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Mitigation of arsenic toxicity and accumulation in hydroponically grown rice seedlings by co-inoculation with arsenite-oxidizing and cadmium-tolerant bacteria

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ABSTRACT

Arsenic (As) contamination of rice grain is a serious problem worldwide. The objective of this study was to mitigate As toxicity and accumulation in hydroponically grown KDML105 rice seedlings using bacteria isolated from heavy metal-contaminated soils. Seven strains (KKU2500-1, −2, −3, −9, −12, −16 and −22) of 24 cadmium (Cd)-tolerant bacteria produced high levels of inorganic sulfide and thiol-rich compounds in As-supplemented media. The strains were allowed to colonize rice seedlings growing in arsenite [As(III)]- or arsenate [As(V)]-supplemented Hoagland's nutrient solutions. Colonization by strains KKU2500-3 and −12 led to increases in plant growth parameters and similarly reduced As translocation into shoots [translocation factor (TF) = 0.05] in the As(V)-supplemented solution. Strains KKU2500-1 and − 12 also greatly reduced As translocation into shoots (TF = 0.16–0.20) in As(III)-supplemented solution. KKU2500-3 and − 12 co-colonized onto seedlings with the As(III)-oxidizing isolates 4.25, 4.27, 4.40 and 4.44, and the strain combinations KKU2500-12/4.25, KKU2500-3/4.25, KKU2500-3/4.27 and KKU2500-3/4.44 resulted in higher growth parameters for plants grown in As [As(III) + As(V)]-supplemented solution than other combinations. Moreover, the combinations KKU2500-3/4.25 and KKU2500-3/4.44 greatly reduced As translocation (TF = 0.15 and 0.12, respectively), and this decreased As accumulation in shoots was significantly correlated with increased sulfide stimulation in roots and nutrient solution. These results indicate that these co-inoculated bacteria can mitigate As toxicity, translocation and accumulation in KDML105 seedlings and thus demonstrate synergistic activity in rice plants, and this effect can be further developed in field trials.

1. Introduction

Heavy metal contamination of soil and water is a primary cause of damage to human health worldwide. Humans are exposed to heavy metals from water and food crops that have accumulated heavy metals via irrigation or cultivation (William et al., 2006; Smith et al., 2008). One such toxic heavy metal contaminant of water and crops is arsenic (As), and the contamination sources of arsenic include natural geochemical cycles and anthropogenic activities, such as industrial factories, mining, and herbicides and pesticides. Thailand is one of several countries contaminated with As due to the extraction processes used by the mining industry. In Ron Phibun District of Nakorn Si Thammarat Province, the water supply has been contaminated with As by the leaching of residue from tin mining and mineral processing into water sources. As a result, more than 200 individuals have presented with symptoms of dermatitis (Williams et al., 1996; Visoottiviseth et al., 2002). Gold mining has resulted in a similar problem in Wangsaphung District, Loei Province, and As contamination has also been observed in soil, water and plants in Thap Khlo District, Phichit Province (Weerasiri et al., 2012).

As is a toxic metal that affects human and plant health. As can be present in organic and inorganic forms, and its toxicity and bioavailability depend on its form. The inorganic forms arsenate [As(V)] and arsenite [As(III)] are more toxic to plants, animals and humans than the organic forms and are more often present in the environment than other forms (Silver and Phung, 2005). Compared with As(V), As(III) is more soluble, mobile, bioavailable and toxic. In humans, As exerts a carcinogenic effect, causing skin tumors and brain, liver, kidney, and

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stomach cancers, as has been reported in many countries, including Bangladesh, China, Japan and Taiwan (Smith et al., 1992; Chen et al., 2016). With respect to crops, the germination, biomass (BM), pigment content and yield production are reduced in plants grown in As-contaminated areas (Rahman et al., 2007; Liu et al., 2012). Rice is the primary crop affected by As contamination, and this contamination decreases the germination percentage, biomass, and shoot and root elongation (Shri et al., 2009). Moreover, rice is more highly susceptible than other cereal crops to As accumulation because it is generally grown under submerged conditions (Williams et al., 2007; Zhao et al., 2009; Wang et al., 2015). The most soluble form, As(III), is predominant under such conditions, resulting in its greater uptake by and translocation in rice compared with those of other forms of As (Abedin et al., 2002; Takahashi et al., 2004). Nonetheless, the level of As in rice depends on the As species, the As concentration in paddy soil, the irrigation method used, and the genotype and physiological properties of the plants grown (Singh et al., 2014).

