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Biosensor for Organoarsenical Herbicides and Growth Promoters

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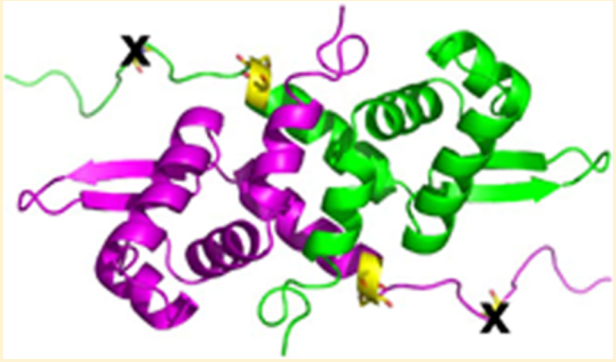
Supporting Information

ABSTRACT: The toxic metalloid arsenic is widely distributed in food, water, and soil. While inorganic arsenic enters the environment primarily from geochemical sources, methylarsenicals either result from microbial biotransformation of inorganic arsenic or are introduced anthropogenically. Methylarsenicals such as monosodium methylarsonic acid (MSMA) have been extensively utilized as herbicides, and aromatic arsenicals such as roxarsone (Rox) are used as growth promoters for poultry and swine. Organoarsenicals are degraded to inorganic arsenic. The toxicological effects of arsenicals depend on their oxidation state, chemical composition, and bioavailability. Here we report that the active forms are the trivalent arsenic-containing species. We constructed a whole-cell biosensor utilizing a modified ArsR repressor that is highly selective toward trivalent methyl and aromatic arsenicals, with essentially no response to inorganic arsenic. The biosensor was adapted for *in vitro* detection of organoarsenicals using fluorescence anisotropy of ArsR−DNA interactions. It detects bacterial biomethylation of inorganic arsenite both *in vivo* and *in vitro* with detection limits of 10^{-7} M and linearity to 10^{-6} M for phenylarsenite and 5×10^{-6} M for methylarsenite. The biosensor detects reduced forms of MSMA and roxarsone and offers a practical, low cost method for detecting activate forms and breakdown products of organoarsenical herbicides and growth promoters.



INTRODUCTION

Arsenic is a ubiquitous environmental carcinogen that comes from both geochemical and anthropogenic sources. It has been linked to multiple health problems, including skin cancer, bladder cancer, diabetes, and cardiovascular and peripheral vascular diseases.[1,2] Consequently, the U.S. Environmetal Protection Agency (EPA) ranks arsenic first on its Superfund List of Hazardous Substances (http://www.atsdr.cdc.gov/cercla/07list.html).

Inorganic arsenic, which is pervasive in the environment from geochemical origins such as volcanoes and hot springs can be either pentavalent (arsenate (As(V)) or trivalent (arsenite (As(III)). Biological activities result in incorporation of arsenic into organic molecules such as arsenobetaine, arsenosugars and arsenolipids, which are found in many marine organisms.[3] Arsenic methylation also contributes to the arsenic biogeocycle.[4] Microbial methylation, catalyzed by ArsM As(III) S-adenosylmethionine methyltransferases, detoxifies inorganic arsenic, producing a variety of less toxic species including MAs(V).[5−8] In addition to biogenesis of methylated arsenicals, MAs(V) is also used as the herbicide MSMA. Approximately 1 360 000 kg (3 000 000 lbs) of MSMA are in commercial use in the USA. Its use has been banned by the EPA after December 31, 2013 except for treatment of cotton because the EPA does not anticipate that arsenic in cotton will end up in the food supply.[9] This may be an erroneous assumption since the herbicide can be degraded by microbial activity to MAs(III) and As(III), both of which are more toxic and carcinogenic than MSMA.[10]

Aromatic arsenicals are also used in animal husbandry to prevent bacterial infections and for growth promotion. For example, derivatives of the pentavalent phenylarsonic acid (PhAs(V)) such as 3-nitro-4-hydroxybenzenearsonic acid (Rox(V)), *p*-arsanilic acid, 4-nitrophenylarsonic acid, and *p*-ureidophenylarsonic acid are all used as additives for animal

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feed due to their antimicrobial properties. Roxarsone is degraded to 4-hydroxy-3-aminophenylarsonic acid [11] and eventually to inorganic arsenic.[12]

