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Microbial oxidation of arsenite: Diversity and distribution of *aox*-like genes in soils and geothermal environments

W.P. Inskeep, R.E. Macur and N. Hamamura

Thermal Biology Institute and Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT, USA, (binskeep@montana.edu / Fax: 406-994-3933 / Phone: 406-994-5077)

Arsenic (As) is an abundant, ubiquitous and dynamic trace element distributed in numerous environmental contexts throughout the globe. The microbial transformation of As and associated genetic regulatory pathways have been studied intensively during the past decade, in part due to recent water quality crises in Bangladesh, India and southeast Asia. Microorganisms posses numerous regulatory genes responsible for various transformations of As, including oxidation, reduction and methylation, all processes that contribute to the fate, transport and biogeochemical cycling of As. The microbial reduction of arsenate [As(V)] and extrusion of arsenite [As(III)] via the *ars* operon (*arsC* and *arsB*, respectively) has been well studied and these genes appear widely distributed throughout the *Bacterial* and *Archaeal* domains. The dissimilatory reduction of arsenate has been confirmed in numerous microorganisms and has recently been shown to be regulated via *arrA* genes. The oxidation of As(III) is commonly observed in soil, sediment and natural waters, and several arsenite oxidases have been characterized. In many cases, organisms do not appear to gain energy via As(III) oxidation, although several As(III) chemolithotrophs have been isolated, and curiously, the Aro proteins implicated in chemolithotrophic organisms are reasonably homologous to other putative Aox proteins, which are thought to be functioning primarily as a detoxification strategy. Consequently, the primary goal of our work was to describe the distribution and importance of *aox*-like genes across different geochemical environments. Extracted DNA from As contaminated soils and geothermal springs was used to amplify bacterial *aox*-like genes using primers designed around known bacterial *aox* genes. To our knowledge this is the first report of successful amplifica-

tion of *aox*-like genes from natural systems. Furthermore, RNA extraction followed by reverse transcription (RT)-PCR showed that these *aox*-like genes are being expressed in geothermal and soil environments, and in pure culture isolates treated with As(III). The expression of *aox*-like genes is consistent with the observed oxidation of As(III) in several systems. In total, nearly 100 different *aox*-like sequences representing the full diversity of bacterial *aox* genes have been sequenced and compared to other known *aox*-genes. Our results suggest that *aox*-like genes are widely distributed among microorganisms, but there are still significant questions regarding the physiological role of arsenite oxidation and why in some organisms these genes appear to be involved in chemolithotrophy, whereas many organisms do not apparently gain energy from oxidation. In either case, the distribution of *aox*-like genes in natural systems suggests that microbial oxidation of As(III) is a critical component of the global As cycle.