Bacterial Populations Associated with the Oxidation and Reduction of Arsenic in an Unsaturated Soil

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Microbial populations responsible for the oxidation and reduction of As were examined in unsaturated (aerobic) soil columns treated with 75 μM arsenite [As(III)] or 250 μM arsenate [As(V)]. Arsenite [As(III)] was rapidly oxidized to As(V) via microbial activity, whereas no apparent reduction of As(V) was observed in the column experiments. Eight aerobic heterotrophic bacteria with varying As redox phenotypes were isolated from the same columns. Three isolates, identified as Agrobacterium tumefaciens-, Pseudomonas fluorescens-, and Variorax paradoxus-like organisms (based on 16S sequence), were As(III) oxidizers, and all were detected in community DNA fingerprints of various As transforming bacteria from soil, although the relevance of arsenite oxidizing bacteria to the environment was not studied (17, 19, 29). For example, several As(V)-reducing bacteria have been found to mediate the reduction of As(V) under highly aerobic conditions resulting in enhanced mobilization of As from limed mine tailings (18). The characterization of several aerobic heterotrophs isolated from these tailings suggested that the probable mechanism of As(V) reduction was As(V) detoxification. Other investigators have also isolated apparent As detoxifying bacteria from soil, although the relevance of these populations to As redox cycling actually occurring in the environment was not studied (17, 19, 30). Given the importance of As(V)–As(III) redox cycling in soil–water systems, very little is known regarding the potential role of various As detoxification strategies versus metabolisms capitalizing on As for energy conservation. Consequently, one of the goals of the current study was to improve our understanding of the possible mechanisms and associated microbial diversity responsible for As(V)/(III) cycling in soil systems. Specifically, the objectives of this study were to (i) utilize cultivation-independent 16S rDNA sequence analysis to identify microbial populations associated with observed As redox transformations occurring in a soil environment, (ii) cultivate As(III)-oxidizing and As(V)-reducing aerobic heterotrophic bacteria from the same soil systems, and (iii) determine if isolates with demonstrated As redox activity correspond to those populations detected with molecular methods. These objectives were addressed by conducting...
unsaturated flow column transport experiments where micromolar concentrations of either As(III) or As(V) were used as enrichment for microorganisms capable of transforming As under aerobic conditions.

**Materials and Methods**

**Column Experiments.** The upper 20 cm of a well-drained, fine loamy, frigid Typic Calciaquoll that contained 2.6 ± 0.8 mg kg⁻¹ As (saturated paste extraction using deionized H₂O) was used as inocula for column studies. Measurements of soluble As concentrations in Madison River Valley pasture in the Madison River Valley (Gallatin County, Montana) and unsaturated flow within the columns, filter-sterilized air was drawn through the column via a syringe needle at the bottom endcap using a peristaltic pump at a rate of 100 mL h⁻¹. Solution applied to the columns drained through the soil/sand matrix via gravimetric flow and exited the bottom of the columns along with the air stream. The volumetric water content (θv) of the soil/sand mixture was maintained at 0.24 cm⁻¹ (53% of saturation). The influent was formulated to enrich for aerobic heterotrophs capable of transforming As and contained NH₄NO₃ (1.25 mM), CaSO₄ (2 mM), MgCl₂ (2 mM), KH₂PO₄ (10 mM), KOH (1.25 mM), and FeCl₃ (50 μM) (33 supplemented with micronutrients (34), vitamins (35), 5 mM glucose (to enrich for heterotrophic organisms), and either 75 μM NaH₂AsO₃ or 250 μM NaH₂AsO₄. These concentrations of As were significantly higher than concentrations shown to induce genes involved in As detoxification (21) but lower than those normally employed for As(III) cometabolism or for anaerobic respiration on As(V) (8, 35).

