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Arsenite Oxidase Also Functions as an Antimonite Oxidase

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Arsenic and antimony are toxic metalloids and are considered priority environmental pollutants by the U.S. Environmental Protection Agency. Significant advances have been made in understanding microbe-arsenic interactions and how they influence arsenic redox speciation in the environment. However, even the most basic features of how and why a microorganism detects and reacts to antimony remain poorly understood. Previous work with *Agrobacterium tumefaciens* strain 5A concluded that oxidation of antimonite [Sb(III)] and arsenite [As(III)] required different biochemical pathways. Here, we show with *in vivo* experiments that a mutation in *aioA* [encoding the large subunit of As(III) oxidase] reduces the ability to oxidize Sb(III) by approximately one-third relative to the ability of the wild type. Further, *in vitro* studies with the purified As(III) oxidase from *Rhizobium* sp. strain NT-26 (AioA shares 94% amino acid sequence identity with AioA of *A. tumefaciens*) provide direct evidence of Sb(III) oxidation but also show a significantly decreased V_{max} compared to that of As(III) oxidation. The *aioBA* genes encoding As(III) oxidase are induced by As(III) but not by Sb(III), whereas *arsR* gene expression is induced by both As(III) and Sb(III), suggesting that detection and transcriptional responses for As(III) and Sb(III) differ. While Sb(III) and As(III) are similar with respect to cellular extrusion (ArsB or Acr3) and interaction with ArsR, they differ in the regulatory mechanisms that control the expression of genes encoding the different Ars or Aio activities. In summary, this study documents an enzymatic basis for microbial Sb(III) oxidation, although additional Sb(III) oxidation activity also is apparent in this bacterium.

The metalloids arsenic (As) and antimony (Sb) are members of group 15 of the periodic table and are ubiquitous in the environment. Both are poisonous and have oxidation states of −3, 0, +3, and +5, with the last two being the most prevalent in the environment (1–5). The release of both As and Sb into the environment can occur either naturally or anthropogenically (e.g., mining), and both are considered by the U.S. Environmental Protection Agency to be priority environmental pollutants (6), with maximum drinking water standards of 10 ppb and 6 ppb for As and Sb, respectively (7). As has received more publicity due to As poisoning that has occurred and that continues (4, 8). However, Sb has emerged as a major contaminant in environments that contain mine tailings, such as those in China, Australia, New Zealand, and parts of Europe (for example, see references 5 and 9–11).

Microorganisms are fundamental to elemental cycling in all environments, and this includes As (12, 13) and presumably Sb, although information for the latter is quite sparse. As cycling has been well documented and at present is thought primarily to involve arsenite [As(III)]⇔arsenate [As(V)] redox transformations and As methylation and demethylation reactions. As(V) is reduced for detoxification purposes (via ArsC) or respiratory energy generation (ArrAB) (14–16). The physiological basis for As(III) oxidation is similar (i.e., detoxification or generation of cellular energy) and can be coupled to the reduction of oxygen or nitrate or to anoxygenic photosynthesis (15–19). The mechanisms of As(V) reduction and As(III) oxidation have been studied in some detail, with some of the enzymes purified and characterized (20–27), and X-ray crystal structures were determined for two As(III) oxidases (encoded by *aioB* and *aioA*) (28, 29). Further, an As methyltransferase (encoded by *arsM*) from a thermophilic eukaryotic alga (30) currently is being characterized (31, 32).

In contrast, our understandings of Sb-microbe interactions are rudimentary at best. Sb(III) oxidation was documented first in a microorganism referred to as *Stibiobacter senarmontii*, described as being capable of oxidizing the mineral senarmontite to form Sb_2O_5 (reviewed in reference 33). No further characterization of this organism or Sb(III) oxidation mechanism(s) was performed. More recently, a diverse array of microorganisms that are highly resistant to Sb(III) or that readily oxidize it have been isolated (34–36), as have organisms capable of reducing Sb(V) (37, 38). However, the mechanisms involved in these Sb redox reactions remain unknown.

