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Research Article

SCREENING OF *FLUORESCENT PSEUDOMONAS* FOR PLANT GROWTH PROMOTING ACTIVITIES

***Apastambh A.R.[1] and Baig M.M.V.[2]**
[1]Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded
[2]Department of Botany and Biotechnology, Yeshwant Mahavidyalaya, Nanded
**Author for Correspondence*

ABSTRACT
Rhizospheric soil sample was collected from different regions of Nanded district. Total ten species i.e. two species from each region were selected for the further studies. Species were identified as fluorescent pseudomonas based on morphological and biochemical activities such as grams nature, oxidase, fluorescent pigment, arginine hydrolysis. These species were studied for their plant growth promoting activities like IAA production, phosphate solubilization, cellulase production, protease production. All the isolates were positive for protease production and phosphate solubilization. Nine isolates were positive for IAA production. Five isolates were positive for cellulase production. All the isolates were able to inhibit the growth of plant pathogenic fungi such as *Aspergillus, Fusarium oxysporum, Alternaria.* YPS1, YPS6, YPS8 isolates were found to most effective inhibiting growth of all fungi

Keywords*: Rhizosphere, Fluorescent Pseudomonas, Plant Growth Promotion*

INTRODUCTION
The organisms that establish positive interactions with plant roots and show observable benefits on the plant growth are collectively called as Plant Growth Promoting Rhizomicroorganisms (PGPRs). Such organisms have a greater role in sustaining agricultural production. The genus *Pseudomonas* encompasses arguably the most diverse and ecologically significant group of bacteria on the planet. Fluorescent pseudomonads are gram negative, rod shaped, chaemoheterotrophic bacteria with polar flagella and are characterized by the yellow green iron chelating low molecular weight siderophores called pyoverdines or pseudobactins that fluoresce under UV light (O'Sullivan and O' Gara, 1992).

They are common inhabitants of rhizosphere and are the most studied group within the genus *Pseudomonas*. They can be visually distinguished from the other *Pseudomonas* species by their ability to produce water soluble yellow green pigments. They comprise of *Pseudomonas aeroginosa*, the type species of the genus, *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. putida* and the plant pathogenic species *P. cichorii* and *P. syringae* (Dwivedi and Johri, 2003). Fluorescent pseudomonads are well recognized as plant growth promoting rhizobacteria, phosphate solubilizers and as biocontrol agents against plant pathogens.

The fluorescent pseudomonads can influence the plant growth either indirectly or directly. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. The direct promotion of plant growth by PGPR for the most part entails either providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients like phosphorus from the environment.

The aim of this study was to study these plant growth promoting activities of *Fluorescent pseudomonas* isolated from different regions of Nanded district.

MATERIALS AND METHODS
Collection of Sample
Rhizospheric soil sample was collected from Banana rhizosphere from different regions of Nanded district i.e., Vagegaon, Ardhapur, Vasmat, Maltakdi, Malegaon

Isolation and Identification of Fluorescent Pseudomonas

The soil was suspended from this loop full suspension was streaked on king B media plates and incubate at 30^0c for 48 hrs. The developed Colonies show the yellow-green fluorescent pigment and these were picked up and different and morphological and biochemical studied studies were carried out including gram staining, aesculin hydrolysis, oxides test, and Arginine Hydrolysis.

Antifungal Activity

Fungal pathogen was grown on a PDA plate. With the help of a sterile cork borer, a disc of fungal growth from this plate was taken and placed at the center of a fresh PDA plate. Twenty four hour old culture of each bacterial strain was then streaked parallelly on either side of the fungal disc 3 cm away from the disc. The plates were kept for incubation at 30^oC for 96 hours. Visual observations on the inhibition of growth of fungal pathogen were recorded after 96 hours of incubation in comparison with the PDA plate simultaneously inoculated with only the fungal pathogen (Ganesan And Gnanamanickam, 1987).

Determination of Indole Acetic Acid

Isolates were inoculated in 100 ml King's B broth supplemented 0.1mg/ml tryptophan and incubated at 27 ± 2 °C for 4 days. Supernatant was centrifuged, acidified to pH 2.5 and extracted with 10 ml of ethyl acetate. Ethyl acetate fraction was evaporated at 40 °C under vacuum and residue was suspended in 2 ml ethanol and mixed with Fe-$HClO_4$ reagent. The absorbance was measured at 530nm after 25 min (Gordon and Weber, 1951)

***Protease Production*:** The active bacterial cultures were spot inoculated on casein containing sterile media plate and incubated at 30^0c for 48 hrs and observed for zone of clearance

***Cellulase Production*:** Prepared active bacterial cultures were spot inoculate on czapek mineral salt media plate and incubated at 30^0c for 4-5 days. After incubation 1 % aqueous solution of Hexadecyl trim ethyl ammonium bromide was flooded observed for zone of clearance

***Phosphate Solubilization*:** The active bacterial cultures were spot inoculate on pikovaskay's media plate and incubated at 30^0c for 5 days and observed for zone of clearance

RESULTS AND DISCUSSION

Isolation and Identification of Fluorescent Pseudomonas: *Fluorescent pseudomonas* was identified on the basis of morphological and biochemical characteristics. Isolates were gram negative rod shaped with fluorescent colonies on King'S B agar plates, positive for oxidase, Aesculin Hydrolysis, Arginine hydrolysis (Table 2).