To confirm that As(III) oxidation was dependent on the presence of microbial cells, culture filtrates from As(III)-oxidizing isolates were tested for oxidation of As(III) (5) by spiking 3 mL of filtrate (0.22 μm) from actively oxidizing cell suspensions (inoculated similarly and grown in the same media described for the oxidation rate experiments) with 20 μM As(III) and measuring oxidation after 3 h. The ability of the As(III)-oxidizing isolates to grow chemolithoautotrophically with As(III) as the sole electron donor and CO₂ as the primary C source was tested using the serum bottle method described above with 25 mL of column influent media (described above) supplemented with 50 μM NaHCO₃ (added to replace glucose as the C source), and 5 μM As (III) (modified from ref 8). The serum bottles were sealed to maintain the partial pressure of CO₂. The potential for the As(V)-reducing isolates to respire on As(V) was tested in N₂ (gas)-purged serum bottles containing column influent media modified to contain 5 mM MOPS buffer (pH 7.0), 50 μM NaH₂PO₄, 1 mg L⁻¹ yeast extract, 4 mM As(V), and 1 mM cysteine; NH₄NO₃ and glucose were replaced with 2.5 mM NH₄Cl and 20 mM lactate, respectively, based on past reports of media used to culture As(V) dissimilatory reducers (35).

**DNA Extraction, PCR, and DGGE Analysis.** Total DNA was extracted from the homogenized soil/sand mixture using the FastDNA SPIN Kit for Soil (Bio 101, Vista, CA). DNA extracts were used as template for polymerase chain reaction (PCR) which targeted a specific 322 bp segment within the 16S rRNA gene. The 1070 forward primer targeted the domain Bacteria (Escherichia coli) positions 1055–1070 and the 1392 reverse-GC primer targeted a universally conserved region (E. coli positions 1392–1406; 36). The reverse primer was modified to contain a 40 bp GC-rich clamp to facilitate analysis by DGGE (37). PCR mixtures (50 μL) contained 1–5 μL of template DNA (2–20 ng), 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.0% Triton X-100, 4.0 mM MgCl₂, 800 μM dNTPs, 0.5 μM of each primer, and 1.25 U Taq DNA polymerase (Promega, Madison, WI). The protocol was 94 °C for 4 min;
30 cycles of 94, 55, and 72 °C each for 45 s, and a final 7-min extension period at 72 °C.

PCR products were separated by DGGE as described by Ferris et al. (37) with the following modifications. A DCode System (Bio-Rad, Hercules, CA) was used to resolve the PCR products in gels consisting of 8% acrylamide and a 40–70% gradient of urea/formamide. Electrophoresis was performed at 60 V at 60 °C for 17 h. DGGE gels were stained with SYBR Green II (Molecular Probes, Eugene, OR) for 30 min and photographed using UV transillumination. DGGE bands of interest were stabbed with a sterile pipet tip and used as template for PCR amplification, purification (repeated PCR amplification and DGGE until a pure band was obtained), and subsequent sequencing reactions. The templates were amplified using primers 1070 forward and 1392 reverse (without the GC clamp) as described above. The product was purified with a QIAquick PCR Purification Kit (Qiagen, Inčoper, fucna, CA), the ARB software package (W. Ludwig et al. (36)). Sequencing reactions and analysis were conducted as described above. The near-full-length 16s rDNA sequences for the isolates obtained in this study have been submitted to GenBank and have been assigned the accession numbers AF010234, and AE008073, respectively. The putative Geobacillus sp. arsC gene was novel and was submitted to GenBank (AF393651). PCR products of all four genes were used as probes for dot blot hybridizations. Probes were labeled with 32P using the Megaprime DNA Labeling System (RPN 1606, Amersham Pharmacall Biotec, Piscatway, NJ). Total DNA from each of the isolates was applied to nylon membranes (GeneScreen Plus, NEN Life Sciences, Boston, MA) using the Bio-Dot microfiltration apparatus (BioRad), and hybridizations with each probe were performed according to the manufacturer’s instructions. Probed membranes were visualized using autoradiography with Kodak BioMax MS film (Rochester, NY).

