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Research Article

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Screening for Plant Growth Promoting Activity (PGPA) of fluorescent *Pseudomonas spp.*

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ABSTRACT

Fluorescent Pseudomonas spp. producing the polyketide antibiotic 2,4-diacetylphloroglucinol (DAPG) are some of the most effective PGPR that control root and seedling diseases. However, fluorescent Pseudomonas spp. have emerged as the largest and potentially most promising group of plant growth-promoting rhizobacteria involved in the biocontrol of plant diseases. The present per suit has taken the work to study the plant growth promoting activity of bacterial population found in soil strata of different districts of Chhattisgarh. After 1: 1000 dilution of soil sample on KMB medium plates, population of Pseudomonas was observed. Preliminarily isolated Pseudomonas spp identified as Pseudomonas fluorescence through different biochemical tests. Seven candidate isolates (high in fluorescein, pyocyanin and siderophore production) and crops belonging to different families (Pigeon pea and Pea (Leguminoseae); Tomato (Solanaceae); Okra (Malvaceae)) were selected to identify the crop responsiveness to different isolates. Seed bacterization had significant response for root development and showed negligible to very low increase in shoot length. Higher increase in root length was observed in Pigeonpea and Pea with isolate # P44, P109, P144, and P195.

Key words: *Soil isolates, Pseudomonas fluorescence, PGPR, Seed bacterization, Rhizosphere*

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are root associated bacteria from many different genera that are able to increase the growth of plants when applied to soil, seeds or vegetatively propagated plant parts[11]. Growth promotion occurs as a result of direct stimulation of the plant, inhibition of plant pathogens, or induction of host defense mechanisms against pathogens[9]. Fluorescent *Pseudomonas* that produce the polyketide antibiotic 2,4- di acetylphloroglucinol (2,4-DAPG) are an important group of PGPR that suppress root and seedling diseases on a variety of crops.

Studies continue on the effects of soil factors and host genotype on colonization and the traits and genes that contribute to rhizosphere competence.Various reactions take place within the group of microorganisms and between microorganisms and plants.

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The composition of microorganisms in the root zone undergoes constant changes, for example under the influence of root exudates. On the other hand, the chemical composition of the substances exudated by the roots is related to the genus, species, cultivar, the age of the plant as well as many other biotic and abiotic factors.

Attempts to use fluorescent Pseudomonas to improve crop yield have enormous potential, but to date they have only limited success which is in part due to the lack of understanding of their population dynamics in the environment and the bacterial traits involved in the process of rhizosphere competence. Therefore this study is an attempt to investigate PGPR of certain soil isolates (collected from damp soil of Chhattisgarh) against the crops of different genus.

MATERIALS AND METHODS

Isolation of fluorescent *Pseudomonas* from soil

Soil samples were collected from randomly selected sites of different geographical locations of Chhattisgarh like cultivated fields, jungles, and termitorium. Non- rhizosphere soil (6" below the top layer). For isolation and enumeration of fluorescent *Pseudomonas*, 100 µl of soil suspension from the final dilution (10^{-3}) was transferred in each plate having 20 ml of King's B medium and gently spread with glass L- shaped spreader to ensure uniform distribution of the soil sample. Plates were incubated at 28^0C for 24 hr. They were examined under UV light and colonies with yellow green and blue white color pigmentation were marked and recorded. Individual florescent colony was picked up with the help of sterilized loop and inoculated on solidified Kings medium B Base (KMB)[2] by zigzag streaking. The plates were incubated at 28^0C for 24 hr. The colony growing at last tip of the zigzag line (which was well isolated and assumed to be developed from a few bacterial cells) was transferred to KMB slants.

Screening the Isolates for fluorescein or pycocyanin production

The fluorescein or pycocyanin pigment producing *Pseudomonas* was detected by growing them in *Pseudomonas* agar medium specific for detection of fluorescein or pycocyanin respectively. Plates were incubated at 28^0C for 24 hr. They were examined under UV light and colonies with yellow green and blue white color pigmentation were marked and recorded.

Biochemical tests for confirmation of fluorescent *Pseudomonas*

The isolates which showed fluorescence against UV light were identified as the putative fluorescent *Pseudomonas* and were screened for following biochemical tests 1) citrate utilization test, 2) gelatin hydrolysis test, 3) starch utilization test, 4) nitrate utilization test and 5) growth at 4^0C .

