Assessing the bioremediation potential of arsenic tolerant bacterial strains in rice rhizosphere interface

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ABSTRACT

The arsenic tolerant bacterial strains Staphylococcus arlettae (NBRIEAG-6), Staphylococcus sp. (NBRIEAG-8) and Brevibacillus sp. (NBRIEAG-9) were tested for their roles in enhancing plant growth and induction of stress-related enzymes in rice (Oryza sativa L. cv. NDR-359) plants at two different concentrations, 30 and 15 mg/kg of As(V) and As(III), respectively. An experiment was conducted to test the effect of these strains on plant growth promotion and arsenic uptake. We found 30%–40% reduction in total As uptake in bacteria-inoculated plants, with increased plant growth parameters compared to non-inoculated plants. Moreover, the bacteria-inoculated plants showed reduced activity of total glutathione (GSH) and glutathione reductase (GR) compared to their respective controls, which suggests the bacteria-mediated reduction of oxidative stress in plants. Thus, these strains were found to be beneficial in terms of the biochemical and physiological status of the plants under arsenic stress conditions. Furthermore, one-way ANOVA and principal component analysis (PCA) on enzymatic and non-enzymatic assays also revealed clear variations. The results support the distinction between control and treatments in both shoots and roots. Therefore, this study demonstrates the potential of rhizobacteria in alleviating arsenic stress in rice plants.

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Introduction

Worldwide arsenic (As) toxicity poses a serious health risk to millions of people (Nordstrom, 2002). Two forms of As mainly exist in the environment, arsenate As(V) and arsenite As(III). As(V) generally occurs in well-oxidized media while As(III) is found predominantly in reducing environments. As enters the environment through natural geological processes and/or anthropogenic activities such as mining, fossil fuel burning, and application of fertilizers and pesticides, and poses long-term risks to human health (Cullen and Reimer, 1989; Chen et al., 2015a; Zeng et al., 2013). Chronic exposure to As results in some of the most serious health risks to mankind such as skin and lung cancers, diabetes, and nervous system and cardiovascular problems (Dopp et al., 2004).

As-affected sites are also the main sites for crop production, particularly in India. The global average concentration of As in soil is about 5 mg/kg. Uncontaminated soil contains <10 mg/kg total arsenic, but the concentration can reach hundreds or thousands of mg/kg in contaminated environments (IARC, 2004). As gets accumulated in rice and finds its way to exposure in humans and other life forms through the food chain. So it is necessary to either remediate these sites or to adapt the crops preventing accumulation in the edible parts of plants. For small areas the former approach is better, but for larger contaminated areas the latter approach is beneficial. For this reason, it was...
realized that a cost-effective and broad-scale applicable technology should be developed for betterment of affected sites. Many researchers have indicated that application of certain microorganisms could considerably reduce heavy metal toxicity in plants and allow them to survive and grow in environments containing high levels of arsenic that would be toxic to most other organisms (Chen et al., 2015b). Anderson and Cook (2004) have reported strains such as Aeromonas, Exiguobacterium, Acinetobacter, Bacillus and Pseudomonas that can tolerate high concentrations of arsenic species (up to 100 mmol/L arsenate or up to 20 mmol/L arsenite). Heavy metal contamination causes oxidative stress to plants, due to the stimulation of free oxygen radical production (Gao et al., 2010) and modification of the activity of various antioxidant enzymes (Wei et al., 2010). It stimulates the production of reactive oxygen species (ROS) like ‘O2 (singlet oxygen), O2− (superoxide radical), OH· (hydroxyl radical) and H2O2 (Andrade et al., 2010; Kafel et al., 2010) and induces oxidative stress resulting in unbalanced redox status in cells and cellular damage in terms of lipid peroxidation (Malondialdehyde (MDA), and consequently reduces crop productivity. To cope up with As-induced stress, plants have well-developed scavenging systems comprising non-enzymatic antioxidants (e.g., glutathione (GSH), ascorbate, carotenoids) and enzymatic antioxidative systems (e.g., superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) (Elstner, 1982). The induction of these antioxidant systems promotes the ability to endure metal-induced oxidative stress (Gratão et al., 2005). The heavy metal stress responses of different genotypes have been extensively investigated in crops such as barley (Wu et al., 2003) and Brassica sp. (Sharma et al., 2010). The mechanism of arsenic toxicity and its natural response in living systems are subjects of major importance today, and the aim of this study is to see how bacteria respond to heavy metal toxicity by modification of antioxidant enzyme activity in plants. Several studies have indicated that using the plant-microbial interface could considerably reduce heavy metal toxicity and its accumulation in crop plants, and thus induce plant growth promotion. Many studies have been conducted on hyper-accumulator plants, as well as on consequences of antioxidant enzyme activity under As stress conditions. However, very much less information is available regarding biochemical alterations (antioxidant defense mechanisms) in the presence of microbes on rice plants under arsenic stress. In this study, three bacterial strains (NBRIEAG-6, NBRIEAG-8 and NBRIEAG-9) were isolated from As-contaminated sites in West Bengal (India). Previous studies have characterized NBRIEAG-6 (JQ388197) as Staphylococcus arletae, NBRIEAG-8 (GU991542) as Staphylococcus sp., respectively (Srivastava et al., 2012, 2013) while NBRIEAG-9 (GU997108) as Brevisbacillus sp. (Srivastava, 2012). The present study assesses the effects of these As-tolerant bacterial strains on plant growth promotion, As uptake and antioxidant defense responses in rice plants (Oryza sativa L. var. NDR-359) subjected to As treatment at two different concentrations.

