Bacterial ability in AsIII oxidation and AsV reduction: Relation to arsenic tolerance, P uptake, and siderophore production

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A B S T R A C T
The relationship between bacterial ability in arsenic transformation, siderophore production, and P uptake was investigated using six arsenic-resistant bacteria isolated from the rhizosphere of arsenic-hyperaccumulator Pteris vittata. Bacterial strains of PG5 and 12 were better arsenite (AsIII) oxidizers (31–46 vs. 6.2–21% of 1 mM AsIII) whereas PG 6, 9, 10 and 16 were better arsenate (AsV) reducers (58–95 vs. 7.5–46% of 1 mM AsV). Increase in AsV concentration from 0 to 1 mM induced 3.0–8.4 times more P uptake by bacteria but increase in P concentration from 0.1 to 1 mM reduced AsV uptake by 17–71%, indicating that P and AsV were taken up by P transporters. Bacteria producing more siderophores (PG5 and 12; >73 lM equiv) showed greater AsIII oxidation and AsIII resistance than those producing less siderophore (PG 6, 9, 10 and 16; <23 lM equiv). This observation was further supported by results obtained from mutants of Pseudomonas fluorescens impaired in siderophore production, as they were 23–25% less tolerant to AsIII than the wild-type. Arsenic-resistant bacteria increased their arsenic tolerance by retaining less arsenic in cells via efficient AsIII oxidation and AsV reduction, which were impacted by P uptake and siderophore production.

1. Introduction

Arsenic is a potent poison and is primarily present as arsenate (AsV) and arsenite (AsIII) in soils. AsV, a chemical analog of P, is taken up by living cells via P transporters (Oremland and Stolz,
2003), and it interferes with phosphorylation reactions and competes for P transport (Oremland and Stolz, 2003; Shi et al., 2004). Similar to P, AsV is often associated with Fe/Al minerals in soils (Oremland and Stolz, 2003). On the other hand, AsIII is present as a neutral species and is transported across cell membranes by aquaporin channels (Liu et al., 2002; Oremland and Stolz, 2003). AsIII reacts with the sulphydryl groups of proteins and inhibit many biochemical pathways. Biologically, AsIII is more reactive than AsV, as it induces gene amplification by binding to thiol proteins and altering metabolic pathways in living cells (Liu et al., 2002; Oremland and Stolz, 2003).

Both AsV and AsIII are toxic to living organisms but some microbes have developed ways to survive in arsenic-rich environments (Hoefl et al., 2010). It is known that most bacteria reduce AsV to AsIII inside the cells through the action of arsenate reductase and exclude AsIII by AsIII efflux pump. Arsenic reduction minimizes AsV competition with P uptake so the cells can maintain normal growth and metabolism. However, AsIII can enter the cell through aquaporins and be methylated and immobilized in the bacterial biomass (Oremland and Stolz, 2003). Bacterial oxidation of AsIII to AsV has long been observed (Stolz and Oremland, 1999; Stolz et al., 2002; Silver and Phung, 2005) but still many microbial isolates lack the ability of AsIII oxidation (Silver and Phung, 2005). Bacterial periplasmic AsIII oxidase is encoded by genes asoA and asoB, which requires Fe-containing small Rieske [2Fe–2S] subunits for their activation. Under aerobic condition, AsIII tolerance in bacteria has not often been observed probably due to the lack of activation of the enzyme (Silver and Phung, 2005).

Even though Fe is the most abundant element in soils its availability is limited especially in calcareous soils. Most bacteria and plants have developed their specific Fe acquisition mechanisms to overcome Fe limitations in soils (Winkelmann, 1992). Siderophores are low molecular weight high-affinity Fe-chelators produced by plants and bacteria under Fe-limiting conditions to sequester Fe in soils (Kraemer, 2004). Bacterial ability to sequester Fe by producing siderophores gives them advantage over the non siderophore-producing bacteria to survive in the environment (Huang et al., 2004). Previous study showed that siderophore production by bacteria enhanced their arsenic tolerance by improving Fe nutrition, which is required for activation of AsIII oxidase (Drewniak et al., 2008). So we hypothesize that better Fe nutrition for bacteria may be important for their ability in AsIII oxidation and AsIII tolerance.

