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PLANT GROWTH PROMOTING (PGP) ACTIVITY OF ACTINOMYCETES ISOLATED FROM THE RHIZOSPHERE OF *CAPSICUM ANNUUM* L.

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ABSTRACT

A total 72 actinomycetes strains were isolated from 17 rhizosphere soils of *Capsicum annuum* L. of Warangal, Khammam, Karimnagar and Mahabubnagar, Telangana, India. Actinomycetes were isolated on chitin medium by serial dilution plate method. Among the 72 actinomycetes, 25 were screened for Plant Growth Promoting traits (PGP) *viz.*, indole acetic acid production, ammonia production, phosphate solubilization and hydrogen cyanide production. These 25 strains showed IAA and ammonia production, 11 strains showed phosphate solubilization and no strain showed HCN production. Only 4 strains (OUA8, OUA17, OUA18 and OUA27) were tested and showed siderophore production and biochemical activity.

KEYWORDS: actinomycetes, *Capsicum annuum*, PGP activities, biochemical activity.

INTRODUCTION

The rhizosphere is much richer in bacteria and actinomycetes than the surrounding bulk soil.[1] Actinomycetes are gram positive bacteria with high G+C content in their DNA. Soil actinomycetes particularly *Streptomyces* sp. enhance soil fertility and have antagonistc activity against wide range of soil borne plant pathogens.[2] Actinomycetes control plant pathogens and play an important role in the decomposition of organic material, production of secondary metabolites of pharmacological and commercial interest. Actinomycetes are unparalleled sources of bioactive metabolites including antibiotics, plant growth factors and other substances.[3] Actinomycetes were not only biocontrol agents but also enhances the

plant growth promoting activities such as ammonia, IAA, HCN productions and phosphate solubilization. Phosphate anions can be immobilized by precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Al^{3+} providing a high phosphorus fixation capacity to soils.[4,5]

There are reports on studies with microorganisms capable of solubilizing phosphate, especially bacteria and fungi which have experimentally demonstrated their capacity to improve phosphorous availability to plants in laboratory, green house and field experiments.[6,7,8,9] Earlier we have reported biocontrol activity of these 25 actinomycetes strains isolated from chilli rhizosphere screened against *Colletotrichum capsici* and *Fusarium oxysporum.*[10] The present study demonstrates that the actinomycetes strains have plant growth promoting activities viz., IAA, ammonia, phosphate solubilization and siderophore production, these are useful for plant growth promotion.

MATERIALS AND METHODS

Sample collection

17 *Capsicum annuum* L. rhizosphere soil samples were collected from chilli cultivating areas of Warangal, Khammam, Karimnagar and Mahabubnagar, Telangana, India (Table 1).

Table 1: Sample collected area

Sample No.	Village	Mandal	District
1	Kapulakanaparthy	Sangem	Warangal
2	Nekkonda	Nekkonda	Warangal
3	Nekkonda	Nekkonda	Warangal
4	Chennaraopet	Chennaraopet	Warangal
5	Narsampet	Narsampet	Warangal
6	Parkal	Parkal	Warangal
7	Jaggaiahpet	Regonda	Warangal
8	Devagiripatnam	Mulugu	Warangal
9	Kaniparthy	Kamalapur	Karimnagar
10	Pangidipally	Kamalapur	Karimnagar
11	Naguram	Jammikunta	Karimnagar
12	Naguram	Jammikunta	Karimnagar
13	Naguram	Jammikunta	Karimnagar
14	Pallipadu	Konijerla	Khammam
15	Pallipadu	Konijerla	Khammam
16	Pallipadu	Konijerla	Khammam
17	Charakonda	Vangoor	Mahabubnagar

These samples were taken from the growing roots up to a depth of 5 cm after removing approximately 3cm of the soil surface. These samples were placed in polythene bags, closed tightly and analysed for actinomycetes.

Isolation of actinomycetes

72 actinomycetes were isolated by serial dilution plate method[11] (Fig 1) on chitin medium[12] and sub cultured on starch casein agar medium[13] slants. 1ml aliquots were added to cool and solidified chitin medium. The plates were incubated at 28±2°C for 8 days and sub cultured on starch casein agar (SCA) slants.

