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Multifunctional *Pseudomonas putida* strain FBKV2 from arid rhizosphere soil and its growth promotional effects on maize under drought stress

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ABSTRACT

In the present study, rhizobacteria were isolated from the rhizosphere of maize, okra, eggplant, tomato, green gram, peanut and red gram grown in arid and semi-arid regions in India and were screened for drought tolerance in Trypticase soy broth (TSB) supplemented with different concentrations of polyethylene glycol 6000 (PEG 6000). Out of 23 isolates, three could tolerate minimal negative water potential of −1.03 MPa and were evaluated for plant growth promoting (PGP) traits under control and drought stress (−1.03 MPa) conditions. *Pseudomonas* spp. strain FBKV2 isolated from eggplant (*Solanum melongeana* L.) rhizosphere, showed multiple PGP traits under both control and drought stress conditions. The strain was identified as *Pseudomonas putida* by 16S rRNA sequence analysis and the sequence was submitted to GenBank under the accession number KT311002.1. The strain was evaluated for growth promotion of maize (*Zea mays* L.) under drought stress. Seedlings inoculated with *P. putida* strain FBKV2 showed better growth in terms of shoot, root length, and dry biomass. Furthermore, inoculation improved cellular metabolites and stomatal conductance in maize seedlings. Scanning electron microscopy confirmed the colonization of *P. putida* strain FBKV2 on the root surface of maize seedlings. The present study demonstrates that the isolation of indigenous drought tolerant *P. putida* strain FBKV2 from stressed ecosystems can be a very useful approach for the development of bio-inoculants for drought stress management in crops.

1. Introduction

Among abiotic stresses, drought is one of the serious problems limiting crop productivity in arid and semiarid regions thus affecting food security (Minakshi et al., 2013). This form of abiotic stress, affects plant water relation at cellular and whole plant level causing specific as well as non-specific reactions and damages (Ali et al., 2014). Growth reduction under drought stress has been studied in several crops such as barley (Samarah, 2005), maize (Kamara et al., 2003), rice (Lafitte et al., 2007) and wheat (Rampino et al., 2006). Furthermore, drought stress influences the availability and transport of soil nutrients, as dissolved nutrients are absorbed by roots. Drought stress, therefore, decreases nutrient diffusion and mass flow of water-soluble nutrients such as nitrate, sulfate, Ca, Mg, and Si (Selvakumar et al., 2012). Drought also induces free radicals affecting antioxidant defenses and Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals resulting in oxidative stress (Vurukonda et al., 2016). At high concentrations, ROS can cause damage to various cellular structures and leads to lipid peroxidation, membrane deterioration and degradation of proteins, lipids and nucleic acids in plants (Hendry, 2005; Nair et al., 2008). Drought also affects biochemical activities like nitrate reductase (NR), due to lower uptake of nitrate from the soil (Caravaca et al., 2005). It also accentuates the biosynthesis of ethylene, which inhibits plant growth through several mechanisms (Ali et al., 2014). Drought a multidimensional stress effect various subcellular cell organelles and whole plant level (Rahdari and Hoseini, 2012). Thus, drought negatively affects quantity and quality of growth in plants. Efforts have been made to develop drought resistant/tolerant plants through breeding and biotechnological approaches, but these methods are cost intensive. Rhizosphere microorganisms, being in intimate interaction with host plant are known to influence plant response to biotic and abiotic stresses.

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The role of microorganisms in plant growth, nutrient management, and biocontrol activity is very well established. These beneficial microorganisms colonize the rhizosphere/ endo-rhizosphere of plants and promote the growth of the plants through various direct and indirect mechanisms (Grover et al., 2011). Furthermore, the role of microorganisms in the management of abiotic stresses is gaining importance. Recently, several reports have been published on microorganism- mediated abiotic stress tolerance in plants, such as drought (Mayak et al., 2004; Sandhya et al., 2009), chilling injury (Ait bakra et al., 2006), salinity (Chakraborty et al., 2011), metal toxicity (Dell' Amico et al., 2008), and elevated temperature (Ali et al., 2009) etc. The possible explanation for the mechanism of plant drought tolerance induced by rhizobacteria include: (1) production of phytohormones like abscisic acid (ABA), gibberellic acid, cytokinins, and IAA; (2) ACC deaminase to reduce the level of ethylene in the roots; (3) induced systemic tolerance by bacterial compounds; (4) bacterial exopolysaccharides or biofilms (Glick, 2004; Yang et al., 2009; Dimkpa et al., 2009; Kim et al., 2013; Timmusk et al., 2014; Vurukonda et al., 2016). Inoculation with phytohormones producing bacteria can improve root growth and/or enhance formation of lateral roots and root hairs resulting in better water and nutrient uptake (Dimkpa et al., 2009; Egamberdieva and Kucharova, 2009). Similarly, rhizobacteria with an ability to produce ACC deaminase enzyme reduce the deleterious effect of ethylene, ameliorating plant stress and promoting plant growth under drought stress (Glick, 2005). Inoculation with rhizobacteria may also influence physiology of the host plant under stress conditions resulting in enhanced accumulation of osmolytes and tolerance to drought stress (Minakshi et al., 2013). Furthermore, EPS production by rhizobacteria has been shown to improve permeability by increasing soil aggregation and maintaining higher water potential around the roots, thereby increasing in the uptake of nutrients by plant with an increase in plant growth and protection from drought stress (Selvakumar et al., 2012).