Six arsenic-resistant bacteria from the rhizosphere of arsenic-hyperaccumulator Pteris vittata were isolated and characterized (Ghosh et al., 2011). The plant’s rhizosphere contains higher amount of arsenic than bulk soils (4.42–235 vs. 0.07–63 mg kg⁻¹), suggesting that the rhizosphere bacteria are arsenic resistant as they survive in the arsenic-rich environment. All siderophore-producing bacteria showed fluorescence under UV, which is an intrinsic property of siderophores produced by bacteria (Ghosh et al., 2011). Furthermore, it was found that fluorescent arsenic-resistant bacteria are more effective in solubilizing arsenic from insoluble FeAsO₄ and AlAsO₄ minerals, resulting in increasing arsenic uptake by P. vittata (Ghosh et al., 2011). However, it is still unclear how bacterial AsIII oxidation or AsV reduction relates to their arsenic tolerance and how arsenic influences bacterial P uptake and metabolism.

The most commonly known soil bacteria producing siderophores are Pseudomonads, and they produce mostly pyochelin or pyoverdin type of siderophores (Kraemer, 2004; Huang et al., 2004). This study was to assess the tolerance of six arsenic-resistant bacteria to both AsV and AsIII with control strain Pseudomonas chlororaphis 63–28. Most environmental bacteria are capable of either AsV reduction or AsIII oxidation (Stolz and Oremland, 1999; Stolz et al., 2002). However, bacteria isolated from the spent growth media of P. vittata are able to oxidize AsIII and reduce AsV (Wang et al., 2012). The objectives of this study were to examine the relationship between bacterial ability in AsIII oxidation and AsV reduction and (1) their arsenic tolerance, (2) their P uptake, and (3) their siderophore production.

2. Materials and methods

2.1. Isolation of bacterial strains

The 6 arsenic-resistant bacteria tolerant to 10 mM AsV used for this study were previously isolated and identified from the rhizosphere of arsenic-hyperaccumulator P. vittata (Ghosh et al., 2011). Another bacterial strain Pseudomonas chlororaphis (PC) isolated from non-contaminated soil was used as a control (Alexander and Zuberer, 1991). The arsenic-resistant bacteria included Pseudomonas sp. (PG5, 6, 9, 10 and 12) and Stenotrophomonas sp. (PG18) based on 16S rRNA sequence (Ghosh et al., 2011). Their arsenic tolerance was measured as percentage of growth reduction at 1 and 10 mM of AsV or AsIII in modified LB medium (half-strength LB at pH 7) containing 0.8 mg L⁻¹ Fe incubated at 30 °C after 24 h shaking at 200 rpm (Ghosh et al., 2011). The bacterial medium components were purchased from Sigma Chemical Co. (St. Louis, Missouri). Their growth was measured as absorbance at 600 nm using a Beckman UV–Vis spectrophotometer.

2.2. Siderophore quantification

The method of Alexander et al. (1991) was used to measure siderophore production in vitro (Schwyn and Neilands, 1987; Alexander and Zuberer, 1991). The bacterial cells were grown at 30 °C for 24 h in 1 mL of modified M9 solution, which had the same composition as Chrome Azurol S (CAS) medium with 5 mM MES–KOH buffer at pH 6.8 (Hartney et al., 2011). After the cultures had grown to a specific OD₆₀₀ value, the cells were pelleted by centrifugation (10,000g for 5 min) and the supernatant was filtered through 0.2 μm filter. Siderophore concentration in the filtrate was measured by mixing 500 μL of modified CAS assay solution with 500 μL filtrate. The standard solutions of deferroxamine mesylate were used for siderophore quantification. Zero absorbance was calibrated with a mixture of modified CAS assay solution and 1.5 mM deferroxamine mesylate. Sterile modified M9 solution was used as a reference solution, which did not contain siderophores. A standard curve was prepared by analyzing the absorbance (630 nm) of the reference solution (A/Asym) as a function of the siderophore concentration.

2.3. Arsenic transformation by bacteria

All arsenic-resistant bacteria plus the control bacterium (PC) were incubated with 1 mM AsV or 1 mM AsIII in modified LB medium at 30 °C shaking at 200 rpm for 24 h. Sodium arsenite (Na₂HAsO₃·H₂O) and sodium arsenate (NaAsO₂) were purchased from Mallinckrodt Baker Inc. (Phillipsburg, New Jersey). The supernatants were analyzed for total arsenic, AsV and AsIII, which were separated using the Sep-Pak™ Plus C18 arsenic speciation cartridge (Ghosh et al., 2011). Concentrations of AsV and AsIII were determined by graphite furnace atomic absorption spectrophotometry (GFAAS, Varian AA240Z, Walnut Creek, CA) (Ghosh et al., 2011).

