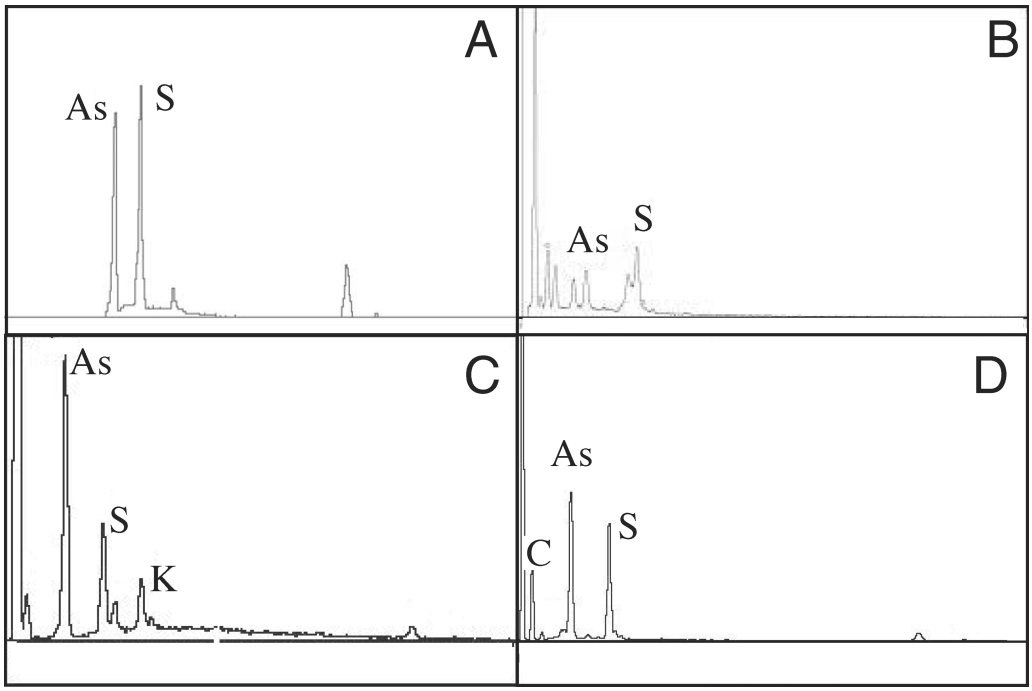

FIG. 7. (A) SEM-EDS spectrum of an electrodense grain in the ulexite matrix of an Ascotán sample (shown in Figures 6A and B). Calculated As/S molar ratio= 0.68 ± 0.07. (B) SEM-EDS spectrum of the laboratory-produced microbial precipitate, specifically from the surface of a cell from an enrichment culture. Calculated As/S molar ratio= 0.78 ± 0.03. (C) SEM-EDS spectrum of the laboratory-produced microbial precipitate, specifically from the bulk precipitate from an enrichment culture. Calculated As/S molar ratio= 1.13 ± 0.26. (D) SEM-EDS spectrum of the laboratory-produced microbial precipitate from an Asc-3 strain. Calculated As/S molar ratio= 0.57 ± 0.01.

this variation could be a characteristic of the different bacteria.

Pure culture experiments using different substrates showed that both sulphate and arsenic reduction are associated with energy conservation. The 16S rRNA analysis revealed that those strains are different enough from *Bacteria* previously described able to precipitate arsenic sulphide in the specified conditions.

In summary, the enrichment and pure cultures showed that bacteria able to carry out the precipitation of arsenic sulfide were in fact present in high numbers in the environment and that such precipitation did not occur in the laboratory in the absence of the bacteria. Moreover, MPN data of arsenic-precipitating bacteria determined in samples from Salar de Surire, Cejar lagoon (Salar de Atacama) and River Loa sediments (data not shown), all in Northern Chile, suggests that these bacteria are not limited to the borate layers described here but, rather, that they are widespread through the different environments associated with the presence of arsenic.

Morphology and Chemistry of the Precipitates. One obvious difference between laboratory and environmental precipitates was the presence of a ulexite matrix in the latter. This was expected since precipitation of borate salts to form ulexite is a chemical process independent of the arsenic precipitation. Likewise, the environmental precipitate was mixed with a number of minerals such as calcite, quartz or halite that could not form in the cultures.

