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Plant Growth Ameliorating and Rhizosphere Competent Native *Acinetobacter pittii* Strain F2 5 from the Rhizosphere of *Zea mays* L.

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ABSTRACT

Background: *Acinetobacter* is an aerobic gram negative, non-spore forming, oxidase negative, catalase positive, non-motile encapsulated coccobacilli. They are ubiquitous in nature mostly isolated from soil, water, food, sewage, animal and human skin, marine water and rhizosphere of maize and wheat. The present study was focused on isolation and characterization of a native strain of rhizospheric *Acinetobacter* for plant growth promoting ability through different microbiological and morphometric parameters under greenhouse conditions.

Methods: Rhizosphere soil samples were collected from maize fields and processed as per the standard microbiological procedure. The morphology was reconfirmed through scanning electron microscopy. DNA extraction was performed by using al. Two sets of primers (universal and specific for *H. pylori*) were used to amplify the *16S ribosomal* gene. Sanger sequencing was applied and the resulted sequences were matched with the sequences of the National Center for Biotechnology Information (NCBI) nucleotide database. The evolutionary aspects were analyzed using MEGA7 software.

Result: The strain exhibited plant growth promoting attributes of ammonia generation, inorganic and organic phosphate solubilization 1-aminocyclopropane-1-carboxylate deaminase activity. The strain was identified as *Acinetobacter pittii* through 16S rRNA sequencing and was designated as *Acinetobacter pittii* strain F2 5 with the accession number KM677194. Scanning electron microscopy was carried out to reconfirm the morphology of the strain. Under laboratory and green house conditions the strain improved the shoot and root length and its biomass of the treated maize seedlings as compared to the uninoculated control underscoring the plant growth potential of these strains in sustainable agricultural practices.

Key words: *Acinetobacter pittii,* 1-aminocyclopropane-1-carboxylate deaminase, PGPR.

INTRODUCTION

The rhizosphere is an ecological niche of large microbial interactions which can affect plant growth both positively and negatively. Among these, a group of microorganisms known as the plant growth promoting rhizobacteria (PGPR) affect the growth of plants positively by employing different mechanisms. Hence screening of potential strains from the rhizosphere of crop plants is critical (Bhavasar and Chopade, 2003). Bacterial genera belonging to *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Pseudomonas* and *Serratia* are reported to enhance plant growth by increasing seed emergence, plant biomass and crop yield (Glick 1995). They augment plant growth by nitrogen fixation from atmosphere, solubilisation of phosphate, creation of siderophores and synthesis of plant growth-promoting hormones, *e.g*., gibberellic acid, indole acetic acid and cytokinin (Sivasakthi *et al.,* 2014). In addition they also act as biocontrol agents by competing for nutrients with the plant pathogens in specific ecological niches and are involved in systemic acquired resistance (Bloemberg and Lugtenberg, 2001). Since then, hundreds of PGPRs have been screened and evaluated for plant growth promoting traits under greenhouse and field conditions. But these trials are not successful always and there is a constant need for searching newer PGPR with enhanced spectrum of activity. In this context, the genus *Acinetobacter* gains significance as it is a strain reported with multifaceted plant growth promoting and biocontrol activity.

Acinetobacter species are reported to be involved in plant growth promotion through production of phytohormones, solubilisation of insoluble tricalcium phosphate, production of siderophores *etc*. (Sachdev *et al.,* 2010). It belongs to the phylum *Proteobacteria* comprising of non-fermentative, non-fastidious, Gram negative coccobacilli. Strains of this genus have been isolated from soil, raw sewage, activated

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sludge, freshwater ecosystems, marine habitats, healthy human skin, foodstuffs, meats, fishes, milk products and vegetables (Towner and Chopade, 1987). Previous studies on *Acinetobacter* highlighted on the growth potential of the strains like , indole-3- acetic acid (IAA) production, inorganic phosphate solubilization and nitrogen fixation) (Indiragandhi *et al.,* 2008). *Acinetobacter calcoaceticus* was recently reported as gibberellins producing bacterium (Kang *et al.,* 2009). Recent literature indicates its positive role in plant growth enhancement and biologically active metabolites production (Indiragandhi *et al.,* 2008, Kang *et al.,* 2009). Thus, using such beneficial strain as biofertilizers instead of synthetic chemicals will not only improve plant growth and development but will also help to enhance soil and crop productivity (O'Connell 1992). Very few studies have been carried out on the growth potential of *Acinetobacter pittii* as a plant growth promoter and a bioinoculant. Hence the present study was focused on isolation and screening of native *A. pittii* strains from the rhizosphere of *Zea mays* L. and to evaluate its plant growth promoting ability under greenhouse and field trials.

