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Native Isolates of *Beijerinckia* from Western Ghats Producing High Amount of Indole Acetic Acid

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A total of sixty five strains of *Beijerinckia* were isolated in the present study from rhizosphere, endorhizosphere and non-rhizosphere soil samples collected from different forest ecosystems of Western Ghats, Karnataka. Representative colonies were purified and microscopic, morphological and biochemical studies were performed. The strains were tentatively ascribed to two species of *Beijerinckia*, namely, *B. derxii* and *B. mobilis*. In order to explore the functional PGPR potential of these strains, all the strains were tested for indole acetic acid and gibberelic acid production. All the strains produced IAA and GA in the range of 6.2 to 99.9 µg /ml and 0.02 to 0.3 µg /ml respectively. Considering the high amount of IAA obtained in the colorimetric assays and ambiguity of the test reagent to react with a range of indole and its derivatives produced by the organism along the pathway, the specific production of indole acetic acid of six strains producing over 75 µg/ ml of IAA, was further analysed by its extraction and detection through HPLC. It was seen that the strain MDBeijXXVIIR321c produced a maximum of 385.7µg/ ml IAA in tryptophan supplemented broth over an incubation period of 7 days with agitation. It can be concluded that the potential strains could be used in raising crop productivity.

Key words: Plant growth promoting rhizobacteria, Indole acetic acid, *Beijerinckia*.

Beijerinckia, a heterotrophic group of proteobacteria mostly inhabits tropical soils [1]. It is one of the potential plant growth promoting rhizobacteria (PGPR), marked by its proven ability to positively influence plant growth and yield[2] by substantially effecting N acquisition by plant or by releasing phytohormones that promote root growth. Thuler *et al*[3] demonstrated that species of *Beijerinckia derxii* release plant growth regulators such as indole acetic acid (IAA) and ethylene. They also release a variety of amino acids in N free media[4]. These functional PGPR attributes *viz*., dinitrogen fixing ability and production of plant active substances are often considered separately as they are independent of each other. Several scientists have also reported gibberelin like substances (GA), originating from PGPR which promote the growth of associated plants [5,6]. The hormones influence the nutrient uptake by plant root surface by increasing phosphatase activity[7].

Representatives of the genus *Beijerinckia* are known to utilize a wide range of multicarbon compounds. Studies on ecology of this genus have shown that this group of bacteria are competent enough to proliferate in soil in large numbers [8] and also influence the survival and aggregation of other non-diazotrophic bacteria[9]. These observations emphasize the significance of this very important group of bacteria in soil community structure and dynamics and the possible role they play in association with plants.

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The aim of this paper is to isolate strains of *Beijerinckia* from rhizosphere, endorhizosphere and non-rhizosphere soil samples from a region often quoted for its rich biodiversity and to study their PGPR traits *viz.*, IAA and GA production apart from their ability to grow on N free medium (dinitrogen fixing ability).

MATERIALS AND METHOD

Isolation of *Beijerinckia*

Soil (non rhizosphere), rhizosphere soil, and root samplings were performed by composite sampling method[10] in different forests of Western Ghats of Uttara Kannada district of Karnataka, India. The different samples collected were associated with plant species such as, *Acacia lantronum, Areca catechu, Ceiba pentandra Eucalyptus tinctorius, Eugenia jambulana, Lantana camara, Musa paradisica, Oryza sativa, Tectona grandis, Terminalia alata, Terminalia paniculata* and *Terminalia arjuna.*

The *Beijerinckia* were isolated using serial dilution of rhizosphere soil samples and plating 1 ml of selected dilution on a selective N free media containing glucose 20g/L, K_2HPO_4 0.8g/L, $MgSO_4.7H_2O$ 0.5 g/L , $FeCl_3.6H_2O$ 0.05g/L, $Na_2MoO_4.2H_2O$ 0.5g/L, agar 20g/L and pH adjusted to 4.5 +1.0. The endorhizospheric bacteria were isolated from the washed and surface sterilized root samples of the plants mentioned above. One ml suspension from the macerated roots was spread uniformly on the surface of selective agar media for isolation of *Beijerinckia* and incubated at 30ÚC. After the required incubation period, the characteristic colonies of bacteria were purified, sub-cultured and maintained in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad for further analysis.

In order to identify the organisms, microscopic, morphological, motility, colonial and other distinguishing biochemical characteristics of the genus *Beijerinckia* were studied according to Bergey's Manual of Systematic bacteriology[11].

Quantification of IAA and GA by colorimetric assays

All the isolates were tested for the production of indole acetic acid and gibberellic acid as described by Gordon and Paleg[12]. The cultures were inoculated in Czapeck's broth amended with 100 ìg/ml of DL tryptophan and incubated at 37ÚC for 24 hours in dark. After incubation, the cultures were spun at 6000 rpm for 20 min. The supernatants wee mixed with Sulkowsky reagent (150 ml conc. H_2SO_4+ 250 ml water +7.5 ml of 0.5*M* $FeCl_3.6H_2O$) and incubated for 1 hr in dark. The pink colour developed was read at 535 nm. The quantity of IAA was determined using a standard IAA curve and expressed as mg of IAA /mL of the medium.

Detection and quantification of IAA through HPLC

Six isolates were grown aerobically in 200 ml Czapek's broth containing 500 µg /mL of DL-tryptophan in dark conditions for seven days. Bacterial cultures were centrifuged at 7,700 rpm for 30 minutes. Indole acetic acid was extracted by following the method described by Tein[13]. Authentic standard for IAA was obtained from Sigma Aldrich, USA. Chromatograms of standard IAA and samples were obtained by injecting 20µL of the filtered extracts in to a reversed phase column (HPLC Waters 515 pump, Germany) column 4.6X250 mm with pore size 5µm) equipped with a Dual ë Absorbance detector 2487 at 280 nm in gradient mode. Methanol: 1% Acetic acid (40: 60) was used as a solvent system to detect IAA. The flow rate was adjusted to 0.8 ml/min. Quantification of IAA was achieved by comparing the peak area of HPLC chromatogram of samples with that of standard.