Analysis of the arsenic minerals showed both similarities and differences between the laboratory and environmental precipitates. First, the environmental precipitate had a molar ratio As/S of 0.68 ± 0.07, consistent with the mineral orpiment. On the other hand, the molar ratio of the laboratory precipitate using enrichment culture was 0.78 ± 0.03 (closer to orpiment) for the material precipitated on cell surfaces, but it was 1.13 ± 0.26 (closer to realgar) for the cell-free precipitate. Arsenic precipitate molar ratio produced by pure cultures was also consistent with orpiment stoichiometry (molar ratio As/S 0.57 ± 0.01). And second, the precipitates showed morphological differences: grains (1–3 μm in diameter) and spicules (0.3–0.5 μm thick) in sediments, fibers (0.2–0.6 μm), spheres (0.3 μm in diameter) and framboids (2 μm in diameter) in enrichment cultures. The pure culture precipitate shows an amorphous appearance composed mainly of grains (1–2 μm in diameter).

These differences can be attributed to the very different environments in which the precipitation occurred. In fact, the different molar ratios of the arsenic precipitated directly onto the cells and of those further away indicate that both minerals could be precipitated by the same bacterial consortium. It is worth noting that *D. auripigmentum* precipitates orpiment alone (Newman et al. 1997), which was the mineral present on cell surfaces in enrichment cultures. Perhaps different bacteria in the enrichments were responsible for the precipitation of the two minerals. Additionally, it could be suggested that the artificial culture conditions select only a fraction of the bacteria able to precipitate the As minerals present in Ascotán. For instance, all the experiments were carried out using lactate as electron donor, but inorganic electron donors may be important for As (V) reduction metabolism in some extreme systems (Oremland and Stolz 2005).

The framboid-like structures observed in the bacterial precipitate obtained in the laboratory (Figure 6C) are common for pyrite. These structures are typically formed via monosulfide precursors (Sawlowicz 1990), mainly in sedimentary rocks. As far as is known, no framboid-like structures composed of arsenic sulfide have been reported before. However, there are also other minerals which, under specific conditions, may reveal similar structures, e.g., copper (Sawlowicz 1990) and zinc (Sawlowicz 2000) sulfides. Different genetic processes have been postulated for these structures, from purely inorganic to the directly biogenic, and including indirect biogenic formation. Recently, a multistage process has been proposed for the formation of framboidal pyrite (Wilkin and Barnes 1997). According to Sawlowicz (2000) a colloidal stage is needed for framboid formation.

In conclusion, even though arsenic precipitates were found in both nature and cultures, the details of molar ratios, and morphology of minerals seemed dependent on the particular environment where the precipitation took place and the microorganisms involved. It cannot be expected that the cultures mimic all aspects of the natural environment. This is especially true if the completely different time scales involved in both processes are considered. Clearly, diagenesis might have occurred in the environmental sample, changing the structure and chemistry of the minerals.

Isotopic Fractionation of Precipitates. It is well known that enzymatic processes tend to enrich products in the lighter isotopes due to kinetic effects. This characteristic has been used extensively to implicate biological activity in geochemical processes (Boschker and Middelburg 2002). The biological arsenic sulfide precipitation is the result of microbial arsenic and sulfate reduction plus precipitation. Sulfate-reducing bacteria produce sulfide depleted in ^{34}S during their metabolism (Canfield 2001) and, thus, the isotopic composition of sedimentary sulfides provides an indication of the activity of sulfate-reducing bacteria in sulfide formation.

Natural sulfides produced from bacterial sulfate-reduction can reach ^{34}S depletion values of about $-50‰$ (Kaplan and Rittenberg 1964). Recently reported information by Palmer et al. (2004), confirmed the presence of δ^{34}S values as low as $-30‰$ in arsenic sulfides (orpiment and realgar) associated with the Emet (Turkey) borate deposits, suggesting microbially mediated sulfate reduction (Table 2). These δ^{34}S values were lighter than in previously reported data for dissolved sulfide in geothermal fluids, and also lighter than the range determined for sedimentary sulfides formed by sulfate-reducing bacteria (Canfield 2001).

