Microbiological Research 167 (2012) 151–156

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.de/micres

Inhibition of microbial arsenate reduction by phosphate

Deanne C. Slaughter[a], Richard E. Macur[b,*], William P. Inskeep[a]

[a] Department of Land Resources and Environmental Sciences, Montana State University – Bozeman, Bozeman, MT 59717, USA
[b] Center for Biofilm Engineering, Montana State University – Bozeman, Bozeman, MT 59717, USA

ARTICLE INFO

Article history:
Received 24 December 2010
Received in revised form 13 May 2011
Accepted 13 May 2011

Keywords:
Arsenite
Arsenate
Phosphate inhibition
Arsenate reductase
Arsenate reduction

ABSTRACT

The ratio of arsenite (As^{III}) to arsenate (As^{V}) in soils and natural waters is often controlled by the activity of As-transforming microorganisms. Phosphate is a chemical analog to As^{V} and, consequently, may competitively inhibit microbial uptake and enzymatic binding of As^{V}, thus preventing its reduction to the more toxic, mobile, and bioavailable form – As^{III}. Five As-transforming bacteria isolated either from As-treated soil columns or from As-impacted soils were used to evaluate the effects of phosphate on As^{V} reduction and As^{III} oxidation. Cultures were initially spiked with various P:As ratios, incubated for approximately 48 h, and analyzed periodically for As^{V} and As^{III} concentration. Arsenate reduction was inhibited at high P:As ratios and completely suppressed at elevated levels of phosphate (500 and 1000 μM; P inhibition constant (K_i) ~ 20–100 μM). While high P:As ratios effectively shut down microbial As^{V} reduction, the expression of the arsenate reductase gene (*arsC*) was not inhibited under these conditions in the As^{V}-reducing isolate, *Agrobacterium tumefaciens* str. 5B. Further, high phosphate ameliorated As^{V}-induced cell growth inhibition caused by high (1 mM) As pressure. These results indicate that phosphate may inhibit As^{V} reduction by impeding As^{V} uptake by the cell via phosphate transport systems or by competitively binding to the active site of ArsC.

Introduction

The activity of soil microorganisms that transform As into different chemical species has significant environmental ramifications because different forms of As exhibit variation in solubility, mobility, bioavailability, and toxicity (Masscheleyn et al. 1991; Nriagu 1994; Inskeep et al. 2002). Arsenic most commonly occurs in soils as As^{III} (arsenite) and As^{V} (arsenate). The predominant As^{III} species in soils is $H_3AsO_3^0$, with an exception that sulfidic environments may yield significant levels of As^{III}–sulfide complexes (Rochette et al. 2000). The weak acid $H_3AsO_3^0$ has a pK_a value of 9.2; consequently, across most environmental systems, As^{III} will exist as an uncharged species (Cullen and Reimer 1989). The neutrally charged As^{III} species can enter cells via aqua-glyceroporins (large pores in the cell membrane) that allow passage of water and uncharged solutes (Rosen 2002). Once internalized, As^{III} toxicity occurs as it binds to the sulfhydryl groups of proteins and impairs their function (Oremland and Stolz 2003).

Pentavalent As is thermodynamically favored in oxic environments as the oxyanion As^{V}, which generally sorbs more strongly and to a wider variety of minerals than As^{III} (Pierce and Moore 1982; Xu et al. 1991). Hence, As^{V} is generally considered to be less mobile and less bioavailable than As^{III}. The predominant forms of As^{V} in soil solutions and natural waters will typically be either $H_2AsO_4^-$ ($pK_a = 7.0$) or $HAsO_4^{2-}$ ($pK_a = 11.5$), although the presence of significant concentrations of thioarsenates in sulfidic environments has also been reported (Planer-Friedrich et al. 2007). Structural similarities between arsenate and phosphate enable As^{V} to enter cells via membrane-associated phosphate transporters (Mukhopadhyay et al. 2002; Oremland and Stolz 2003). Arsenate is detrimental to basic cell function when it is substituted for phosphate in cell metabolic processes such as oxidative phosphorylation (Mukhopadhyay et al. 2002; Oremland and Stolz 2003).

The relative abundance of As^{III} and As^{V} in soil environments is influenced by microbial transformations including detoxifying or energy-yielding redox pathways (Inskeep et al. 2002). Most microorganisms in culture have been shown to possess at least one type of As-transforming mechanism. While As^{V} is often the predominant valence state in oxidized environments (Oremland and Stolz 2003), microbial reduction to As^{III} in both aerobic and anaerobic systems is an important factor increasing the mobility and potential bioavailability of As (Macur et al. 2001; Harvey et al. 2002).