***In vitro* assay for PGP activities**

IAA production

Actinomycetes strains were grown in starch casein broth supplemented with L-tryptophan (1 µg ml^{-1}) and incubated for 6 days. The cultures were centrifused at 10,000 rpm for 10 min. and the supernants were collected. 1 ml of culture filtrate was allowed to react with 2ml of Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) at 28°C and incubated for 30 min. At the end of the incubation development of pink colour indicated the presence of IAA. IAA quantification was done by measuring the absorbance in a spectrophotometer at 530 nm. A standard curve was plotted to quantify the IAA (µg ml^{-1}) present in the culture filtrate.[14]

Ammonia production

Isolated actinomycetes strains were inoculated in 10 ml peptone water in each test tube and incubated for two to three days at 28°C. 0.5 ml Nessler's reagent was added to each test tube and observed for the development of brown to yellow color, considered as positive for ammonia production.[15]

Phosphate solubilization

Actinomycetes strains were streaked on Pikovskaya's agar medium containing tricalcium phosphate (Glucose 10.0g, $Ca_3(PO_4)_2$ 5.0, $(NH_4)_2SO_4$ 0.5, KCl 0.2, $MgSO_4.7H_2O$ 0.1, $MnSO_4$ 0.002, $FeSO_4$ 0.002, yeast extract 0.5, agar 16.0, distilled water 1 lit) and incubated at 30°C for 6 days. The presence of a clear halo zone around the culture indicated the phosphate solubilization capacity of the strain.[16]

Siderophore Production

Actinomycetes isolates were assayed for siderophore production on the chrome azurol S agar medium described by Schwyn and Neilands.[17] Chrome azurol S agar plates were prepared and divided in two equal sectors and spot inoculated with test organism and incubated at

28±2ºC for 48-72h. Development of yellow-orange halo around the colony was considered as positive for siderophore production.

HCN production

Nutrient agar was amended with 4.4g glycine/L and streaked on modified agar plate. A Whatman filter paper No. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate. Plates were sealed with parafilm and incubated at 28°C for 6 days. Development of orange to red colour indicated HCN production.[18]

RESULTS

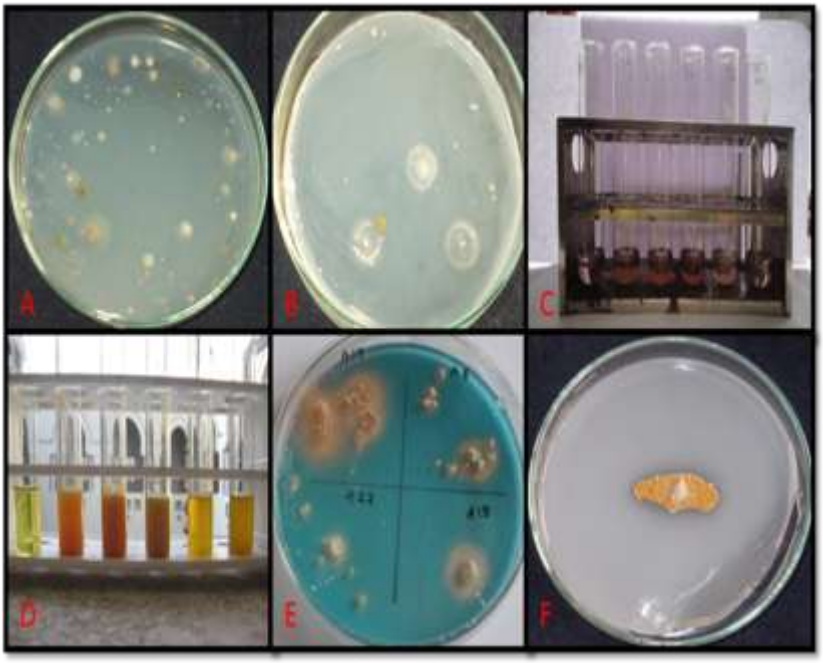

Fig 1: A and B) 10^{-5}, 10^{-6} dilution plates; production of C) IAA; D) ammonia; E) siderophore- OUA17, OUA8, OUA27 and OUA18; F) Phosphate solubilization- OUA17.