1. Materials and methods

1.1. Soil properties

The physico-chemical analysis of soil was carried out after incubation for 5 days according to the procedure described by Kalra and Maynard (1991). Soil samples were collected from pots and sieved (<2 mm). For dehydrogenase activity (DHA), soil was stored at –20°C and analysis was completed within a week. Dehydrogenase activity (DHA) was examined following the method of Pepper et al. (1995) through the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) and expressed in μg triphenyl formazan “per gram soil per hour.” The pH was measured in aqueous soil solution of 1:2.5 (W/V dry weight basis) soil: water ratio at room temperature. Total organic carbon was analyzed by the Walkley and Black (1934) method. Available phosphorus was analyzed by following the method proposed by Olsen (1954).

1.2. Experimental setup and biological treatment

A pot experiment was conducted under greenhouse conditions (18°C (night) and 24°C (day), 80% relative humidity, 11 hr photoperiod) at CSIR-NBRI Lucknow, India. Each pot (25 kg capacity) was filled with 12 kg autoclaved soil and supplemented with 30 mg/kg As(V) and 15 mg/kg As(III). The rice seeds (O. sativa L. var. NDR-359) were surface-sterilized in 0.1% sodium hypochlorite for 5 min and rinsed twice with sterile water. For seedling preparation, the seeds were grown in pots. After two weeks, when seedlings became 15 cm tall, 6 uniform seedlings were transplanted at 10 cm distance in each pot. Each experiment was done in triplicate.

The strains were grown in 500 mL Erlenmeyer flasks containing 250 mL nutrient broth at 30°C, for 24 hr on a rotary shaker, at 200 r/min. Cells in the exponential phase were collected by centrifugation at 12,000×g for 15 min at 4°C, washed with sterile distilled water and again followed by centrifugation. Bacterial inoculums were prepared by re-suspending pellet cells in sterile distilled water to obtain an inoculum density of approximately 7.5 × 10⁶ CFU/mL, giving an absorbance of 0.5 at 600 nm. After one week, the seedlings were inoculated with 50 mL/pot bacterial suspension in control treatment pots. The plants were harvested 90 days after transplanting and growth parameters like shoot length, root length and dry weight were recorded.

1.3. As estimation in plant and soil samples

Plants were harvested carefully after 3 months from the pots and the root surface was cleaned twice with distilled water. Growth parameters such as root and shoot length and dry weight of the plants were measured. For arsenic estimation of soil and plant samples (root, shoot, husk and grain), the samples were oven-dried at 55°C and 0.1 g samples were digested with a mixture of nitric acid and perchloric acid (5:1, V/V) in a BURGHOF-speedwave-MSW-3+ digestion system. Digested material was maintained with 30 mL of MilliQ water and As content was estimated by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Agilent-7500 cx, Model No. G3160B, Germany). A translocation factor (TF, mg/kg) was calculated by Eq. (1):

\[ TF = \frac{A_{metal\_shoot}}{A_{metal\_root}} \]

where \( A_{metal\_shoot} \) (mg/kg) is the mean accumulation of metal by the shoot part, and \( A_{metal\_root} \) (mg/kg) is the mean accumulation of metal by the root part.
1.4. Antioxidant response in rice