MATERIALS AND METHODS

Soil sampling and isolation of the organism

The study was carried out at Karunya University, Coimbatore, Tamil Nadu in the year 2018. Rhizosphere soil samples were collected from maize fields and processed as per the standard procedure of serial dilution and spread plate method. Strains producing distinct circular, convex, smooth, slightly opaque colonies were taken for the study and stored as glycerol stock at -20°C for further analysis. Gram's staining revealed gram negative non sporulating rods occurring in pairs or in chains.

Scanning electron microscopy

Based on Gram's staining the isolate was subjected to Scanning electron microscopy to confirm the morphology. The bacterial cells were harvested and pre-fixed with buffered 4% formaldehyde (pH 7.2) overnight. The cells were then centrifuged and the air dried pellet was used for the analysis. The cells were mounted on the aluminium stub, coated with gold and examined under a scanning electron microscope (Zeiss, Sigma) operating at a voltage of 3 kV at X21000 magnification.

16S rRNA Sequencing

The 16S rRNA gene was targeted in the genomic DNA using the primers (F)-AGTTTGATCCTGGCTCAG and (R)-ACGGCTACCTTGTTACGACTT. Amplification reactions contained 50 ng of genomic DNA, 1× *Taq* DNA polymerase buffer, 1 U of *Taq* DNA polymerase, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 10 pM of each primer. PCR was performed in a Verti Thermo cycler (Applied Biosystems) at 95°C for 5 min, followed by 30 cycles of 1min at 95°C, 1 min at 50°C and 2 min at 72°C with an extension of 72°C for 10 min. The amplicons were visualised in a 1.4% agarose gel under a UV transilluminator. The amplicon was sequenced with ABI PRISM Big Dye terminator cycle sequencing ready reaction kit on an ABI Prism3100 Genetic Analyser. It was edited using Finch TV ver 1.4.0 and phylogenetically compared with those of the type strains of all the species from closely related genera, which were retrieved from NCBI GenBank BLAST program (Altschul *et al.,* 1997). The phylogenetic tree was constructed by the neighbour-joining method with *Pseudomonas plecoglossicida* strain NBRC 103162 as the out group (Saitou and Nei, 1987) using MEGA version 6.06 software (Tamura *et al.,* 2011).

Screening for plant growth promoting attributes

Production of ammonia was determined by adding Nessler's reagent to 48-h-old bacterial culture grown in peptone water (Cappucino and Sherman, 1992). Phosphate solubilisation potential of the strains was determined by growing the strain in modified Pikovskaya's agar containing 0.4% bromophenol blue and incubating the culture at 37°C for 48-72 hrs (Gupta *et al.* 1984). 1-aminocyclopropane-1-carboxylate deaminase activity of the strain was detected on plates with DF minimal medium containing 1-aminocyclopropane-1-carboxylate (ACC) as the sole source of nitrogen (Johri *et al.* 1999).

Plant growth promoting ability of the strain under greenhouse conditions

Pot trials were carried out in triplicates to determine the ability of the test strain to promote the growth of the maize seedlings. Maize seeds of the variety F1 hybrid sweet corn (SUGAR-75) were used which was surface sterilized 2% sodium hypochlorite solution for 2-3 min followed by repeated washing in sterile distilled water. The test strain was inoculated in nutrient broth and incubated until the cells reached an optical density of $1x10^8$ cfu/ml. The seeds were then coated with the bacterial culture by placing the culture flasks in an orbital shaker for 2 hrs (Nandakumar *et al.* 2001). Seeds treated with sterile nutrient broth served as control. After the treatment, the seeds were dried in laminar air flow in sterile Petri plates. The experiment was carried out in triplicates with 15 seeds per treatment. The soil was sterilized by autoclaving and filled in plastic pots. The pots were damped to 2/3 of maximal retention capacity of the soil 24hrs before the seedling. The seeds were placed at equidistance and watered regularly with tap water. The plants were harvested at the end of 15^{th} day to record the biometric observations.