A total of 72 actinomycetes strains isolated from chilli rhizosphere were tested for antifungal activity among these 25 strains showed antifungal activity in our earlier work.[10] All the 25 potent antifungal strains tested for the production of IAA, ammonia, siderophore and phosphate solubilization are presented in Table 2 (Fig 1). The 25 strains showed IAA and ammonia activity out of which OUA37 showed maximum production of IAA whereas OUA14 showed least production. Out of 25 ammonia production strains, 8 were (OUA3, OUA5, OUA7, OUA8, OUA12, OUA14, OUA30, OUA32) showed maximum production, 8 strains (OUA15, OUA28, OUA29, OUA37, OUA39, OUA40, OUA41, OUA50) showed

moderate and 9 strains (OUA9, OUA16, OUA17, OUA18, OUA27, OUA31, OUA33, OUA36, OUA38) exhibited least ammonia production activity (Table 2).

Out of 25 strains only 11 strains (OUA3, OUA5, OUA8, OUA9, OUA12, OUA17, OUA18, OUA27, OUA29, OUA30 and OUA40) showed maximum phosphate solubilization activity, No strain exhibited HCN production. Four strains (OUA8, OUA17, OUA18 and OUA27) were tested and showed siderophore production.

Table 2: **PGP activities of actinomycetes strains.**

Strain number	IAA P (OD)	AP			PS	SP
		L	M	H		
OUA3	0.0766			+	+	NT
OUA5	0.220			+	+	NT
OUA7	0.0390			+	-	NT
OUA8	0.103			+	+	+
OUA9	0.0470	+			+	NT
OUA12	0.104			+	+	NT
OUA14	0.005			+	-	NT
OUA15	0.171		+		-	NT
OUA16	0.070	+			-	NT
OUA17	0.0126	+			+	+
OUA18	0.008	+			+	+
OUA27	0.024	+			+	+
OUA28	0.0518		+		-	NT
OUA29	0.2118		+		+	NT
OUA30	0.1008			+	+	NT
OUA31	0.165	+			-	NT
OUA32	0.014			+	-	NT
OUA33	0.163	+			-	NT
OUA36	0.173	+			-	NT
OUA37	0.2855		+		-	NT
OUA38	0.028	+			-	NT
OUA39	0.0599		+		-	NT
OUA40	0.0391		+		+	NT
OUA41	0.1373		+		-	NT
OUA50	0.015		+		-	NT

IAA P: IAA production, **AP:** ammonia production, **L:** low, **M:** medium, **H:** high, **PS:** phosphate solubilization, **SP:** siderophore production, **NT**: not tested