Plant samples were homogenized with 0.1 mol/L sodium phosphate buffer (pH 7.5) and EDTA 0.5 mmol/L in a chilled mortar and pestle. The homogenate was centrifuged at 12,000 x g for 15 min, and the resulting supernatant was used for enzyme assay and protein estimation. The MDA content was determined through the method of Heath and Packer (1968). The absorbance of the MDA was examined by glutathione-dependent oxidation of NADPH (Ahmad et al., 2008) enzymes, siderophores, and antibiotics (Glick, 2010). In addition, they also solubilize inorganic phosphorous and mineralize organic phosphorous, which is a necessary element for plant growth (Khan et al., 2009). Further, several studies have identified plant growth promoting characteristics in certain heavy-metal-resistant bacteria (Marques et al., 2013). This result leads to the conclusion that the above-mentioned strains have plant-growth-promoting characteristics that help to induce plant growth.

2.2. Effect of As-tolerant bacteria on rice plant development

The potential of certain metal-resistant bacteria in mitigating the toxic effects of metals and subsequently improving plant growth promotion has been documented in many studies (Glick, 1995). In this study, we used three bacterial strains, NBRIEAG-6 and NBRIEAG-9 isolated from arsenic-contaminated sites in West Bengal, India (Srivastava, 2012; Srivastava et al., 2012). The bacterial inoculation with As was found to significantly promote plant growth with respect to arsenic-treated plants. The root and shoot lengths were increased by 47% and 35% and the biomass of roots and shoots were increased by 42% and 47% respectively (Table 1), while no significant difference was observed in plant growth promoting ability among these bacterial strains. Previous studies showed that plant-growth-promoting bacteria have the potential for improving plant growth by means of synthesizing phytohormone precursors (Ahmad et al., 2008) enzymes, siderophores, and antibiotics (Glick, 2010). In addition, they also solubilize inorganic phosphorous and mineralize organic phosphorous, which is a necessary element for plant growth (Khan et al., 2009). Further, several studies have identified plant growth promoting characteristics in certain heavy-metal-resistant bacteria (Marques et al., 2013). This result leads to the conclusion that the above-mentioned strains have plant-growth-promoting characteristics that help to induce plant growth.

2.3. Arsenic accumulation in rice

Generally, in response to high levels of metal stress, plants adopt two basic strategies, accumulation and exclusion (Vogel-Mikuš et al., 2005). In this study we found that the coexistence of arsenic and arsenic-tolerant bacterial strains increased the exclusion potential of rice. Moreover, NBRIEAG-6 and NBRIEAG-8 cumulatively reduced arsenic accumulation in rice plants (root, shoot husk and grain) by ~30%, while the NBRIEAG-9 strain decreased As accumulation in husk and grain by ~11% and ~21% respectively, but no significant differences were observed in roots and shoots in comparison to As-treated plants (Fig. 1). The results showed similarities with previous studies where the presence of metal-tolerant bacterial strains resulted in decreased accumulation in host plants (Jiang et al., 2008). In other studies, Hasnain and Sabri (1997) observed the presence of rhizobacteria (Pseudomonas sp.), which reduced chromium accumulation in wheat (Triticum aestivum L.); and in another report, bacterial (Burkholderia sp. J62) inoculation resulted in decreased cadmium concentration in maize plants compared to non-inoculated plants (Jiang et al., 2008).

In this study, As-tolerant bacterial strains reduced metal uptake in rice plants, since bacteria can share the metal load and prevent accumulation in plants (Delorme et al., 2001). Moreover, the mechanisms responsible for decreasing As uptake include binding of the metal to functional groups of bacteria or chelation of metal with bacterial extracellular polymers, siderophores and organic acids (Glick, 2010). In addition, bacterial inoculation can also alter the metal
biosavailability by producing ammonia or organic bases, which can enhance the formation of metal precipitates in the root zone, or by metal reduction/oxidation (Chen and Cutright, 2003).

