

SHORT COMMUNICATION

Arsenite-dependent photoautotrophy by an *Ectothiorhodospira*-dominated consortium

Charles R Budinoff¹ and James T Hollibaugh

Department of Marine Sciences, University of Georgia, Athens, GA, USA

Over the past decade numerous lineages of bacteria have been shown to obtain energy for growth through redox transformations of arsenic. However, phototrophic growth using reduced arsenic as an electron donor has not been described. Here we report the light-dependent oxidation of arsenite to arsenate, coupled with autotrophic growth, by an *Ectothiorhodospira*-dominated consortium of bacteria from alkaline, hypersaline Mono Lake, California. Pure cultures of the Mono Lake *Ectothiorhodospira* were not capable of phototrophic arsenite oxidation under the culture conditions tested. Electron micrographs of the culture showed a close association between consortia members, although the specific contribution of the individual bacteria is currently unknown. This report extends the list of compounds known to support anoxygenic photosynthesis and documents a previously unknown pathway in arsenic geochemistry.

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Soda lakes are alkaline, saline water bodies in which carbonate and sulfate are the dominant anions. These lakes are widely distributed throughout the world, typically the result of an endorheic drainage basin and a dry climate, where evaporation is greater than freshwater inflow. The salt composition of these lakes is mainly determined by the composition of inflowing water and differential precipitation of certain salts. Inputs from geothermal springs and the weathering of volcanic rock can enrich these lakes with elements such as arsenic (Maest *et al.*, 1992). Saline lakes and wetlands of the Great Basin region of the western US are frequently enriched in arsenic, with concentrations ranging from 20 to 3000 μM (Oremland *et al.*, 2004).

Soda lakes are highly productive environments; primary production is often an order of magnitude greater than in many other aquatic habitats (Grant *et al.*, 1990). Phototrophic primary production in these ecosystems is driven by a variety of species

including cyanobacteria, eukaryotic algae (Roesler *et al.*, 2002) and purple sulfur bacteria, such as the anoxygenic phototrophic bacteria of the family Ectothiorhodospiraceae (Cloern *et al.*, 1983; Imhoff, 2006). This elevated primary production supports dense populations of organotrophic bacteria and can cause anoxia when it sinks below the photic zone in deeper water bodies, leading to dependence on alternate terminal electron acceptors such as sulfate and arsenate for energy generation. The reduced inorganic sulfur and arsenic compounds generated from carbon mineralization can then be oxidized by chemoautotrophic bacteria to obtain energy for growth (Oremland *et al.*, 2004).

Chemolithoautotrophic growth of bacteria capable of using inorganic arsenic compounds is well documented (Stolz *et al.*, 2006). Energy conservation and carbon fixation have been demonstrated in arsenite oxidizers and arsenate reducers under both aerobic and anaerobic conditions (Huber *et al.*, 2000; Santini *et al.*, 2000; Oremland *et al.*, 2002; Hoefl *et al.*, 2003; Salmassi *et al.*, 2006). Given the breadth of arsenic redox transformations mediated by bacteria, the high concentration of arsenic in many Great Basin soda lakes, and recent observations that anoxygenic phototrophs could use electron donors other than sulfur compounds in energy generation (Widdel *et al.*, 1993; Griffin *et al.*, 2007), we sought

Correspondence: JT Hollibaugh, Department of Marine Sciences, University of Georgia, Athens, GA 30602-3636, USA.
E-mail: aquadoc@uga.edu

¹Current address: Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA.

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to determine if phototrophic bacteria from these lakes might be capable of using reduced arsenic as an electron donor for phototrophic growth.

Anoxic, sulfidic sediment from the central basin of Mono Lake, California (Station 6, 37° 57.822' N, 119° 01.305' W) was incubated anaerobically in the light, resulting in prolific growth of purple sulfur bacteria (See Supplementary Material). Samples from these incubations were inoculated into a mineral medium containing 3 mM H₂S, placed in the light and transferred regularly under anaerobic conditions. Once growth was consistent (about 3 transfers, 1.5 months), cells were transferred to mineral media devoid of reduced sulfur compounds, but instead containing 3 mM arsenite. Growth became apparent after ~30 days and the enrichment was transferred regularly on the same arsenite medium thereafter. This culture has been maintained in our laboratory for nearly 3 years.

