# BREATHING ARSENATE: MICROBES, MINERALS, MONO LAKE, AND MAYBE EVEN MARS.

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Oxyanions of arsenic occur in waters and soils either as a consequence of human activities or from natural sources associated with the weathering or dissolution of arsenic-bearing minerals. This toxic element has a complex biogeochemical cycle that is in part mediated by microorganisms, including both oxidation and reduction reactions involving arsenite and arsenate, respectively. An important discovery was that certain diverse and novel bacterial species achieve anaerobic growth using arsenate as a respiratory electron acceptor for the oxidation of organic substrates or $H_2$, quantitatively forming arsenite as the reduction product (Newman and others, 1998; Stolz and Oremland, 1999; Oremland and Stolz, 2000; Oremland and others, in press). This phenomenon, termed dissimilatory arsenate reduction (DAsR), has geochemical and ecological significance, influencing the speciation, mobility, and toxicity of arsenic in the environment. Currently held in pure culture, there are seven novel and taxonomically/physiologically diverse species of anaerobes from the domain Bacteria that achieve anaerobic growth by DAsR. Recently, two species of hyperthermophiles from the domain Archaea have been reported to achieve chemoautotrophic growth with arsenate as their electron acceptor (Huber and others, 2000). Bacterial reduction of arsenate in culture has been studied primarily when it occurs as a free ion in solution, however, significant reduction of adsorbed arsenate also occurs when it is associated with solid mineral phases like Fe(OOH) or $Al(OH)_3$ (Zobrist and others, 2000). The phenomenon of DAsR may also be exploited someday to remove arsenate from contaminated waters and soils, thereby achieving their practical remediation.

We have focused much of our investigations on Mono Lake, CA because it has high levels of hydro-thermally derived arsenate (~ 200 uM) in its alkaline (pH = 9.8) and hypersaline (salinity = 75 – 90 g/l) waters. The arsenate-respiring haloalkaliphilic bacteria *Bacillus arsenicoselenatis* and *B. selenitireducens* were isolated from Mono Lake's bottom mud, and were demonstrated to be well adapted to the chemically extreme conditions therein (Switzer Blum and others, 1998). Culturable arsenate-respiring bacteria outnumber sulfate-reducers in the lake's anoxic waters by a factor of 10 (Oremland and others, 2000). Preliminary experiments using Denaturing Gradient Gel Electrophoresis techniques upon arsenate-enriched lakewater failed to detect DNA from the above listed bacilli, but instead identified clones that aligned near the *Sulfurospirillum* and *Desulfovibrio* genera of the epsilon and delta Proteobacteria (Hoeft and others, in preparation), each of which have representatives capable of DAsR. Bacterial reduction of arsenate in Mono Lake's anoxic waters was experimentally demonstrated to be responsible for the observed shift from arsenate to arsenite as the dominant species in the anoxic monimolimnion. Indeed, a rather surprising discovery was that DAsR mineralizes as much as 14 % of the lake's annual planktonic primary production achieved during periods of meromixis, making it the second most important anaerobic electron acceptor after sulfate (Oremland and others, 2000). This finding represents the first reported instance in which the respiration of arsenate makes a significant contribution to the carbon budget of an ecosystem, albeit in this instance a rather uncommon and extreme case.

At present, this field of research is really in its infancy. There are some fundamental questions that need addressing in the years to come. For example, does DAsR represent an ancient means of microbial respiration, reaching back >3.5 billion years ago to perhaps a primordial hot spring biome of the Earth's Archaen era? Did the respiratory arsenate reductases found in bacteria and archaea arise from a common ancestor and propagate into the diversity microbes we observe today by such means as lateral gene transfer, or did these genes arise independently? Is DAsR related to the *"arsC"* system present in a number of bacteria that has a detoxifying but not a respiratory function (Cervantes and others, 1994)? To address questions like these, we must know considerably more than we do at present about the respiratory arsenate reductase enzymes that occur both in bacteria and archaea: their structure, and the genes that encode for their expression and regulation. Currently, only the periplasmic arsenate reductase from *Chrysiogenes arsenatis* has been partially characterized (Krafft and Macy, 1998), and work on the cell membrane-bound arsenate reductase from *Sulfurospirillum barnesii* is nearing completion as summarized by Oremland and others (in press). The initial data suggests that these two enzymes are quite different.