2.4. Non-enzymatic and enzymatic assay determination

Lipid peroxidation (degradation) is the indicator of oxidative damage in plants growing under stressed environments (Zenk, 1996). Measurement of MDA levels, the final product of lipid peroxidation, is routinely applied as a perceptive index of oxidative stress (Choudhary et al., 2007). In this study, the MDA content under As(V) and As(III) treatments was increased, owing to excessive production of ROS, which leads to lipid peroxidation (Fig. 2A). MDA content was reduced in all bacteria-inoculated plants compared to As treated plants. This result indicates that the bacterial strain helps to reduce As stress in plants, by which varying levels of reduction in MDA content are observed as compared to arsenic treatment. Several authors have reported increased contents of MDA under heavy metal stress conditions (Singh et al., 2010; Raj et al., 2011). Moreover, the effects of As on soluble protein content were also measured (Fig. 2B). As toxicity decreased the protein content in shoots (45% and 49%), whereas bacterial inoculation with As could potentially enhance the protein content in shoots, while the MDA content under all treatments was almost the same in roots. Furthermore, the data concerning the soluble protein content confirmed the results, indicating that these bacterial strains improved plant health by reducing metal stress on roots and shoots, confirmed by the observed changes in protein content. In the case of enzymatic activity, there was not much variation (except in a few cases) between inoculated and uninoculated treatments. Inoculated treatments showed an increase in antioxidant enzyme (APX and CAT) activity, but the increase was slightly less compared to that in uninoculated treatments, on exposure to arsenic stress. Moreover, bacterial incorporation resulted in variable responses to GSH content in treatments, on exposure to arsenic stress. Moreover, bacterial inoculation with As(V) reduced the formation of GSH, while inoculation with As(III) induced the formation of GSH. Such an observation was probably either due to enhanced sequestration of As(V) in the rhizosphere or lower availability of a phosphate transporter for As(V) transport inside the root, which leads to lower GSH formation. However, As(III) transport takes place via aquaporins, which may lead to an increase in GSH formation. Some other studies also reported a decrease in the level of GSH as a response to Cu stress (Nagalakshmi and Prasad, 2001). The maximum increase was observed in GR activity on exposure to arsenic stress showed the lowest GR activity. Therefore, the enzymatic activity showed reduced expression of GR in bacteria-inoculated plants compared to As-treated plants. GR is involved in reduction of As(V) to As(III), which ultimately is either sequestered inside vacuoles or transported

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length (cm) Root</th>
<th>Shoot</th>
<th>Dry weight (g) Root</th>
<th>Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.33 ± 11.23</td>
<td>75.33 ± 6.80</td>
<td>9.73 ± 0.45</td>
<td>20.36 ± 2.34</td>
</tr>
<tr>
<td>As(V) only</td>
<td>19.0 ± 5.26</td>
<td>58.33 ± 3.51</td>
<td>4.6 ± 1.77</td>
<td>7.77 ± 1.15</td>
</tr>
<tr>
<td>As(V) + NBRIEAG-6</td>
<td>30.33 ± 3.51</td>
<td>80.67 ± 8.14</td>
<td>8.13 ± 1.41</td>
<td>14.13 ± 4.41</td>
</tr>
<tr>
<td>As(V) + NBRIEAG-8</td>
<td>28.0 ± 3.60</td>
<td>70.67 ± 14.01</td>
<td>6.03 ± 2.03</td>
<td>10.49 ± 2.96</td>
</tr>
<tr>
<td>As(V) + NBRIEAG-9</td>
<td>24.67 ± 8.38</td>
<td>82.33 ± 8.02</td>
<td>9.73 ± 1.70</td>
<td>17.56 ± 3.70</td>
</tr>
<tr>
<td>As(III) only</td>
<td>17.33 ± 4.04</td>
<td>60.67 ± 4.17</td>
<td>6.13 ± 1.84</td>
<td>11.30 ± 1.91</td>
</tr>
<tr>
<td>As(III) + NBRIEAG-6</td>
<td>26.0 ± 4.35</td>
<td>83.33 ± 6.11</td>
<td>6.3 ± 0.86</td>
<td>16.90 ± 2.95</td>
</tr>
<tr>
<td>As(III) + NBRIEAG-8</td>
<td>38.0 ± 8.88</td>
<td>87.76 ± 4.35</td>
<td>8.33 ± 1.9</td>
<td>12.30 ± 1.91</td>
</tr>
<tr>
<td>As(III) + NBRIEAG-9</td>
<td>25.33 ± 8.96</td>
<td>89.33 ± 8.02</td>
<td>8.43 ± 1.26</td>
<td>13.8 ± 0.44</td>
</tr>
</tbody>
</table>

* Values are mean ± S.D.

