Glutathione and glutathione-S-transferase activity in *Jatropha curcas* in association with pyrene degrader *Pseudomonas aeruginosa* PDB1 in rhizosphere, for alleviation of stress induced by polyaromatic hydrocarbon for effective rhizoremediation

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ABSTRACT

The strategy of rhizosphere mediated bioremediation of Polyaromatic hydrocarbons (PAH) contaminated soil is designed around plant-bacterium symbiosis. For effective application of this strategy, it is important that the bacterium should have efficient PAH degradation ability and plant should be able to tolerate the stress induced by the contaminant. The aim of this study was to investigate the ability of bacterial isolates to degrade pyrene, in addition to promote growth of *Jatropha curcas*, and reduce the stress response induced by pyrene in host plant for effective rhizoremediation. Among 31 bacterial strains isolated from rhizospheric soil of contaminated sites, *Pseudomonas aeruginosa* PDB1 was selected based on its excellent ability to degrade pyrene (74%), chrysene and benzo-a-pyrene as estimated by GC–MS analysis, in addition to plant growth promoting attributes including indole acetic acid (IAA) release, phosphate solubilization and siderophore production, which was quantitively estimated in presence and absence of pyrene.Also, glutathione-S-transferase and catechol-2,3-dioxygenase activities were induced in this strain when grown on pyrene as sole source of carbon. Pyrene negatively affected the growth of *Jatropha curcas*, as 28% decrease in total biomass was observed with 80 mg/Kg of pyrene, while treatment with PDB1 promoted the growth of *J. curcas* even in presence of pyrene. Glutathione content and glutathione-S-transferase of roots of the *J. curcas* was estimated in pyrene contaminated soil, and with PDB1 in presence of pyrene. The GST level increased with increase in pyrene contamination. However, GST activity was significantly in same range of the plants treated with PDB1 in presence of high dose pyrene (80 mg/Kg) and plants without pyrene (control) which confirmed the effectiveness of bacteria in removal of pyrene induced stress in host plants. Therefore, *P. aeruginosa* PDB1 is not only efficient degrader but also potential rhizospheric bacteria for reducing the PAH stress on *J. curcas*, and therefore useful for rhizosphere mediated remediation of pyrene contaminated soil.

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1. Introduction

PAH are one of the important pollutants, which are infamous for carcinogenic and mutagenic properties, and toxic effects such as neurodevelopmental defects of fishes (He et al., 2012), reproductive stress and growth of earthworm (Wu et al., 2012), children's oxidative stress (Singh et al., 2008),serious DNA damage (Park et al., 2006), decreased cell viability (Xin et al., 2012), carcinogenic potential and ubiquitous presence in the environment (Flowers et al., 2002). There are reports that suggest amount of human exposure to PAH through soils are relatively higher as compared to air or water (Menzie et al., 1992). Therefore, considering the potential hazard of PAH to human health and to living organisms, finding an effective strategy for PAH removal from the environment remains a challenge and an important concern (Techer et al., 2012).

The use of plant-microbe interaction in rhizosphere for bioremediation (rhizoremediation) of PAH in soil is described as an effective alternative to other remediation technologies (Pant et al., 2016). In rhizoremediation, inoculation of PAH degrading rhizobacteria is facilitated through the root systems of host plant that acts as injection system while penetrating through contaminated soils. This strategy depends heavily on physiological effectiveness of PAH degrading rhizobacteria. However, the plant stress response to the toxic compounds at the site of the contamination remains a major

concern for the success of the in situ rhizoremediation strategy. In plants, the detoxification of toxic compounds generally involves transformation, conjugation with glutathione and sequestration in the vacuole or cell wall (McCutcheon and Schnoor, 2003). The role of plant glutathione-S-transferases have been remarkably implicated in various physiological processes. The plant GSTs are well known for stress response to detoxify herbicides and other xenobiotic compounds (Ezaki et al., 2004; Reade et al., 2004). GSTs are induced by various environmental stimuli, including biotic stresses (Edwards et al., 2000), and abiotic stresses such as herbicides (Cummins et al., 1999, dehydration (Kiyosue et al., 1993), cold (Seppanen et al., 2000; Anderson and Davis, 2004)), metals (Marrs, 1996; Ezaki et al., 2004), heat shock, and high salt, and hormone treatments such as ethylene (Zhou and Goldsbrough, 1993). Yet, a detail description of plant response to PAH, is poorly understood (Cobbett and Meagher, 2002; Harvey et al., 2002). It has been reported that, in *Arabidopsis thaliana*, presence of phenanthrene upregulates the production of glutathione-S-transferase, which binds and transports the phenanthrene to vacuolar system (Weisman et al., 2010). Further, the benefits of rhizoremediation strategy may be enhanced by utilizing rhizobacteria with plant growth promoting attributes. Selecting a bacterium that promotes growth of host plant is expected to result in a healthy root system, and therefore better rhizoremediation (Bisht et al., 2015). Rhizobacteria provide plant growth-promoting benefits through, IAA, nitrogen fixation, acquisition of phosphorus, siderophore synthesis, HCN production, and enzyme ACC deaminase. Few rhizobacteria (e.g., *Burkholderia* spp., *Pseudomonas* spp.) have ability to act both as plant growth promoters and contaminant degraders (Hontzeas and Glick, 2004; Cherian and Oliveira, 2005; Compant et al., 2005). An ideal organism for rhizoremediation of PAH should have attributes for plant growth promotion, as well as ability to synthesize enzyme such as ring hydroxylating dioxygenases.