Measurements taken during growth of the culture clearly show the oxidation of arsenite to arsenate coupled to CO₂ fixation, occurring only in live replicates exposed to light (Figures 1a and b; Supplementary Figure 1). Approximately 3.6 moles of arsenite were oxidized to arsenate for every 1 mole of carbon fixed, compared to a theoretical requirement of two moles needed to supply 4 e⁻ to reduce CO₂ to CH₂O. The standard redox potential of the HAsO₄²⁻/H₃AsO₃ couple was calculated to be +54 mV (Supplementary Material), which is a weaker reductant than the couple S⁰/HS⁻ (-270 mV) (Brune, 1989), but stronger than the couples Fe(OH)₃+HCO₃⁻/FeCO₃ (+200 mV) (Widdel *et al.*, 1993) and NO₃⁻/NO₂⁻ (+430 mV) (Griffin *et al.*, 2007).

Sequences (*n* = 30) of cloned 16S rDNA amplicons from late exponential phase cultures revealed the presence of four unique ribotypes. The library was dominated by clones containing amplicons most similar to *Ectothiorhodospira* sp. strain 'Bogoria Red' (*n* = 24, >99% similarity, GenBank accession number EU341300). Small numbers of sequences related to other bacteria were also found: *Alkalilimnocola ehrlichii* (*n* = 3, 99% similarity, GenBank accession number EU341303); an uncultured Bacteroidetes (*n* = 2, 92% similarity GenBank accession number EU341301); and *Spirochaeta bajacaliforniensis* (*n* = 1, 93% similarity, GenBank accession number EU341302); (Supplementary Figures 2–4). The relative abundances of sequences in libraries constructed from samples of other arsenite-grown cultures were similar to this distribution, although clone libraries from cultures in prolonged stationary phase gave a significant greater number of sequences (up to 30%) of the Bacteroidetes ribotype.

Attempts to isolate components of the culture via serial dilution and direct plating using arsenite as the sole electron donor were not successful. An apparently pure culture of the *Ectothiorhodospira* sp. was obtained by plating on mineral medium containing thiosulfate (GenBank accession number

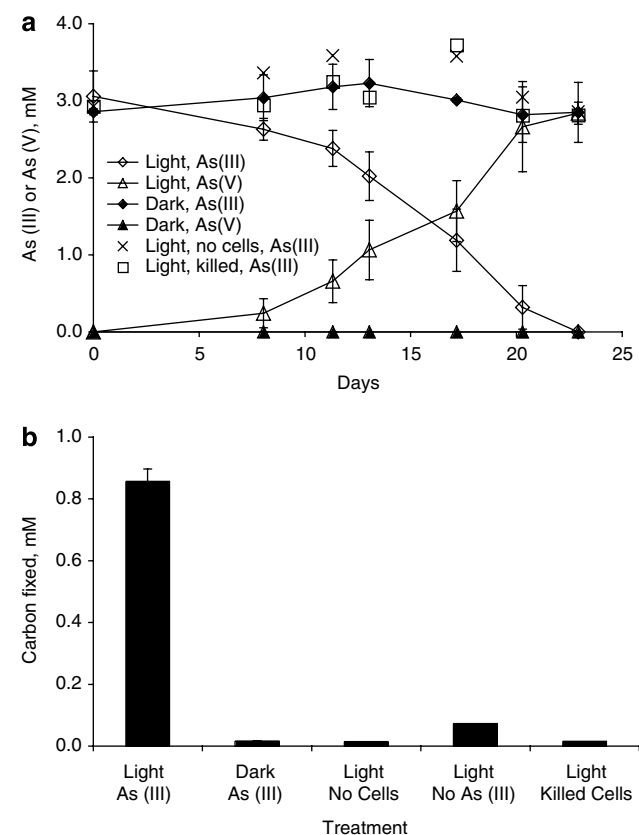


Figure 1 (a) Phototrophic oxidation of arsenite to arsenate by an enrichment culture dominated by *Ectothiorhodospira* sp. Means and s.d. of replicate incubations (*n* = 3) are shown. (b) Carbon fixed after 23 days of growth. Error bars (where shown) are s.d. of triplicate incubations.

EU341299). Repeated attempts to induce light-dependent arsenite oxidation by the pure culture failed, nor was a pure culture of strain *Ectothiorhodospira* sp. 'Bogoria Red' capable of phototrophic arsenite oxidation under these conditions.