Screening the isolates of fluorescent *Pseudomonas* for siderophore production on iron deficient succinate medium.

The flasks containing 20 ml of SM broth were inoculated with young cultures of individual isolates of fluorescent *Pseudomonas* growing on King's Medium B base. The flasks were incubated for 48 hr at 28^0C on rotating shaker at 120 rpm. The flask were visualized under UV light and categorized as B (blue), BG (blue green), G (green), LB (light blue) and N (for Nil).

Screening of isolates of fluorescent *Pseudomonas* for siderophore production following universal chrome azurols assay

Siderophore producing ability of isolates of fluorescent *Pseudomonas* was assessed by universal chrome azurole assay[8] with slight modification. Spot inoculation of bacterial isolates was done on the CAS solution supplemented King's B medium, incubated at 28^oC and was observed at regular intervals at 24 hr, 48hr, and 72hr respectively. Observation was recorded for production of siderophores by and measuring the development of yellow hallow surrounding the colonies

Screening for plant growth promoting activity

The isolates of *Pseudomonas* having shown reasonably good in fluorescein or pyocyanin production and high in siderophore production were screened to assess their PGPA. The isolates

were grown at 28^0C for 48 hrs on KMB medium in sterilized Petri plates.

Seed bacterization

Among several methods developed for application of Biocontrol agents (BCA's), the seed bacterization is common one. Seeds were rolled on culture for uniform coating with bacteria. After 10-15 minutes seeds were planted in sterilized soil in different polythene bags. The plants were uprooted after 15 days after sowing. While uprooting utmost care was taken to avoid damage of roots. All the seedlings per bag/treatment were taken as one replication. For taking observations of root and shoot length, plants were washed and stretched fully on fixed clean transparent surface. Shoot length was measured from the base of the shoot to the upper most leaf, while root length has taken as the length of the longest root.

RESULTS AND DISCUSSIONS

Screening the isolates for fluorescein or pycocyanin production

When the fluorescent *Pseudomonas* colonies were visualized under UV light, the colonies with yellow-green fluorescence and those with blue-white illumination represented fluorescein and pycocyanin producing *Pseudomonas* respectively. Based on the degree of fluorescein and pyocyanin production as observed via illumination against UV light, isolates were categorized as high, medium and low pigment producers (Table1).

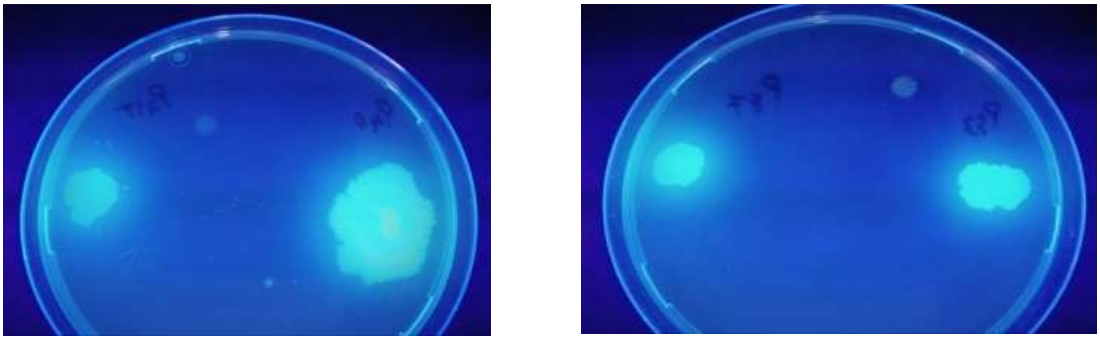

Fig. 1: Blue white illumination indicating the Pyocyanin production by fluorescent *Pseudomonas* on *Pseudomonas* Agar Medium (for pyocyanin)

Screening the isolates of fluorescent *Pseudomonas* for siderophore production

Isolates which showed blue-green / green illumination in succinate medium were identified as high siderophore producers, whereas isolates with light blue or blue illumination were low siderophore producers (Table1). Siderophore producing ability of isolates of fluorescent *Pseudomonas* was assessed by universal chrome azurole assay by and measuring the development of yellow hallow surrounding the colonies (Table1).