Some provocative questions remain about the biogeochemical cycle of arsenic that is of relevance to the field of "astrobiology." Is a functioning arsenic cycle pertinent to the possibility that life once developed on an early, climatically-clement period of the planet Mars, as may have been prevalent during its late Noachian and early Hesperian epochs (~ 3.6 – 4.2 billion years ago)? In order to sustain a functioning DAsR-based ecosystem, the arsenate ion must be regenerated. Aerobic arsenite-oxidizing bacteria are active in terrestrial hot-springs (Wilkie and Hering, 1998). Some of these organisms are chemoautotrophs that employ arsenite as their energy-supplying electron donor, using $CO_2$ as their carbon source from which they form their more complex organic makeup (Santini and others, 2000). However, these processes have biogeochemical significance on the Earth's current surface where oxygen molecules are abundant. On the ancient Earth (or Mars) with an oxygen-free atmosphere, they would have been absent. However, the electrochemical potential of the arsenate/arsenite redox couple is relatively low, and therefore stronger oxidants like ferric, manganic, and nitrate ions can oxidize arsenite back to arsenate in the absence of oxygen. This can happen either chemically or biologically. Indeed, we have observed a bacterial re-oxidation of arsenite with nitrate in anoxic Mono Lake water (Hoeft and others, in preparation). Thus, if microbial life existed on the volcanically active surface of Mars, some 4.0 billion years ago, it is not unreasonable to hypothesize an anoxic redox couple of arsenate/arsenite as a basis for its sustenance. Indeed, a good case can be made for a final Martian, arsenic-based biome being typified by that of a shrinking, increasingly saline soda lake, akin to modern-day Mono Lake.

References:

Cervantes, C. and others. 1994. Resistance to arsenic compounds in microorganisms. FEMS Microbiol. Rev. 15: 355 – 367.

Hoeft, S.E. and others. Characterization of bacterial arsenate reduction in the anoxic bottom waters of Mono Lake, California. In preparation.

Huber, R. and others. 2000. Respiration of arsenate and selenate by hyperthermophilic Archaea. Syst. Appl. Microbiol. 23: 305 – 314.

Krafft, T., and J.M. Macy. 1998. Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. Eur. J. Biochem. 255: 647 – 653.

Newman, D.K., D. Ahmann, and F.M.M. Morel. 1998. A brief review of microbial arsenate respiration. Geomicrobiol. J. 15: 255 – 268.

Oremland, R.S., and others. In press. Bacterial respiration of arsenate and its significance in the environment. *In* W.T. Frankenberger Jr. (ed) Environmental Chemistry of Arsenic, Marcel Dekker, New York.

Oremland, R.S., and J.F. Stolz. 2000. Dissimilatory reduction of selenate and arsenate in nature. pp. 199 – 224 *In* D.R. Lovley (ed) Environmental Metal-Microbe Interactions, Amer. Soc. Microbiol. Press, Washington, D.C.

Oremland, R.S. and others. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. Geochim. Cosmochim. Acta 64: 3073 – 3084.

Santini, J.M., and others. 2000. A new, chemoautolithotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. Appl. Environ. Microbiol. 66: 92 – 97.

Stolz, J.F. and R.S. Oremland. 1999. Bacterial respiration of arsenic and selenium. FEMS Microbiol. Rev. 23: 615 – 627.

Switzer Blum, J., and others. 1998. *Bacillus arsenicoselenatis* sp. nov., and *Bacillus selenitireducens* sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch. Microbiol. 171: 19 – 30.

Wilkie, J.A., and J.G. Hering. 1998. Rapid oxidation of geothermal arsenic (III) in streamwaters of the eastern Sierra Nevada. Environ. Sci. Technol. 32: 657 – 662.

Zobrist, J., and others. 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. Environ. Sci. Technol. 34: 4747 – 4753.