The objective of this study was to develop a biosensor that could specifically sense the reduced forms of MSMA (MAs(III)) and roxarsone (Rox(III)) without interference from inorganic arsenic. We demonstrate here that MAs(III) and Rox(III) are the active forms of the herbicide and antimicrobial growth promoter and are also obligatory intermediates in their breakdown, so the ability to sense the trivalent species is important to understanding their environmental impact. Current detection methods for total organic arsenicals in biological samples involve oxidative digestion of the organic matrix into inorganic arsenic, which is quantified by analytical laboratory techniques such as inductively coupled mass spectroscopy (ICP−MS). These laboratory-based spectroscopic methods are time-consuming, costly, and require skillful operators. Commercial chemical field test kits are used in countries such as Bangladesh and India with varying degrees of success.[13] The principle of these kits is the formation of volatile arsine gas (AsH_3) to separate arsenic from the aqueous matrix and subsequent colorimetric detection on a paper strip.[14] However, these test kits have low precision, poor reproducibility, high rates of false positives and negatives, and accuracy is limited to concentrations between the EPA maximum containment level and World Health Organization (WHO) maximum allowable concentration for arsenic in drinking water of 10 ppb (0.13 μM) up to 100 ppb (1.33 μM).[15] Most critically, these methods cannot distinguish between inorganic and organic arsenic species.

Whole-cell bacterial biosensors have been proposed as an alternative, rapid, cost-effective, and high-throughput method to measure arsenic in aquatic samples.[16,17] Bacterial biosensors rely on the ability of cells to produce a detectable signal that can serve as a reporter of a particular environmental condition. Most reported arsenic biosensors utilize the As(III)-responsive transcriptional repressor ArsR, first described from our laboratory,[18,19] or other members of the ArsR family to control expression of reporter genes or electrochemical responses.[20−22] ArsR binds to the *ars* operator/promoter, preventing transcription of the *ars* genes for arsenic detoxification. When bacterial cells are exposed to environmental As(III) or Sb(III), ArsR binds the trivalent metalloid, which produces a conformational change that results in dissociation of the repressor from the DNA, and, in turn results in expression of the genes driven by the *ars* promoter.

However, currently available bacterial whole-cell biosensors detect only trivalent inorganic arsenic. We have previously shown that PhAs(III) induces expression of the *ars* operon of *E. coli* [23] by binding to the ArsR repressor,[24] suggesting that ArsR might be suitable for construction of an organoarsenical biosensor. This observation formed the basis for construction of a two-plasmid biosensor with selectivity for PhAs(III) = Rox(III) > MAs(III) > As(III) using the AfArsR of *Acidithiobacillus ferrooxidans* [25] to control *gfp* expression.[26] Purified *A. ferrooxidans* AfArsR binds to the *ars* operator/promoter. The binding of As(III) to AfArsR residues Cys95, Cys96, and Cys102 is predicted to produce a conformational change in the C-terminal dimerization domain helix that results in its dissociation from the *arsO* operator/promoter. When Cys102 was changed to serine, the repressor still bound to DNA but exhibited significantly reduced response to inorganic As(III).[25] Here the results of *in vitro* DNA binding assays and *in vivo gfp* expression assays with AfArsR C102S demonstrate that the mutant repressor exhibits greatly reduced binding of inorganic As(III) without affecting binding of organoarsenicals. In addition, the biosensor strain could be used for *in situ* detection of methylation activity when grown in a mixed culture with a strain of *E. coli* expressing an *arsM* gene for As(III) methylation. In this mixed culture, the biosensor was responsive to As(III), demonstrating that methylation activity can be sensed. Finally, the biosensor strain can detect MSMA reduction *in situ*. The biosensor strain was incubated with MSMA in a mixed culture with *Burkholderia* sp. MR1, which reduces MAs(V) to MAs(III).[10] In coculture, the biosensor strain became responsive to MAs(V), which indicates that it sensed the reduction of MSMA to MAs(III) by *Burkholderia* sp. MR1 *in situ*. Thus this genetically engineered biosensor may prove useful in detecting environmental biotransformation of arsenical herbicides and animal growth promoters.