The percentage of AsIII oxidation and AsV reduction were calculated based on the remaining fraction of the initial and final concentration of the particular arsenic species in the growth media before and after bacterial incubation.
### 2.4. Phosphate and AsV uptake by bacteria

Two arsenic-resistant bacteria (PG5 and 16) were selected based on their difference in siderophore production and arsenic tolerance. PG5 is highly AsIII tolerant with high siderophore-production (73.2 μM equiv) while PG16 is highly AsV tolerant with low siderophore-production (10.8 μM equiv) (Ghosh et al., 2011) (Table 1). Control bacterium PC is moderate in siderophore production (18.6 μM equiv) and tolerant to AsV. P utilization rates and AsV reduction were measured in 1 mL modified LB medium containing 0, 0.1 or 1 mM AsV and 1 mM P at 4 and 8 h time intervals. Phosphate concentration was measured by modified molybdenum blue method (Ghosh et al., 2011). Bacterial growth and arsenic speciation were measured as described before.

### 2.5. Determination of P utilization index and arsenic in bacterial cells

In addition to AsV reduction and bacterial growth, we also measured P utilization by bacteria. The P utilization index (PUI) was calculated by dividing P utilized (changes in P concentration in the medium before and after bacterial growth) by the dry bacterial biomass corresponding to OD units of growth. The dry weight conversion factor for bacterial biomass was calculated by growing individual bacteria in bulk and then measuring the weight of cells/OD value before drying down in the oven at 100 °C. The number was obtained by the difference in weight before and after drying/OD value of cells.

To measure the amount of arsenic immobilized in cell biomass, the arsenic remaining in the media after bacterial growth was measured per OD bacterial cells. Here the conversion factor between the cell OD value and their dry weight was used. To determine the conversion factor between OD value of cells and dry weight of biomass, 50 mL cell cultures were grown, absorbance determined, then centrifuged down and the cell pellets were dried to constant weights at 37 °C for 2 d. The bacterial cells were centrifuged down and washed again with fresh modified LB media to remove arsenic immobilized on outer cell surface.

### 2.6. Arsenic tolerance by two mutants of Pseudomonas fluorescens

To test the role of siderophores in arsenic tolerance and transformation, the wild type (LK099) and two mutants of Pseudomonas fluorescens PF-5, JL4900 (Apvdl/ApchC) and LK032 (ApcHAApvdl) with differing ability in siderophore production were evaluated (Hartney et al., 2011). Arsenic tolerance of all three strains was measured as percentage of growth reduction at 0, 1 and 10 mM of AsV or AsIII in modified LB medium incubated at 30 °C for 24 h shaking at 200 rotations min⁻¹ (rpm). Growth was measured as absorbance at 600 nm on Beckman UV–Vis spectrophotometer.

### Table 1

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Siderophore (μMol DFOM equiv/cell OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG12</td>
<td>115 ± 0.02</td>
</tr>
<tr>
<td>PG5</td>
<td>73.2 ± 0.01</td>
</tr>
<tr>
<td>PG6</td>
<td>22.7 ± 0.01</td>
</tr>
<tr>
<td>PG16</td>
<td>15.8 ± 0.02</td>
</tr>
<tr>
<td>PG9</td>
<td>10.8 ± 0.04</td>
</tr>
<tr>
<td>PG10</td>
<td>9.47 ± 0.02</td>
</tr>
<tr>
<td>P. chlororaphis</td>
<td>18.6 ± 0.02</td>
</tr>
</tbody>
</table>

DFOM stands for deferroxamine mesylate.

### 2.7. Statistical analysis

All bacterial experiments were conducted with three replicates for each treatment and every experiment was done at least twice. The analysis of variance (ANOVA) and Tukey’s mean grouping were used to determine significance of differences between the treatment means. All statistical analyses were performed with SAS statistical software (SAS Inst., Cary, North Carolina, USA).

### 3. Results and discussion

A study on the functional gene diversity of bacteria in arsenic-rich rhizosphere of P. vittata showed that the fern maintained a variety of microbes in its rhizosphere compared to only arsenic-tolerant convergent microbial community in typical arsenic-contaminated soil (Xiong et al., 2010). This may be due to the continuous arsenic solubilizing and uptake activity taking place by the rhizobacteria in the rhizosphere. In soils, AsV is often the predominant form and as such soil bacteria are more tolerant to AsV than AsIII (Rodriguez and Fraga, 1999; Wang et al., 2011). However, it is possible that AsV tolerance requires bacterial ability in AsV reduction whereas AsIII tolerance requires bacterial ability in both AsIII oxidation and AsV reduction.

