Search for Arsenite Oxidizing Anoxygenic Photolithoautotrophs

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Abstract

Arsenic introduced by natural processes or human activities can result in the contamination of water. The presence of arsenic in drinking water is detrimental to human life due to its high toxicity. Arsenic exists in the environment in two oxidation states, As^{V} (arsenate) and As^{III} (arsenite). Microorganisms mediate various geochemical processes in arsenic cycling. Although numbers of dissimilatory arsenate-reducing chemolithoheterotrophs, and arsenite oxidizing chemolithoautotrophs and chemolithoheterotrophs have been reported, arsenite oxidizing photolithoautotrophs have not been found. The objective of this research was to isolate and identify arsenite oxidizing anoxygenic photolithoautotroph. Two different (As_2S_3) orpiment degraders that utilize sulfide from orpiment were found, although arsenite oxidizing photolithoautotrophs were not.

Introduction

Arsenic is a ubiquitous metalloid that is toxic to human. Arsenic can be introduced into natural environment by both natural and anthropogenic processes (Nriagu, 2002). Many areas worldwide are facing serious health problem due to elevated concentration of arsenic in drinking water.

Arsenic has four oxidation states (-3, 0, +3, and +5), and its speciation is often directly or indirectly influenced by microbially mediated redox processes. Predominant species of arsenic in the environment are inorganic As^{V} and As^{III} (Francesconi and Kuehnelt, 2002). Arsenate can be directly used as respiratory oxidant, and arsenite may be used to derive energy as a reductant coupled with oxidant such as nitrate (Oremland and Stolz, 2003). Speciation of arsenic strongly impact its mobility since arsenate is more strongly adsorbed on minerals common in natural soil and sediments (e.g. Fe (hydr)oxides) compared to arsenite (Raven et al., 1998). Hence, comprehensive understanding of microbially mediated arsenic redox cycling is essential.

Although numbers of dissimilatory arsenate-reducing chemolithoheterotrophs, and arsenite oxidizing chemolithoautotrophs and chemolithoheterotrophs have been reported, arsenic oxidizing photolithoautotrophs has not been found (Oremland and Stolz, 2003). The objective of this study is to isolate and identify arsenite anoxygenic photolithoautotroph, and to obtain its cell morphology, photopigment spectra, and phylogenetic characteristics.

Materials and Methods

Sources of Inoculums. Inoculums were taken from six different sources as discussed in Table 1. Each sample was collected in sterile conical tubes on the day of inoculations.

Table 1. Sources and types of inoculums used in the enrichment study.

Location	Type
Eel pond	Green mat scraped from the wood by the dock
Trunk river	Green and puple sandy layers
Sippewissett	Green and puple sandy layers
Plum island sound	Surface of dark orange mat
GSB enrichment	Puple sandy layer
PSB enrichment	Purple berries from Sippewisett

Synthesis of orpiment. Orpiment was synthesized by mixing sodium arsenite ($NaAsO_2$) and sodium sulfide (Na_2S) in deionized water. Its pH was adjusted to 6 by adding concentrated HCl, and the product was washed three times with deionized water. Orpiment was stored in 1.6 M suspension in anaerobically sealed serum bottle.

Enrichment and Monitoring. Enrichments were prepared using the modified seawater base media discussed in the appendix with 0.5 mM sodium arsenite, 1 mM phosphorus acid, or 5mM orpiment as an electron donor in Pfennig bottles with pea-sized headspace. Upon addition of pea size inoculums, enrichments were first incubated in dark for three hours and positioned under fluorescent, incandescent, or 880 nmLED lights at room temperature. Additional media and electron donor was provided to enrichments upon noticeable change in the turbidity. Sub sample taken from enrichments were filtered through 0.2 μm filter, and arsenic was speciated using molybdenum blue color metric method (Carvalho et al., 1998). The pH value of the filtrate was also obtained. Furthermore, photopigment spectra was obtained using VARIAN CARY 50 Bio UV-Visible spectrophotometer upon the development of any color in the enrichments.