The bacterium *Agrobacterium tumefaciens* strain 5A oxidizes both As(III) (39, 40) and Sb(III) (34). Since Sb(III) and As(III) both can interact with ArsR to derepress the *ars* operon required for optimal As(III) and Sb(III) resistance (41) and both are ef-

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fluxed by ArsB and Acr3 (41–43), it was assumed that As(III) and Sb(III) were similar enough to share the same redox biochemistry. However, *A. tumefaciens* strain 5A mutants unable to oxidize As(III) were found to oxidize Sb(III) (34). Specifically, genes inactivated in these mutants were *aioR*, which codes for the transcriptional regulator of *aioBA*, and *mrpB*, which encodes part of a multisubunit Na^+:H^+ antiporter required for As(III) oxidation. This suggested that the biochemistries of As(III) and Sb(III) oxidation are indeed different.

The current study is one of several we are conducting that aim to unravel the genetics and cellular activities underlying Sb(III) resistance and oxidation in bacteria, using *A. tumefaciens* 5A as one potential model organism. Here, we demonstrate *in vivo* and *in vitro* evidence that As(III) oxidase can oxidize Sb(III), although the V_{max} is significantly reduced compared to that of As(III). We conclude that our data are not in conflict with the previous report by Lehr et al. (34).

MATERIALS AND METHODS

Bacterial strains and plasmids. *A. tumefaciens* strain 5A was used for transposon mutagenesis studies and *in vivo* Sb(III) resistance and oxidation experiments. This strain and its routine cultivation in liquid minimal mannitol ammonium ($MMNH_4$) medium have been described previously (44), although the $MMNH_4$ medium was modified to include only 50 μM phosphate and MOPS (4-morpholinepropanesulfonic acid) as a pH buffer. An *aioA*::Tn*5* mutant was isolated from the wild-type strain using methods we have previously described (40) and was characterized (see below). *Rhizobium* sp. strain NT-26 was the genetic source for cloning of the *aioBA* genes, which encode the As(III) oxidase in NT-26 and are close homologues to *aioBA* in other aerobic As(III)-oxidizing microorganisms. *Escherichia coli* strain S17-1 (45) was used as a conjugation donor to introduce the transposon or for mutant complementation work. We previously described the construction of the *aioB*::*lacZ* reporter that allows for tracking expression of the *aioBA* genes transcribed from the *aioB* proximal promoter that involves AioR and RpoN (46, 47). *A. tumefaciens* strain 5A contains four *arsR* genes (48) that are well known to be induced by both metalloids (49); thus, as an experimental control, we also selected one of these genes, *arsR4*, to examine As(III) and Sb(III) regulatory effects. The *lacZ* reporter gene for *arsR4* was constructed in a manner similar to that for *aioB*::*lacZ*. The 5′ region of *arsR4* along with 269 bp of upstream DNA was PCR amplified and directionally cloned into plasmid pLSP-KT2*lacZ*, which carries a promoterless *lacZ* gene. The reporter plasmid was maintained in 5A with 500 μg/ml kanamycin on $MMNH_4$ agar.

Transposon Tn*5*-B22 (45) was used for mutagenesis screening. Plasmid pDK402 was used to complement the *aioA*::Tn*5*-B22 mutant generated in this study and was previously constructed and described by Kashyap et al. (40). Briefly, it is pCPP30 that carries a 7,397-bp XbaI fragment cloned from strain 5A and that contains the complete coding regions for *aioSRBA-cytC* genes. This cloned fragment also contains the 3′ region of *aioX* (bp 369 to 918) upstream of *aioS* and the first 269-bp 5′ region of *moeA* downstream of *cytC* (40). Plasmid pDK402 was used to complement the *aioA*::Tn*5* mutant for As(III) oxidation activity. Plasmid pCPP30 was used as the control plasmid.

Mutagenesis and complementation studies. Strain 5A transconjugants first were selected on $MMNH_4$ agar containing gentamicin ($MMNH_4$ Gent agar; 20 μg/ml) and then transferred individually (sterile toothpick) to $MMNH_4$ Gent agar with and without 100 μM potassium antimonyl tartrate [antimonite; here referred to as Sb(III)]. Transconjugants displaying reduced growth in the presence of 100 μM Sb(III) compared to growth in the absence of Sb(III) were selected for additional study. The Tn*5*-B22 insertion site in the *aioA*::Tn*5*-B22 mutant was characterized as previously described (40). For complementation studies, *E. coli* S17-1 was used to transfer pDK402 to the *aioA*::Tn*5*-B22 mutant by conjugation.

