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Harnessing the potential of *Pantoea* sp. F4-12 as a plant growth promoter and antagonism towards *Fusarium moniliforme*

Reena Josephine.C.M and Jibu Thomas*

Algae Biomass Research Laboratory, Department of Biosciences and Technology, Karunya University, Coimbatore-641114, Tamil Nadu, India

Abstract : Bacterium *Pantoea* sp. F4-12 isolated from agricultural soil was evaluated for its potential as a plant growth promoting agent through various plate and broth assays. The isolate tested positive for ammonia production indicating its ability to fix atmospheric nitrogen. In tryptophan containing medium the isolate produced a red coloration indicating indole acetic acid production. On blue CAS agar, the colonies were yellow in color highlighting the production of siderophores, an iron chelator. The strain grew well on DF medium containing ACC, a precursor to indicate ACC deaminase activity. The strain was tested for its antagonistic activity towards the fungal pathogen of crop plants, *Fusarium moniliforme.* The strain was also evaluated for its ability to hydrolyse different fungal cell wall degrading enzymes like cellulase and pectinase. 16S rRNA phylogeny revealed that the strain showed a close similarity to the genera *Pantoea* sp. Thus, the multiple PGP activities along with biocontrol activity of the isolate characterized its potential to be used as a commercial agricultural formulation.
Keywords : *Pantoea* sp.,ammonia,siderophores,*Fusarium moniliforme.*

Introduction

Plant microbe interactions are a vital step in the growth of plants as the microbes may affect the growth of plants in a positive, negative or neutral way. A variety of microorganisms including members of the genera *Azospirillum, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Methylobacterium, Pantoea, Pseudomonas, Serratia* have been reported to promote the growth of the plants positively . Such beneficial bacteria that affect the growth of plants in a beneficial way are collectively called as plant growth promoting rhizobacteria. Among these, *Pantoea* sp are gaining importance due to the environment versatility and adaptability of these strains. They are group of gram negative bacteria comprising nearly twenty species of the family *Enterobacteriaceae* distinct from *Enterobacter* species found associated with plants, soil, water, animals and humans[1].

Analysing the plant beneficial property of this strain, it is reported to solubilise inorganic phosphate[2], produce several phytohormones, fix atmospheric nitrogen[3], 1-aminocyclopropane- 1-carboxylate (ACC) deaminase activity[4] and biological control activity[5]. It has been identified as an efficient antagonist against several pathogenic bacteria and fungi indicating its role in systemic resistance. *Pantoea* sp. strain EA106, isolated from the rhizosphere of rice, abates arsenic uptake in rice by a high siderophore (iron binding) activity, thereby preventing arsenic accumulation in plant tissues[6].*Pantoea* sp. NII-186 strain, exihibited multiple plant growth-promoting attributes, such as phosphate solubilization activity, as well as indole acetic acid (IAA),

siderophore, and HCN production[7]. In addition, this genus is also known to possess biodegradation potential capable of hydrolysing recalcitrant compounds like petroleum hydrocarbons and toxic metals.

Hence, the present study was focused on the isolation of *Pantoea* sp., from the rhizosphere of agricultural farm soil, evaluating its plant growth potential, and its biocontrol activity against the fungal pathogen, *Fusarium moniliforme*. Identifying the versatile nature of this genus will pave way for its application in sustainable agriculture.

Materials and Methods

Isolation of the organism

The soil used was from an agricultural field which was processed as per the standard serial dilution method. The dilutions were then plated on nutrient agar, and incubated at 37°C for 24hrs for the isolation of discrete colonies. Creamy white to yellow pigmented colonies were then isolated by subculture and stored as glycerol stock at -20°C. Only the organisms that were Gram negative, motile rods were considered for the study.