Because phosphate and arsenate are structural analogs, it would be expected that high levels of P can inhibit enzymatic processes involving As^{V}. Phosphate has been shown to compete with the cell uptake of As^{V} in numerous biological species (Rothstein and Donovan 1963; Harold and Baarda 1966; Willsky and Malamy

* Corresponding author. Tel.: +1 406 994 1849; fax: +1 406 994 6098.
E-mail address: rmacur@montana.edu (R.E. Macur).

0944-5013/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2011.05.007

1980; Thiel 1988); therefore, it is hypothesized that high concentrations of P may completely inhibit microbial uptake of As^V, thus preventing its reduction to As^{III} via the ArsC (cytoplasmic arsenate reductase) encoded in the *ars* operon.

The *ars* operon is widely distributed phylogenetically, including the three genera of particular interest for this study: *Agrobacterium*, *Arthrobacter*, and *Bacillus* (Silver and Phung 2005; Jackson and Dugas 2003). Other studies further support that microorganisms capable of reducing As^V are ubiquitous across myriad soil environments (Macur et al. 2001, 2004). Previous research (Gladysheva et al. 1994; Ji et al. 1994; Mukhopadhyay et al. 2000; Zhou et al. 2004) indicates that the ability of phosphate to inhibit arsenate reductase activity is relatively low and varies by species. For example, phosphate did not inhibit the *in vitro* activity of the arsenate reductase in *Leishmania major*, LmACR2 (Zhou et al. 2004); the *Saccharomyces cerevisiae* arsenate reductase, Acr2p (Mukhopadhyay et al. 2000); or the arsenate reductase encoded on plasmid pI258 of *Staphylococcus aureus* (Ji et al. 1994). However, phosphate was shown to be a weak competitive inhibitor of As^V reduction by the ArsC encoded on the *Escherichia coli* plasmid R773 (Gladysheva et al. 1994). The degree to which phosphate may affect the uptake of As^V or the activity of ArsC in countless other organisms is yet to be determined.

Consequently, the overall goal of the current study was to evaluate the effects of phosphate on the microbial transformations of arsenic in five As-transforming soil bacteria. Specific objectives were to (i) determine the effects of phosphate:arsenic ratios on arsenate reduction and cell growth in organisms known to possess *arsC* genes (e.g., known aerobic As^V reducers); (ii) evaluate whether high phosphate:arsenate ratios can inhibit the expression of *arsC* in a specific As^V-reducing microorganism, namely *Agrobacterium tumefaciens* str. 5B; and (iii) evaluate the oxidation of arsenite by several known As^{III}-oxidizing organisms as a function of P:As ratio.

Materials and methods

Isolate selection and preparation

Five As-transforming microorganisms isolated from soils with long-term As contamination were selected for use in liquid culture experiments. Specifically, the microorganisms were three known As^V-reducing organisms – *A. tumefaciens* str. 5B (accession #: AF388030), *Bacillus*-like str. S18 (EU787020), and *Arthrobacter*-like str. S6 (EU787019) – and two known As^{III}-oxidizing organisms – *Variovorax* sp. str. RM1 (AF388028) and *A. tumefaciens* str. 5A (AF388033). Three of these organisms (*Variovorax* sp. str. RM1 and *A. tumefaciens* strs. 5A and 5B) were isolated previously (Macur et al. 2004) from aerobic column experiments conducted using agricultural soil with prior exposure to As-rich irrigation water from the Madison River (Gallatin County, MT). The remaining two organisms (*Bacillus*-like str. S18 and *Arthrobacter*-like str. S6) were isolated from soil samples impacted by aerial As contamination from several copper smelters near Anaconda, MT (N 46.10313° W 112.87296°; Masur et al. 2007).

Prior to their use as inoculum in As transformation experiments, these microorganisms were grown in autoclaved centrifuge vials containing 200 mL of synthetic soil solution media (SSE), which was modified from Macur et al. (2004) to contain NH_4NO_3 (1.25 mM), $MgCl_2$ (1 mM), KH_2PO_4 (0.05 mM), KOH (0.25 mM), $FeCl_3$/Na_2EDTA (0.02 mM), $CaSO_4$ (2 mM), glucose (5 mM), yeast extract (2 mg L^{-1}), MOPS buffer (5 mM), and 1 mL L^{-1} trace metals solution modified from Newman et al. (1997) to exclude $FeCl_3$. Cells in the logarithmic growth phase were pelleted (centrifuged at 5000 × *g*, 40 min) and re-suspended in approximately 30 mL of SSE media without KH_2PO_4. Cells were enumerated using light microscopy and epifluorescent microscopy of DAPI-stained cells; cell numbers were then correlated to optical density measurements (A_{500}).