EXPERIMENTAL PROCEDURES

Strain, Plasmids, Medium, and Reagents. Unless otherwise noted, cells of *E. coli* were grown aerobically in Luria−Bertani (LB) medium [27] at 37 °C supplemented with 100 μg/mL ampicillin, 12.5 μg/mL tetracycline, or 34 μg/mL chloramphenicol, as required. The biosensor strain consisted of *E. coli* AW3110(DE3) (Δ*arsRBC*) [28] bearing two plasmids, pACYC184-p*arsO-gfp* and pBAD*arsR* (either wild type or C102S *afarsR* genes).[25,26] Bacterial growth was monitored by measuring turbidity at A_{600} nm. For resistance assays, 10^8 cells of the indicated bacteria were spread onto solid agar plates with M9 medium with 0.2% glucose [27] and allowed to dry. Sterile filter disks were applied to the surface, 10 μL of each organoarsenical was applied to the disks, and the plates were incubated overnight at 37 °C. All trivalent organoarsenicals were stable during this period of time (data not shown). All reagents were obtained from commercial sources. PhAs(III) (phenylarsine oxide) was obtained from Sigma-Aldrich, St. Louis, MO. The herbicide MSMA (Target 6.6) was a generous gift from Luxembourg-Pamol, Inc., Houston, TX. MSMA and Rox were reduced by the method of Reay and Asher.[29]

***In Vitro* Biosensor Assays.** Wild type AfArsR and the C102S derivative were purified from cells of *E. coli* strain Top10 (Invitrogen, Grand Island, NY) bearing pBAD*arsR* plasmids, as described previously.[25] AfArsR binding to DNA and dissociation by inducers was assayed by fluorescence anisotropy using a Photon Technology International spectrofluorometer fitted with polarizers in the L format.[25] Changes in anisotropy were calculated after each addition using the supplied Felix32 software. Complementary 30-mer oligonucleotides, one of which was labeled at the 5′ end with fluorescein, were synthesized (Integrated DNA Technologies, Inc., Coralville, IA) with the following sequences: 6-FAM-5′-ATCCACGAA-TATTTCTTGCAGTATTGACAA-3′ and 5-TAGGTGCTTA-TAAAGAACGTCATAACTGTT-3′.[25] Desalted DNA was annealed at 94 °C for 5 min, cooled to room temperature, and stored in aliquots at −20 °C. Anisotropy measurements were performed in a buffer consisting of 10 mM MOPS-NaOH, pH7.5, containing 0.1 M NaCl, 15% glycerol, and 250 nM DNA. AfArsR was titrated with 50 nM fluorescein-labeled *arsO* DNA and metalated by mixing 1 mol equiv of AfArsR with the indicated concentrations of inorganic As(III) or organoarsenicals. All solutions were deaerated by bubbling with argon.

***In Vivo* Biosensor Assays.** Transcriptional activity of the biosensor was estimated from arsenical-responsive expression

of *gfp*.[26] Cultures of the biosensor (*E. coli* strain AW3110 bearing plasmids pBAD*arsR* and pACYC184-p*arsO-gfp*) were grown to midexponential phase in LB medium at 37 °C with 100 μg/mL ampicillin and 34 μg/mL chloramphenicol with shaking. Glucose (0.2%) was added for constitutive expression of *gfp*. Wild type or C102S *afArsR* gene was induced by addition of the indicated concentrations of arabinose for 5 h. Derepression was produced by the simultaneous addition of arabinose and arsenicals for 5 h. The trivalent organoarsenicals were stable to oxidation over this period of time. Cell densities were normalized by dilution or suspension to the same A_{600} nm, and expression of *gfp* was assayed from the fluorescence of cells using a Photon Technology International spectrofluorometer with an excitation wavelength of 470 nm and emission wavelength of 510 nm. In Figure 4C the cells were not normalized to a constant cell density because the organoarsenicals inhibited growth at elevated concentrations. Qualitative *gfp* expression was visualized at 365 nm with a hand-held ultraviolet lamp.

For *in situ* methylation of As(III), the biosensor strain was cocultured with *E. coli* AW3110(DE3) pET28C*rarsM*, which expressed the *Chlamydomonas reinhardtii* As(III)-S-adenosylmethionine methyltransferase *arsM* gene encoding the CrArsM As(III) S-adenosylmethionine (SAM) methyltransferase.[30] For *in situ* reduction of MAs(V), the biosensor strain was cocultured with *Burkholderia* sp. MR1 [10] in M9 medium with 0.2% glucose at 23 °C with shaking. The ratio of inoculation size of *Burkholderia* to *E. coli* was 1.5. *Burkholderia* grows more slowly than *E. coli*, so following growth, the population sizes were approximately equal.