Sb(III) sensitivity and oxidation. Cultures were grown in liquid $MMNH_4$ containing tetracycline (20 μg/ml; for plasmid maintenance) and Sb(III), with concentrations of the latter depending on the experiment (see figure legends). At each sampling time, culture samples were measured for turbidity (Spectramax 384 Plus microtiter plate reader; Molecular Devices), and 1.0-ml aliquots of the cultures were centrifuged to pellet the cells. The supernatant then was analyzed for Sb(III) and Sb(V) using high-performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS; 986A; Beijing Puxi General Instrument Co., Beijing, China) (50).

Heterologous expression and purification of As(III) oxidase. The recombinant As(III) oxidase (here referred to as Aio) from *Rhizobium* sp. strain NT-26 was expressed and purified as described previously (29), except with the following modifications: (i) after eluting the enzyme from the Ni-charged affinity column, the eluent was buffer exchanged into 50 mM Tris-HCl, 100 mM NaCl (pH 8), and the sample was concentrated; (ii) size exclusion chromatography was carried out using 50 mM Tris-HCl, 100 mM NaCl (pH 8). Fractions containing Aio were pooled and concentrated as described previously (29).

Aio assays. Aio enzyme assays were done at 25°C at 550 nm with 20 μM equine heart cytochrome *c* (≥95% purity; Sigma-Aldrich) [$\varepsilon_{550(red\text{-}ox)}$ (reduced-minus-oxidized extinction coefficient at 550 nm) = 21.1 mM^{-1} cm^{-1}] as the electron acceptor (in 50 mM Tris-HCl, pH 8) and 1.2 to 1.3 nM Aio for the As(III) assays or 160 to 190 nM for the Sb(III) assays (based on an M_r of 113,252 as calculated by mass spectrometry). Concentrations of As(III) (as sodium arsenite) and Sb(III) were varied as described below. V_{max} is reported as μM As(III) or nM Sb(III) oxidized min^{-1} mg^{-1} $protein^{-1}$ and is based on 1 mol As/Sb being required to reduce 2 mol cytochrome *c*. The results of the kinetics are from three independent experiments (i.e., with three different enzyme preparations). Protein concentrations were determined as described previously (29).

RESULTS

Characterization of an Sb(III)-sensitive *aioA*::Tn*5* mutant. Preliminary experiments determined that the wild-type parental strain 5A was sensitive to Sb(III), with maximum Sb(III) tolerance levels in $MMNH_4$ agar of 100 μM. At higher Sb(III) levels, fast-growing colonies that presumably represented mutations resulting in enhanced Sb(III) resistance (e.g., reduced uptake or enhanced extrusion) spontaneously arose. This was in contrast to Sb(V), where concentrations as high as 200 μM had no apparent effect on growth (data not shown). Therefore, it was concluded that, at a minimum, Sb(III) oxidation in this strain provided a detoxification mechanism and that a transposon mutagenesis screen at 100 μM Sb(III) could be a reasonable strategy to identify genes/operons involved in Sb(III) oxidation and/or resistance.

One transconjugant of interest, and the focus of this study, was interrupted in *aioA*, which encodes the large subunit of the Aio (i.e., AioA). The involvement of the Aio was unexpected, given our previous work that suggested Sb(III) and As(III) oxidation involved separate genes and metabolic pathways (34). However, this Sb(III)-sensitive mutant was verified to be negative for As(III) oxidation using a qualitative silver nitrate staining technique described previously (40). In liquid $MMNH_4$ containing 75 μM or 100 μM Sb(III), growth of the mutant was constrained relative to that of the mutant carrying the complementing *aioSRBA-cytC* DNA fragment (Fig. 1). Consistent with our previous observations (34), Sb(III)-naive cells required significant time to adjust to the Sb(III), with the lag phase being longer with increasing levels of Sb(III) (Fig. 1). At lower levels of Sb(III) (i.e., ≤25 μM) used for Sb(III) oxidation assays, a lag phase still was apparent, although growth of the mutant did not differ from that the wild type or the mutant carrying pDK402 (Fig. 2A).