Table 1
Concentrations of phosphate and arsenic, and corresponding P:As ratio, used in experiments to examine effects of phosphate on As^{III}-oxidizing or As^V-reducing isolates. A phosphate:arsenic ratio of 0.5 (baseline treatment) was used to confirm each organism's As phenotype.

P (μM)	As (μM)	P:As
50	100	0.5
50	1000	0.05
500	100	5
1000	100	10
50	10	5
50	5	10
1000	1000	1

Liquid culture experiments

Duplicate autoclaved glass 118 mL culture vials were filled with sterile SSE media (80 mL without KH_2PO_4) and spiked with either As^V (Na_2HAsO_4) or As^{III} ($NaAsO_2$) plus phosphate (KH_2PO_4) at one of the P:As ratio combinations used in the experiments (Table 1). Vials were inoculated with cells grown in the absence of As to obtain similar initial cell densities among treatments. Cultures and sterile controls were incubated at 30 ± 1 °C for approximately 48 h on an orbital shaker (120 rpm). Two 1–5 mL solution samples were extracted and filtered (0.22 μm) at 0, 8, 24, and ~48 h; samples were utilized to measure As^V and As^{Total} using the sodium-borohydride method described in Macur et al. (2004). Arsenic was analyzed using hydride generation-atomic absorption spectrometry (HG-AAS; Varian VGA 77, Palo Alto, CA; Perkin Elmer 3100, Waltham, MA) or inductively coupled plasma spectrometry (ICP-OES, Model 5500Perkin Elmer) as detailed in Macur et al. (2004). The As^{III} concentration was calculated from the difference between measured As^V and As^{Total} values. At each sampling time-point, samples were also obtained for optical density measurement and for subsequent analysis of mRNA transcripts (cell suspensions were centrifuged and immediately frozen at −80 °C).

Amplification of arsC mRNA transcripts

The effect of phosphate and As concentration on the expression of *arsC* during growth of *Agrobacterium* str. 5B was evaluated by extracting RNA from cells using the FastRNA Pro Blue Kit (MP Biomedicals, Irvine, CA). A 346 bp fragment of *arsC* mRNA was PCR-amplified from the total RNA extracts (~10 ng RNA) using 1 μM each of the *Atume-arsC* forward (5′-ACCCTCGCACTCATTGAGC-3′) and reverse (5′-ACCTGCTCGCCGTCTTCT-3′) primers. The primer design was based on the known *arsC* sequences in the *Agrobacterium* str. 5B (Macur et al. 2004; accession #: AY286230). The initial generation of cDNA using the Access RT-PCR System (Promega, Madison, WA) was followed by a PCR protocol of 95 °C for 2 min, 40 cycles of 95 °C for 45 s, 50 °C for 45 s, 72 °C for 50 s, and final extension of 72 °C for 5 min. To verify that the correct target sequences were amplified, purified PCR products were cloned into the pGEM-T Vector System (Promega) and the clones were sequenced.

Results and discussion

Effects of phosphate on microbial reduction of arsenate

All three As^V-reducing isolates (*Agrobacterium* str. 5B, *Arthrobacter* str. S6, and *Bacillus* str. S18) exhibited efficient reduction of As^V to As^{III} within 48 h in the presence of 100 μM As^V and 50 μM phosphate (referred to as the baseline treatment; Fig. 1). The