Microscopy. Sub samples were taken from enrichments to monitor microbial community, cell morphology and its arrangement around orpiment aggregate using ZEISS Imager M1. In order to closely observe the distribution of cells around orpiment aggregate, samples were stained with DAPI in dark for 3 minutes to be examined under epifluorescent with ZEISS Imager M1.

Scanning electron microscope (SEM) images of the selected enrichment was obtained using JOEL JSM-840 SEM. Cells were fixed on the 0.2 μm nucleopore membrane filter using 2% glutaraldehyde and 1.5% formaldehyde for 4 hours at room temperature. Following the fixation process, the filer was washed with deionized water and dehydrated with DMP. The filter was then stored in absolute ethanol. Specimen was critical-point dried, and sputter coated with gold.

Transmission electron microscope (TEM) images of the selected enrichment was obtained using ZEISS 10CA TEM. A drop of enrichment sample was place on Formvar coated TEM grid, and was left for 15 minutes for cells to settle on the grid. Cells were then stained with 1% ammonium molybdate at pH 7, and air-dried.

Results and Discussions

After three days, clearing of orpiment color (bright yellow) and the change in its flocculation were observed. Larger aggregates of orpiment compared to the control were observed on the bottom of the Pfennig bottles. All of the enrichments with orpiment were successfully transferred to secondary and tertiary stages. Turbidity in As^{III} and P^{III} enrichments was not observed; therefore, these enrichments were not transferred further. Two treatments, Trunk river inoculum under Fluorescent light (TR-FL) and PSB enrichment inoculum under incandescent light (PSB-IN) were selected to study closely.

TR-FL enrichment. Clearing of the orpiment color and formation of larger aggregate started after 3 days. In addition, evolution of gas was noticed from the primary enrichment with agitation. Secondary transfer was positioned under fluorescent light and in dark to evaluate the light dependency of the metabolism. Clearing of the orpiment color and formation of larger aggregate was observed in both light and dark treatments, which suggested the metabolism in this enrichment was light independent. Furthermore, oxidation of As^{III} to As^{V} was not detected by molybdenum blue colormetric method. The pH was stable throughout the study.

Rod-shaped cells were consistently found in the secondary and tertiary enrichment regardless of the light treatment, and their dimensions were 0.5 X 2 μm on the average (Figure 1-c). Orpiment aggregate was covered with the rod-shaped cells (Figure 1-a and c, and Figure 2). Microscopic study revealed close association between orpiment mineral and cells.

Proposed metabolism in TR-FL is denitrification utilizing sulfide from orpiment and nitrate from the media. Nitrate was readily available as its concentration in the media was 5 mM. Denitrification is thermodynamically feasible as $\Delta G^{o'}$ of the first step of denitrification, $NO^{3-} + HS^{-} + H^{+} \rightarrow NO^{2-} + S^{o} + H_2O$, is -131.2 kJ/mol. Therefore, the gas evolved in this enrichment is expected to be N_2O or N_2.

Figure 1. TR-FL enrichment. a. phase contrast image of orpiment and cells. b. DAPI stained cells around orpiment aggregate. c. phase contrast image of rod shaped orpiment degraders (cell dimension: 0.5 X 2 μm).