Identification through 16S rRNA Sequencing

The genomic DNA was isolated from the organism using a modified method[8]. The 16S rRNA universal primers (F)-AGTTTGATCCTGGCTCAG and (R)-ACGGCTACCTTGTTACGACTT were used to amplify the target region. The reaction mix consisted of 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. The cycle was run 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 55°C and 2 min at 72°C with an extension of 72°C for 10 min which was then run in an 1.2% agarose gel to observe the amplified product.Sequencing was performed in ABI PRISM Big Dye terminator cycle sequencing ready reaction kit on an ABI Prism3100 Genetic Analyser. The sequence obtained was edited using Chromas Lite 2.1 and compared using BLAST in NCBI. Phylogenetic analysis was performed using the MEGA 6.0 software package with *Pseudomonas plecoglossicida* strain NBRC 103162 as the outgroup[9].

Ammonia and IAA production

Ammonia production was detected in peptone broth (0.3g/1000ml) by inoculating a loopful of test strain and incubating at 37°C for 24-48hrs in a shaker. After incubation, Nessler's reagent was added drop by drop to the culture to observe a color change of yellow to brown[10]. The isolate was grown in nutrient broth amended with (100µg/ml) L-tryptophan at 30°C for 72hrs. The culture was centrifuged at 3,000 rpm for 30 min. To 2ml of the supernatant, 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution) and 100µL of orthophosphoric acid was added for color development[11].

Siderophore and ACC deaminase activity

The test isolate was inoculated in blue CAS agar medium. Development of yellow orange halo on Chromeazurol S agar (CAS) after 48-72hrs of incubation indicated a positive reaction[12]. The isolate was spot inoculated on Petri plates containing DF salts minimal medium[13]supplemented with 3mM 1-Aminocycolpropnaecarboxylic acid (ACC). Plates containing DF minimal medium without ACC served as negative control and with $(NH_4)_2SO_4$ (2.0 g/ l) as a nitrogen source served as positive control. The plates were incubated for 3-4 days at 28°C. The ability of the isolate to grow well on ACC supplemented medium indicated its ability to hydrolyse ACC.

Synthesis of cell wall degrading enzymes

The ability of the isolate to hydrolyse cellulose was estimated qualitatively using M9 medium amended with 10.0g of cellulose and 1.2g of yeast extract per liter[14]. After 8 days of incubation, the plates were flooded with Gram's iodine to observe a clear zone of hydrolysis. Pectinase activity was determined by plating the isolate in M9 medium containing 4.8g pectin and 1.2g of yeast extract per liter[15]. After 3 days, the plates were flooded with iodine to observe a zone of hydrolysis around the colonies.

Antagonistic Studies using *Fusarium moniliforme*

*Fusarium moniliforme*is a fungal pathogen that is known to cause stalk and ear rot in cereal crops like maize. Hence a qualitative assay known as dual culture technique[16] was performed to analyse the antagonistic potential of the isolated strain. The fungal plugs were inoculated on one side of the medium and incubated for 48hrs. The bacterial isolate was inoculated on the other side of the agar plate and incubated till a zone of inhibition was formed between the fungus and the bacterial strain. A control plate inoculated only with the fungus was used for comparing the zone of inhibition.

Results and discussion

Isolation and Identification of the Organism

The rhizosphere sample contained many organisms when plated on nutrient agar. The organism *Pantoea* sp. was identified as a colony that was cream to yellow colour, circular 2-4mm diameter on nutrient agar after incubation. Gram staining revealed the isolates as Gram negative rods under microscopic observation. The isolate had 96% homology with the related *Pantoea* sp., available in the NCBI database (Fig1).The phylogenetic tree constructed using 16S rRNA sequences of the related *Pantoea* sp., revealed that the isolate formed a close association with *Pantoea anthophila* strain LMG 2558. The sequence data has been deposited in the NCBI GenBank database under the accession number KT735223.