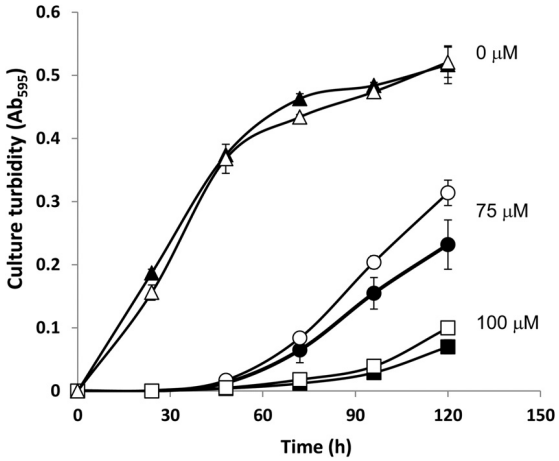

FIG 1 Sensitivity of the *aioA*::Tn*5*-B22 mutant to Sb(III). Growth profiles are shown for $MMNH_4$ media containing 0, 75, or 100 mM Sb(III). Filled symbols, *aioA*::Tn*5*-B22 mutant (pCPP30, control plasmid); open symbols, *aioA*::Tn*5*-B22 mutant (pDK402 is pCPP30 containing *aioSRBA-cytC*). The media also contained 10 ppm tetracycline for plasmid maintenance. Error bars (where visible) represent the range of duplicate cultures.

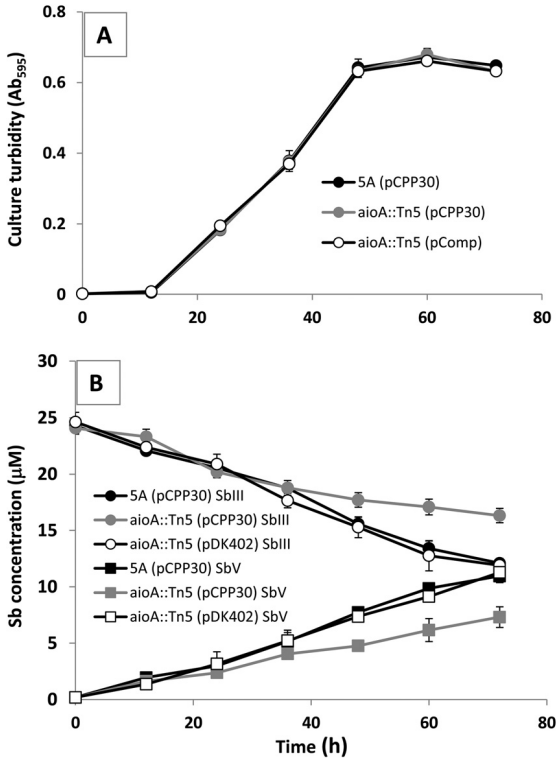

FIG 2 *In vivo* evidence of Sb(III) oxidation via the *A. tumefaciens* AioBA As(III) oxidase. (A) Growth profiles of all three strains illustrating no differences in apparent sensitivity to 25 mM Sb(III). (B) Concentrations of Sb(III) (circles) and Sb(V) (squares) in culture supernatants as a function of culture age. In both panels, error bars (where visible) represent ± one standard deviation.

Sb(III) oxidation was directly assayed in the *aioA*::Tn*5* mutant, the wild-type strain, and the mutant carrying the *aioSRBA-cytC* operon in *trans* on plasmid pCPP30 that was shown to complement As(III) oxidation in an *aioR*::Tn*5*-B22 mutant (40). As controls, the wild-type strain and the mutant carried the pCPP30 vector. For these assays, lower, noninhibitory Sb(III) levels were used so that oxidation activity comparisons would not be complicated by cell biomass differences. There was no apparent difference in Sb(III) oxidation during the initial 36 h of growth in $MMNH_4$ containing 25 μM Sb(III) (Fig. 2B); however, thereafter the mutant differed from the wild-type and complemented mutant strains ($P < 0.01$, which is statistically significant). Using best-fit regression of Sb(III) oxidation versus time, Sb(III) oxidation profiles were nearly linear, with R^2 values of 0.99 for all three strains. Averaged over the course of the 72-h experiment for the 100-ml cultures ($n = 3$ each), the mutant oxidized 103 ± 7 pmol Sb(III) · h^{-1}, compared to 155 ± 4 pmol · h^{-1} and 152 ± 5 pmol · h^{-1} for the wild-type and complemented mutant, respectively. Thus, under the culturing conditions employed, it appears that the Aio accounted for roughly one-third of the Sb(III) oxidized by wild-type cells during the time period spanning 36 to 72 h.