An unrooted arsC phylogenetic tree was constructed using sequences of 14 characterized and putative arsC genes obtained from GenBank and from sequences described in this study. Sequences were aligned using the neighbor joining procedure as described above; only positions that were unambiguous for all sequences were used, and gaps were ignored.

**Results and Discussion**

**Column Experiments.** Arsenate was the predominant species of As eluted from nonsterile unsaturated columns after 3 d, regardless of whether they received As(III) or As(V) (Figure 1). The value of the column-derived first-order rate constant (k) for As(III) oxidation in treatments receiving 75 μM As(III) was determined to be >0.60 h−1 (t1/2 < 1.2 h) using an analytical solution to the advection–dispersion equation (eq 9 in ref 41), assuming that all As(III) was converted to As(V) (detection limit = 0.05 μM). Although the oxidation of As(III) to As(V) is thermodynamically favored under oxic conditions (42), sterilized treatments did not result in detectable conversion of As(III) to As(V). Consequently, the rapid oxidation of As(III) observed in the nonsterile columns was mediated by microbial processes.

**Molecular Analyses.** DGGE was used to obtain DNA fingerprints of microbial populations present in the original soil inoculum and in each of the column enrichments. Replicate DGGE profiles of the untreated soil appeared identical, exhibited numerous bands, and suggested that numerous microorganisms were present in the original soil inoculum (Figure 2). The As(III) and As(V) treatments produced somewhat different banding patterns, indicating that enrichment conditions, including As speciation and/or concentration, influenced the selection of soil microbial populations. Replicate columns of each As treatment yielded similar DGGE banding patterns; however, differences in band intensity between the replicates were observed, indicating potential variation in inoculum and/or subsequent column conditions. As with all defined enrichment environments, the chemical and physical attributes of the media select for organisms that are competitive or highly adapted to such conditions. Consequently, the use of glucose as a potential C and energy source and the relatively high As concentrations used in these experiments do not necessarily enrich for organo-
isms that may predominate in natural soil environments containing other C substrates and lower As concentrations.

Purification and sequencing of selected bands in the DGGE gels and comparison with sequences in GenBank revealed that the two most prominent bands in the columns supplemented with As(III) represented populations that were 99.4% similar to Pseudomonas fluorescens (band 3) and 99.6% similar to Alcaligenes sp. (band 8, GenBank Accession No. AF536820) (Figure 2). Less conspicuous bands in this treatment that were successfully purified and sequenced most closely matched Variovorax paradoxus (99.5% identity, band 1), A. tumefaciens (100% identity, band 5), and A. vitis (100% identity, band 9, GenBank Accession No. AF536821). Major bands in columns supplemented with As(V) represented P. fluorescens (band 3) and A. tumefaciens (band 5), while minor bands represented Alcaligenes sp. (band 8) and A. vitis (band 9)-like populations. Thus, all of the sequenced bands were found in each of the two treatments with the exception of V. paradoxus, which was only observed in the As(III)-supplemented columns. The most apparent differences between treatments were decreased intensity of the Alcaligenes sp. band (no. 8) and increased intensity of the A. tumefaciens band (no. 5) in the As(V)-treated columns relative to the As(III)-treated columns. These differences in band intensity suggested that the Alcaligenes sp.-like population may have been favored in the presence of As(III), and conversely, the A. tumefaciens-like population was favored in the presence of As(V).