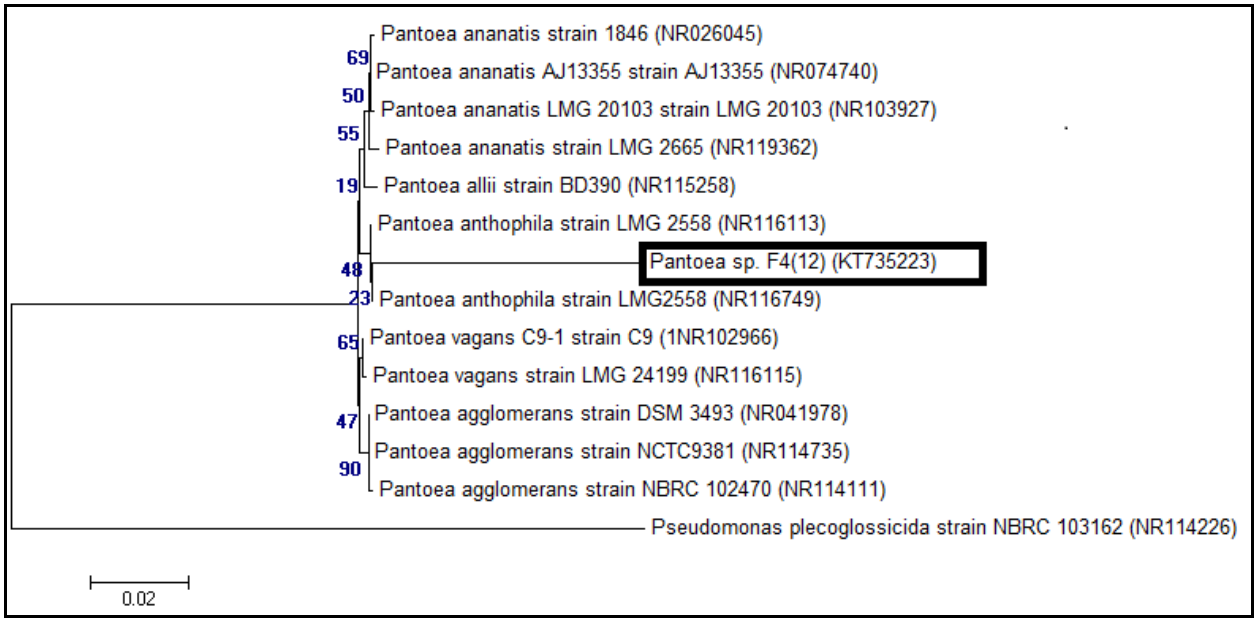

Fig 1Phylogenetic dendrogram based on 16S rRNA sequences showing neighbour-joining relationships between *Pantoea* sp. F4 12 and related taxa

PGPR activity of the test isolate

The isolate produced ammonia developing a deep yellow color in the broth upon the addition of Nessler's reagent. Pink color developed in the culture supernatant after the addition of Salwoski's reagent indicated IAA production (Fig 2). The activities tested *in vitro* highlighted the importance of this isolate in biological nitrogen fixation and phytohormone production thereby leading to an increase in nutrient uptake by the plants. Similar results were obtained in studies involving a native *Pantoea* sp. NII-186 strain, from Western Ghats, which showed multiple plant growth-promoting attributes, such as phosphate solubilization activity, as well as indole acetic acid (IAA), siderophore, and HCN production[7].

