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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

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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 $^1O_2$ (singlet oxygen), O_2^- (superoxide radical), OH^- (hydroxyl radical) and H_2O_2 (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 arlettae*, NBRIEAG-8 (GU991542) as *Staphylococcus* sp., respectively (Srivastava et al., 2012, 2013) while NBRIEAG-9 (GU997108) as *Brevibacillus* 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 7.5×10^8 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):

$$TF = A_{metal-shoot}/A_{metal-root} \quad (1)$$

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.

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