After ~6 months of transfers in liquid media containing thiosulfate, our purified culture of the Mono Lake *Ectothiorhodospira* sp. regained the ability to oxidize arsenite. Analysis of a clone library constructed from an early stationary phase arsenite-grown culture revealed the presence of other ribotypes. This library was also dominated by *Ectothiorhodospira* sp., and contained sequences identical to the Bacteroidetes and the *Alkalilimnocola ehrlichii*-like organisms retrieved previously. From this we conclude that phototrophic arsenite oxidation under the conditions of our experiments involves a consortium of two or more of these organisms. Previous reports of cultures capable of using NO₂⁻ or FeCO₃ as electron donors for anoxygenic photosynthesis (Heising *et al.*, 1999; Griffin *et al.*, 2007), may also have involved consortia because these authors reported the absence of, or dramatically reduced rates of, phototrophic oxidation in pure cultures of consortium members, relative to the original mixed-culture enrichments.

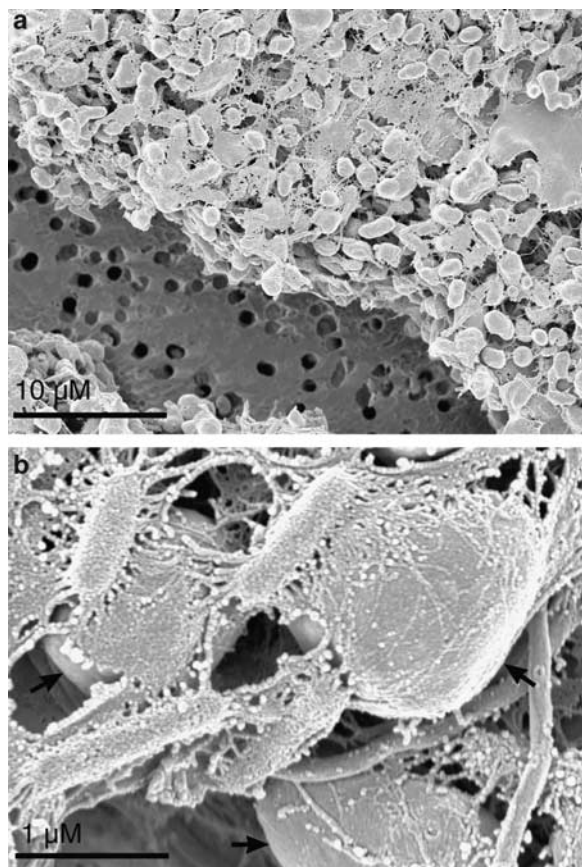


Figure 2 Scanning electron microscopy of an *Ectothiorhodospira* sp.-dominated enrichment culture growing in the light on arsenite. (a) Low magnification view of clumping cells. (b) Higher magnification micrograph emphasizing the close association of bacteria in the consortium and the organic matrix enclosing them. Arrows indicate *Ectothiorhodospira* sp. cells.

In general, *Ectothiorhodospira* sp. cultures grew more slowly on arsenite than on reduced sulfur compounds. The cells tend to be less motile and cultures contained clumps of cells with abnormal morphology (relative to growth on thiosulfate) held together by an extracellular matrix (Figure 2a). Cells were found in close association with each other within the matrix (Figure 2b). We assume that the *Ectothiorhodospira* sp. is responsible for C fixation and that the other bacteria contributed in some way to phototrophic arsenite oxidation, possibly by providing a shuttle compound, cofactors needed for growth, or by removing toxic by-products; however, we have not yet been able to determine the mechanism of this process. Additionally, we do not know whether the Bacteroidetes and the *Alkalilimnicola ehrlichii*-like organisms are unique in their ability to facilitate this metabolism.

The capability for phototrophic arsenite oxidation in Mono Lake microbes may result from a combination of factors associated with the water chemistry of Mono Lake and similar soda lake environments. The concentration of Fe (II) in the water column of Mono Lake is low (10 µM) compared to that of arsenite

(200 µM) and is controlled by Fe monosulfide precipitation (Domagalski *et al.*, 1990; Maest *et al.*, 1992). In contrast, arsenic sulfides are soluble in the anoxic waters of Mono Lake (Hollibaugh *et al.*, 2005). Sulfide oxidation and formation of thioarsenic compounds at the base of the photic zone reduces the pool of free sulfide there (Hollibaugh *et al.*, 2005), which could force anoxygenic phototrophic bacteria to rely on weaker electron donors. Arsenite is the most abundant, suitable inorganic compound in this zone. Given the redox potential of the $\text{HAsO}_4^{2-}/\text{H}_3\text{AsO}_3$ couple, the slow abiotic oxidation of arsenite (Oscarson *et al.*, 1980), and the low concentrations of Fe (II) and nitrite in Mono Lake, arsenite could provide a suitable alternative to reduced sulfur compounds for anoxygenic phototrophic growth in arsenic-rich soda lakes.

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