To test this hypothesis, we tested bacterial ability in transforming 1 mM AsIII or AsV. Among the 6 bacteria tested, their ability in AsV oxidation and AsV reduction varied (Fig. 1A). Based on their ability in arsenic transformation, the 6 bacteria were separated into two groups: AsV reducers (PG6, 9, 10 and 16) and AsV oxidizers (PG5 and 12) (Fig. 1A). The AsV reducers reduced 58–95% AsV to AsIII in 24 h, which were more effective than the AsIII oxidizers (7.5–23%) (Fig. 1A). On the other hand, the AsIII oxidizers oxidized 31–46% AsIII to AsV in 24 h, which were more effective than the AsV reducers (6.2–21%) (Fig. 1A). The control bacterium PC reduced all AsV to AsIII in 24 h but showed little oxidation ability.

### 3.1. Arsenic tolerance linked to bacterial arsenic oxidation and reduction

To link bacterial tolerance to their ability in AsIII oxidation and AsV reduction, we tested their tolerance to 1 and 10 mM AsIII or AsV. The AsIII oxidizers (PG5 and 12) showed higher tolerance to AsIII than the AsV reducers (PG6, 9, 10 and 16), with the control strain PC being the least tolerant (Fig. 2). For example, at 10 mM AsIII, the growth reduction of PG5 and 12 were <30% compared to 42–50% for PG6, 9, 10 and 16 (Fig. 2A). All six arsenic-resistant bacteria were tolerant to 1 mM AsV with no significant reduction in growth after 24 h (Fig. 2B). At 10 mM AsV, the AsV reducers (PG6, 9, 10 and 16) showed little growth reduction, but AsIII oxidizers (PG5 and 12) showed more reduction (10–52%) (Fig. 2B). Under aerobic conditions, AsV may be reduced to AsIII by the ars operon of arsenic-resistant bacteria, which is observed in most bacteria (Silver and Phung, 2005; Wang et al., 2011). The AsIII oxidizers with lower AsV reduction ability showed more growth reduction in AsV treatment. Thus there was a link between AsIII oxidation and AsIII tolerance, and AsV reduction and AsV tolerance in these bacteria.

Arsenic-resistant bacteria can expel arsenic out of the cells utilizing AsIII efflux pump after its reduction from AsV to AsIII via the ars operon. This process helps bacteria to keep arsenic out of the cells when AsV is the dominant species in the medium. However, eventually most of AsV will be reduced to AsIII in the media. So, it is also important for arsenic-resistant bacteria to deal with AsIII entering through the aquaporins. Oxidation of AsIII to AsV would balance the other half of the transformation from AsV to AsIII. When exposed to 1 mM AsIII, the AsIII-oxidizers (PG5 and 12;
AsV oxidizers and AsV reducers. We hypothesized that this uptake occurred during the first 4 h where there was minimum arsenic reduction using bacteria PG16 and PG5. Phosphate transporters. We studied the impact of P and arsenic on their uptake and those with better reducing ability were more tolerant to AsV.

3.2. Lower P uptake linked to better As resistance

Fig. 1B) retained less arsenic than AsV-reducers in the biomass (PG6, 9, 10 and 16; Fig. 1B). On the other hand, at 1 mM AsV, the AsV-reducers retained less arsenic than AsIII oxidizers in the biomass. It is known that bacteria efflux AsIII out to reduce arsenic toxicity within the cells. However, our research clearly showed that AsIII oxidation to AsV under aerobic condition was also utilized by bacteria to reduce their intracellular stress by AsIII. So, bacteria with better oxidizing ability were more tolerant to AsIII and those with better reducing ability were more tolerant to AsV. Control strain was highly efficient in reducing 1 mM AsV to AsIII (Fig. 1A) so it was not tolerant to AsIII. The growth reduction in PC was ~20% at 1 mM AsV (Fig. 2B) compared to 70% at 1 mM AsIII (Fig. 2A). So our results supported the hypothesis that AsV tolerance required bacterial ability in AsV reduction whereas AsIII tolerance required bacterial ability in both AsIII oxidation and AsV reduction.