Table 1 - Root and shoot length and dry weight of two arsenic (As(V) and As(III)) treated groups noninoculated (As only) and inoculated (As + bacterial strains NBRIEAG-6, NBRIEAG-8 and NBRIEAG-9) at two concentrations, As(V) (30 ppm) and As(III) (15 ppm) respectively.

Table 2 - Changes in pH, dehydrogenase enzyme, total organic carbon and available phosphorous levels in two arsenic (As(V) and As(III)) treated groups noninoculated (As only) and inoculated (As + bacterial strains NBRIEAG-6, NBRIEAG-8, NBRIEAG-9) at two concentrations, As(V) (30 ppm) and As(III) (15 ppm) respectively*.

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Parameter</th>
<th>As(V) of 30 mg/kg</th>
<th>As(III) of 15 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>8.46 ± 0.05</td>
<td>8.53 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Control (As only)</td>
<td>8.53 ± 0.11</td>
<td>8.73 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-6</td>
<td>8.66 ± 0.05</td>
<td>8.73 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-8</td>
<td>8.56 ± 0.05</td>
<td>8.66 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-9</td>
<td>2.53 ± 0.68</td>
<td>5.47 ± 1.13</td>
</tr>
<tr>
<td></td>
<td>Dehydrogenase enzyme (μg/g)</td>
<td>5.85 ± 0.19</td>
<td>2.83 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>Control (As only)</td>
<td>4.60 ± 0.97</td>
<td>4.79 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-6</td>
<td>4.06 ± 0.56</td>
<td>5.73 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-8</td>
<td>0.62 ± 0.02</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-9</td>
<td>0.90 ± 0.08</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Total organic carbon (%)</td>
<td>0.72 ± 0.02</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Control (As only)</td>
<td>1.00 ± 0.02</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-6</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-8</td>
<td>0.14 ± 0.01</td>
<td>0.14 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-9</td>
<td>0.15 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Available phosphorous (μg/g)</td>
<td>0.18 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Control (As only)</td>
<td>16.65 ± 1.12</td>
<td>19.51 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-6</td>
<td>10.39 ± 1.00</td>
<td>12.53 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-8</td>
<td>4.25 ± 0.80</td>
<td>11.05 ± 0.96</td>
</tr>
<tr>
<td></td>
<td>NBRIEAG-9</td>
<td>7.69 ± 0.84</td>
<td>7.39 ± 0.85</td>
</tr>
</tbody>
</table>

* Values are mean ± S.D.
to shoots (Mishra et al., 2008). Reduced GR activity in bacteria-inoculated plants as compared to As-treated plants is probably due to lower As(V) transport inside roots (Fig. 3A). This result is in agreement with our above data, in which GSH
activity was lower in bacteria-inoculated plants. On exposure to bacterial strains, GR activity was reduced in roots and shoots, which allowed a decrease in GSH level in all bacteria-inoculated plants, and reduced GSH concentration in the presence of bacteria indicates lower arsenic accumulation in roots and shoots, allowing the plant to withstand As-induced oxidative stress. In another study it was reported that glutathione reductase is the main controlling aspect of As-tolerance in bacteria (Mukhopadhyay et al., 2002). However, APX and catalase activity did not show a significant role related to bacteria-mediated As remediation (Fig. 3B, C). The microbial effect on plant heavy metal uptake varies considerably, and screening of appropriate plant-microbe combinations in technical applications such as bioremediation is an important step. When we applied arsenic-tolerant microbial strains, we observed wide-scale reduction of total As uptake by rice, and concurrently the promotion of plant growth. As our previous study documented, lower transport of As(V) through phosphate transporters is probably due to the phosphate solubilizing property of these bacterial strains (Srivastava, 2012; Srivastava et al., 2012). Several other studies also documented the dual behavior (improvement of host plant growth and mitigation of toxic effects of metals) of certain heavy metal resistant bacteria (Rajkumar and Freitas, 2008). According to Marques et al. (2013), the inoculation of Helianthus annuus (Sunflower) with a metal-resistant strain leads to an increase in bacterial diversity along with plant growth promotion. Recent studies report the role of oxalic acid in the uptake of Cd and participation in the detoxification process in Phanerochaete chrysosporium (Xu et al., 2015a). For instance, Xu et al. (2015b) have also shown the induction of hydrogen peroxide accumulation by means of cadmium and enzymatic antioxidant responses in P. chrysosporium. In this study we found an overall 30%–40% reduction of the total As uptake after applying bacterial strains in rice plants. This result was comparable to those of other bioremediation studies where inoculation of rice with metal resistant microbial strains led to significant reduction of metal in upper plant parts. In another study when Cd-tolerant microbial strains were grown with rice at 200 mmol/L CdCl₂, 6%–61% reduction in Cd accumulation was observed (Siripornadulsil and Siripornadulsil, 2013). Our microbial strains (NBRIEAG-6, NBRIEAG-8 and NBRIEAG-9) can be an excellent choice for improving the bioremediation potential of plants. Another important feature of these strains is their adaptability to various environments, i.e., As-contaminated and uncontaminated soil.