Nevertheless, the isotopic composition of sedimentary sulfides depends on variables such as temperature, availability of dissolved sulfate and organic substrates (the last two factors

modify the specific rate of sulfate reduction), and substrate type (Harrison and Thode 1958; Kaplan and Rittenberg 1964; Kemp and Thode 1968; Chambers et al. 1975). Thus, the relevant variable is the enrichment ε, which shows the changes in the isotopic composition of the product of the biological process with respect to the substrate. Under optimal conditions (adequate temperature and abundant organic substrate) fractionation can be minimized due to the fact that the reaction controlling the rate of SO_4^{2-} reduction is its transformation to APS (adenosine-5′-phosphosulfate), that does not involve fractionation (Kemp and Thode 1968). This may happen in cultures, where fractionations are, commonly, smaller than under natural conditions (Canfield 2001). Under other conditions, fractionation may be extreme. Thus, sulfate-reducing bacteria in cultures have been shown to generate sulfide with ^{34}S enrichment values of $-4‰$ to $-40‰$ compared to sulfate (Kaplan and Rittenberg 1964; Chambers et al. 1975).

The laboratory arsenic sulfide precipitation experiments described in this work showed $\delta^{34}S$ –0.56 and –0.79, while the $\delta^{34}S$ of the initial sulfate added to the culture medium ranged between +2.67 and +3.35. Therefore, the ^{34}S enrichment values ranged between −3.5 and −3.9‰, which are in the lower range of reported values (Table 2). Sulfate $\delta^{34}S$ data obtained from the Salar de Ascotán brines ranged between 2.9 and 4.3‰ and sulfide precipitated in Ascotán showed $\delta^{34}S$ about −0.55‰. Thus the ^{34}S enrichment values ranged between −3.5 and −4.8‰ compared to sulfate (Table 2). Both laboratory and Ascotán precipitates, therefore, showed the same enrichment values. These were significantly different from chemically precipitated arsenic sulfide (without sulfate-reduction) that showed an enrichment value of +2.5 (Table 2). This strongly supports the involvement of microorganisms in the origin of the arsenic sulfides in Salar de Ascotán, and that natural conditions of sulfate-reduction in the salar were similar to the experimental ones, probably reflecting availability of organic substrates in both cases.

Considering the evidence presented here, the involvement of bacteria in the precipitation of arsenic minerals in Salar de Ascotán seems clear. It is very likely that the same is true for similar borate deposits in other parts of the world, such as the Argentinian side of the Andes, North America or Turkey. Thus, the microbial reduction of arsenic becomes a biogeochemically relevant process. Steps will be taken to characterize the in situ microbial community and to attempt to isolate in pure culture and identify the bacteria responsible for most of the in situ activity.

REFERENCES

Ahmann D. 2002. Microbially-mediated arsenic reduction and its implications for arsenic sorption and mobility. Abstracts Amer Chem Soc 223:U599.

Ahmann D, Krumholz LR, et al. 1997. Microbial mobilization of arsenic from sediments of the Aberjona Watershed. Environ Sci Technol 31(10):2923–2930.

Alonso R. 1986. Ocurrencia, posición estratigráfica y génesis de los depósitos de boratos de la Puna Argentina. Salta, Universidad Nacional de Salta, p 196.

Arroyo A, Alonso H, et al. 1999. Análisis de los eventos ocurridos en el Río Loa Marzo de 1997. XIII Congreso de Ingeniería Sanitaria y Ambiental AIDIS Chile, Antofagasta.

Ball P. 2005. Arsenic-free water still a pipedream. Nature 436(7049):313.

Boschker HTS, Middelburg JJ. 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol Ecol 40(2):85–95.

Canfield DE. 2001. Isotope fractionation by natural populations of sulfate-reducing bacteria. Geochim Cosmochim Acta 65(7):1117–1124.

Chambers L, Trudinger P, et al. 1975. Fractionation of sulfur isotopes by continuous cultures of *Desulfovibrio desulfuricans*. Can J Microbiol 21:1602–1607.