3.3. Arsenic-resistant bacteria retained less As in biomass

The amount of AsV and AsIII immobilized in the cell biomass may be related to their arsenic tolerance. AsIII after getting into the cells needs to be expelled out; otherwise it would complex with proteins and is immobilized in the cells. Hence, microbes detoxify AsIII via AsIII oxidation (Lloyd and Oremland, 2006; Chang et al., 2007), which may be expelled out of the cells or mod-

Fig. 1. Arsenic oxidation and reduction (A) and arsenic immobilized in cell biomass (B) by 6 arsenic-resistant bacteria (PG5, 12, 6, 9, 10, and 16) and control strain P. chlororaphis (PC) in modified LB medium spiked at 1 mM AsV or AsIII. The bacteria were grown for 24 h at 30 °C under 200 rpm. The bars represent mean ± SE of three replicates. We used Tukey’s mean grouping test and same letter denotes means belonging to the same group.

Fig. 2. Arsenic tolerance of 6 As-resistant bacteria (PG5, 12, 6, 9, 10, and 16) and control strain P. chlororaphis (PC) measured as % growth reduction of the control in 1 ml modified LB medium spiked with 1 and 10 mM AsIII (A) and AsV (B). The bacteria were grown for 24 h at 30 °C under 200 rpm. The bars represent mean ± SE of three replicates. We used Tukey’s mean grouping test and same letter denotes means belonging to the same group.

ify AsIII via methylation (Silver and Phung, 2005). Since AsV tolerance by bacteria is through its uptake, reduction to AsIII and subsequently expulsion of AsIII out of the cells (Beppu and Arima, 1964), lower AsV in biomass may imply better arsenic tolerance. Among the 6 arsenic-resistant bacteria, the AsV reducers (PG6, 9, 10 and 16) (Fig. 1A), which were more tolerant to AsV (Fig. 2A), retained lower amount of arsenic at 1 mM AsV than the AsIII oxidizer (PG5 and 12) (Fig. 2B).

As a group, the oxidizers (PG5 and 12) were slightly more tolerant to AsV than the reducers (PG6, 9, 10 and 16) though the separation was not as clear as AsV tolerance. The group more tolerant to AsIII was also more efficient in siderophore-production (Table 1) and AsIII oxidation (Table 2). Arsenic in cell biomass was lower in AsV treatment than the AsIII treatment. All 6 arsenic-resistant bacteria were capable of both AsV reduction and AsIII oxidation under aerobic culture conditions. This probably is because arsenic transformation in soil is mainly mediated by bacteria and arsenic species change between AsV and AsIII depending upon the environment (Beppu and Arima, 1964). So, bacteria surviving in arsenic-rich environment need to be tolerant to both AsV and AsIII, which may be a unique feature of arsenic-resistant bacteria. Only reducing AsV to AsIII may not be effective in detoxifying high arsenic in arsenic-contaminated soil as both AsV and AsIII are toxic, so the bacteria surviving in the arsenic-rich rhizosphere developed both properties. However, this was not the case with the control bacterium, which was only effective in AsV reduction (Fig. 1A).

The AsIII oxidation and AsV reduction help bacteria in expelling arsenic out of their cells. AsV entering the cell is reduced to AsIII which was expelled out through AsIII efflux pump. Previous study on P. pseudomallei with radio-labeled AsIII showed that AsIII tolerance is correlated with retention of less AsIII in the cells during their growth (Beppu and Arima, 1964). Similar to AsV, AsIII is also highly toxic to the cells and enters cells through aquaporins (Silver and Phung, 2005). AsIII oxidation and AsV reduction helped in expelling arsenic species out of the bacterial cell. Hence, AsIII-oxidizing bacteria (PG5 and 12) retained less As in 1 mM AsIII treatment whereas AsV-reducing bacteria (PG6, 9, 10 and 16) retained less As in 1 mM AsV treatment (Fig. 2B).

3.4. P. fluorescens siderophore mutants were less tolerant to arsenic

The 6 arsenic-resistant bacteria were separated into two groups based on their ability in siderophore production: low siderophore-production (PG6, 9, 10 and 16; 9.5–22.7 μM equiv) and high-siderophore production (PG5 and 12; 73.2–115 μM equiv) (Table 1), with the control bacterium PC being moderate (18.6 μM equiv).