The Sb(III) oxidation profiles for the mutant did not diverge from the those of the wild type and the complemented mutant until well into the culture growth cycle, suggesting that the *aioBA* genes were not expressed during the earlier time points. To examine this further, we used an *aioB*::*lacZ* reporter construct to conveniently assess whether Sb(III) would induce expression of the *aioBA* genes. Initial experiments using 25 μM Sb(III) failed to detect any *aioB*::*lacZ* induction during 48 h of incubation. Given our evidence that Sb(III) was toxic to strain 5A at low concentrations and recent studies showing that excessive As(III) could inhibit *aioB*::*lacZ* expression (43), the induction experiments were repeated, but this time adding a low level of Sb(III) (5 μM) at time zero. Cell growth of the wild-type strain carrying the reporter constructs appeared slightly inhibited by 100 μM As(III) and 5 μM Sb(III) relative to that of the control culture (Fig. 3A). No growth was observed in the cultures containing 25 μM Sb(III), consistent with the prolonged lag phase that was exhibited by Sb(III)-naive cells (Fig. 1 and 2) and that we have documented previously (39). However, while *aioB*::*lacZ* was strongly induced with 100 μM As(III), no expression was detected with 5 μM Sb(III) (Fig. 3B). These results stand in contrast to significant reports in the literature demonstrating that Sb(III) can substitute for As(III) in inducing As(III)-sensitive regulatory proteins, such as ArsR (for example, see reference 49). Consequently, a *lacZ* reporter was constructed for one of the four *arsR* genes in this strain and tested for induction with As(III) and Sb(III). The *arsR4*::*lacZ* reporter responded rapidly to both 100 μM As(III) and 25 μM Sb(III) (Fig. 3B), illustrating the same phenomenon as that previously observed (49), as well as serving to verify that the null results with the *aioB*::*lacZ* reporter were reliable. We note that *arsR4*::*lacZ* induction in 25 μM Sb(III) occurred even though cell growth was not detected (Fig. 3A and B).

***In vitro* evidence of arsenite oxidase functioning as an antimonite oxidase.** Subsequent work then sought to directly address whether the Aio can oxidize Sb(III). The Aio from NT-26 (24) and that of *A. tumefaciens* strain 5A share 94% amino acid sequence identity (40); thus, the availability of the enzyme from NT-26 provided an expedient opportunity to directly test whether the purified enzyme will oxidize Sb(III). Preliminary assays illustrated Aio-mediated Sb(III) oxidation. For comparison, reaction kinetics were characterized for both As(III) and Sb(III) (Fig. 4). Using equine cytochrome *c* as the electron acceptor and As(III) as the electron donor, the V_{max} was 120.4 ± 6.0 μmol^{-1} min^{-1} mg^{-1}

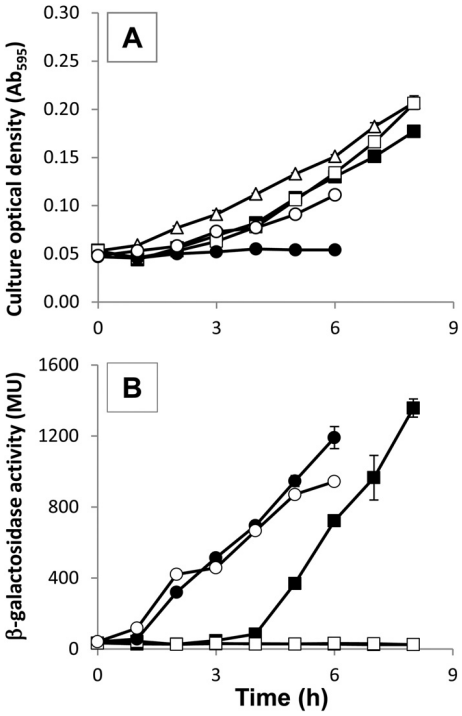

FIG 3 Influence of As(III) and Sb(III) on transcriptional activation of *aioB*::*lacZ* and *arsR4*::*lacZ* reporter genes. (A) Cell growth. As was measured by culture optical density. (B) Reporter gene β-galactosidase activity. Symbols and error bars (where visible) represent the means ± ranges from duplicate cultures. △, Zero As/Sb controls; ■, *aioB*::*lacZ* with 100 mM As(III); □, *aioB*::*lacZ* with 5 mM Sb(III); ●, *arsR4*::*lacZ* with 25 mM Sb(III); ○, *arsR4*::*lacZ* with 100 mM As(III).