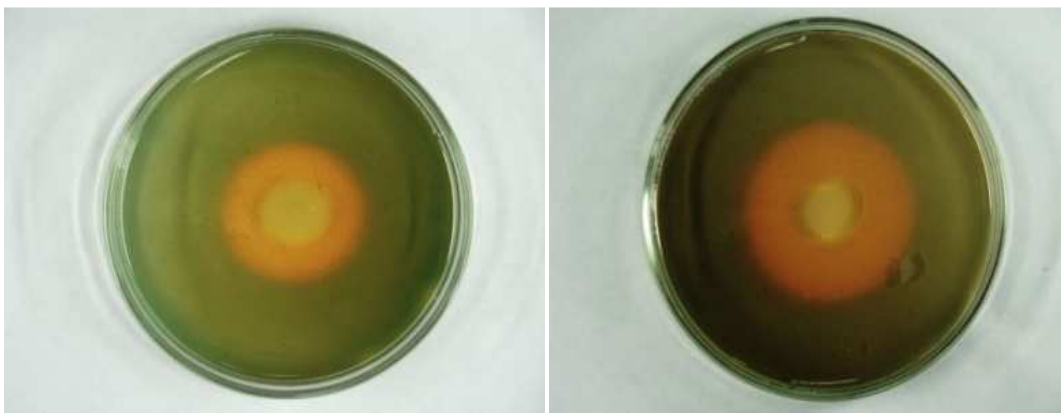

Fig. 2: Siderophore productions by fluorescent Pseudomonas on CAS medium indicating as yellow hallow

Table 1 List of potential candidate isolates of fluorescent *Pseudomonas* with high Fluorescein and Pyocyanin production ability

S.No.	Isolates	Fluorescein	Pyocyanin	Succinate medium	Avg dia in CAS medium(mm)
1	P44	**	*	BG	29.33
2	P109	**	***	BG	35
3	P144	***	***	LB	29.67
4	P179	*	N	BG	23.67
5	P184	***	***	G	41.67
6	P76	***	N	BG	27
7	P195	**	***	LB	26.3

Screening of candidate *Pseudomonas* isolates for plant growth promoting activity

Seven candidate isolates (high in fluorescein, pyocyanin and siderophore production) and crops belonging to different families (Pigeon pea and Pea (Leguminoseae); Tomato (Solanaceae); Okra (Malvaceae)) were selected to identify the crop responsiveness to different isolates and a candidate isolate which is able to induce significant increase in plant growth in all crop species.

Treated (Bacterized) and untreated (control) plants were uprooted after 15 days and observations were recorded on shoot and root lengths (in cm) (Table 2). In case of **Pigeon pea**, maximum increase in shoot length is 5 cm, and minimum is 0.75 cm against the isolates P44 and P195 respectively. Same in the case of root length maximum increase (5.95 cm) against isolate P109 and minimum (0.75 cm) against P184. **Tomato**: Maximum increase in shoot length (2cm) for the isolates P70 and P195, and in root (3cm) for P179. P44 having negative affect on shoot growth where as P184 in root growth on Tomato plants. **Okra:** Maximum increase in shoot length (1.5cm) for P44 and root (4cm) for P179; whereas isolate P184 have negative affect on root growth (-0.5cm). **Pea:** Maximum increase in shoot length is 1.75 cm (P109) and in root length 3cm (P195) over the control. P184 have negative effect (-0.5cm) in shoot growth. The performances of the seven isolates on tested crops are summarized in Table 2.

Table 2 Effect of seed bacterization with candidate fluorescent *Pseudomonas* on shoot and root length of Pigeon pea, Tomato, Okra and Pea

S. No.	**Isolates**	**Shoot length**	**Increase over control**	**% of increase over control**	**Root length**	**Increase over control**	**% of increase over control**
Pigeon pea							
1	P44	22	5	29.4	18.25	5.45	42.5
2	P109	21	4	23.5	18.75	5.95	46.5
3	P144	20.5	3.5	20.5	18.5	5.7	44.5
4	P179	19.75	2.7	16.2	15	2.2	17.2
5	P184	21.25	4.25	24	13.5	0.75	4
6	P76	21	4	23.5	17.5	4.7	36.7
7	P195	17.75	0.75	4.5	14.5	6.7	52.3
8	control	17		Avg =20.22	12.8		Avg =34.81
Tomato							
	P44	11.5	-0.75	-6.1	12.5	0.5	4.2
	P109	13.5	1.25	10.2	14.5	2.5	20.8
	P144	13.75	1.5	12.2	15.25	0.25	2
	P179	13.5	1.25	10.2	15	3	25
	P184	12.75	0.5	4.08	11.5	-0.5	-4.2
	P76	14.25	2	16.3	13.5	1.5	12.5