**Isolates.** Traditional cultivation methods were used to isolate microorganisms from the As-treated columns. Serial dilutions of the soil/sand mixture obtained after column enrichment were plated on R2A media and after 72 h, 24 colonies representing 10 different colony morphologies were picked from the most dilute plates (10⁰–10⁴ dilutions) and tested for their ability to either oxidize As(III) or reduce As(V). Because all of these isolates were obtained from plates inoculated with dilute serial suspensions, they represented the cultivatable organisms that were enriched as a result of the As(III) or As(V) treatments. As with any cultivation method, the microorganisms selected under specific enrichment conditions, in this case on R2A media, do not necessarily represent all the populations that were important in the soil columns. Of the 24 bacteria isolated from plates, 10 were capable of oxidizing As(III) and 10 were capable of reducing As(V). Four others grew poorly in the solution media and were therefore dropped from the experiment. Both the As(III)-oxidizing and the As(V)-reducing isolates were obtained from columns that received either As(III) or As(V), indicating that As oxidizers or reducers were not limited to a specific treatment (Table 1). The fact that both oxidizers and reducers were obtained from the same treatments suggests that As oxidation and reduction may have been occurring simultaneously. These organisms were identified by near-full-length sequencing of their 16S rRNA genes, revealing that a total of three different As(III)-oxidizing populations and five different As(V)-reducing populations were represented by these isolates (Table 1). BLAST searches showed that the closest matches to the three As(III) oxidizers were P. fluorescens, V. paradoxus, and A. tumefaciens. The closest GenBank matches to the five As(V)-reducing isolates were Flavobacterium heparinum, A. tumefaciens, Microbacterium sp., Arthrobacter aurescens, and Arthrobacter sp. The
Pseudomonas and Agrobacterium genera contain members that were previously shown to have As-transforming capabilities (11, 21). Interestingly, the two A. tumefaciens isolates that exhibited opposite As redox phenotype have identical 16S rDNA sequences (across ~1400 bp). Further discussion regarding these two strains follows below. The similarity in colony morphology between the A. tumefaciens isolates as well as among other isolates prevented attempts to enumerate specific populations from either column based on colony forming units (cfu).

**Correlating Isolates with Column Populations.** Sequences of 16S rDNA fragments amplified from nucleic acid extracts of column samples were compared to sequences of As(III)-oxidizing isolates listed above. These comparisons showed that three DGGE bands present in the column treatments represented 16S rDNA sequences that were 100% identical to the V. paradoxus (band 1), P. fluorescens (band 3), and A. tumefaciens (band 5)-like isolates (Figure 2), all of which were As(III) oxidizers. However, with exception of the A. tumefaciens band (no. 5), no DGGE bands corresponding to other As(V)-reducing isolates were observed in DGGE profiles. The direct identification of As(III)-oxidizing populations in the column environments using molecular methods was consistent with the microbial oxidation of As(III) observed during solute transport. However, the fact that two strains of A. tumefaciens [an As(III) oxidizer and an As(V) reducer] could not be differentiated by DGGE and that other As(V) reducers were isolated from these columns preclude a conclusion that only As(III)-oxidizing microorganisms were relevant to the net As redox output observed in these columns. Template bias (43) may have reduced the sensitivity of the molecular approach and may explain the lack of detection of As(V)-reducing populations using PCR-DGGE. Furthermore, detection of the As(V)-reducing populations using DGGE was potentially limited by the addition of ~100 ng of total DNA per lane; it is possible that higher quantities of DNA may have allowed detection of these bands, although overall band resolution may have suffered. Regardless of the actual population size of As(V)-reducing organisms in the soil columns, the combined metabolic activity of the As(V) reducers failed to dominate the net As redox activity in the column communities.

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### Rates of As Transformation by Bacteria

Rates of As(III) oxidation or As(V) reduction by each of the eight isolates cultivated from the column environments were measured in continuously aerated serum bottles (Figure 3). Rates of As(III) oxidation during logarithmic growth of the three As(III)-oxidizing isolates varied from 0.7 to 1.7 μmol d⁻¹ per 10⁹ cell with corresponding As(III) half-lives ranging from 0.2 to 0.5 d (Table 1). In comparison, rates of As(V) reduction by the five As(V)-reducing isolates ranged from 0.5 to 3.3 μmol d⁻¹ per 10⁹ cell (half-lives of 0.1–0.6 d; Table 1). Assuming that As(III) oxidation was the only transformation process in the soil column experiments, the number of As(III)-oxidizing organisms required to support the column-derived As(III) oxidation rates was estimated to range from 1 to 3 × 10⁹ cells/g of soil, based on the As(III) oxidation rates obtained for each of the As(III)-oxidizing isolates (Table 1). Although this estimate assumes that the physiological status of the isolates was similar in pure culture to that under column conditions, it suggests that either of the three oxidizing isolates would be capable of supporting the oxidation of As(III) observed in the soil columns, either acting individually or in concert.

