Journal of Applied Research on Medicinal and Aromatic Plants 3 (2016) 71–77

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

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

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 2 February 2016
Accepted 8 February 2016
Available online 22 February 2016

Keywords:
Phyllanthus amarus
Acinetobacter sp
Bacillus sp
Salt stress
Radical scavenging activity
Antioxidative enzymes

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 160 mM 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.

1. 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). Morover, salinity is an important cause of oxidative stress and plants produce both enzymatic and nonezymatic 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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http://dx.doi.org/10.1016/j.jarmap.2016.02.003
2214-7861/

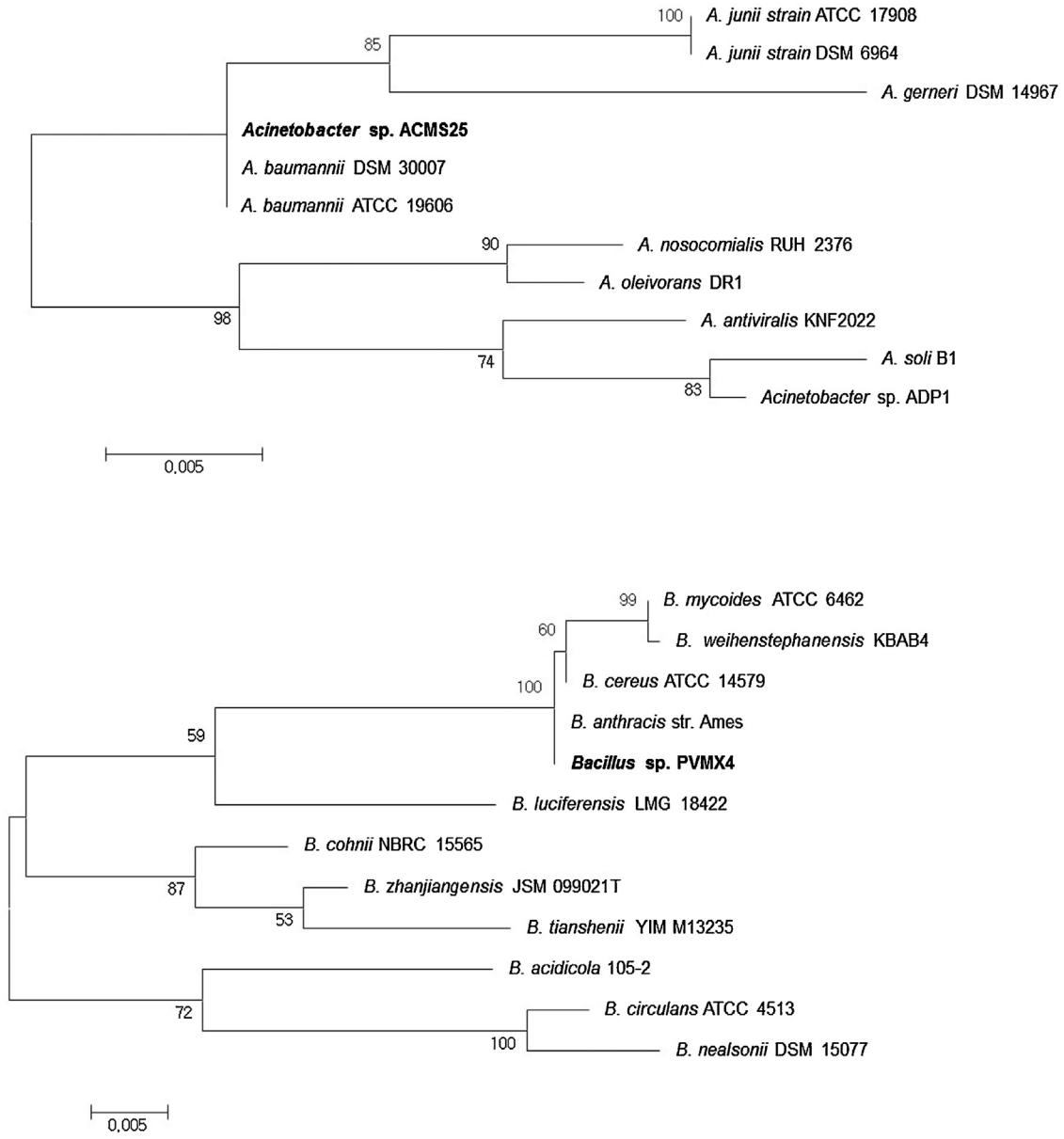

Fig. 1. Phylogenetic analysis of the 16S rRNA gene sequences of *Acinetobacter* sp. ACMS25 (Genbank ACC No. KJ921622) and *Bacillus* sp. PVMX4 (Gen Bank ACC No. KJ921623) strains within the species complex. The tree was constructed using MEGA version 6.0 and the Distance matrices were determined based on Jukes–Cantor model and dendrogram was elaborated with the Maximum Likelihood method. Scale bar indicates 0.5% substitution of nucleotide.

2. Materials and methods

2.1. 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 160 mM NaCl, incubated at $28 \pm 1\,^{\circ}C$, for 72 h and zone of clearance were observed. Positive colonies showing zone of clearance were subjected to further studies. These strains were tested for phosphate solubilization by plate assay method using NBRIP media supplemented with 1.5% agar (Nautiyal, 1999).

2.2. Bacterial identification

Genomic DNA was isolated using HiPurA^{TM} Bacterial Genomic DNA Purification Kit (Himedia, India) by following the manufacturer's instructions. Amplification of 16s ribosomal RNA (rRNA) gene fragment was carried out with the universal primers 27F and 1492R (Jiang et al., 2006) by a Palm Cycler PCR system CG9600 (Genetix biotech). The PCR product was purified using Column based PCR purification kit (Himedia, India). Sequencing of 16s ribosomal RNA was performed with ABI Prism 3730 DNA analyzer. The 16s rRNA gene sequences were compared with sequences in nucleotide database (NCBI) and the sequences were aligned by CLUSTAL W and phylogenetic trees were generated by maximum likelihood (ML) method using MEGA 6 software.