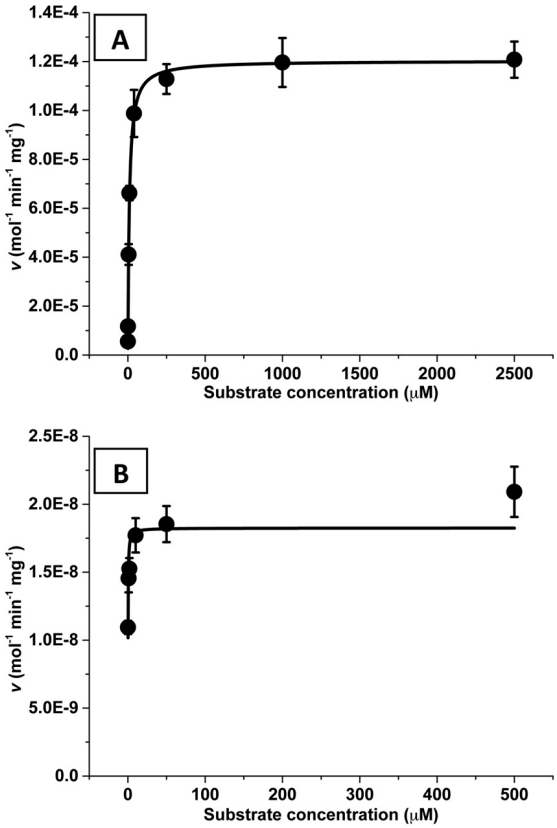

FIG 4 Comparison of Michaelis-Menten kinetic data obtained for arsenite (A) and antimonite (B) for the recombinant *Rhizobium* sp. strain NT-26 arsenite oxidase with 20 μM horse heart cytochrome *c* as the electron acceptor. Data points and error bars represent the means and standard deviations from three replicate experiments, each conducted from separate arsenite oxidase purifications. Reaction velocity (*v*) is expressed as mol substrate oxidized per min per mg of purified Aio.

and the K_m for As(III) was 9.3 ± 1.5 μM. In contrast, when Sb(III) was used as the electron donor, the V_{max} was significantly lower, at 18.4 ± 1.2 nmol^{-1} min^{-1} mg^{-1}, and the K_m for Sb(III) also was lower, at 163 ± 8 nM (Fig. 4). The kinetics experiments showed that Aio has a higher affinity for Sb(III) but that reaction velocities were reduced. This is further supported by the result of Aio activity assays when both electron donors were included in assays. At equimolar amounts (500 μM), activity increased by about 300-fold compared to that of Sb(III) alone but was reduced by about 10-fold compared to that of As(III) alone (specific activity was 5.8 μmol^{-1} min^{-1} mg^{-1}). Other assays examined Aio behavior when the two substrates were added disproportionately. The specific activity with 500 μM As(III) and 100 μM Sb(III) was 10.5 μmol^{-1} min^{-1} mg^{-1} and 29 nmol^{-1} min^{-1} mg^{-1} for 500 μM Sb(III) plus 100 μM As(III). In summary, these results confirmed that the Aio is capable of oxidizing Sb(III), establishing a link between the *in vivo* Sb(III) sensitivity and oxidation experiments and providing a biochemical explanation for the *in vivo* data (Fig. 2B).

DISCUSSION

This study documents an enzymatic basis for microbial Sb(III) oxidation. This was facilitated by the screening of *A. tumefaciens* Tn*5*-B22 mutants for Sb(III) sensitivity, followed by *in vivo* characterization with HPLC-HG-AFS, and then *in vitro* studies with the purified Aio. In previous work, Lehr et al. (34) concluded that Sb(III) oxidation used a unique mechanism relative to that employed for As(III) oxidation. Their conclusion was based on the finding that two different types of mutants that lacked As(III) oxidation still could oxidize Sb(III) at rates indistinguishable from that of the wild-type strain. In the current study, the initial transconjugant screen linking Sb(III) sensitivity to an *aioA*::Tn*5*-B22 insertion mutation was unanticipated. Furthermore, Shi et al. (35) found that only a minor proportion of Sb(III)-oxidizing organisms contained *aioBA* homologues, providing additional evidence that these oxidation systems are fundamentally different. Nevertheless, the initial observations with the mutant spurred efforts to reassess the findings of Lehr et al. (34). In so doing, we optimized expression of *aioBA* by reducing the phosphate content of the medium (see reference 47) to encourage a timely expression of *aioBA* (within 5 h) (Fig. 3B), assuming an *aioBA* inducer was present, and we extended culture incubations beyond that employed previously (34). When the sampling was extended beyond log phase and well into the stationary phase (>36 h), the influence of the Aio was evident, accounting for roughly a third of the Sb(III) oxidation in the wild-type and complemented mutant strains during the course of the experiment (Fig. 2). The link between Aio and Sb(III) oxidation was confirmed by using purified recombinant Aio (Fig. 4). To summarize this element of the study, we conclude that the failure of Lehr et al. (34) to identify differences in Sb(III) oxidation between the wild-type and mutant strains incapable of oxidizing As(III) was a coincidence of culture and assay conditions.