Chong G. 1984. Die Salare in Nordchile—Geologie, Struktur und geochimie. Goetekt, Forschung 67:146.

Chong G, Pueyo JJ, et al. 2000. The borate deposits in Chile. Revista Geológica de Chile 27(1):99–119.

Demergasso C, Escudero L, et al. 2003. Análisis de factores microbiológicos que participan en el control de la concentración, distribución en especies y movilidad del arsénico en aguas superficiales de la Región de Antofagasta. XXV Congreso Chileno de Microbiología, Antofagasta, Chile.

Eary L. 1992. The solubility of amorphous As_2S_3 from 25 to 90°C. Geochim Cosmochim Acta 56:2267–2278.

Harrington J, Fendorf S, et al. 1998. Biotic generation of Arsenic (III) in metal(loid)-contaminated fresh water lake sediments. Environ Sci Technol 32:2425–2430.

Harrison A, Thode H. 1958. Mechanisms of the bacterial reduction of sulfate from isotope fractionation studies. Trans Faraday Soc 53:84–92.

Heidelberg JF, Paulsen IT, et al. 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nature Biotechnol 20(11):1118–1123.

Helvaci C. 1995. Stratigraphy, mineralogy, and genesis of the bigadic borate deposits, Western Turkey. Econ Geol Bull Soc Econ Geol 90(5):1237–1260.

Helvaci C, Orti F. 1998. Sedimentology and diagenesis of Miocene colemanite-ulexite deposits (Western Anatolia, Turkey). J Sediment Res 68(5):1021–1033.

Helvaci C, Orti F. 2004. Zoning in the Kirka borate deposit, western Turkey: primary evaporitic fractionation or diagenetic modifications? Can Mineral 42:1179–1204.

Jensen M, Nakai N. 1962. Sulfur isotope meteorite standards, results and recommendations. Biogeography of sulphur isotopes. J. ML, Natl Sci Found Symp:30–35.

Kaplan I, Rittenberg S. 1964. Microbiological fractionation of sulphur isotopes. J Gen Microbiol 34:195–212.

Karthikeyan S, Beveridge TJ. 2002. *Pseudomonas aeruginosa* biofilms react with and precipitate toxic soluble gold. Environ Microbiol 4(11):667–675.

Kashefi K, Tor JM, et al. (2001). Reductive precipitation of gold by dissimilatory Fe(III)-reducing bacteria and archaea Appl Environ Microbio l 67(7):3275–3279.

Kemp A, Thode H. 1968. The mechanism of the bacterial reduction of sulphate and of sulphite from isotope fractionation studies. Geochim Cosmochim Acta 32:71–91.

Labeda D. 2000. International Committee on Systematic Bacteriology; IXth International (IUMS) Congress of Bacteriology and Applied Microbiology. Int J Syst Evol Microbiol 50(6):2245–2247.

Liu A, Garcia-Dominguez E, et al. 2004. A novel arsenate respiring isolate that can utilize aromatic substrates. FEMS Microbiol Ecol 48(3):323–332.

Lovley DR. 2001. Bioremediation—Anaerobes to the rescue. Science 293(5534):1444–1446.

Macalady J, Banfield JF. 2003. Molecular geomicrobiology: genes and geochemical cycling. Earth Planet Sci Lett 209(1–2):1–17.

Macy JM, Santini JM, et al. 2000. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch Microbiol 173(1):49–57.

Marchant HJ, Thomas DP 1983. Polylysine as an adhesive for their attachment of nanoplankton sybstrates for electron microcopy. J Microsc 131:127–129.

Mardones-Pérez L. 1997. Flux et évolution des solutions salines dans les systemes hydrologiques des salars d'Ascotán et d'Atacama. Paris, Université Paris-Sud / Orsay: 203.

Mariotti A, Germon J, et al. 1981. Experimental determination of nitrogen kinetic isotope fractionation: Some principles: Illustration for the denitrification and nitrification processes. Plant Soil 62:413–430.

Meng XG, Jing CY, et al. 2003. A review of redox transformation of arsenic in aquatic environments. In: Cai Y, Braids, OC, editors. Biogeochemistry of Environmentally Important Trace Elements. Washington, DC: American Chemical Society. 835**:**70–83.

