arrA Is a Reliable Marker for As(V) Respiration

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Bacteria play an important role in controlling the geochemistry of arsenic. A tragic example is the case of Bangladesh, where microorganisms have been implicated in the release of arsenic into drinking water supplies and the exposure of millions of people to chronic arsenic poisoning (*1*–*3*). Arsenate [As(V)] respiration [i.e., the oxidation of organic carbon, hydrogen, or sulfide coupled with As(V) reduction to arsenite] is one of the microbial processes that contributes to arsenic mobilization (*4*). It has been difficult to monitor the activity of As(V)-respiring bacteria because they are phylogenetically diverse, and this metabolic capability is not consistently present within any given clade. Here we report that a conserved functional gene, *arrA*, can be used to detect As(V)-respiratory activity in the environment.

The *arrA* gene from the Gram-negative γ-Proteobacterium, *Shewanella* species strain ANA-3, encodes for a reductase that catalyzes respiratory As(V) reduction (*5*). *arrA* is a well-conserved gene, having 61 to 100% similarity at the amino acid level when compared with seven phylogenetically diverse As(V)-respiring bacteria (supporting online text). The ArrA proteins form a unique group within the dimethyl sulfoxide (DMSO) reductase family of molybdenum-containing enzymes, which includes other terminal reductases used in microbial respiration (fig. S1).

Because of the high degree of conservation within the ArrA protein subfamily, we designed degenerate polymerase chain reaction primers, ArrAfwd (5′-AAGGTGTATGGAATAAAGCGTTTgtbgghgaytt-3′) and ArrArev (5′-CCTGTGATTTCAGGTGCCcaytyvggngt-3′), to amplify a diagnostic region of *arrA*. These primers were tested on 13 phylogenetically diverse As(V)-respiring bacteria, one As(V)-respiring archaeon, and five negative-control strains that cannot respire As(V) but that possess other genes within the DMSO reductase family (*6*). Twelve of the 13 As(V)-respiring bacteria tested positive for *arrA*; no fragments were amplified from the As(V)-respiring archaeon or negative-control strains (fig. S2). ArrAfwd and ArrArev thus appear to be reliable markers for *arrA* for the majority of As(V)-respiring bacteria.

Poorly crystalline ferric (hydr)oxide [$Fe(OH)_3$] has been shown to be the most critical sedimentary phase in controlling arsenic mobility in a variety of locales, including anaerobic sediments of the Haiwee Reservoir in Olancha, California (*7*), and Bengal delta aquifers (*8*). Accordingly, we prepared As(V)-saturated $Fe(OH)_3$ for experiments with strain ANA-3 and the mutant strain ANA-3Δ*arrA* (*6*). Both strain ANA-3 and strain ANA-3Δ*arrA* are capable of respiratory Fe(III) reduction and As(V) reduction for the purpose of detoxification using the ArsC As(V) reductase, but only ANA-3 is capable of respiratory As(V) reduction using ArrA.

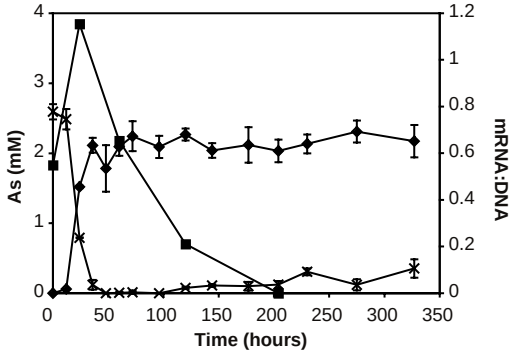

Fig. 1. *arrA* is required for As(V) reduction under iron-rich conditions. Concentrations of total As(V) (crosses) and As(III) (diamonds) are shown for samples containing *Shewanella* sp. strain ANA-3 in the presence of As(V)-saturated $Fe(OH)_3$. *arrA* expression (i.e., the mRNA:DNA ratio) is also indicated (squares). Data represent the average and standard deviation of triplicate samples, except in the case of the mRNA:DNA ratio, for which a representative data set is shown.

Reduction of As(V) occurred in samples incubated with strain ANA-3, but not in the uninoculated samples or in samples inoculated with strain ANA-3Δ*arrA*, showing that As(V) reduction is not mediated abiotically or in the absence of *arrA*, even when *arsC* is present. With strain ANA-3, the maximal expression of *arrA* (the ratio of *arrA* mRNA transcript per *arrA* gene copy number) corresponded to the fastest rate of As(V) reduction (Fig. 1). This shows that *arrA* is required to catalyze the conversion of As(V) to arsenite [As(III)] in iron-rich systems and that ArrAfwd and ArrArev can be used to track *arrA* expression.

Geochemical studies of Haiwee sediments have shown that arsenic is sorbed to $Fe(OH)_3$ and that As(III) predominates below a few centimeters (*7*). Using ArrAfwd and ArrArev, *arrA* fragments were amplified from DNA and total RNA extracted from these sediments, revealing that *arrA* is present and expressed at this site (*6*). Seven representative DNA fragments and 14 mRNA fragments were sequenced to confirm that these products were bona fide *arrA* gene fragments. These fragments ranged from ~62 to 97% identity to *arrA* sequences from *Bacillus selenitireducens*, *Chrysiogenes arsenatis*, and strain ANA-3 (fig. S3).

It is intriguing from an evolutionary perspective that *arrA* is so well conserved. On a practical level, it enables a simple molecular assay to be used to determine whether respiratory As(V) reduction is contributing to the speciation and mobilization of arsenic in a variety of environments.

References and Notes

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9. We thank R. Oremland, F. Rosenzweig, and C. House for sending us strains. All new *arrA* gene sequences have been deposited in GenBank. Accession numbers are available in supporting online material. Supported by the Luce and Packard Foundations (D.K.N.) and NSF award nos. BES-0201888 (J.G.H.) and DBI-0200145 (C.W.S.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/306/5695/455/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
References and Notes

6 July 2004; accepted 16 August 2004

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