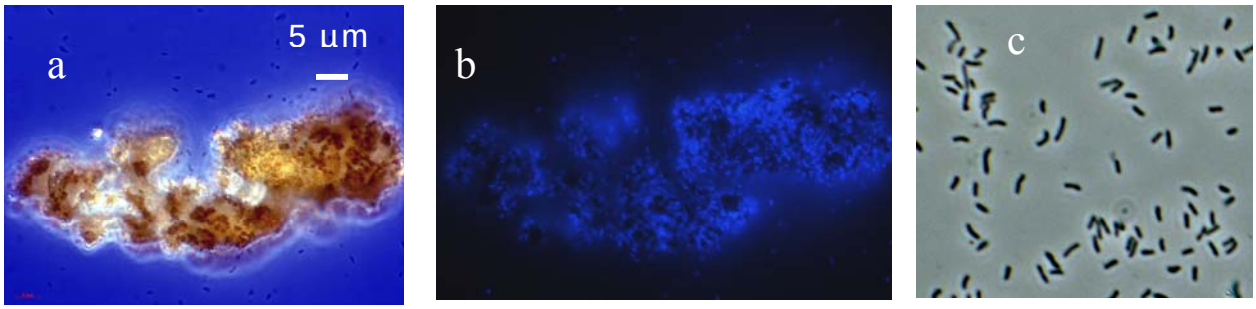
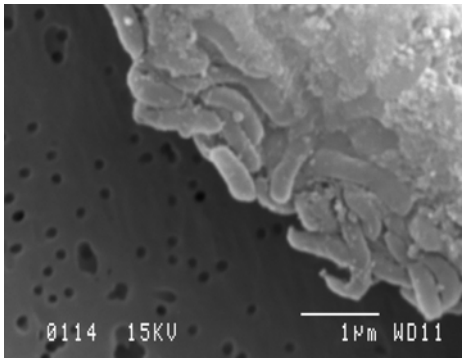


Figure 2. SEM image of cells and orpiment from TR-FL enrichment.



PSB-IN enrichment. Development of purple color was noticed after 7days. Secondary transfer was placed under incandescent light and in dark to examine the light dependency of the metabolism. Although the secondary transfer stored under the light showed clearing of orpiment color and change in orpiment aggregation, dark treatment transfer did not show change in color or aggregate. This result confirmed the metabolism was light dependent. Photopigment analysis indicated the light absorptions at 857, 799, 591, and 435 nm, which were indicative of bacteriochlorophyll *a* (Figure 3). Oxidation of As^{III} to As^{V} was also not detected by molybdenum blue colormetric method. The pH of the enrichment was stable throughout the study.

Oval-shaped cells were consistently found in the secondary and tertiary enrichment placed under incandescent light, and cell dimensions were 2.5 X 4 μm on the average (Figure 4 and Figure 5). Cells were motile, and their movement towards orpiment was observed under phase contrast microscope. Orpiment aggregate was heavily covered with the oval-shaped cells (Figure 4). Microscopic study revealed close association between orpiment mineral and cells.

Proposed mechanism of the orpiment degradation is sulfide oxidation by purple photoautotroph. Sulfide within orpiment could be utilized as an electron donor to reduce Cyt C_2 during the light cycle of the purple sulfur bacteria. This process could result in dissolution of orpiment mineral in the enrichment.

Figure 3. Pigment analysis of primary PSB-IN enrichment.

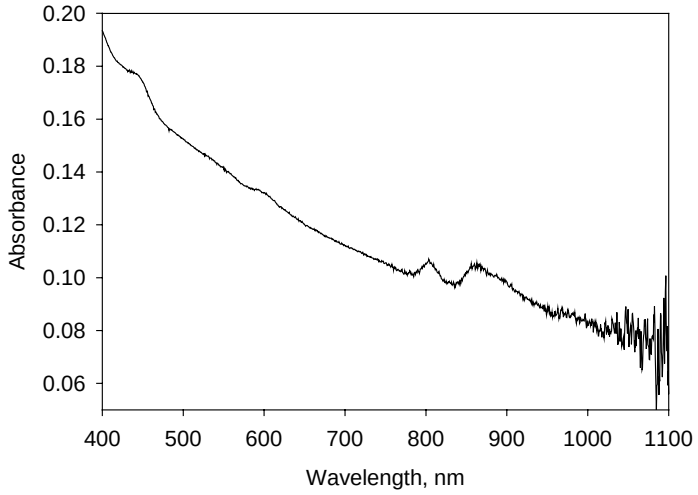


Figure 4. Cell arrangement of PSB around orpiment. a. phase contrast. b. edge contrast image showing sulfur globular. c. DAPI stained cell around orpiment aggregate.