RESULTS AND DISCUSSION

The Active Forms of the Herbicide MSMA and the Antimicrobial Growth Promoter Roxarsone Contain Trivalent Arsenic. Both the herbicide MSMA and the antimicrobial poultry growth promoter roxarsone are pentavalent organic arsenicals. These soluble pentavalent arsenicals are more stable to atmospheric oxidation than trivalent arsenicals, which is why they can be successfully applied in agriculture and animal husbandry. In the cytosol of most animal, plant and bacterial cells, inorganic As(V) is reduced to the more toxic As(III).[31−33]

However, neither MSMA (Figure 1A) nor the aromatic arsenicals PhAs(V) (Figure 1B) and Rox(V) (Figure 1C) are toxic to a variety of bacteria, including *Eschericha coli* (Figure 1), and environmental isolates of *Bacillus* and *Burkholderia* (data not shown). This indicates that those pentavalent organoarsenicals are not the true pesticides or antimicrobial growth promoters but must first be reduced. We hypothesize that trivalent organoarsenical species are the bioactive forms of the herbicide and antimicrobial growth promoters. To test that hypothesis, the effect of the reduced organoarsenical on growth of a variety of microbes was examined. PhAs(III) which was purchased from Sigma Chemical Co. as the reduced species, and MSMA and Rox(V) were chemically reduced. The reduced species MAs(III) (Figure 1A), PhAs(III) (Figure 1B) and Rox(III) (Figure 1C and Supporting Information, Figure S1) were shown to be toxic at micromolar concentrations to *E. coli* (Figure 1) and the environmental isolates (data not shown). Although no additional species were observed on high performance liquid chromatography (HPLC), it is possible that reduction produces highly toxic minor species even though in control assays the reducing agent alone had no effect on growth. While theses studies were performed in bacteria, MSMA is taken up by plant roots, where the low redox potential the cytosol results in its reduction to more toxic MAs(III).[34]

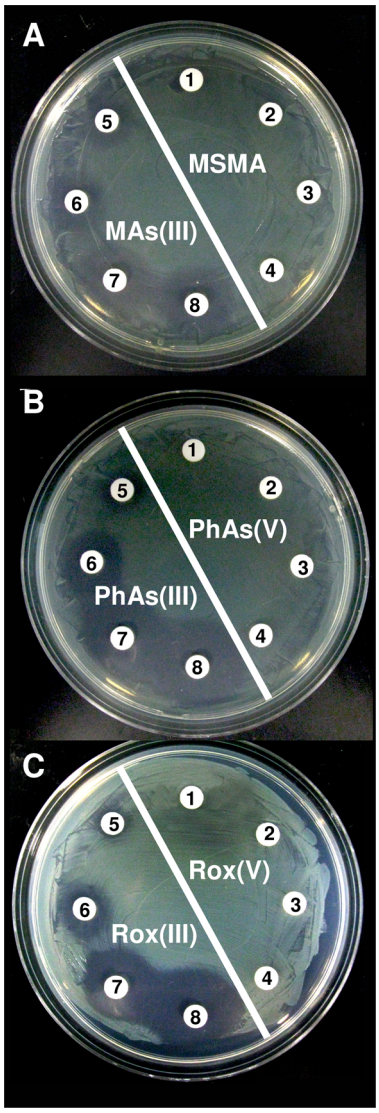

Figure 1. Reduced MSMA and roxarsone are the toxic species. The effects of pentavalent and trivalent organoarsenicals on the growth of *E. coli* AW3110 grown in M9 medium overnight at 37 °C were compared as described under Experimental Procedures. (A) 1, no addition; 2, 20 μM MSMA; 3, 30 μM MSMA; 4, 40 μM MSMA; 5, 10 μM MAs(III); 6, 20 μM MAs(III); 7, 30 μM MAs(III); 8, 40 μM MAs(III). (B) 1, no addition; 2, 5 μM PhAs(V); 3, 10 μM PhAs(V); 4, 15 μM PhAs(V); 5, 2 μM PhAs(III); 6, 5 μM PhAs(III); 7, 10 μM PhAs(III); 8, 15 μM PhAs(III). (C) 1, no addition; 2, 5 μM Rox(V); 3, 10 μM Rox(V); 4, 15 μM Rox(V); 5, 2 μM Rox(III); 6, 5 μM Rox(III); 7, 10 μM Rox(III); 8, 15 μM Rox(III).

