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A Novel Biosensor Selective for Organoarsenicals

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Organoarsenicals used as herbicides and growth promoters for farm animals are degraded to inorganic arsenic. Available bacterial whole-cell biosensors detect only inorganic arsenic. We report a biosensor selective for the trivalent organoarsenicals methylarsenite and phenylarsenite over inorganic arsenite. This sensor may be useful for detecting degradation of arsenic-containing herbicides and growth promoters.

Arsenic is a ubiquitous environmental carcinogen that is linked to skin cancer, bladder cancer, and diabetes, as well as cardiovascular and peripheral vascular diseases (1, 10). Consequently, it ranks first on the Superfund List of Hazardous Substances (http://www.atsdr.cdc.gov/spl/index.html). Methylarsenate [MAs(V)] is used as an herbicide; approximately 3,000,000 pounds (1,360,000 kg) are in commercial use in the United States. The herbicide is degraded by bacteria to more-toxic and -carcinogenic methylarsenite [MAs(III)] and inorganic As(III) (12). Derivatives of the aromatic arsenical phenylarsenite (PAO), such as 3-nitro-4-hydroxybenzenearsonic acid (Roxarsone), are used in animal husbandry to prevent bacterial infections and for growth promotion. 3-Nitro-4-hydroxybenzenearsonic acid is degraded to 4-hydroxy-3-aminophenylarsonic acid (4) and eventually to inorganic arsenic (9). Diphenylchloroarsine (Clark I), which was used as a chemical warfare agent in World Wars I and II, is also degraded by microbial activity (2, 6). Monitoring the prevalence of these environmentally pervasive arsenicals requires methods for detection of organic arsenic species in the field.

Reported arsenic biosensors utilize the As(III)-responsive transcriptional repressor ArsR, first described from our laboratory (8, 11), to control expression of reporter genes in response to inorganic arsenite. The objective of this study was construction of a biosensor with the capability of, first, sensing organic arsenicals and, second, exhibiting selectivity between the inorganic and organic species. A two-plasmid biosensor that utilizes the *Acidithiobacillus ferrooxidans arsR* gene (for convenience termed *AfarsR*) encoding the As(III)-responsive repressor from reference 7 to control expression of the *Aequorea victoria gfp* gene for green fluorescent protein was constructed. Plasmid pBAD*arsR* (7) has the *AfarsR* gene from *A. ferrooxidans* under the control of the arabinose promoter (see Fig. S1A in the supplemental material), and plasmid pACYC184-p*arsO-gfp* has an enhanced *gfp* reporter gene fused to the *A. ferrooxidans arsO* promoter (see Fig. S1B in the supplemental material). A dual-control design involving separate regulation of *arsR* and *arsO*::*gfp* allowed for metabolite-specific, tunable control of *gfp* expression (see below). Expression of genes from the *ara* promoter in pBAD*arsR* is regulated by AraC, the regulator of the arabinose operon (5). In the presence of glucose and the absence of arabinose, AraC is a negative regulator that represses expression of genes behind P_{ara}, in this case, of *AfarsR*, resulting in *gfp* expression (see Fig. S1C in the supplemental material). 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 increase sensitivity, the arsenic-hypersensitive *Escherichia coli* strain AW3110 (*ars*::*cam*), which is unable to extrude As(III) (3), was utilized as a host for the plasmids. Expression of *gfp* was visibly constitutive when *AfarsR* was repressed by growth on glucose (see Fig. S2 in the supplemental material) and was able to be quantified spectrofluorometrically (Fig. 1). When *AfarsR* was induced by addition of 0.2% arabinose, *gfp* was repressed and the cells were not fluorescent. Addition of 20 μM sodium arsenite dissociates AfArsR from P_{ars}, derepressing *gfp* expression. The intensity was proportional to the concentration of As(III), which makes it possible to accurately quantify the concentration of As(III) in environmental samples. The decrease

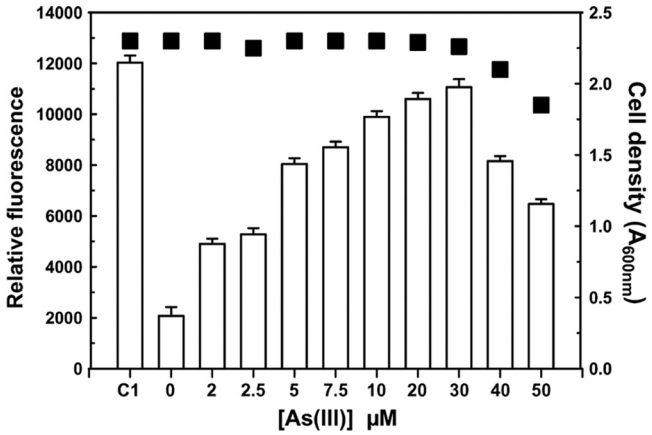

FIG 1 Response of the bacterial biosensor to As(III) concentration. Cells were grown in LB medium in the absence of arabinose (C1) or with 0.2% arabinose and the indicated concentrations of inorganic As(III). Cell density (■) was monitored after 14 h of incubation, and the fluorescence intensities (open bars) were measured by spectrofluorometry with an excitation wavelength of 470 nm and an emission wavelength of 510 nm.