Biochemical tests

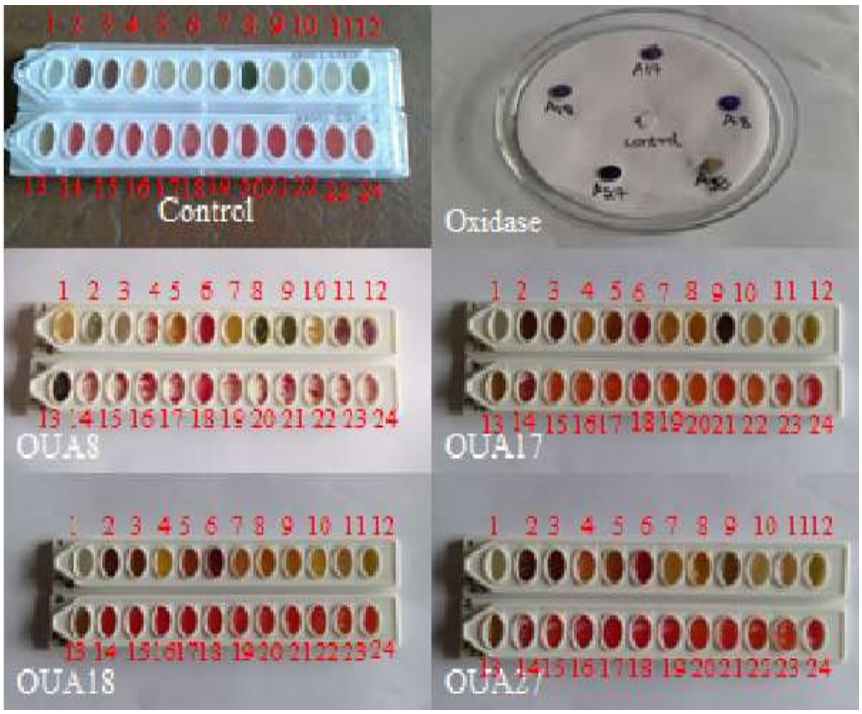

Fig 2: Four selected actinomycetes showing biochemical activity on Himedia strips.

Table 3: Four selected actinomycetes showing biochemical activity.

Strip number	Test	OUA8	OUA17	OUA18	OUA27
1	ONPG	+	-	-	-
2	Lysine utilization	-	+	+	+
3	Ornithin utilization	-	+	+	+
4	Urease	+	-	-	+
5	Phenylalanine deamination	-	-	-	-
6	Nitrate reduction	+	+	+	+
7	H_2S Production	-	-	-	-
8	Citrate utilization	-	-	-	-
9	VogesProskauer's	-	-	-	-
10	Methyl red	-	-	-	-
11	Indole	-	-	-	-
12	Malonate utilization	-	-	-	-
13	Esculin hydrolysis	+	-	-	-
14	Arabinose utilization	-	-	-	-
15	Xylose utilization	-	+	-	-
16	Adonitol utilization	-	+	-	-
17	Rhamnose utilization	-	+	-	-
18	Cellobiose utilization	-	-	-	-
19	Melibiose utilization	-	+	-	-
20	Saccharose utilization	-	+	-	+
21	Raffinose utilization	-	-	-	-
22	Trehalose utilization	-	+	-	+

23	Glucose utilization	-	+	-	+
24	Lactose utilization	-	-	-	-
Oxidase		+	+	+	+

Biochemical activity test for 4 actinomycetes strains (OUA8, OUA17, OUA18 and OUA27) was conducted using Himedia strips (No. 1-24) (Table 3 and Fig 2). OUA8 was positive for ONPG, urease and esculin hydrolysis. OUA17, OUA18 and OUA27 were positive for lysine and ornithine utilization. OUA17 and OUA27 had saccharose, trehalose and glucose utilization. OUA17 had xylose, adonitol, rhamnose and melibiose utilization. OUA27 and OUA8 had urease activity. All four strains showed nitrate reduction and oxidase activity. All four strains were negative for phenylalanine deamination, H_2S production, citrate utilization, vogesproskauer's, methyl red test, indole, malonate, arabinose, cellobiose, raffinose and lactose utilization.

DISCUSSION

All the 25 potential antifungal strains showed positive for IAA and ammonia production activity. IAA producing microorganisms are known to promote root elongation and plant growth.[19] Only 11 strains showed phosphate solubilization activity that could be attributed to promote plant growth activity. The actinomycetes strains have the ability to solubilize available inorganic phosphate sources in the soil. The phosphate solubilizing activity characterizes the microorganisms with the ability to produce and release metabolites such as organic acids that chelate the cations bound to phosphate (mainly calcium), converting them into soluble forms.[20] None of the actinomycetes strains showed HCN production. Four strains were tested and showed siderophore production. Selection of indigenous fluorescent Pseudomonad isolates from maize rhizospheric soil in Vojvodina as possible PGPR.[21] Similarly, actinomycetes from chilli rhizosphere soil with antagonistic activities against phytopathogenic fungi and plant growth promoting character with the purpose for further field application.

CONCLUSION

Among 72 isolated actinomycetes strains, 25 selected strains showed IAA and ammonia production, 11 strains showed phosphate solubilization, 4 strains (OUA8, OUA17, OUA18 and OUA27) were tested and showed siderophore production. No strain showed HCN production. Actinomycetes have not only biocontrol activity but also plant growth promoting activity.

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