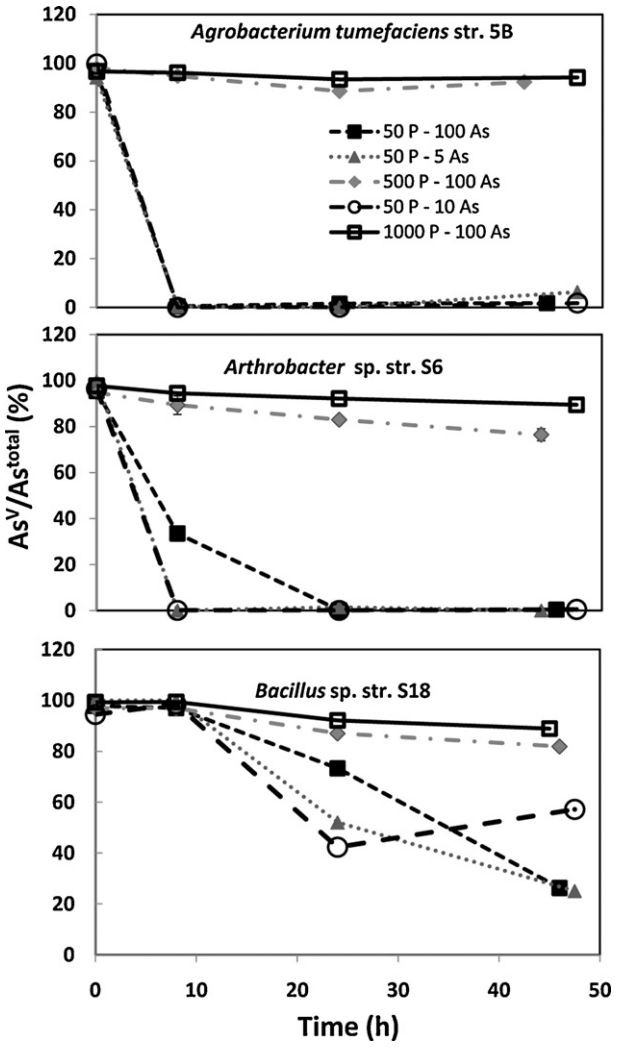

Fig. 1. Percent of As^V relative to As^{Total} plotted as a function of time in P:As^V ratio experiments by three As^V-reducing strains – *Agrobacterium tumefaciens* str. 5B, *Arthrobacter* sp. str. S6, or *Bacillus* sp. str. S18. Error bars indicate standard deviation of duplicate cultures; when apparently absent, error bars are contained within the symbol. Legend values in μM.

Agrobacterium str. 5B and *Arthrobacter* str. S6 showed near complete reduction (>98%) of As^V within 24 h, while the *Bacillus* str. S18 isolate showed approximately 74% As^V reduction by 48 h. Apparently, under these conditions, the *Bacillus* str. S18 is a less vigorous As^V-reducing strain when compared to the *Agrobacterium* and *Arthrobacter* strains used in this study. Sterile controls sampled over the same time-frame exhibited less than 4% reduction to As^{III}.

At 100 μM As^V, reduction was almost completely inhibited (Fig. 1) when P concentrations were increased to 500 and 1000 μM (P:As ratios of 5 and 10). These effects were observed for all three As^V-reducing isolates, suggesting that high P to As^V ratios in natural systems may decrease the likelihood that As^V will become reduced to As^{III} via microbial detoxification processes. However, results from experiments conducted at identical P:As ratios (5 and 10) but with relatively low As^V concentrations (5 and 10 μM, respectively) and a constant P concentration (50 μM), showed no significant differences ($\alpha = 0.05$; univariate ANOVA) in As^V reduction compared to the baseline treatment (50 μM P and 100 μM As^V) where nearly 100% of the As^V was reduced (Fig. 1). Consequently, high P:As ratios alone are not sufficient to inhibit microbial reduction of As^V; the absolute concentration of P is also an important factor. Finally, none of the three As^V-reducers showed significant As^{III} oxidation when grown in media containing only As^{III}. Growth curves and As totals remained uniform, respectively, for each bacterial species across the five P:As ratio treatments.

Effects of phosphate on microbial oxidation of arsenite

In all five of the P:As ratio experiments that utilized the two As^{III}-oxidizing strains (*Variovorax* str. RM1 and *Agrobacterium* str. 5A), the amount of As^{III} decreased to less than 10% of the initial concentration by the ~48-h time-point (Fig. 1, Supporting Information). Significant differences ($\alpha = 0.05$; univariate ANOVA) in the amount of As^{III} oxidized as a function of P concentration were minimal at the conclusion of each experiment. These results suggested that the presence of phosphate had little apparent effect on the oxidation process. Sterile control trials showed average abiotic oxidation of 1.3%.

Effects of phosphate on As^V reduction and cell growth in the presence of high arsenic

Isolates were subjected to additional experiments in the presence of either 1000 μM As^V or As^{III} (for reducing- or oxidizing-isolates, respectively) plus 50 μM phosphate (P:As = 0.05) to query each organism's ability to transform significant levels of As. All As-oxidizers (Fig. 2, Supporting Information) and As-reducers (Fig. 2), except the *Bacillus* str. S18, continued to exhibit their expected As-phenotype at this elevated As concentration. However, a lag in As^V reduction (Fig. 2) or As^{III} oxidation (Fig. 2, Supporting Information) was noted when plotted as a percent of As^{Total}. The actual maximum rates of As^V reduction in the 50 μM P:1000 μM As experiments for the *Agrobacterium*, *Arthrobacter*, and *Bacillus* strains were 4.46×10^{-11}, 1.64×10^{-11}, and 1.75×10^{-9} mmol As^V reduced h^{-1} $cell^{-1}$, respectively, and each was higher than the maximum rate of reduction calculated in the baseline experiments.