Table 1: Isolates codes and region of isolation

Sr.no.	Name of Isolate	Region
1	YPS1 & YPS2	Malegaon
2	YPS3 & YPS4	Vanegaon
3	YPS5 & YPS6	Ardhapur
4	YPS7 & YPS8	Dongarkada
5	YPS9 & YPS10	Maltekdi

Table 2: Morphological and biochemical characteristics of isolates

Isolate code	Gram Nature	Morphology	Oxidase test	Aesculin Hydrolysis	Arginine dihydrolysis	Fluorescent Pigment
YPS1	Gram negative	Rod Shaped	+	+	+	+
YPS2	Gram negative	Rod Shaped	+	+	+	+
YPS3	Gram negative	Rod Shaped	+	+	+	+
YPS4	Gram negative	Rod Shaped	+	+	+	+
YPS5	Gram negative	Rod Shaped	+	+	+	+
YPS6	Gram negative	Rod Shaped	+	+	+	+
YPS7	Gram negative	Rod Shaped	+	+	+	+
YPS8	Gram negative	Rod Shaped	+	+	+	+
YPS9	Gram negative	Rod Shaped	+	+	+	+
YPS10	Gram negative	Rod Shaped	+	+	+	+

Plant Growth Promoting Activities of Isolates: All the isolates were studied for their plant growth promoting activities such as protease production, cellulose production, IAA production. All the isolates were positive for protease production and phosphate solubilization. The ability of some microorganisms to convert insoluble phosphorus (P) soluble form, like orthophosphate, is an important trait in a PGPR for increasing plant yields (Rodriguez *et al.*, 2006). Nine isolates were positive for IAA production. Five isolates were positive for cellulase production (Table 3). Prokryl *et al.*, (1985) have reported production of IAA and some other auxins in liquid culture of *P. fluorescens* isolated from maize and bean rhizosphere promoted plant growth directly. Production of fungal cell wall degrading enzymes is the mechanism involved indirect plant growth promotion (Ongena *et al.*, 2007).

Table 3: Plant growth promoting activities of isolates

Isolate code	Cellulase production	Protease production	IAA production	Phosphate solubilization
YPS1	+	+	+	+
YPS2	_	+	+	+
YPS3	_	+	+	+
YPS4	+	+	+	+
YPS5	_	+	+	+
YPS6	+	+	+	+
YPS7	_	+	_	+
YPS8	+	+	+	+
YPS9	_	+	+	+
YPS10	+	+	+	+

Antifungal Activities of Fluorescent Pseudomonas: All the isolates were able to inhibit the growth of plant pathogenic fungi such as *Aspergillus, Fusarium oxysporum, Alternaria.* YPS1,YPS6, YPS8 isolates

were found to most effective inhibiting growth of all fungi (Table 4). The fact that all *Fluorescent pseudomonas* strains exhibit antifungal activity, as there are many mechanisms that work against fungi like production of 2, 4-diacetylphloroglucinol (Thompson *et al.*,1994), production of siderophore (Ahmadzadeh and Afsharmanesh, 2006), production of HCN (David and O'Gar, 1994).

Table 4: Antifungal activities of *Fluorescent pseudomonas*

Isolate code	Zone of inhibition in mm		
	Aspergillus sp	***Fusarium oxysporum***	***Alternaria sp***
Yps1	++	++	++
Yps2	+	+	+
Yps3	+	+	+
Yps4	++	++	+
Yps5	+	+	+
Yps6	+++	++	++
Yps7	+	++	+
Yps8	+++	++	+++
Yps9	+	+	+
Yps10	+++	+++	+

'+' low inhibition (1-10mm), '++' medium inhibition (11-20mm), +++ strong inhibition (21mm and above)

Conclusion

Total of the ten isolates were selected for plant growth promoting activities. All were found to be gram negative rods, oxidase positive and yielded fluorescent colonies on King's B agar plates. All possess potential plant growth promoting activities i.e. IAA production, phosphate solubilization, protease production etc. Of the ten isolates, Nine isolates were positive for IAA production. Five isolates were positive for cellulase production. YPS1, YPS4, YPS6, YPS8, YPS8 possess all the plant growth promoting activities. All the isolates were able to inhibit the growth of fungi such as *Aspergillus, F.oxysporum, Alternaria.*YPS6, YPS8, YPS10 possess strong antifungal activity against *Aspergillus* YPS8 posses strong antifungal activity against *Alternaria*

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