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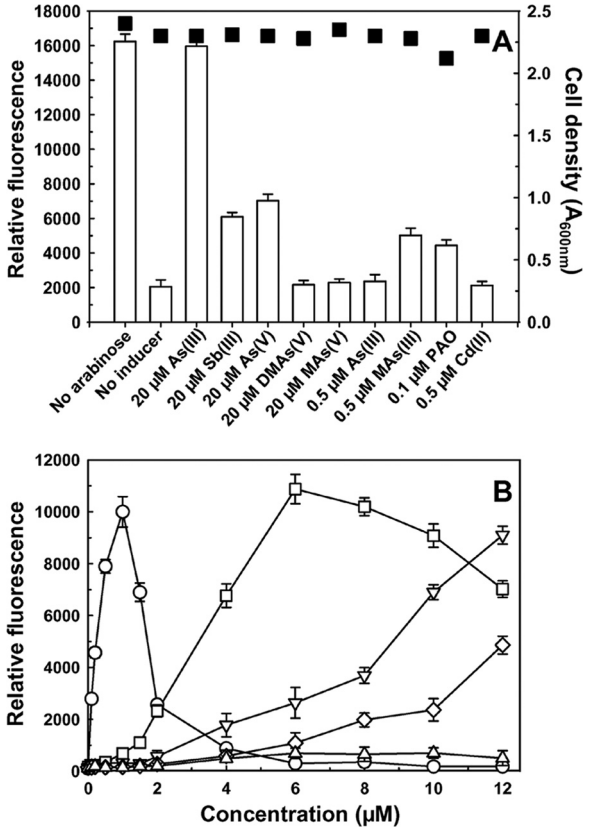

FIG 2 Comparison of the responses of the bacterial biosensor to inorganic and organic arsenicals. (A) Cells were grown in LB medium in the absence of arabinose or with 0.2% arabinose and the indicated concentrations of inducers. Cell density (■) was monitored after 14 h of incubation, the concentrations of inducers were adjusted to prevent toxicity, and the fluorescence intensities (open bars) were measured by spectrofluorometry. (B) Cells were grown in LB medium with 0.2% arabinose and the indicated concentrations of inducers. ○, PAO; □, MAs(III); ▽, As(III); ◇, Sb(III); △, Cd(II).

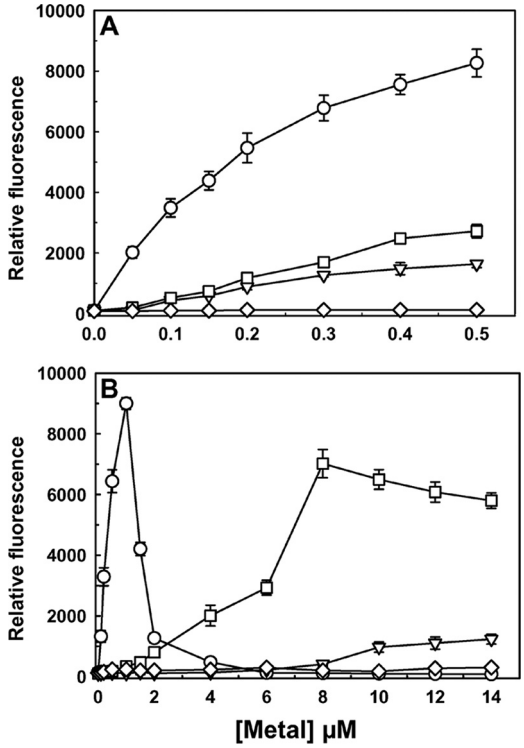

FIG 3 Tuning the bacterial biosensor for either higher sensitivity or greater selectivity. (A) At a low arabinose concentration (0.05%), the biosensor was highly selective for PAO. (B) At a high arabinose concentration (0.5%), the sensor was highly selective for MAs(III) over As(III). ○, PAO; □, MAs(III); ▽, As(III); ◇, MAs(V).

in cellular fluorescence and growth at 40 μM As(III) or higher is due to the hypersensitivity of AW3110. However, this corresponds to over 500 ppb, higher than the amount of arsenic found in most environmental situations, and should not limit the utility of the biosensor.

The response of the *AfarsR-gfp* biosensor to subtoxic concentrations of a variety of potential inducers was examined (Fig. 2A). PAO, MAs(III), As(III), and Sb(III) each induced *gfp* expression, while Cd(II), MAs(V), and DMAs(V) were comparable to the control. DMAs(III) was too unstable to test. The affinity of the biosensor for trivalent metalloids was quantified (Fig. 2B). Although each metalloid became toxic at higher concentrations, the apparent half-maximal concentrations required for induction were approximately 0.25 μM for PAO, 3 μM for MAs(III), and 10 μM for As(III). These results demonstrate that the *AfarsR-gfp* sensor is highly selective for aromatic and methylated trivalent arsenicals over inorganic arsenic in the order PAO≫MAs(III)>As(III).