A 10–82% decrease in the cell growth of As-reducers (Fig. 3) and As-oxidizers (Fig. 3, Supporting Information) was observed in experiments containing 1000 μM As. However, it is noteworthy that all isolates, with the exception of *Bacillus* str. S18, remained surprisingly vigorous despite this extreme As pressure. To determine if increased P concentration would ameliorate high As-induced reduction in cell growth, the As^V-reducing bacteria were subjected to an additional treatment: 1000 μM As and 1000 μM P. This high P treatment did enhance growth rates in all isolates and appeared to have the greatest effect on the growth of *Bacillus* str. S18. This organism was clearly sensitive to As^V at concentrations of 1000 μM; however, the addition of 1000 μM P appeared sufficient to alleviate this toxicity (Fig. 3). Increased phosphate concentrations of 1000 μM also successfully inhibited As^V reduction for all three As^V-reducing isolates (Fig. 1).

Possible mechanisms controlling phosphate inhibition of arsenate reduction

The inhibition of As^V reduction by phosphate could be the result of several possible factors. One of the hypotheses tested was that increased P:As^V causes As^V-reducing isolates to down-regulate the production of ArsC at, or prior to transcription. However, an analysis of *arsC* mRNA using gene-specific PCR primers revealed that high P:As^V failed to inhibit transcription of *arsC* in *Agrobacterium* str. 5B, even when a high absolute P concentration (1000 μM) was used (Fig. 4). A similar result was reported in a previous study (Saltikov et al. 2005), where three different P:As^V ratios (0.02, 0.12, and 1) failed to reduce *arsC* expression in *Shewanella* sp. str. ANA-3.

In the current study, expression of the arsenate reductase gene was not observed at T_0, suggesting that either *arsC* is not a consti-

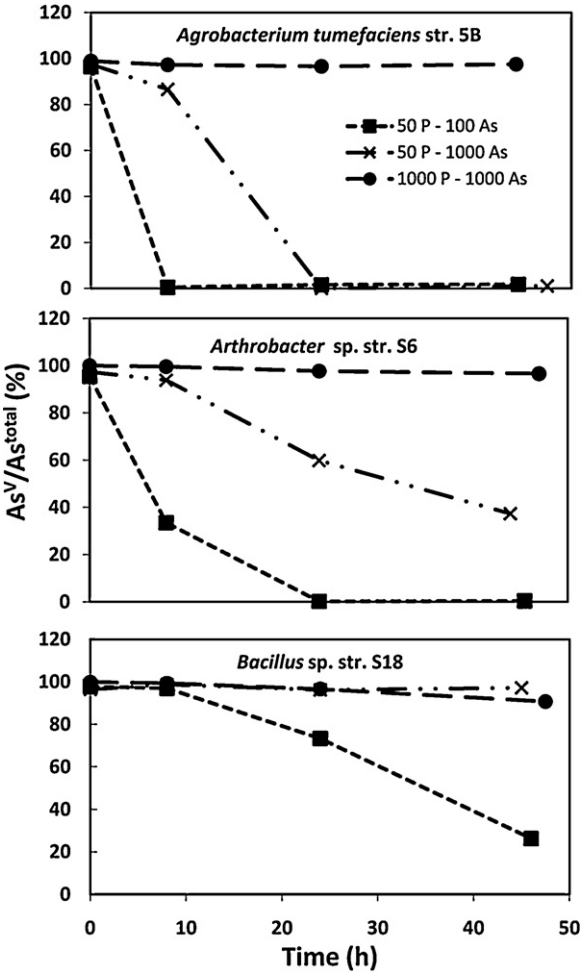

Fig. 2. Reduction of As^V by three As^V-reducing isolates – *Agrobacterium tumefaciens* str. 5B, *Arthrobacter* sp. str. S6, and *Bacillus* sp. str. S18 as a function of AsV and P concentrations, including high levels of As^V (1000 μM) and phosphate (1000 μM). The 0.5 ratio study (50 μM P:100 μM As) serves as each organism's baseline reduction trend. Error bars indicate standard deviation of duplicate cultures; when apparently absent, error bars are contained within the symbol. Legend values in μM.