Inoculating Sb-naive 5A into $MMNH_4$ containing ≥5 μM Sb(III) results in prolonged lag phases (Fig. 1), as reported previously (34), clearly indicating that the cells need to make adjustments to overcome Sb(III) toxicity. The length of the adjustment phase appeared proportional to the Sb(III) concentration (Fig. 1), but at Sb(III) levels exceeding 25 μM, growth of cells lacking Aio was further constrained (Fig. 1), providing evidence that is consistent with the conclusion that, to at least some extent, Sb(III) tolerance by *A. tumefaciens* 5A is linked to the presence of a functional Aio that oxidizes Sb(III) to the less toxic Sb(V) (Fig. 1).

The promoter and regulatory system that drives and governs the expression of *aioSRBA-cytC* in the absence of As(III) has yet to be defined. In experiments involving lower inhibitory Sb(III) levels, the effect of Aio on Sb(III) oxidation was delayed until well into the culture growth cycle (i.e., 36 h) (Fig. 2), implying that Sb(III) did not induce *aioBA* transcription, which otherwise should have occurred within ~4 h if an AioXSR/RpoN regulatory pathway (see references 46, 47, and 51) is capable of recognizing Sb(III), as it does As(III) (Fig. 3B). As we have shown previously, the *aioB*::*lacZ* reporter was induced with As(III) (47) but not with Sb(III) under what were otherwise the same culture conditions (Fig. 3B). Since the Sb(III) oxidation activity was shown to be depleted in the *aioA*::Tn*5*-B22 mutant but restored by the *aioSRBA-cytC* fragment, the evidence clearly argues that there must be a separate, active promoter somewhere within this fragment that influences *aioBA* expression other than the As(III)-sensitive promoter upstream of *aioB* that involves RpoN and AioR (46, 47). We suggest that this promoter is upstream of *aioS* based on the following observations. First, in previous reverse transcriptase PCR work with this strain of *A. tumefaciens*, we showed that *aioSRBA-cytC-moeA* are cotranscribed (40), illustrating that there must be a promoter upstream of *aioS*. Second, the complementing fragment in pDK402 contains only the 3′ region of *aioX*, the gene directly upstream of *aioS* (48); i.e., the 5′ region of *aioX* and its promoter are absent. We do not yet understand which environmental cue(s) activates this promoter; however, we have previously reported on two potential regulatory systems. Kashyap et al. (40) showed that quorum sensing can be involved in regulating *aioSRBA* expression independently of As(III), and more recently, Kang et al. (48) showed how *aio* gene expression is integrated into the phosphate stress response. Specifically, *aioB*::*lacZ* upregulation is coordinated with *phoA* (endogenous reporter for the phosphate stress response), and *aioSR* transcripts are significantly reduced in Δ*phoB2*, Δ*pstS1*, and/or Δ*phoU1* mutants. These *pho* and *pst* genes are located in an adjacent, divergently expressed operon and encode foundational aspects of the phosphorus stress response in Gram-negative bacteria. In the current study, involvement of the Pho regulatory system in regulating the expression of *aioSRBA-cytC* would not be inconsistent with the low phosphate content (50 μM) of the $MMNH_4$ medium used in these experiments to promote the expression of *aioBA*.