RESULTS AND DISCUSSION

Sixty five strains of *Beijerinckia* were isolated from rhizospheric, endorhizospheric and non rhizosphere soil samples collected from different forest ecosystems of Western Ghats, Karnataka. Characteristic colonies were initially semitransparent, turning to buff and amber brown colour after ageing. The cells were gram negative, straight or curved rods and weakly motile. Additional distinguishing biochemical test results such as growth on casein agar, utilization of asparagine and carbon sources like lactose, propanol and benzoate as per Bergey's Manual of Systematic bacteriology revealed that the isolates belonged to two species of the genus *Beijerinckia* namely, *B. derxii* and *B. mobilis*.

Sixty two isolates scored positive for IAA production in colorimetric assay, a quantity ranging from 6.6 to 99.9 ìg /ml of IAA was produced by the isolates. Eleven isolates produced notable amount of GA ranging from 4.5 to 7.7 ìg / 25 ml of broth. Very high production of IAA as witnessed in preliminary colorimetric, Sulkowsky reagent based method by these *Beijerinckia* isolates intrigued us to further test for their ability to produce the plant active substance indole-3 acetic acid exclusively. This was accomplished by extraction and quantitative detection of IAA through HPLC. Yields of 130 to 385.7 ìg /ml IAA were recorded in ethyl acetate extracts of six of the most promising isolates during the Fig

stationary phase. Colorimetric assays for IAA quantification did not correlate with the results of IAA quantification by HPLC. This could only suggest the presence of intermediates of indole acetic acid pathway, the various indolic compounds produced during the initial log phase of growth of the organisms, which react with Sulkowsky reagent. Reversed phase HPLC chromatograms and the maximum amount of IAA produced from the four isolates are shown in Fig 1 (A -D).

IAA is the most common plant growth hormone which can directly enhance root growth. In plant growth promoting rhizobacteria it is mainly produced by tryptophan dependent pathway. Several rhizobacteria producing IAA and GA, and their plant growth promotional activity have been documented[14, 15]. However, studies on *Beijerinckia*, a highly effective group of free living diazotrophs, so also, endorhizospheric strains producing high amount of IAA are seldom addressed. In the present study six strains of *Beijerinckia* namely- MDBeijXXVIIE314a, MDBeijXXVIIR315b, MDBeijXXVIIR315c, MDBeijXXVIIS317, MDBeijXXVIIR321c and MDBeijXXVIIE321a have proved highly efficient IAA producers, which could be used as potential bioinoculants. Also, the role of IAA as one of the effectors in antagonism elicited by PGPR[16] is an indication of the possible purpose of high IAA production by these strains, as several studies have witnessed marked reduction of incidence of pathogenesis after PGPR treatment[17]. However, facts like threshold of any plant growth hormone required by the plant and pathway of indole acetic acid production chosen by the high IAA producers and purpose of high IAA production when these strains are in close

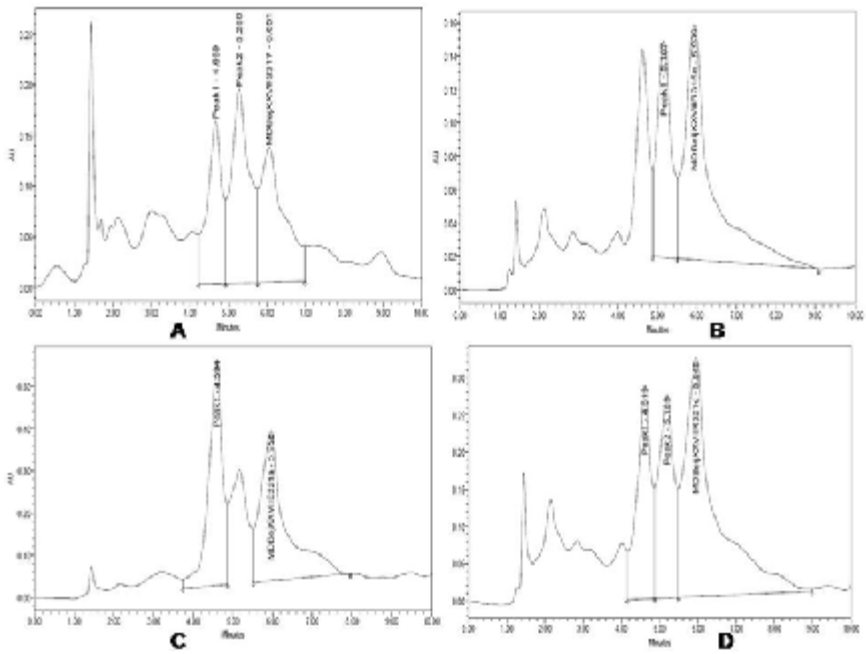

Fig 1. A: HPLC chromatogram of acidic extract of **A**: strain MDBeijXXVIIS317 producing 122.9 µg/mL of IAA, **B**: strain MDBeijXXVIIR315c producing 161 µg/mL of IAA, **C**: strain MDBeijXXVIIE321a producing 335.7 µg/mL of IAA, **D**: strain MDBeijXXVIIR321c producing 385.7 µg/mL of IAA

association with plants, as in endorhizospheric bacteria need to be dealt with. None the less, extensive study is awaited in these directions and to explore the natural and innate potential of plant growth promoting rhizobacteria to develop novel bioinoculants for sustainable agriculture.

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