AfArsR C102S Responds *in Vitro* Selectively to Trivalent Organoarsenicals. Biotransformation is essential for degradation of these organoarsenical herbicides and antimicrobials, where trivalent species are formed prior to breakdown into inorganic arsenic.[10,12] There are no current methods for monitoring these environmentally pervasive organic arsenic species in the field. Our initial cell-based biosensor exhibited high selectivity to organic over inorganic trivalent arsenicals (PhAs(III) = Rox(III) > MAs(III) >

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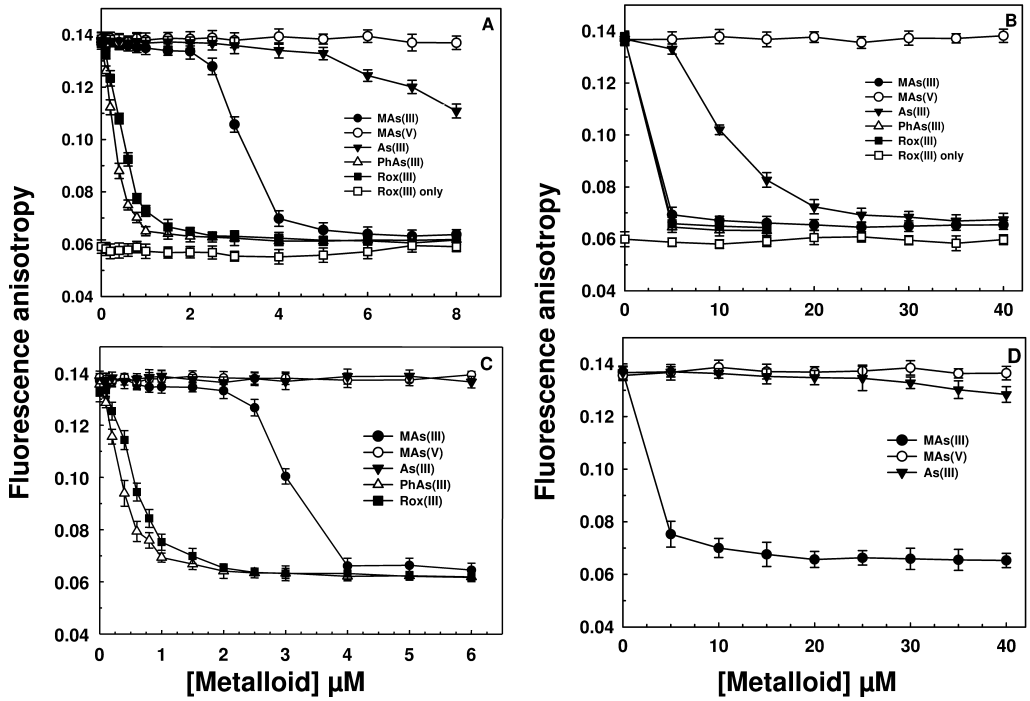

Figure 2. Effect of arsenicals on AfArsR binding to DNA. DNA binding assays were performed as described under Experimental Procedures. (A, B, C, and D) AfArsR dissociation from the DNA was induced by addition of the indicated concentrations of arsenicals: (●) MAs(III); (▼) As(III); (○) MAs(V); (△) PAO; (■) Rox(III). As controls, the effect of each arsenical on the anisotropy of the fluorescently labeled DNA was measured in the absence of AfArsR, shown only for (□), Rox(III). The data are the mean ± SE (n = 3).

As(III)).[26] Although the biosensor senses nanomolar concentrations of aromatic arsenicals, it still responds to micromolar concentrations of inorganic As(III). The basis of sensing is the binding of trivalent arsenic to AfArsR cysteine residues Cys95, Cys96, and Cys102, which releases the repressor from *ars* operator/promoter DNA (Supporting Information, Figure S2A).[25] We previously observed that substitution of Cys102 with a serine residue reduced affinity for As(III) by nearly an order of magnitude without affecting binding to DNA. From the results of X-ray absorption spectroscopy, high affinity binding to wild type AfArsR was shown to involve three sulfur ligands (Supporting Information, Figure S2B), while only two-coordinate binding was observed with the C102S derivative (Supporting Information, Figure S2C). Since the binding of MAs(III), PhAs(III), or Rox(III) would be expected to require only two sulfur ligands, we considered the possibility that a biosensor with C102S AfArsR would respond with high affinity to trivalent organoarsenicals but not inorganic As(III). If so, the mutant could form the basis of a highly selective organoarsenic biosensor.