In addition to the above-described short-term *aioB*::*lacZ* reporter assays, prolonged (48-h) induction incubations with 25 μM Sb(III) failed to promote the expression of *aioB*::*lacZ*. The differential response of the *aioB*::*lacZ* and *arsR4*::*lacZ* reporters to As(III) and Sb(III) (Fig. 3) suggests that Sb(III) does not interact with the As(III)-sensing system, which thus far is understood to involve AioX and AioS (51), or at least not at the proficiency necessary to readily detect induction of the *aioB*::*lacZ* reporter. The Bonnefoy group has documented similar differential As(III) and Sb(III) regulatory effects on the expression of the *Thiomonas arsenitoxydans aioBA-cyc1-aioF-cyc2* operon (52–54), apparently due to the lack of Sb(III) interaction with the *aioBA* transcriptional activator in this organism, AioF (55). At present, our understanding of Sb(III) detection and the associated changes to transcriptional profiling is quite poor, but the differential influences of As(III) and Sb(III) on induction of *aioB* and *arsR4* (Fig. 3) illustrate that there are significant differences yet to be uncovered with respect to the regulation of Sb(III) oxidation.

Experiments summarized in this study clearly demonstrate that the Aio will oxidize Sb(III); however, a comparison of the Sb(III) and As(III) reaction kinetics makes it equally clear that rates differ by orders of magnitude. Although likely cometabolic in context, it would be expected that this also would be reflected by microbial Sb(III) oxidation activities in the environment. Aio contributions to Sb(III) oxidation might be most meaningful in relatively static environments, where Sb(III) residence time is sufficient to allow for quantitative oxidation (e.g., slow-moving ground water scenarios), but less likely in swiftly flowing environments (e.g., acid mine drainage flows). Importantly, while the *aioB* proximal promoter is not activated by Sb(III) (Fig. 3), *aioBA* upregulation in nature could be induced by As(III) that often cooccurs with Sb(III), facilitating Sb(III) oxidation, albeit competing with and perturbing As(III) oxidation. We also have observed Sb(III) oxidation in other strains that contain *aioBA* (35), but the proportional contribution of Aio to the Sb(III) oxidation in these bacteria is unknown at present.

A direct kinetic comparison between Aio and a putative Sb(III) oxidase(s) awaits the identification and cloning of the relevant gene(s). Other enzymes capable of oxidizing Sb(III) obviously are present in strain 5A and in the organisms isolated by Shi et al. (35) and Hamamura et al. (36). The genome sequence of the Sb(III)-oxidizing bacterium *Comamonas testosteroni* S44 does not contain a recognizable *aioBA* (56) and is consistent with the inability of this strain to oxidize As(III). Such organisms offer convenient modeling opportunities free of potential background Aio influences and are targets of current efforts.

To summarize, we suggest that the results of the different experimental components detailed above are internally consistent with one another and the overall assessment that (i) Aio is capable of Sb(III) oxidation, (ii) Sb(III) oxidation serves as a detoxification mechanism in *A. tumefaciens* strain 5A, and (iii) depending on environmental conditions and the composition of the extant microbial community, Aio may account for an appreciable proportion of Sb(III) oxidation and measurably contribute to Sb(III) cycling in nature. Being able to directly associate Sb(III) oxidation with Aio at least suggests the potential of Sb(III) as an electron donor for generating cellular energy, as has been shown previously (17, 25, 53). With *A. tumefaciens* strain 5A, however, Sb(III) toxicity was observed at levels below that likely to be required for growth. It will be interesting to determine if this is the case with all Sb(III)-oxidizing organisms, including the Arx-based As(III) oxidizers, and indeed whether the dissimilatory arsenate reductase (Arr) is capable of reducing Sb(V), contributing to Sb biogeochemical cycling.

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ERRATUM

Erratum for Wang et al., Arsenite Oxidase Also Functions as an Antimonite Oxidase

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Volume 81, no. 6, p. 1959–1965, 2015. Page 1961, Fig. 1 legend, line 2: ". . . or 100 mM Sb(III)" should read ". . . or 100 μM Sb(III)."

Page 1961, Fig. 2 legend, line 3: ". . . sensitivity to 25 mM Sb(III)" should read ". . . sensitivity to 25 μM Sb(III)."

Page 1962, Fig. 3 legend, lines 5 to 7: ". . .■, *aioB*::*lacZ* with 100 mM As(III); □, *aioB*::*lacZ* with 5 mM Sb(III); ●, *arsR4*::*lacZ* with 25 mM Sb(III); ○, *arsR4*::*lacZ* with 100 mM As(III)" should read ". . .■, *aioB*::*lacZ* with 100 μM As(III); □, *aioB*::*lacZ* with 5 μM Sb(III); ●, *arsR4*::*lacZ* with 25 μM Sb(III); ○, *arsR4*::*lacZ* with 100 μM As(III)."

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