### Mechanisms of As Oxidation/Reduction

No oxidation of As(III) was observed in sterile controls or in experiments using cell-free filtrate taken from isolate suspensions that were actively oxidizing As(III), indicating that As(III) oxidation required the presence of microbial cells. In addition, none of the As(III)-oxidizing isolates grew in media designed for chemolithotrophic metabolism using As(III) as the sole electron donor [same medium as used during column enrichment except glucose was replaced by CO₂ and As(III) was increased to provide adequate energy source]. This result taken together with the fact that As(III) oxidation profiles for each isolate corresponded with microbial growth (Figure 3) suggests that the mechanism of As(III) oxidation by these isolates was related to As detoxification rather than energy generation. Likewise, none of the As(V)-reducing isolates could grow in media designed for dissimilatory As(V) reduction, where lactate served as the primary C and energy source and As(V) was present as the primary electron acceptor. This result was not surprising considering that the isolated organisms were enriched under aerobic conditions. Although these findings must be tempered with the possibility

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### Table 1. Closest GenBank Neighbors, Sequence Similarities, and As-Transforming Characteristics of Isolates Cultivated from Unsaturated Soil Columns

<table>
<thead>
<tr>
<th>isolate accession no.</th>
<th>closest GenBank neighbor (% similarity)³</th>
<th>DNA sequences detected in soil columns¹²</th>
<th>column treatment from which isolates were obtained⁴</th>
<th>As(III) oxidation/As(V) reduction parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rate (μmol d⁻¹ 10⁹ cell⁻¹) half-life (d)</td>
</tr>
<tr>
<td>As(III)-Oxidizing Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 AF388028</td>
<td>Variovorax paradoxus (99.3)</td>
<td>yes</td>
<td>√</td>
<td>1.7</td>
</tr>
<tr>
<td>3 AF388027</td>
<td>Pseudomonas fluorescens (99.1)</td>
<td>yes</td>
<td>√</td>
<td>1.4</td>
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<tr>
<td>5A AF388033</td>
<td>Agrobacterium tumefaciens (99.9)</td>
<td>yes</td>
<td>√</td>
<td>0.7</td>
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<tr>
<td>As(V)-Reducing Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 AF388029</td>
<td>Flavobacterium heparinum (94.9)</td>
<td>no</td>
<td>√</td>
<td>0.5</td>
</tr>
<tr>
<td>4 AF388031</td>
<td>Microbacterium sp. (98.1)</td>
<td>no</td>
<td>√</td>
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</tr>
<tr>
<td>5B AF388030</td>
<td>Agrobacterium tumefaciens (99.9)</td>
<td>yes</td>
<td>√</td>
<td>3.3</td>
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<tr>
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<td>Arthrobacter aurescens (99.6)</td>
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<tr>
<td>7 AF388034</td>
<td>Arthrobacter sp. (97.8)</td>
<td>no</td>
<td>√</td>
<td>1.6</td>
</tr>
</tbody>
</table>