The binding of wild type AfArsR to DNA and dissociation upon binding of arsenicals was assayed by fluorescence anisotropy.[25] In this assay, when AfArsR binds to fluorescein-labeled *ars* operator/promoter DNA, the rotation of the larger protein−DNA complex is slower than the rotation of free DNA, producing an increase in anisotropy. Anisotropy increased as a function of the concentration of wild type AfArsR, with a K_d value of 0.9 μM (Supporting Information, Figure S3). The effects of the addition of As(III), MAs(V), MAs(III), PhAs(III), and Rox(III) on the dissociation of wild type AfArsR were compared (Figure 2A and B). There was no effect of MAs(V) (Figure 2B) (or inorganic As(V); data not shown) on anisotropy of the wild type AfArsR−DNA complex. MAs(III) decreased anisotropy with a half-maximal concentration of approximately 2.5 μM (Figure 2A), while approximately 10 μM As(III) (Figure 2B) was required, a 4-fold difference in affinity. PhAs(III) and Rox(III) decreased anisotropy with a half-maximal concentration of approximately 0.3 μM for each (Figure 2A). These results demonstrate that the PhAs(III), Rox(III), or MAs(III) bind to AfArsR and effect dissociation from DNA in the order PhAs(III) = Rox(III) > MAs(III) > As(III).

The binding properties of the C102S derivative were examined (Figure 2C,D). In contrast to wild type AfArsR, C102S exhibited little response to As(III) up to 40 μM (Figure 2C,D). The response to MAs(III), Rox(III), and PhAs(III) was slightly higher than the wild type but still in the submicromolar range (Figure 2C compared with Figure 2A). These results demonstrate that AfArsR could be engineered for increased selectivity for organoarsenicals by substitution of Cys102.

A C102S Mutant Biosensor Loses the Ability to Respond to Inorganic As(III). We have previously reported construction of a two-plasmid biosensor utilizing wild type AfArsR.[26] The objective of this study was to modify wild type AfArsR so that it would no longer respond to inorganic As(III). Since purified AfArsR C102S was shown to exhibit increased selectivity for trivalent organoarsenicals over inorganic As(III), a similar biosensor was constructed with the mutated gene for C102S. The biosensors incorporate either wild type or mutant *afarsR* gene under control of the arabinose promoter in one plasmid, pBAD*arsR*, and the *A. ferrooxidans ars* operon promoter (p_{ars}) controlling expression of *gfp* on a second plasmid, pACYC184-*parsO-gfp*. As the concentration of arabinose is increased, AraC becomes a positive regulatory protein that drives expression of *AfarsR*, and consequently expression of *gfp* from pACYC184-*parsO-gfp* is decreased. To

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increase sensitivity, the arsenic-hypersensitive *E. coli* strain AW3110 (*ars:cam*), which is unable to extrude As(III),[28] was utilized as host for the plasmids. Expression of *gfp* was visibly constitutive when either mutant or wild type *afarsR* was repressed by growth on glucose (Supporting Information, Figure S4, lanes 1 and 4) and could be quantified by spectrofluorometry (Figure 3, lanes 1 and 4). When the repressors were induced by addition of 0.2% arabinose, *gfp* was repressed, and the cells were not fluorescent (Supporting Information, Figure S4 and Figure 3, lanes 2 and 5). The biosensor with wild type *afarsR* exhibited derepressed *gfp* expression upon addition of 20 μM sodium arsenite (Supporting Information, Figure S4 and Figure 3, lanes 6). In contrast, the biosensor with the C102S mutant was not responsive to addition of arsenite (Supporting Information, Figure S4 and Figure 3, lanes 3).

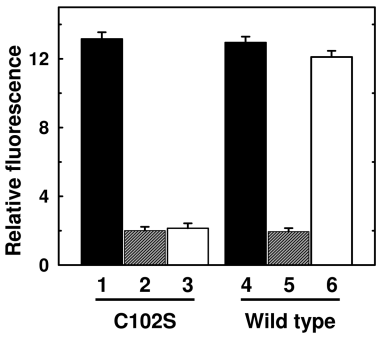

Figure 3. Comparison of the response of the wild type and C102S biosensor to As(III). Expression of the *gfp* reporter gene was assayed as described under Experimental Procedures. Cells were grown in LB medium for 14 h. Fluorescence intensities of the cell suspensions were quantified by spectrofluorometry. (1 and 4) no arabinose; (2 and 5) 0.2% arabinose; (3 and 6) 0.2% arabinose + 20 μM inorganic As(III). The data are the mean ± SE (n = 3).