2.5. Statistical analyses

The statistical evaluation was carried out by analysis of variance (ANOVA) following the Bonferonni method between control, addition of arsenic species and arsenic-resistant bacteria (Tables S1 and S2). PCA was applied on the quantified values from enzymatic and non-enzymatic assays of control, addition of arsenic species and arsenic-resistant bacteria. PCA
was performed separately on assays of root and shoot samples that were responsible for the observed separation between the scores of control and treated samples in the PCA score plots. PCA loadings plots were constructed to identify the assays responsible for separation. The clustering between control and treated in the PCA of enzymatic and non-enzymatic assays showed significant variations in shoots (Fig. 4a1). The PC1 loading plot contributed 71% of the explained variance (Fig. 4b1). Positive scores in PC1 are related to high amounts in APX and low amounts in protein assays, whereas negative scores are related to catalase, lipid peroxidation and GR assays. Glutathione assays do not play a major role in separation of PC1 and PC2 components. The PC2 loading plot contributed 19% of the explained variance (Fig. 4c1). The PC2 loading plot showed that the cluster separation occurred due to positive loadings of high amounts in GR and Catalase assays and low amounts in APX assays, whereas negative loadings were from lipid peroxidation and protein assays. The score plot of control and treated assays clustered into eight groups, viz. control, As(III), As(III) with NBRIEAG-6, As(III) with NBRIEAG-8, As(III) with NBRIEAG-9, As(V) with NBRIEAG-6 and As(V) with NBRIEAG-8 in separate groups and As(V) and As(V) with NBRIEAG-9 in a single group. The PCA score plot of root samples is presented in (Fig. 4a2). The PC1 loading plot contributed 78% of the explained variance (Fig. 4b2). Positive scores in PC1 are related to high amount in GR and low amounts in lipid peroxidation assays, whereas negative scores are due to APX and catalase assays. The PC2 loading plot contributed 18% of the explained variance (Fig. 4c2). The PC2 loading plot showed that the cluster separation is because of positive loadings of high amounts in catalase assays and low amounts in APX, GR and lipid peroxidation assays. The protein and glutathione assay did not play a major role in the separation of PC1 and PC2 components. The score plot of controlled and treated assays clustered into eight groups, viz. control, As(III), As(V) with NBRIEAG-6 and As(V) with NBRIEAG-6, As(III) with NBRIEAG-8, As(III) with NBRIEAG-9, As(V) with NBRIEAG-8 and As(V) with NBRIEAG-9 in a single group.

3. Conclusions

In the present study, arsenic-tolerant bacteria were isolated from heavily As-contaminated soil. The bacterial strains (NBRIEAG-6, NBRIEAG-8 and NBRIEAG-9) not only decreased the As uptake in rice plants, but also reduced the damage induced by As and improved plant growth promotion. Moreover, the bacterial mediation reduced the activity of total glutathione (GSH), and glutathione reductase (GR) showed the reduction of As transport in roots. Hence, the study reveals that bacterial inoculation may influence the biochemical and physiological parameters of rice plants, and helps plants to tolerate As stress compared to uninoculated plants. Further investigation is needed to explore this phenomenon.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.12.034.

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