¹ Isolates were obtained from treatments indicated with checkmark, and detection of their 16S rDNA sequences in soil columns is indicated. ² Closest GenBank neighbors and similarities of near full-length 16S rDNA sequences of isolates were determined using BLAST (38). ³ 16S rDNA sequences in columns were analyzed by purifying and sequencing DGGE bands derived from PCR amplification of DNA extracts. ⁴ Rates of As(III) oxidation and As(V) reduction demonstrated by isolates were measured during logarithmic growth under aerated serum bottle conditions. Rates normalized to cell number using an empirically developed relationship between cell enumeration with phase contrast microscopy and optical density (OD) measurements (A⁵₀₀) of cell suspensions. ⁵ Apparent half-lives (t₁/₂) are dependent on experimental conditions and are estimated based on rates assuming pseudo-first-order reaction dependent on As(III) (oxidizing isolates) or As(V) (reducing isolates).
that some or even all of these bacteria may utilize As for energy metabolism in different media, under the conditions tested here, none of these organisms demonstrated energy-conserving reactions with As. Thus, all of the bacteria isolated from the As-supplemented columns demonstrated the capacity to either oxidize As(III) or reduce As(V), apparently for detoxification purposes.

Hybridization experiments using \( \text{arsC} \) probes derived from \( \text{P. aeruginosa} \), \( \text{Geobacillus} \), \( \text{E. coli} \), and \( \text{A. tumefaciens} \) for dot blots of DNA obtained from the soil isolates revealed significant homology between only the \( \text{A. tumefaciens arsC} \) probe and the DNA from the \( \text{A. tumefaciens} \) isolates (Figure 4A). To characterize the apparent \( \text{arsC} \) homologues in these A. tumefaciens isolates, the homologues were PCR amplified and sequenced (GenBank Accession Nos.: AY286230 for isolate 5B, AY286231 for isolate 5A). The nucleotide sequences of these putative \( \text{arsC} \) genes (375 nucleotides) were 99.2% identical to each other, and their inferred amino acid sequences were 80% identical and 88% similar to the annotated \( \text{arsC} \) in \( \text{A. tumefaciens} \) (across 124 amino acids). Amino acid alignments (Figure 4B) showed that the cloned \( \text{A. tumefaciens arsC} \) genes shared extensive homology with the well-characterized \( \text{arsC} \) from \( \text{E. coli} \), including the highly conserved amino acids Cys12, Ser15, Arg60, Arg94, and Arg107.

**FIGURE 3.** Microbial biomass (as OD 500 nm, ◆) and As(III) (▲) or As(V) (▲) concentrations as a function of time for three As(III)-oxidizing isolates and five As(V)-reducing isolates incubated under aerated serum bottle conditions. Total concentrations of As in solution ([total As] = [As(III)] + [As(V)]) remained nearly constant during each experiment. Isolates are named after their nearest match found in the GenBank Database. Error bars represent standard errors of three replicate experiments for \( \text{P. fluorescens} \) and two replicate experiments for all other isolates. [Preliminary results for \( \text{P. fluorescens} \) were presented in a review chapter (39) based on results of Macur et al. (40).]
 Positive controls using total DNA extracts from (44). acids experimentally shown to be essential for enzyme function (9). A. tumefaciens soil column isolates (5A and 5B) as compared to strain C58, Geobacillus, E. coli, P. aeruginosa. Positive hybridizations column isolates probed with selected arsC genes (A. tumefaciens FIGURE 4. (A) Dot blot hybridizations of total DNA from the eight conditions (65 °C to any of the four arsC genes unambiguously placed both as sharing a partial homology between the A. tumefaciens arsC genes; the amino acids alignments of the arsC homologue PCR cloned from the geobacillus arsC gene probe are shown as an example of the consensuses positions of homology between the A. tumefaciens arsC genes; the amino acids in bold and gray boxes show positions of conservation across all three arsC genes, and asterisks (*) note conservation of amino acids experimentally shown to be essential for enzyme function (44). (E. coli p773 arsC numbering) found to be essential for activity in several ArsCs (44). Phylogenetic analysis of the cloned partial arsC genes unambiguously placed both as sharing a common node with the A. tumefaciens strain C58 arsC and separate from the other closely related arsC genes (Figure 5).