Presently, it is not clear to what extent PAH exposure triggers stress signaling pathways in host plants, common to other abiotic or biotic stresses in plants, and whether signaling components specific to PAH stress exist. Therefore, in the present work, difference in the activity level of Glutathione-S-transferase (GST) and glutathione content (GSH) was estimated, that was induced under the response to the pyrene contamination, in presence and absence of rhizobacteriumhaving pyrene degradation ability, in addition to plant growth promoting attributes.

2. Materials and methods

2.1. Enrichment culture and isolation of PAH degrading bacteria

One gram of contaminated soil was added in sterile half strength nutrient broth suspended with pyrene (50 mg/L) and incubated in rotary shaker at 120 rpm for 48 h. The PAH degrading bacteria were isolated by spread plate technique on the Bushnell Haas (BH) minimal medium (Magnesium sulphate 0.20 g, Calcium chloride 0.02, Monopotassium phosphate 1.0 g, Dipotassium phosphate 1.0 g, Ammonium nitrate 1.0 g, Ferric chloride 0.05 g, pH 7.0 ± 0.2, agar 15 g) amended with 0.005% pyrene, chrysene, or benzo-a-pyrene respectively, as sole source of carbon, incubated at 30 °C for 48 h. The ability of isolates to grow on PAH amended medium was confirmed by successive culturing in Bushnell Haas medium. The selected strain was identified on the basis of its 16S rDNA sequence analysis. The 16S rDNA was amplified using genomic DNA of the strain as template and primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) under condition – initial denaturation- 94 °C for 3 mins, denaturation at 94 °C for 30 s. annealing at 54 °C for 1 min, extension at 72 °C for 1.30 mins

and final extension at 72 °C for 10 mins. Sequences were identified using the Basic Local Alignment Search Tool for Nucleotide sequences (BLASTn) facility (http://www.ncbi.nlm.nih.gov/blast) of the National Center for Biotechnology Information (NCBI) with the GenBank non-redundant database.

2.2. PAH degradation

Bacterial inoculum was prepared by growing the bacterial isolates in peptone water. Cells were harvested and washed with phosphate buffer and re-suspended in sterile water to give absorbance of 0.4 at 600 nm. This inoculum was added to minimal medium BH broth containing 0.005% pyrene, chrysene, or benzo-a-pyrene respectively, incubated at 30 °C and 140 rpm for 216 h. The growth of the culture was determined at every 72 h of incubation using UV spectrophotometer. At the end of the experiment the broth culture was taken with equal volume of ethyl acetate and the residual amount of PAH was extracted three times with acidification of the broth to pH 2–3 with concentrated HNO_3 (Hesham et al., 2014). Culture medium containing PAH but without bacterial inoculum served as control. The residual amount of PAH was detected by GC–MS and percent degradation was determined for each PAH. GC–MS analysis was performed using an HP 6890 gas chromatograph with an HP 5973 mass spectrometer system. The column was a TR-5MS (5% phenyl polysilphenylene siloxane) (30 m × 0.25 mM diameter, 0.25 uM film thickness). Helium was the carrier gas, at 1 ml/min constant flow. The column temperature was held at 70 °C for 5 min, increased at a rate of 4 °C/min to 290 °C, and held for 10 min. To remove any remaining compounds, the analysis was finished with a ramp of 20 °C/min to 320 °C held for 20 min. The mass spectrometer was operated in electron impact (EI) mode at 70 electrons volts (EV) in the full scan mode from 85 to 450 m/z over 6.5–85 min. Injector and detector temperatures were 270 °C and 280 °C, respectively. Limit of detection was kept instrument noise multiplied by 3 for each PAH compound.

2.3. Enzyme activity in bacterial isolate

The enzyme activity was determined in the minimal medium (BH broth amended with 0.005% pyrene and 1% glucose respectively) as described below (2.3.1–2.3.2). From the broth culture, 10 ml cells (O.D.$_{600}$ = 0.5) were harvested by centrifugation (8000 rpm for 10 mins.). The pellets were washed twice and re-suspended in Tris buffer (50 mM). Cell free extracts were prepared by treating the cells with GTE buffer, lysozyme and SDS solution, incubated for 30 mins at 37 °C with intermittent vortexing and centrifugation at 10,000 rpm for 10 min at 4 °C. The cell free supernatant was used as crude enzyme for determining the enzyme activities in bacterial culture for the following enzymes.

2.3.1. Enzyme assay of catechol-2,3-dioxygenase
Catechol 2,3 dioxygenase (C-2,3-D) was assayed spectrophotometrically by measuring the rate of production of 2-hydroxy muconic semi-aldehyde as a metabolite from catechol as described (Silva et al., 2013). Test solution was prepared with enzyme- 0.05 ml, TrisHCl Buffer- 1.40 ml, catechol-0.05 ml and the absorbance was monitored spectrophotometrically at 375 nm by using Schimadzu U-2800 spectrophotometer.

2.3.2. Glutathione-S-transferase activity
Glutathione–S–transferase (GST) was assessed by the method of Habig et al. (1974). The enzyme was assayed by its ability to conjugate GSH (glutathione) and CDNB (1-chloro-2,4-dinitrobenzene), the extent of conjugation causing a proportionate change in the absorbance at 340 nm. The reaction was initiated by the addition of crude enzyme with phosphate buffer and equal amount of CDNB