Nealson KH, Belz A, et al. 2002. Breathing metals as a way of life: Geobiology in action. Antonie Van Leeuwenhoek Inter J Gen Mol Microbiol 8(1–4):215–222.

Newman DK. 2004. *The Genetics of Geochemistry*. Eleventh International Symposium on Water-Rock Interaction, Saratoga Springs, New York: A.A. Balkema Publishers.

Newman DK, Beveridge TJ, et al. 1997. Precipitation of arsenic trisulphide by *Desulfotomaculum aurpigmentum*. Appl Environ Microbiol 63:2022–2028.

Newman DK, Kennedy EK, et al. 1997. Dissimilatory arsenate and sulfate reduction in Desulfotomaculum auripigmentum sp. nov. Arch Microbiol 168(5):380–388.

Nriagu J. 2002. Arsenic poisoning through the ages. In: Frankenberger WT, Environmental Chemistry of Arsenic. New York, Marcel Dekker, pp 1–26.

Oremland RS, Dowdle PR, et al. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. Geochim Cosmochim Acta 64(18):3073–3084.

Oremland RS, Kulp TR, et al. 2005. A microbial arsenic cycle in a salt-saturated, extreme environment. Science 308(5726):1305–1308.

Oremland RS, Stolz JF. 2003. The ecology of arsenic. Science 300(5621):939–944.

Oremland RS, Stolz JF. 2005. Arsenic, microbes and contaminated aquifers. Trends Microbiol 3(2):45–49.

Palmer MR, Helvaci C, et al. 2004. Sulphur, sulphate oxygen and strontium isotope composition of Cenozoic Turkish evaporites. Chem Geol 209(3–4):341–356.

Popova V, Polykov V. 1986. Alacranite As8S9; a new mineral. Zap Vses Mineral Obshchest 115:360–368.

Redman AD, Macalady DL, et al. 2002. Natural organic matter affects arsenic speciation and sorption onto hematite. Environ Sci Technol 36(13):2889–2896.

Roberts AC, Ansell HG, et al. 1980. Pararealgar, a new polymorph of AsS, from British Columbia. Can Mineral 18:525–527.

Robertson WJ, Franzmann PD, et al. 2000. Spore-forming, *Desulfosporosinus*-like sulphate-reducing bacteria from a shallow aquifer contaminated with gasoline. J Appl Microbiol 88(2):248–259.

Sawlowicz Z. 1990. Primary copper sulfides from the Kupferschiefer, Poland. Mineralium Deposita 25(4):262–271.

Sawlowicz Z. 2000. Framboids: From Their Origin to Application. Kraków, Poland, Institute of Geological Sciences, Jagiellonian University.

Schippers A, Neretin LN, et al. 2005. Prokaryotic cells of the deep subseafloor biosphere identified as living bacteria. Nature 433(7028):861–864.

Shen Y, Buick R, et al. 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. Nature 410(6824):77–81.

Simu K, Holmfeldt K, et al. 2005. Culturability and coexistence of colony-forming and single-cell marine bacterioplankton. Appl Environ Microbiol 71(8):4793–4800.

Son MK, Shin HD, et al. 2005. Novel cationic microbial polyglucosamine biopolymer from new Enterobacter sp BL-2 and its bioflocculation efficacy. J Microbiol Biotechnol 15(3):626–632.

Stackebrandt E, Schumann P, et al. 2003. Reclassification of *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov. Intern J System Evolution Microbiol 53(Pt 5):1439–1443.

Stoertz GE, Ericksen GE. 1974. Geology of salars in northern Chile. US Geological Survey Professional Paper, 811–876.

Tanner LH. 2002. Borate formation in a perennial lacustrine setting: Miocene-Pliocene Furnace Creek Formation, Death Valley, California, USA. Sediment Geol 148(1–2):259–273.

Whitman WB, Coleman DC, et al. 1998. Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 95(12): 6578–6583.

Wilkin RT, Barnes HL 1997. Formation processes of framboidal pyrite. Geochim Cosmochim Acta 61(2): 323–339.