The sensitivity of the biosensor can be increased by lowering the AfArsR levels.[26] When produced in lower amounts, wild type AfArsR does not repress *gfp* expression as tightly, allowing the sensor to respond to lower concentrations of arsenical inducer. To improve the sensitivity of mutant biosensor, conditions for regulating AfArsR synthesis were examined (Figure 4). As the concentration of arabinose is increased, AraC becomes a positive regulatory protein that drives expression of *afarsR*, which consequently decreases expression of *gfp* from pACYC184-*parsO-gfp*. Wild type and mutant *afarsR* exhibited similar repression by increasing concentrations of arabinose, with 0.05% sufficient for maximal repression (data not shown). At 0.05% arabinose, the *in vivo* response of the mutant biosensor was examined at subtoxic concentrations of a trivalent arsenicals (Figure 4A). Cellular *gfp* derepression by the trivalent organoarsenicals PhAs(III), Rox(III), and MAs-(III) was proportional to the concentration of the inducer with both wild type and mutant repressor. Cells with either biosensor were unresponsive to MAs(V) and Rox(V). The difference between cells with the wild type and mutant repressor was that the wild type was induced by inorganic As(III) (Figure 3, lane 6) while the mutant did not respond to As(III) (Figure 3 and 4A, lanes 3). The response of the mutant biosensor to organoarsenicals was quantified (Figure 4B). The apparent half maximal concentrations required for induction were approximately 0.25 μM for PhAs(III) or Rox(III) and 3 μM for MAs(III). In the arsenic hypersensitive strain AW3110 the aromatic arsenicals became toxic at concentrations in excess of 2 μM, and MAs(III) was toxic at concentrations in excess of 10 μM, which accounts for the decline in *gfp* expression at higher concentrations. Of significance, the mutant biosensor exhibited no response to As(III) even at 10 μM (Figure 4A). As a proof of concept that the biosensor could be used in the field, the response to PhAs(III) was visible to the eye under ultraviolet light (Supporting Information, Figure S5A, lanes 4 and 5), These results demonstrate that the C102S biosensor is highly selective for trivalent aromatic and methylated arsenicals over inorganic arsenic in the order PhAs(III) = Rox(III) > MAs(III) ≫ As(III).

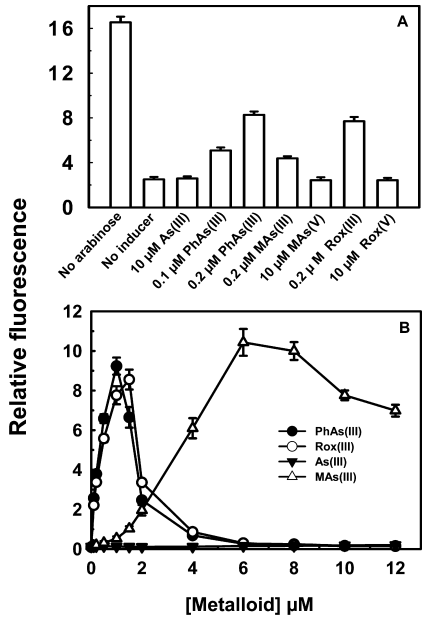

Figure 4. Comparison of the response of the bacterial biosensor to inorganic and organic arsenicals. (A) Cells were grown as described in Experimental Procedures in LB medium in the absence of arabinose or with 0.05% arabinose and the indicated concentrations of arsenicals. The concentration of inducers was adjusted to prevent toxicity, and fluorescence intensities were measured by spectrofluorometry. Column 1, no arabinose; column 2, no inducer; column 3, 10 μM As(III); column 4, 0.1 μM PhAs(III); column 5, 0.2 μM PhAs(III); column 6, 0.2 μM MAs(III); column 7, 10 μM MAs(V); column 8, 0.2 μM Rox(III); column 9, 10 μM Rox(V). (B) Cells were grown in LB medium with 0.2% arabinose and the indicated concentrations of inducers: (△) MAs(III); (●) PAO; (○) Rox(III); (▼) As(III). The data are the mean ± SE (n = 3).