The data in Fig. 2 demonstrate the capability of the biosensor, but both the sensitivity and selectivity must be improved if it is to be useful under environmental conditions or to be able to detect specific species of arsenicals. We considered the possibility that if AfArsR were produced in smaller amounts (by decreasing the arabinose concentration), it should not repress *gfp* expression as tightly, allowing the sensor to respond to lower levels of arsenical inducers, leading to increased sensitivity. We also predicted that higher levels of ArsR (by increasing the arabinose concentration) would repress more tightly, so inducers with higher affinity would be required to derepress *gfp* expression, thus decreasing sensitivity but increasing selectivity.

To explore these possibilities, conditions for variably regulating AfArsR levels were determined. Expression of *AfarsR* from the *ara* promoter in pBAD*arsR* is regulated by AraC, the regulator of the arabinose operon. In the presence of glucose and the absence of arabinose, AraC is a negative regulator that represses expression of *AfarsR*, and lower levels of AfArsR facilitate *gfp* expression (Fig. 3C). 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* (see Fig. S3A in the supplemental material). Since arsenicals that bind to AfArsR would be expected to derepress *gfp* expression, the effect of inorganic As(III) concentration at several concentrations of arabinose was examined. At 0.02% arabinose repression, sensitivity to low concentrations of inorganic arsenic increased, but at the expense of a higher endogenous background level of *gfp* expression (see Fig. S3B in the supplemental material). At 0.05% arabinose, the background was lower but

the level of induction was about the same (see Fig. S3C in the supplemental material).

When the effect of arsenical inducers was examined at 0.05% arabinose, the response was roughly linear with inorganic As(III), MAs(III), and PAO over the range of 0.1 to 0.5 μM (Fig. 3A). The signal with PAO was much greater than the signals with the other two, and there was little discrimination between MAs(III) and As(III). Thus, at low arabinose, the biosensor is highly selective for PAO. To increase selectivity for MAs(III) over As(III), the concentration of arabinose was increased to 0.5% (Fig. 3B). With this level of arabinose, the response to inorganic arsenic was decreased, but the selectivity was increased. The signal with PAO was lost due to the toxicity of the phenyl derivative, but the response to MAs(III) was nearly linear over the range of 2 to 8 μM, with practically no response to inorganic As(III). Thus, at the higher level of arabinose, the biosensor becomes selective for MAs(III). In conclusion, this is the first biosensor selective for organoarsenicals, with little response to inorganic arsenic, and it may prove useful in detecting the breakdown products of arsenical herbicides and animal growth promoters and their mobility in agricultural settings.

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REFERENCES

1. **Abernathy CO, Thomas DJ, Calderon RL.** 2003. Health effects and risk assessment of arsenic. J. Nutr. **133**:1536S–1538S.
2. **Arao T, Maejima Y, Baba K.** 2009. Uptake of aromatic arsenicals from soil contaminated with diphenylarsinic acid by rice. Environ. Sci. Technol. **43**:1097–1101.
3. **Carlin A, Shi W, Dey S, Rosen BP.** 1995. The *ars* operon of *Escherichia coli* confers arsenical and antimonial resistance. J. Bacteriol. **177**:981–986.
4. **Cortinas I, et al.** 2006. Anaerobic biotransformation of roxarsone and related N-substituted phenylarsonic acids. Environ. Sci. Technol. **40**: 2951–2957.
5. **Guzman LM, Belin D, Carson MJ, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J. Bacteriol. **177**:4121–4130.
6. **Maejima Y, Arao T, Baba K.** 2011. Transformation of diphenylarsinic acid in agricultural soils. J. Environ. Qual. **40**:76–82.
7. **Qin J, et al.** 2007. Convergent evolution of a new arsenic binding site in the ArsR/SmtB family of metalloregulators. J. Biol. Chem. **282**:34346–34355.
8. **San Francisco MJ, Hope CL, Owolabi JB, Tisa LS, Rosen BP.** 1990. Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon. Nucleic Acids Res. **18**:619–624.
9. **Stolz JF, et al.** 2007. Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by Clostridium species. Environ. Sci. Technol. **41**:818–823.
10. **Tchounwou PB, Centeno JA, Patlolla AK.** 2004. Arsenic toxicity, mutagenesis, and carcinogenesis—a health risk assessment and management approach. Mol. Cell. Biochem. **255**:47–55.
11. **Wu J, Rosen BP.** 1991. The ArsR protein is a trans-acting regulatory protein. Mol. Microbiol. **5**:1331–1336.
12. **Yoshinaga M, Cai Y, Rosen BP.** 2011. Demethylation of methylarsonic acid by a microbial community. Environ. Microbiol. **13**:1205–1215.