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Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus* Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress

Manoharan Melvin Joe^{a,c}  , Shalini Devaraj^a, Abitha Benson^{b,c}, Tongmin Sa^c  

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Highlights

- Endophytic isolates Acinetobacter sp. and Bacillus sp., were isolated from *P. amarus*.
- Strains were positive for most of plant growth promoting traits and hydrolytic enzyme.
- Isolates under salt stress promoted higher plant growth and P content.
- Plant phenolic content, radical scavenging and antioxidative activity, were improved

Abstract

In the present study, two salt tolerant endophytic and phosphate solubilizing bacteria ACMS25 and PVMX4 isolated from *Phyllanthus amarus* are identified as Acinetobacter sp. and Bacillus sp. based on 16s rRNA sequencing. Both the strains were found to be positive for most of plant growth promoting traits evaluated and hydrolytic enzyme studied. Under *in vitro* conditions at 160mM NaCl, both the endophytes alone or in combination promoted a higher vigor index, germination (%), plant biomass, P content, plant phenolic content, radical scavenging and antioxidative activity, compared to the standard strain *Bacillus megaterium* MTCC446 and un-inoculated control.

Introduction

Phyllanthus amarus Schum & Thonn belonging to the family Euphorbiaceae and is used in the treatment of various ailments such as diarrhoea, dysentery, gastropathy, fevers, ophthalmopathy, ulcers and wounds in India (Patel et al., 2011). Globally, in particular in India, there has been an increased interest in organic based cultivation of medicinal plants, which are free from chemical based pesticides and fertilizers. For this organic cultivation, biofertilizer and biopesticide application plays an important role in improving the growth and yield of agricultural, horticultural and medicinal plants (Lugtenberg and Kamilova, 2009).

For successful biofertilizer application, numerous studies have been conducted on diversity of plant growth promoting bacteria (PGPB) associated with medicinal plants including *Aloe barbadensis*, *Aloe vera*, *Catharanthus roseus*, *Coleus forskohlii*, *Ocimum sanctum* and *Withania somnifera* (Thosar et al., 2005, Karthikeyan et al., 2008, Elango and Rajasekar, 2011).

Among these PGPB studies, research on the metabolic association between the endophytic PGPB and the host medicinal plants has gained tremendous interest due to their ability to produce different bioactive components apart from promoting growth in inoculated plants (Bhore et al., 2010). Moreover, salinity is an important cause of oxidative stress and plants produce both enzymatic and nonenzymatic enzymes to contend with this stress (Carrasco-Ríos and Pinto, 2014).

Taking this into account the present study was devised to isolate salt tolerant endophytic bacterial strains capable of phosphate solubilization from *P. amarus*. Strains identified by 16S r-RNA sequencing were further screened for plant growth promotion traits and hydrolytic enzyme production. Finally, the ability of these strains to promote plant growth and antioxidant activity in *P. amarus* plants in the presence of salt stress was tested under *in vitro* conditions.

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Section snippets

Isolation of phosphate solubilizing endophytic bacteria

Roots of *P. amarus* were collected from the foot hills of Western Ghats of Kalakad region, Tamilnadu, India. The collected roots were washed and surface sterilized using 70% ethanol followed by two per cent sodium hypochlorite and sterile water. The root was cut uniformly (1 cm) and homogenized using sterile pestle and mortar with 1 M phosphate buffer. The resulting suspensions were plated in NBRIP medium (Nautiyal, 1999) supplemented with 160mM NaCl, incubated at 28 ± 1 °C, for 72 h and zone of

Isolation of phosphate solubilizing bacteria

From a total of thirty five isolates, two isolates that showed a higher zone of clearance and solubilization index when compared with standard strain (*B. megaterium* MTCC446) in the presence of 160mM NaCl. We have used *B. megaterium* MTCC as a standard strain since various *B. megaterium* strains were to known for their phosphate solubilization and plant

growth promotion efficiency in many plants (Chen et al., 2006, Tao et al., 2008, Verma et al., 2013). Strains ACMS2 and PVMX4 are identified as

Conclusion

In the present study two salt tolerant endophytic bacterial strains *Acinetobacter* sp. ACMS25 and *Bacillus* sp. PVMX4 were isolated from the medicinal plant *P. amarus*. These two strains were found to be positive for most of the plant growth promoting activities evaluated such as P solubilization, IAA production, siderophore production, and ACC deaminase activity and hydrolytic enzymes such as cellulose, protease, and pectinase responsible for endophytic colonization. These strains in the presence

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...At the time of writing, several studies have been performed on plant growth-promoting rhizosphere bacteria to assess their potential role in alleviating salinity stress (Bashan et al., 2014; Egamberdieva and Lugtenberg, 2014; Kang et al., 2014a, 2014b). However, only a few plant growth-promoting bacterial endophytes are known to ameliorate the impact of salinity stress (Ali et al., 2014; Joe et al., 2016; Khan et al., 2016, 2017). Bacterial endophytes are bacteria that live inside plant tissues and have the potential to colonize plant inner tissues without causing disease or having detrimental effects on the plant (Sturz et al., 2000)....

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