Bacteria with high siderophore-production were better AsIII oxidizers (PG5 and 12) (Fig. 1A), so they were more resistant to AsIII (Fig. 2A). On the other hand, bacteria with low siderophore-production were also better AsV reducers (PG6, 9, 10 and 16) (Fig. 1A) so they were more resistant to AsV (Fig. 2B).

These results suggest that microbial ability in both AsIII oxidation and AsV reduction was important for their arsenic tolerance (Drewniak et al., 2008; Cai et al., 2009). AsV reduction and AsIII oxidation helped in expelling arsenic out of the cell. Siderophore-producing bacteria (PG5 and 16) were more efficient in AsV oxidation (r = 0.77, p = 0.01) and more tolerant to AsIII (r = 0.62, p = 0.01), whereas there was inverse relationship with AsV reduction (r = −0.81, p = 0.01). Production of siderophores seemed linked to efficient AsIII oxidation by arsenic-resistant bacteria. Siderophores may help sequester Fe, which is needed to activate AsIII oxidase as Fe is unavailable at pH 7 (Kraemer, 2004). So, higher availability of Fe may aid higher AsIII oxidation by AsIII oxidizers (PG5 and 16), although the mechanistic details remain unclear.

To test the hypothesis that siderophore production had a role in arsenic tolerance, arsenic tolerance of two P5 mutants (pchA-partially impaired in siderophore production, and pchA-completely impaired in siderophore production) with the wild-type strain

Table 2

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Growth time (h)</th>
<th>P utilization (PUI)* (mg g⁻¹)</th>
<th>AsV reduction in media (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>PG16</td>
<td>4</td>
<td>0.53 ± 0.020abc</td>
<td>1.61 ± 0.003c</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.15 ± 0.013d</td>
<td>0.18 ± 0.003e</td>
</tr>
<tr>
<td>PC</td>
<td>4</td>
<td>4.62 ± 0.011f</td>
<td>14.3 ± 0.003g</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.21 ± 0.005i</td>
<td>0.23 ± 0.003i</td>
</tr>
<tr>
<td>PG5</td>
<td>4</td>
<td>4.58 ± 0.010f</td>
<td>4.66 ± 0.003F</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.29 ± 0.005j</td>
<td>0.26 ± 0.003j</td>
</tr>
</tbody>
</table>

* Same letter denotes not significantly different based on Tukey’s mean grouping. Lower case letters denote PUI and upper case letters for AsV reduction in media.

** Approximate 90% of the total P in the growth media was taken up by the bacterial cells within 8 h.
were compared (Hartney et al., 2011). The pchC gene construct was made by using GmR–gfp cassette to interrupt the pchC gene. The pchA gene construct was made by deleting the middle portion of the pchA gene. The pchC mutant produces residual amount of enantiopyochelin siderophores while the pchA mutant does not (Hartney et al., 2011). When exposed to 1 mM AsV, the two mutants were 16% less tolerant than the wild type (Fig. 3A). However, this was not the case with 1 mM AsV where pchC and pchA was 6% and 23% less tolerant than the wild type (Fig. 3B). At 10 mM AsIII, pchA was the least tolerant with 9% reduction compared to the wild type. This indicated that siderophore in pchC (partially-impaired) helped its AsIII tolerance as it had similar rates of AsIII oxidation as the wild type (Fig. 4). Mutant PchA impaired in siderophore production showed no AsIII oxidation (Fig. 4). The data suggested that siderophore production was important for AsIII oxidation as it was required to activate AsIII oxidase, i.e., bacteria with more siderophore production were more tolerant to AsIII.

4. Conclusions

AsIII oxidation and AsV reduction were important for arsenic-resistant bacteria to minimize arsenic accumulation in bacteria. Bacteria producing more siderophores were more efficient in AsIII oxidation and bacteria producing less siderophores were better AsV reducers. The bacteria had the ability to reduce AsV to AsIII and expel AsIII to the media so that AsV does not interfere with P uptake. Presence of AsV in the media stimulated P uptake, indicating arsenic probably up-regulated P transporters. Arsenic-resistant bacteria had the ability to oxidize AsIII to AsV for detoxification in a cyclical manner. In soil, bacteria possessing the dual ability of AsV oxidation and AsIII reduction benefit from reducing arsenic toxicity and thriving better in arsenic-contaminated rhizosphere. So these bacteria dominating in contaminated soils will maintain a cycle between AsV and AsIII transformation in the microenvironment. This cycle may also change the soil environment in the rhizosphere compared to the bulk soil.

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References