Data analysis

The data (expressed as the mean± standard deviation of the mean of three replicates) were calculated using GraphPad version 6.01 and unpaired *t* test was performed to analyse the significant differences ($P \leq 0.05$) in the morphometric observations between the treated and untreated plantlets.

RESULTS AND DISCUSSION

The present study was aimed at isolating native *Acinetobacter*

species from the rhizosphere of maize and to evaluate its plant growth potential through microbiological and green house studies. Any isolate that was Gram negative, non-motile, oxidase negative, catalase positive, coccobacillus or short rods, was taken for the study and reconfirmed through scanning electron microscopy and 16S rRNA gene sequencing which was considered as conclusive.

Isolation and characterization of the test strain

The strain produced colonies which were circular, convex, smooth and entire. It was Gram negative, non motile short rods or coccobacilli, characteristic of *Acinetobacter* species. Coccobacillary forms were observed under scanning electron microscopy reconfirming the morphology of the strain to *Acinetobacter* species (Fig 1). Molecular analysis based on 16S rDNA homology of 1421-bp partial sequence identified the strain as *Acinetobacter pittii*. In the phylogenetic tree, the test strain and other groups of *Acinetobacter* were grouped at 99% similarity (Fig 2). The strain was named as *Acinetobacter pittii* strain F2 5 with the accession id KM677194.

Determination of plant growth promoting traits

The strain showed a positive result for the accumulation of ammonia by a change in color of the broth from yellow to brown upon the addition of Nessler's reagent indicating its role in biological nitrogen fixation. There are indirect evidences of usefulness of free living N_2 fixing bacteria in crop improvement under tropical and sub-tropical conditions especially with strains excreting a high amount of ammonia (Aggarttal *et al.* 1986). Indole-3-acetic acid (IAA), a principal phytohormone, controls several crucial physiological processes of plants. It ameliorates plant growth by stimulating cell elongation, root initiation, seed germination and seedling growth. Alteration of IAA level by plant growth promoting rhizobacteria leads to varied impacts on plant growth and development (Bishnu *et al.* 2020). Mineral solubilization and increasing mineral availability are regarded as the most important traits directly associated with PGPR (Suresh *et al.* 2010). The maximum yield is obtained only when phosphate is available in sufficient quantity to plants. But, these inorganic phosphates are considered a non-renewable depleting resource, as they are unavailable resources present in the ecosystem. Hence, it has become necessary to find alternative resources to make this phosphate available which is mediated through phosphate solubilising bacteria (PSB) increasing the crop yield (Wani *et al.* 2007). Phosphate solubilizing microorganisms (PSM) are considered as most important traits associated with plant phosphate nutrition and growth (Bandi and Jain, 2021) .The *Acinetobacter* strain reported in this study showed phosphate solubilization efficiency on modified Pikovskaya's medium supplemented with the pH indicator bromophenol blue dye, as reported in previous literature (Gadagi and Sa, 2002). The solubilisation of phosphate was evident by formation of yellow halo zones surrounding the colonies indicating the hydrolysis of inorganic phosphates. Previously it was reported that *A. calcoaceticus* showed a higher phosphate solubilization in National Botanical Research Institute's Phosphate (NBRIP) growth media plates (Kang *et al.* 2009). The bacterial strain exhibited a confluent growth in DF medium with ACC as the sole source of nitrogen as compared to the negative control and positive control with only ammonium sulphate as the nitrogen source. The bacteria producing ACC deaminase

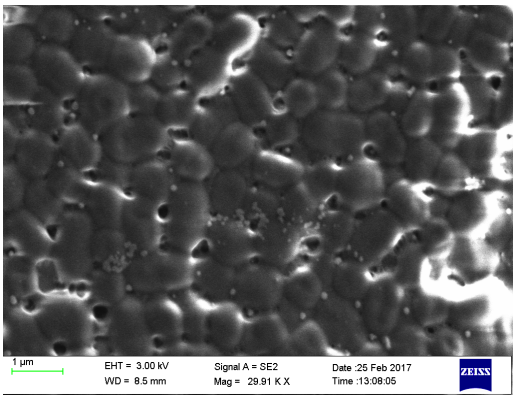

Fig 1: Scanning electron microscopy of *Acinetobacter pittii* strain F2 5.