No other soil isolates obtained in this study hybridized to any of the four arsC probes under low stringency wash conditions (65 °C, 2x SSC; data not shown). Conversely, positive controls using total DNA extracts from A. tumefaciens, P. aeruginosa, Geobacillus, and E. coli produced strong hybridization signals with their respective probes. The failure of these arsC probes to hybridize with DNA from the soil isolates is consistent with the enormous diversity of known arsC sequences (e.g., refs 9 and 23–27; Figure 5) and the fact that this diversity may preclude detection of arsC homologues using probes designed from phylogenetically different organisms. Interestingly, both of the A. tumefaciens isolates were shown to contain a putative arsC, despite the fact that isolate 5A did not exhibit As(V) reduction. The apparent lack of As(V) reduction in the As(III)-oxidizing A. tumefaciens isolate may be due to a variety of reasons including point mutations in arsC or separation of arsC from its As derepressible promoter. Given that the two arsC genes cloned from the different A. tumefaciens isolates shared 100% amino acid identity across that portion of the gene amplified and sequenced in this study, any putative mutation would have to be external to this part of the arsC coding region. An alternative explanation may be that the arsC gene in the As(III)-oxidizing isolate became separated from its As derepressible promoter due to a genomic rearrangement event that are now known to be common in bacteria (45, 46), including the Rhizobiaceae (47, 48). It is also possible that the opposite As redox activity of the two A. tumefaciens strains is due to the presence, absence, or differential expression of an As(III) oxidase gene. The As(V)-reducing strain either may lack the genes required for As(III) oxidation or may not have significantly expressed these genes under the conditions studied herein. Studies are currently under way to examine the genetic differences between these A. tumefaciens strains and to assess whether mutation, lateral gene transfer events, or other factors may explain the different As phenotypes.

Implications for As Cycling in Soils. Eight heterotrophic, aerobic As-transforming bacteria representing diverse genera in the Gram-positive, flavobacteria, and proteobacteria kingdoms were isolated from soil column enrichments and characterized. None of the five As(V)-reducing isolates grew on media designed for dissimilatory As(V) reduction, and the three As(III)-oxidizing isolates did not grow in media with As(III) as the primary electron donor for chemolithotrophic metabolism. On the basis of the reported mechanisms of As oxidation—reduction activity among microorganisms (44, 49), these results suggest that both the As(V)-reducing and As(III)-oxidizing isolates were transforming As via detoxification mechanisms as opposed to energy generation. Although we were able to cultivate both As(V)-reducing and As(III)-oxidizing bacteria from the column environments, As(III)-oxidizing populations apparently dominated the observed net As redox activity and represented the primary 16S rDNA sequences that were detected in column samples using molecular methods. The coexistence of both As(III)-oxidizing and As(V)-reducing aerobic populations in the same soil suggests that
the relative numerical and or metabolic dominance of these populations will influence the predominant As valence state. The isolation of both As(III)-oxidizing and As(V)-reducing A. tumefaciens strains from the same culture enrichment (Table 1) shows that the ability to either oxidize As(III) or reduce As(V) is variable even among strains that proliferate under the same environmental conditions. Because of such variation in phenotype within similar organisms, phylogenetic identification of microorganisms based on 16S rRNA sequence analysis is not sufficient to predict the As-transforming capabilities of specific bacterial populations. Further, As contamination in oxic environments may not select for microorganisms capable of utilizing As in energy metabolism but rather may shift the microbial community structure to favor organisms capable of detoxification either via As oxidation or reduction. Specifically, the broad phylogenetic distribution of arcA genes suggests that the importance of nondissimilatory As(V) reduction may be underestimated as a mechanism of As redox cycling in natural systems. Results from the current study as well as other recent examples (17, 18) support the hypothesis that the oxidation and reduction of As occurs in phylogenetically diverse soil bacteria via mechanisms that are not directly associated with respiration or chemolithothrophic metabolism.

Acknowledgments

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Literature Cited