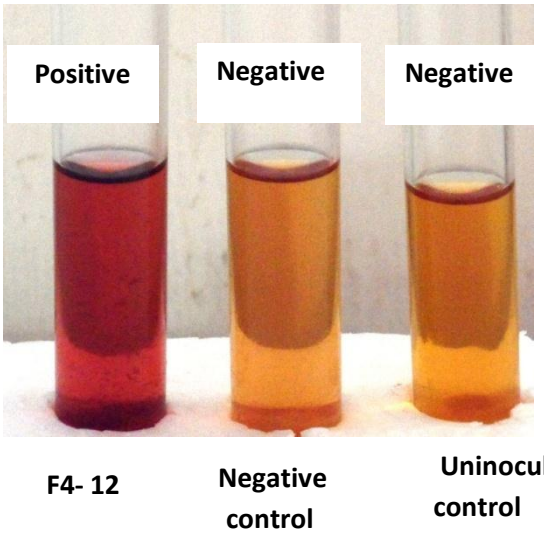

Fig 2 IAA production by the test isolate

Siderophore and ACC deaminase activity

The isolate produced a yellow halo in the blue CAS agar medium indicating siderophore production, which help in the acquisition of iron from the surroundings under iron deficient conditions (Fig 3). It also grew well on medium containing 3mM ACC indicating its ability possess ACC deaminase activity that helps in the regulation of ethylene biosynthesis delaying premature senescence in the plant tissues. Similar results were obtained with the ability of *Pantoea dispersa* 1A to produce siderophores, isolated from a sub alpine soil in the Himalayas[17]. It has been reported that the *Pantoea* sp., obtained from cardamom tissue had the ability to produce ACC deaminase[18].

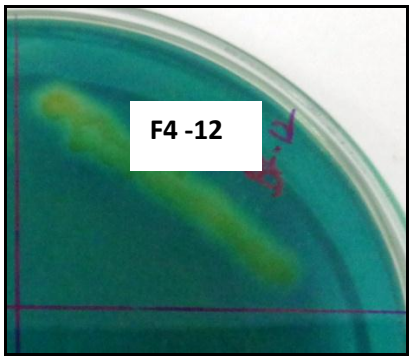

Fig 3 Siderophore production by the test isolate

Synthesis of cell wall degrading enzymes

The isolate produced a clear zone of hydrolysis upon the addition of Gram's iodine exhibiting cellulase and pectinase production, which are important traits that determine the biocontrol activity of the organism. The results are coherent with the study that *P.agglomerans* strains isolated from deepwater rice produced these enzymes enabling penetration and providing nutrients to the distant parts of the plant[19]. These enzymes serve a dual purpose by helping penetration into the plant tissues and hydrolysing the cell wall of the plant pathogens.

Antagonistic studies using *Fusarium moniliforme*

A clear zone of inhibition was observed between the test isolate and the fungal pathogen highlighting its role as an effective biocontrol agent.*Pantoea* sp are found to be antagonistic to a number of bacterial and fungal pathogens like *Pseudomonas syringae*, *Xanthomonas campestris*, *Fusarium* sp., *Rhizctonia solani* etc.

This enables its use as a biocontrol agent thereby significantly reducing the application of chemical pesticides[20]. The results of the plant growth promoting and biocontrol activity of the isolate are summarized in Table 1.

Table 1 PGPR and biocontrol activities of the isolate *Pantoea* sp. F4-12

Isolate No	NH_3	IAA	Siderophore	Protease activity	Starch Hydrolysis	Antagonistic Studies	Cellulase Activity	Pectinase activity	ACC deaminase
F4 12	++	++	++	-	+	++	++	+++	++

+++, ++ - Strongly positive, + - Positive, - - Negative
NH_3 – Ammonia, IAA – Indole Acetic Acid

Conclusion

Rhizosphere region is a rich niche where many associations of plant beneficial microorganisms are observed of whichfew studies have been carried out using *Pantoea* as PGPR. This study demonstrated the ability of *Pantoea* sp. F4 -12 as a potential plant growth promoting and a biocontrol agent. The organism was able to produce ammonia and siderophores. ACC deaminase activity implies its role in delaying plant senescence. Antagonistic studies against *Fusarium moniliforme* and ability to hydrolyse cellulose and pectin highlights its role as an antagonistic agent in combating the fungal pathogens. The versatile nature of *Pantoea* sp. F4 -12 with multi beneficial traits could be deployed as a bioinoculant for plant growth promotion in sustainable agricultural practices.

Acknowledgements

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