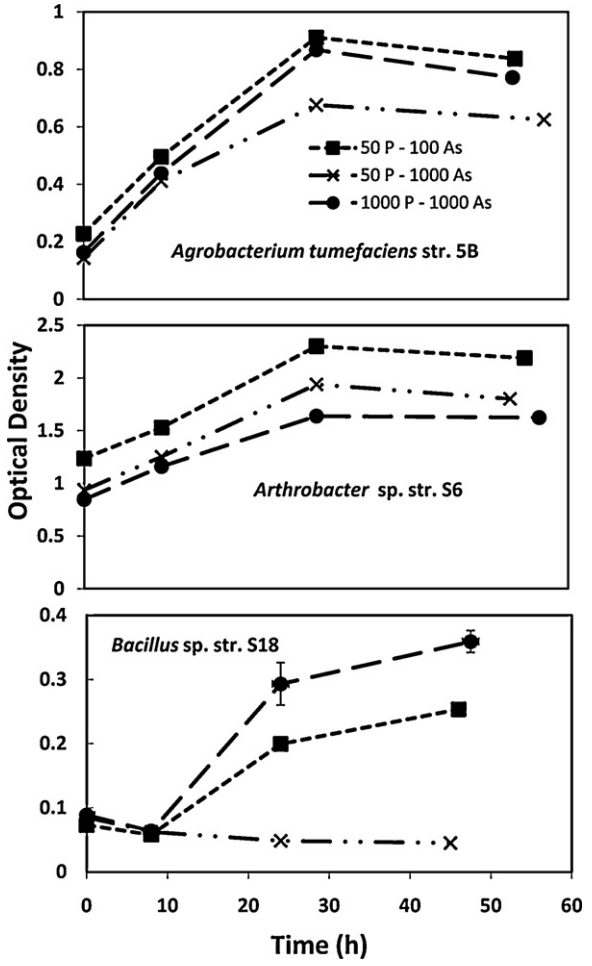

Fig. 3. Cell growth of As^V-reducing isolates subjected to three treatments – 50 μM P:100 μM As, 50 μM P:1000 μM As, and 1000 μM P:1000 μM As. Error bars indicate standard deviation of duplicate cultures; when apparently absent, error bars are contained within the symbol. Legend values in μM.

tutively expressed gene in this organism or that the concentration of ArsC mRNA in the cells was below detection using this protocol. However, expression was observed at the 8- and 24-h time-points for both the 50 μM P:100 μM As and 1000 μM P:100 μM As experiments, despite the fact that essentially no As^V reduction was observed in the latter experiment. These results suggest that transcription of the isolate's *arsC* gene is not substantially inhibited by either high P to As^V ratios or a high P concentration and that the decreased reduction of As^V observed in the high P:As^V experiments (ratios of 5 and 10) was the result of some other mechanism, such as competitive inhibition at the enzymatic binding site during the uptake and or the reduction step.

Enzyme kinetics

The inhibitory effect of phosphate in the P:As^V experiments with *A. tumefaciens* str. 5B, *Arthrobacter* sp. str. S6, and *Bacillus* sp. str. S18 was modeled using Michaelis–Menten enzyme kinetic expressions. The maximum velocity of As^V reduction (V_{MAX}) and the Michaelis constant (K_M) for each isolate were estimated using Eq. (1), which was optimized with MicroSoft Excel® Solver optimization software. Input values included As^V-reduction velocities (v, in mmol As^V h^{-1} $cell^{-1}$) and corresponding As^V concentrations (S_1, in mM) from the four P:As^V experiments that used an initial P concentration of 50 μM (Table 1):

$$v = \frac{V_{MAX}}{1 + (K_M/[S_1])} \quad (1)$$

The predicted V_{MAX} and K_M values for each isolate were then used to estimate the phosphate inhibition constant (K_i, in mM) in Eq. (2). Input values included P concentrations (I, in mM) for the three P:As experiments that used an initial As^V concentration of 100 μM (S_2, in mM; Table 1):

$$v = \frac{V_{MAX} \cdot [S_2]}{K_M(1 + ([I]/K_i)) + [S_2]} \quad (2)$$

The results of this modeling effort (Table 2) confirm that phosphate is a strong competitive inhibitor of As^V reduction in *Agrobacterium* str. 5B (K_i = 0.024 mM), *Arthrobacter* str. S6 (K_i = 0.090 mM), and *Bacillus* str. S18 (K_i = 0.105 mM). Although this data supports the idea that phosphate acts as a competitive inhibitor of As^V reduction in these As^V-reducing isolates, it does not specify whether inhibition occurs during phosphate transport or As^V reduction. A previous study has documented phosphate's ability to competitively inhibit As^V reduction by the ArsC in *E. coli* (Gladysheva et al. 1994); in that case however, P was shown to only weakly inhibit ArsC activity (inhibition constant, K_i = 30 mM). It should be noted however, that direct comparison of the kinetic parameters presented here and those reported by Gladysheva et al.