Sensing MSMA Reduction in a Microbial Community. As shown above, MSMA became bioactive when reduced chemically. We propose that environmentally activation occurs either in planta or by rhizosphere bacteria. An environmental isolate was used to examine whether the biosensor can detect activation under simulated environmental conditions. MSMA (MAs(V)) is broken down environmentally in microbial communities.[10] Breakdown appears to require at least two step, (1) reduction of the pentavalent arsenic to trivalency and (2) cleavage of the As−C bond to form inorganic arsenic. Some organisms can carry out both steps,[35] while in other microbial communities individual organisms carry out individual reactions such as the environmental isolate *Burkholderia* sp. MR1 that reduces MAs(V) to MAs(III) and a *Streptomyces* isolate that demethylates MAs(III) to As(III).[10] To examine whether the

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biosensor can detect the breakdown of MSMA in a simulated microbial community, the *Burkholderia* isolate was cocultured with the *E. coli* biosensor strain in the presence and absence of MAs(V) (Supporting Information, Figure S5). Alone the biosensor strain did not reduce MAs(V), while *Burkholderia* sp. MR1 reduced MAs(V) to MAs(III), either alone (Figure S5B, curve 3) or in coculture with the biosensor strain (Figure S5B, curve 5). In coculture, MAs(V) addition resulted in *gfp* expression (Supporting Information, Figure S5B and C, column 3) that was comparable to the biosensor strain alone with MAs(III) (Figure S5C, column 8). Alone the biosensor strain does not respond to MAs(V) (Figure 2D). On the other hand, there was no response to either roxarsone or PhAs(V) even in coculture (Figure S5C, columns 4 and 5). Note that the *Burkholderia* isolate does not reduce either to their trivalent forms (data not shown), so the response of the biosensor results from *in situ* reduction of the herbicide.

Sensing As(III) Methylation. Many microbes use ArsM methyltransferases to biotransform arsenic into less toxic DMAs(V), TMAs(V), and TMAs(III) gas,[7] and these methylated species are transported to the grain more rapidly than inorganic arsenic and hence increase arsenic in the food supply.[34] We examined whether the biosensor can sense methylated arsenicals produced either *in vitro* or *in vivo*. Purified CrArsM from the common environmental alga *Chlamydomonas reinhardtii* [30] added to the AfArsR-DNA complex, and the effect of the addition of 10 μM As(III) was determined. In the absence of CrArsM, there was no decrease in fluorescence anisotropy (Supporting Information, Figure S6, lane 3). When CrArsM was added, there was a significant and enzyme-concentration dependent reduction in anisotropy (Figure S6, lanes 4 and 5). Under similar conditions, CrArsM converted As(III) into a combination of MAs(III) and DMAs(V) with a similar enzyme-concentration dependence (Supporting Information, Figure S7).

To examine for sensing methylation *in vivo*, the *C. reinhardtii arsM* gene was added to the C102S mutant biosensor (Supporting Information, Figure S8) to create an *E. coli* strain that could generate methylated arsenicals in cells that could respond by *gfp* expression. The addition of As(III) to this modified biosensor exhibited significant induction of *gfp* by As(III) (Supporting Information, Figure S9, lane 2) compared with the C102S biosensor lacking the *crarsM* gene (Supporting Information, Figure S9, lane 4). These results are a proof of concept that the C102S biosensor can detect methylated arsenicals generated environmentally. The biosensor will require optimization such as elimination of the two-plasmid system by integration of the repressor-reporter genes into the chromosome before it could be used in the field, and further development is in progress.

■ ASSOCIATED CONTENT

Supporting Information

Plasmid construction, expression, and chemical analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

As(V)	arsenate
As(III)	arsenite
MSMA	monosodium methylarsenate
MAs(V)	methylarsenate
MAs(III)	methylarsenite
DMAs(V)	dimethylarsenate
PhAs(V)	phenylarsenate
PhAs(III)	phenylarsenite
Rox(V)	roxarsone (3-nitro-4-hydroxybenzenearsonic acid)
Rox(III)	roxarsone with reduced As(III)
HPLC	high performance liquid chromatography
ICP−MS	inductively coupled mass spectroscopy
EPA	U.S. Environmental Protection Agency
WHO	World Health Organization

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