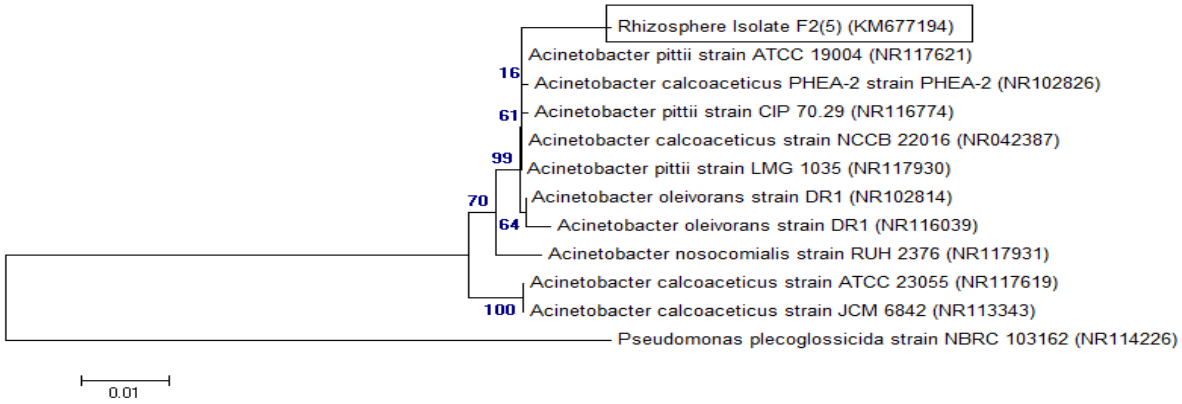

Fig 2: Phylogenetic tree showing the genetic relatedness of the strain F2 5 based on 16S rRNA sequence using Neighbour- joining algorithm.

Table 1: Effect of the seed treatment with *Acinetobacter pittii* strain F2 5 on the morphometric parameters of the maize seedlings.

Growth parameters	Control	F2 5
Shoot length(cm)	33.1±2.49	**40.52±1.90**
Root length (cm)	6.68±1.190	8.28±1.97
No of leaves	4.4±0.4	4.6±0.24
No of roots	5.4±0.67	6±0.77
Shoot fresh weight (g)	0.81±0.16	**1.20±0.17**
Root fresh weight (g)	0.02±0.01	0.04±0.01
Shoot dry weight (g)	0.07±0.01	**0.11±0.01**
Root dry weight (g)	0.02±0.01	0.016±0.00

Values are means of triplicates followed by standard deviation. Statistically significant values according to *t* test at 5% probability are indicated in bold.

are known to promote root elongation and plant growth by lowering the ethylene level, as also observed in the current studies (Rodríguez *et al.* 2008). It has been reported that *Acinetobacter* sp. strains from the larval guts of the Diamondback moth are positive for siderophore production and ACC-deaminase activity (Indiragandhi *et al.* 2008).

Influence on plant growth

A significant increase on plant growth was observed with inoculation using *Acinetobacter pittii* strain F2 5 with the maize seedlings grown in pots under controlled conditions (Table 1). There was a significant increase in the shoot fresh and dry weight and length of the plantlets as compared to the uninoculated control plants.

The ability of the *Acinetobacte*r strain F2 5 to promote the growth of maize seedlings was evaluated through pot trials. It significantly increased the length of the shoot and root and its biomass. There was a 22% increase in shoot length, 24% increase in root length, 50% increase in shoot fresh weight and 57% shoot dry weight as compared with untreated seedlings. Earlier, an increase of 41% in root length and 30% in dry weight of canola plants by *Acinetobacter* sp. PSGB04 compared with the control was reported (Indiragandhi *et al.* 2008). It has been reported that *A. calcoaceticus* application resulted in 66.58% and 40.87% higher shoot lengths of cucumber plants in comparison with distilled water and nutrient broth (Kang *et al*., 2012). With its multiple plant growth promoting activity, high rhizosphere competence, *Acinetobacter pittii* strain F2 5 appears to be a promising agent in sustainable agricultural practices.

CONCLUSION

It can be concluded from the present study that *Acinetobacter pittii* strain F2 5 is a novel PGPR due to its multiple mechanism of action Though many studies have been conducted on the role of *Acinetobacter* as a PGPR there are no literatures available on the role of this strain as a PGPR. It showed a good result in terms of ammonia production, phosphate solubilisation which are the highly limiting nutrients for plant growth. In terms of promoting the growth of plants it showed a significant result in terms of increasing the plant height and its biomass as compared to the untreated plantlets emphasizing the role of this strain as a biofertilizer in eco-friendly farming practices.

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