Table 2
Calculated kinetic values for three known As^V-reducing isolates (*Agrobacterium* str. 5B, *Arthrobacter* str. S6, and *Bacillus* str. S18) based on Michaelis–Menten modeling. Calculated parameters included maximum As reduction velocity (V_{MAX}), Michaelis constant (K_M), and phosphate inhibition constant (K_i).

Isolate	V_{MAX} (mmol h^{-1} $cell^{-1}$)	K_M (mM)	R^2 (%)	K_i (mM)	R^2 (%)
Agrobacterium str. 5B	5.0×10^{-11}	0.055	99.9	0.024	97.1
Arthrobacter str. S6	1.9×10^{-11}	0.070	99.0	0.090	88.7
Bacillus str. S18	1.75×10^{-9}	0.075	98.8	0.105	88.7

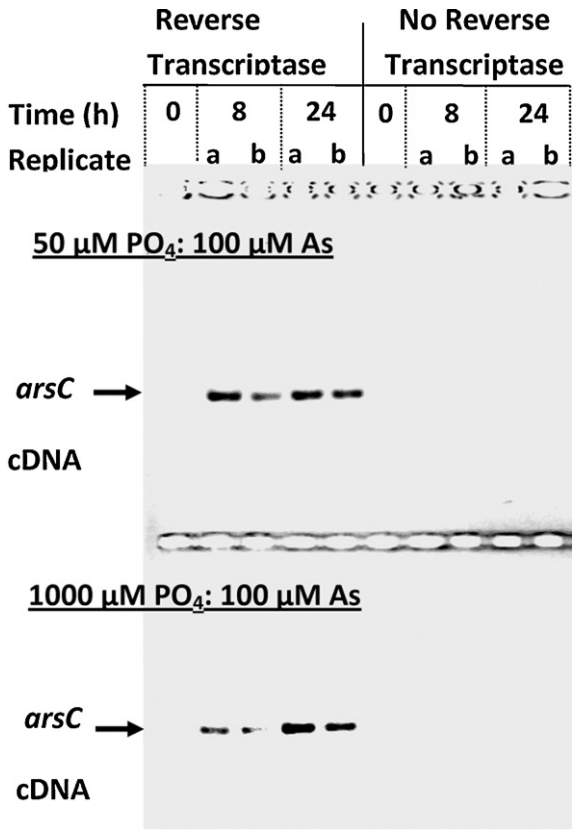

Fig. 4. Expression of the arsenate reductase gene, *arsC*, in *Agrobacterium tumefaciens* str. 5B as a function of time (0–24 h) and phosphate concentration (50 and 1000 μM).

(1994) are not possible because the parameters presented here are based on cell numbers and not on enzyme concentrations. Furthermore, since enzyme concentrations were not measured in this study, direct conversion from a per cell rate to a specific enzymatic rate is not possible.

Conclusions

High phosphate concentrations were shown to inhibit the microbial reduction of As^V to the more toxic As^{III} form both empirically and through Michaelis–Menten enzyme kinetic modeling. Conversely, P:As ratios had no effect on the microbial oxidation of arsenite by known As^{III}-oxidizing organisms. The absolute concentration of P was important as well as the ratio of P to As^V. It appeared that for the three As^V-reducing isolates, a P concentration threshold must be exceeded to effectively inhibit reduction; based on the current study, this threshold is between 50 and 500 μM. The inhibition of As^V reduction observed in this study was not a result of inhibited arsenate reductase expression. Rather, a decreased amount of As^V was microbially reduced to As^{III} via the *ars* operon because the elevated amount of phosphate ions present in the media (i) allowed less As^V to be taken into the cells due to competition for phosphate membrane transporters and or (ii) prevented As^V from binding to the ArsC active site.

All five isolates included in this study showed decreased growth and As-transforming capability when grown under high As conditions (1000 μM), though the *Arthrobacter* sp. str. S6 appeared to be the least affected. With the addition of a high P concentration (1000 μM), cell growth was enhanced and As^V reduction was inhibited in the As^V-reducing isolates, likely by limiting cell exposure to high As^V through competition for cell uptake.

The findings of this study add justification to the theory that phosphate concentration in soils or natural waters may be an important parameter indirectly limiting the formation of As^{III} via microbial processes. A better understanding of the factors that control formation of the more toxic, mobile, and bioavailable As^{III} form in soils and natural waters has implications for reducing chronic human As poisoning, increasing compliance with the US drinking water regulation for As, and for bioremediation of As-contaminated lands.