Overall, As contamination of rice is a pervasive problem because this crop is a staple and daily food for a large fraction of the world's population, particularly in Asia (Meharg and Rahman, 2003; Zhu et al., 2008; Ma et al., 2016). At present, several mitigation technologies are used to reduce As toxicity and accumulation in rice, and these approaches include (i) screening for highly As-tolerant rice cultivars and/or those that show reduced accumulation; (ii) water management during cultivation, which could control the forms of As in paddy fields (Hu et al., 2015); and (iii) chemical and physical technologies, including absorption and filtration, to reduce and/or restore environmental properties. However, these approaches can be time-consuming and costly and can produce secondary waste (Kartinen and Martin, 1995). The formation of insoluble As complexes within minerals is commonly observed in nature. However, As can be released into soil and water by geochemical (altering the soil chemical properties, including redox potential and pH) and microbial transformation processes (oxidation, reduction, and methylation), resulting in increased As uptake and translocation in plants due to the enhanced As availability and mobility in the environment (Zhao et al., 2010). Bacterial bioremediation is a new method used to mitigate arsenic toxicity that utilizes microbial activity in the rhizosphere, which is a key factor that controls As bioavailability to crops (Fitz and Wenzel, 2002; Jia et al., 2014). Furthermore, bioremediation is cost-effective and eco-friendly (Srivastava et al., 2013). Many reports have shown that As(III)-oxidizing bacteria are able to detoxify As(III) by oxidizing it to As(V), which is strongly absorbed onto oxides/hydroxides of aluminum (Al), manganese (Mn) or iron (Fe) and aluminosilicates, making it less toxic and less soluble (Goldberg, 2002; Das et al., 2016). The growth parameters of rice inoculated with As(III)-oxidizing bacteria have been shown to be greatly increased (Yang et al., 2015). However, oxidation processes are not sufficient to solve the problem of As uptake and translocation in plants. Some studies have shown that sulfide (S) can decrease As translocation by enhancing iron plaque formation around the root surface, resulting in As sequestration and thereby inhibiting As (V) transport (Hu et al., 2007). However, the overuse of S fertilizer could produce secondary product pollution in soil and be costly. Moreover, bacterial sulfate reduction (sulfide production) is able to precipitate highly soluble As(III) into an insoluble form, such as trisulfide (As_2S_3) and arsenic sulfide (AsS) (Rittle et al., 1995; Newman et al., 1997; Ledbetter et al., 2007; Yang et al., 2015). However, despite this previous work, the knowledge concerning combinations of methods that would decrease As toxicity or translocation that restrict As near the roots of plants remains lacking. To address this knowledge gap, the present study focused on bacterial co-inoculation to mitigate As toxicity and accumulation in rice plants via As oxidation by As(III)-oxidizing bacteria and S application by Cd-tolerant bacteria. In a previous study, Cd-tolerant bacteria isolated from a Cd-contaminated paddy field in Tak Province, Thailand, were able to produce inorganic sulfide and a thiol-rich compound (organic sulfide) under heavy metal stress (Siripornadulsil and Siripornadulsil, 2013). Therefore, in this study, we investigated the efficacy of co-inoculating these strains with As(III)-oxidizing bacteria to mitigate As toxicity and accumulation in rice plants.

2. Materials and methods

2.1. Microorganisms and culture conditions

As(III)-oxidizing bacteria were isolated from an As-contaminated gold mining area in Loei Province, Thailand. The strains were evaluated for As(III)-oxidizing capacity using the qualitative potassium permanganate ($KMnO_4$) screening method described by Salmassi et al. (2002), and the As(V) quantity was measured using the molybdenum blue method according to Lenoble et al. (2003). The highest oxidation was observed for the As(III)-oxidizing strains 4.25, 4.27, 4.40 and 4.44, which were used in our current study. Twenty-four Cd-tolerant bacterial strains (KKU2500-1 to KKU2500-24) and the four As(III)-oxidizing bacterial strains were grown in nutrient broth (NB) medium at 30 °C for 18 h.

2.2. Measurements of inorganic sulfide and thiol-rich compounds in bacterial cells

The 24 Cd-tolerant bacterial strains (KKU2500-1 to KKU2500-24) were grown for 18 h in NB medium supplemented with or without 200 μM (14.984 mg/L) As(III) or As(V) [As(III) from $NaAsO_2$ and As(V) from Na_2HAsO_4] at 30 °C in a shaker incubator (JSSI-100C, JS Research, Korea) at 150 rpm. The cells were harvested by centrifugation at 5000 × *g* for 10 min using a refrigerated centrifuge (Himac CR20B2, Hitachi, Japan) to measure the concentrations of inorganic sulfide and thiol-rich compounds as indicators of heavy metal detoxification.

The inorganic sulfide contents of the samples were determined according to Harry et al. (1982). Briefly, 100 μL of 10^9 cfu/mL cell suspensions in sterile distilled water (dH_2O) was added to 300 μL of 6% (w/v) sodium hydroxide (NaOH), and the mixture was incubated at 95 °C for 15 min. After cooling at room temperature, 200 μL of dH_2O and 250 μL of 2.6% (w/v) zinc acetate were added, and the mixtures were incubated at room temperature for 1 min. After mixing with 125 μL of 0.1% (w/v) N,N-dimethyl-p-phenylenediamine monohydrochloride ($(CH_3)_2NC_6H_4NH_2$·HCl) in 5 M hydrochloric acid (HCl) and 50 μL of 0.0115 M ferric chloride ($FeCl_3$) in 6 M HCl for 1 min, the mixtures were incubated at room temperature for 30 min. The samples were then mixed with 425 μL of sterile dH_2O and centrifuged for 1 min at 12,000 × *g*. The supernatants were measured at 670 nm with a spectrophotometer (Genesys 10 s UV–VIS spectrophotometer, Thermo Fisher Scientific, USA), and the inorganic sulfide content was evaluated using a calibration curve prepared using sodium sulfide (Na_2S).

Thiol-rich compounds were measured according to Ellman (1959). Briefly, the cell pellets were resuspended with sterile phosphate buffered saline (PBS; 50 mM, pH 6.8) and lysed through three to four freeze/thaw cycles (−70 °C for 30 min and 60 °C for 10 min). The total protein concentrations were determined using Bradford the Bio-Rad Protein Assay (Bio-Rad). Next, 20 μL of Ellman's reagents [4% (w/v) of 5′-dithionitrobenzoic acid (DTNB) in absolute ethanol] was added to the protein solutions, and the mixtures were then incubated at room temperature for 20 min. Finally, the solution was measured at 412 nm with a spectrophotometer. The contents of thiol-rich compounds in samples were calculated via the following equation:

$$A = €bc$$

A = absorbance at 412 nm;
€ = 13, 600 M absorbability (L/mol/cm);
b = path length of the sample;
c = concentration of the compound in solution (mol/L).