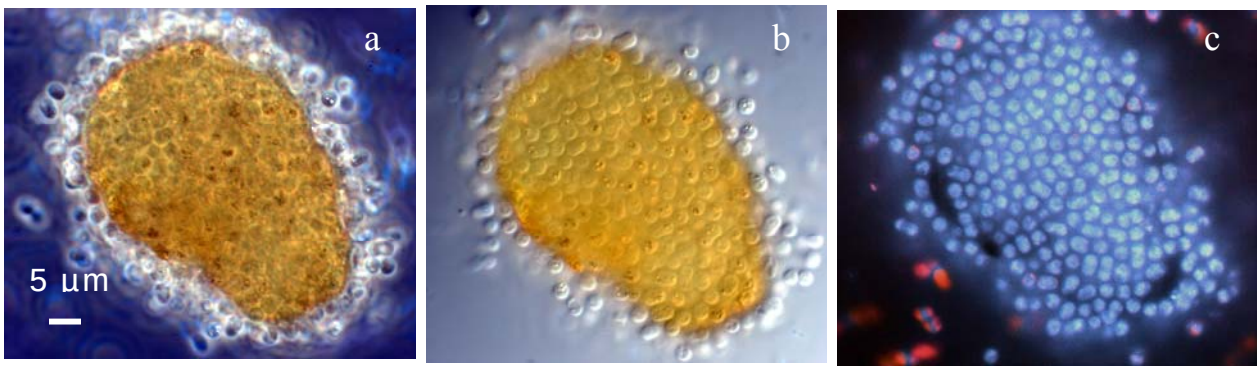
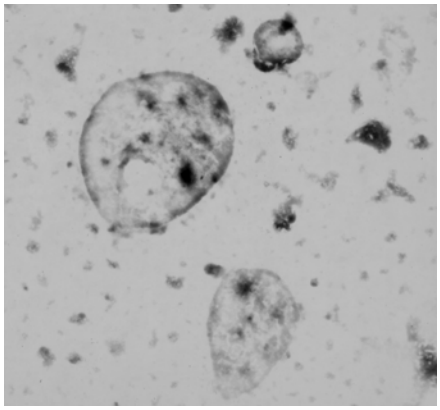


Figure 5. TEM image of phototroph from PSB-IN enrichment at X20,000.



Conclusion and Environmental Significance

This study did not result in enrichment of arsenite oxidizing phototroph. However, two different types of orpiment degraders were founded. The TR-FL and PSB-IN enrichments contained chemotrophic and phototrophic sulfide oxidizers respectively that are capable of utilizing sulfide from orpiment. Sulfide oxidizers that are capable of oxidizing sulfide from orpiment have not been previously found at circum neutral pH.

Oxidation of sulfide from orpiment result in destabilization of orpiment structure; therefore, it may result in mobilization of arsenic. Although orpiment is referred as potential sink for arsenic in the reduced sediments, orpiment degraders could potentially release arsenic into environment by reducing sulfide. This process may be a crucial step in the arsenic biogeochemical cycling to better understand the mechanism of arsenic release into groundwater.

Appendix

1 X SW-Base	
Water	20 liter
NaCl	400 g
$MgCl_2 \cdot 6H_2O$	60 g
$CaCl_2 \cdot 2H_2O$	3 g
KCl	10 g

Media	
1 X SW-base	4 L
1 M $NaNO_3$	20 mL
1 M NH_4Cl solution	10 mL
1 M Na sulfate	1 mL
1 M MOPS buffer, pH 6.8	10 mL
EDTA-chelated trace elements	4 mL
Autoclave in Widdel vessel, and cool under stream of CO_2 gas.	
Add following solutions upon cooling.	
12-vitamine solution	1 mL
vitamine B12 solution	1 mL
1 M bicarbonate	30 mL
DCMU	50 mL
Dispense in sterile Pfennig bottles leaving pea sized bubble, cap tightly.	
In each Pfennig bottle, add following solutions.	
Add K phosphate with Na arsenite and As_2S_3 treatment only.	
150 mM K phosphate solution	0.275 mL
1 M Na arsenite	0.0275 mL
1.6 M As_2S_3	0.165 mL
1 M phosphorous acid	0.250 mL

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