Acknowledgments

This research was supported in part by the United States Department of Agriculture (NRICGP no. 2002-35107-12268), the National Science Foundation (Graduate Research Program Fellowship), and the Montana Agricultural Experiment Station (911398).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.micres.2011.05.007.

References

Cullen WR, Reimer KJ. Arsenic speciation in the environment. Chem Rev 1989;89:713–64.
Gladysheva TB, Oden KL, Rosen BP. Properties of the arsenate reductase of plasmid R773. Biochemistry 1994;33:7288–93.
Harold FM, Baarda JR. Interaction of arsenate with phosphate-transport systems in wild-type and mutant *Streptococcus faecalis*. J Bacteriol 1966;91:2257–62.
Harvey CF, Swartz CH, Badruzzaman ABM, Keon-Blute N, Yu W, Ashraf Ali M, et al. Arsenic mobility and groundwater extraction in Bangladesh. Science 2002;298:1602–6.
Inskeep WP, McDermott TR, Fendorf S. Arsenic (V)/(III) cycling in soils and natural waters: chemical and microbiological processes. In: Frankenberger WT, editor. Environmental chemistry of arsenic. New York: Marcel Dekker; 2002. p. 183–215.
Jackson CR, Dugas SL. Phylogenetic analysis of bacterial and archaeal *arsC* sequences suggests an ancient, common origin for arsenate reductase. BMC Evol Biol 2003;3:18–25.
Ji G, Garber EAE, Armes LG, Chen CM, Fuchs JA, Silver S. Arsenate reductase of *Staphylococcus aureus* plasmid pI258. Biochemistry 1994;33:7294–9.
Macur RE, Jackson CR, Botero LM, McDermott TR, Inskeep WP. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. Environ Sci Technol 2004;38:104–11.
Macur RE, Wheeler JT, McDermott TR, Inskeep WP. Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. Environ Sci Technol 2001;35:3676–82.
Masscheleyn PH, Delaune RD, Patrick Jr WH. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. Environ Sci Technol 1991;25:1414–29.
Masur DC. Microbial and geochemical processes controlling the oxidation and reduction of arsenic in soils. Masters thesis. Montana State University-Bozeman; 2007.
Mukhopadhyay R, Rosen BP, Phung LT, Silver S. Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol Rev 2002;26:311–25.
Mukhopadhyay R, Shi J, Rosen BP. Purification and characterization of Acr2p, the *Saccharomyces cerevisiae* arsenate reductase. J Biol Chem 2000;275:21149–57.

Newman DK, Beveridge TJ, Morel FM. Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*. Appl Environ Microbiol 1997;63:2022–8.
Nriagu JO, editor. Arsenic in the environment, Part II: Human health and ecosystem effects. New York, NY: John Wiley and Sons, Inc.; 1994.
Oremland RS, Stolz JF. The ecology of arsenic. Science 2003;300:939–43.
Pierce ML, Moore CB. Adsorption of arsenite and arsenate on amorphous iron hydroxide. Water Res 1982;16:1247–53.
Planer-Friedrich B, London J, McCleskey RB, Nordstrom DK, Wallschlager D. Thioarsenates in geothermal waters of Yellowstone National Park: determination, preservation, and geochemical importance. Environ Sci Technol 2007;41:5245–51.
Rochette EA, Bostick BC, Li G, Fendorf S. Kinetics of arsenate reduction by dissolved sulfide. Environ Sci Technol 2000;34:4714–20.
Rosen BP. Biochemistry of arsenic detoxification. FEBS Lett 2002;529:86–92.
Rothstein A, Donovan K. Interactions of arsenate with the phosphate-transporting system of yeast. J Gen Physiol 1963;46:1075–85.
Saltikov CW, Wildman RA, Newman DK. Expression dynamics of arsenic respiration and detoxification in *Shewanella* sp. strain ANA-3. J Bacteriol 2005;187:7390–6.
Silver S, Phung LT. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol 2005;71:599–608.
Thiel T. Phosphate transport and arsenate resistance in the cyanobacterium *Anabaena variabilis*. J Bacteriol 1988;170:1143–7.
Willsky GR, Malamy MH. Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. J Bacteriol 1980;144:356–65.
Xu H, Allard B, Grimvall A. Effects of acidification and natural organic materials on the mobility of arsenic in the environment. Water Air Soil Pollut 1991;57:269–78.
Zhou Y, Messier N, Ouellette M, Rosen BP, Mukhopadhyay R. *Leishmania major* LmACR2 is a pentavalent antimony reductase that confers sensitivity to the drug pentostam. J Biol Chem 2004;279:37445–51.