We therefore, hypothesized that rhizobacteria isolated from arid rhizosphere soil may mitigate and support plant growth under drought stress condition. To address this hypothesis, we isolated drought tolerant and ACC deaminase producing *Pseudomonas* spp. strain FBKV2 from eggplant arid rhizosphere soil and evaluated for growth promotion of maize seedlings under drought stress.

2. Material and methods

2.1. Isolation and screening of drought tolerant Pseudomonas spp

Rhizobacteria were isolated from rhizosphere soils of maize (*Zea mays* L.), okra (*Abelmoschus esculentus* L.), eggplant (*Solanum melongeana* L.), tomato (*Solanum lycopersicum* L.), green gram (*Vigna radiate* L.), peanut (*Arachis hypogaea* L.) and red gram (*Cajanus cajan* L.) collected from arid and semi-arid regions in India with a precipitation range of 129–243 mm (during summer season, 2015) across the sampling sites (DES, Telangana, 2015). The crops were grown under rain-fed production system and plants at flowering stage were uprooted and the bulk soil was removed by gently shaking the plants. The root adhering soil (RAS) was collected by dipping the roots in containers containing sterile normal saline followed by shaking for 30 min. The soil suspensions were serially diluted, and the appropriate dilutions were spread plated on solid King's B medium. The plates were incubated at 28 ± 2 °C and morphologically different colonies were picked and purified on respective media. The pure cultures were maintained on agar slants under refrigerated conditions for further experiments.

In order to screen the isolates for drought stress tolerance, TSB with different water potentials (-0.05, -0.15, -0.30, -0.49, -0.73, and -1.03 MPa) was prepared by adding appropriate concentrations of PEG 6000 (Sandhya et al., 2009) and inoculated with the overnight-grown broth cultures adjusted to optical density (OD) of 0.5 at 600 nm. Growth of the isolates at various stress levels was estimated by measuring the OD at 600 nm after incubation at 28 °C for 24 h, under shaking conditions.

2.2. Screening for plant growth promoting activities

Isolates which able to grow at maximum negative water potential (-1.03 MPa) level were tested for plant growth promoting traits under control and drought stress condition. To determine phosphate solubilization under control, Pikovskaya's broth (Himedia, India) was inoculated with 1% of overnight culture (0.5 OD at 600 nm) raised in Luria Bertani (LB) broth and for drought stress Pikovskaya's broth with desired water potential (-1.03 MPa) was inoculated and incubated for seven days at 28 °C on an incubator shaker. The cells were harvested by centrifugation at 2655 *g* for 5 min and the supernatant thus obtained was used for the quantitative estimation of phosphate (Fiske and Subbarow, 1925).

2.3. Indole-3-acetic acid

LB broth (control and drought stress) amended with 5 mmol tryptophan was inoculated with 1% of overnight culture (0.5 OD at 600 nm) raised in LB broth and incubated at 28 °C for 48 h on incubator shaker. Cells were harvested by centrifugation at 2655 *g* for 5 min and the supernatant was mixed with Salkowsky reagent, followed by incubation for 1 h at room temperature under dark conditions. The absorbance of pink color was read at 530 nm (Gordon and Weber, 1951). The concentration of proteins in the pellet was determined by Bradford method (Bradford, 1976), and the amount of IAA produced was expressed as μg/mg cell protein.

2.4. Siderophore and HCN production

To determine siderophore production under control and drought stress Chrome Azurol S (CAS) broth cultures were prepared, inoculated with 1% bacterial cultures, incubated at 28 °C for five days and checked for development of orange color (Schwyn and Neilands, 1987). HCN production under control and drought stress was tested in King's B broth amended with 0.4% glycine and Whatmann No.1 filter paper strips soaked in 0.5% picric acid in 2% sodium carbonate were hanged in test tubes, sealed with Para film and incubated at 28 °C for four days. Conversion of strips from yellow to brown color is positive for HCN production (Bakker and Schipper, 1987).

2.5. 1-Aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity

For qualitative analysis bacterial isolates were grown in LB broth and cell pellets were collected by centrifugation, washed, suspended in sterile water and spot inoculated on Dworkin and Foster (DF) salt minimal medium (Dworkin and Foster, 1958) alone (negative control), DF supplemented with 3 mmol ACC as the main source of nitrogen and DF amended with $(NH_4)_2SO_4$ (positive control). In order to screen ACC deaminase activity under control and drought stress selected isolates were grown individually in liquid DF minimal medium alone, DF+ACC and DF+$(NH_4)_2SO_4$ and their growth were measured at 600 nm.

To measure ACC deaminase activity, isolates were grown in 5 mL of LB broth at 28 °C until they reach stationary phase. To induce ACC deaminase activity under control and drought stress conditions, the cells were collected by centrifugation, washed