	P195	14.25	2	16.3	13.5	1.5	12.5
	control	12.25		Avg =9.02	12		Avg =10.4
Okra							
	P44	17.25	1.5	9.5	16.75	1.5	9.8
	P109	16.9	0.25	1.5	15.75	0.5	3.3
	P144	17.75	2	12.7	15.75	0.5	3.3
	P179	15.75	0	0	19.25	4	26.2
	P184	16.75	1	6.3	14.75	-0.5	-3.3
	P76	15.75	0	0	17.5	2.25	14.8
	P195	16	0.25	1.5	15.25	0	0
	control	15.75		Avg =4.5	15.25		Avg =7.72
Pea							
	P44	25	0.25	1.01	18.5	2	12.12
	P109	26.5	1.75	7.07	19.25	2.75	16.66
	P144	25.3	0.55	2.22	19	2.5	15.15
	P179	25.5	0.75	3.03	18.75	2.25	13.63
	P184	24.5	-0.25	1.01	18	1.5	9.09
	P76	24.75	0	0	18.5	2	12.12
	P195	26	1.25	5.05	19.5	3	18.18
	control	24.75		Avg =2.77	16.5		Avg =13.85

It was observed that seed bacterization had poor response for shoot development. Seed bacterization with different isolates showed negligible to very low increase in shoot length ranging from 1.25 to 5 cm or 6.3 to 29 % increase as compared to control.

Differences in root length was observed after seed bacterization with different isolates. Seed bacterization with different isolates showed negligible to higher increase in root length. Higher increase in root length was observed in pigeonpea when the seeds were bacterized with isolate# P44, P109, P144, and P195 and the minimum and maximum increase in root length ranged from 5.45 to 6.7 cm or 42.5 to 52.3 % increase as compared to control (Fig-1).Tomato (Fig-2) and Pea (Fig-3) shown medium response where as Okra was least responsive against all the isolates tested (Fig-4).

Pigeon pea

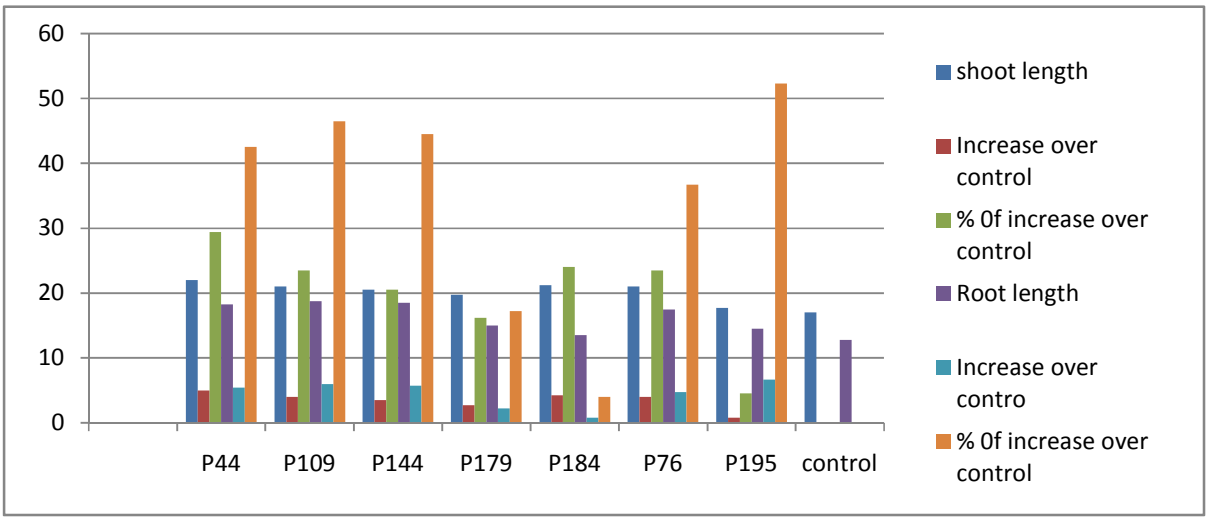

Fig. 1: Efficacy of isolates of fluorescent *Pseudomonas* for plant growth promoting response in terms of shoots and root length in Pigeon pea

Tomato

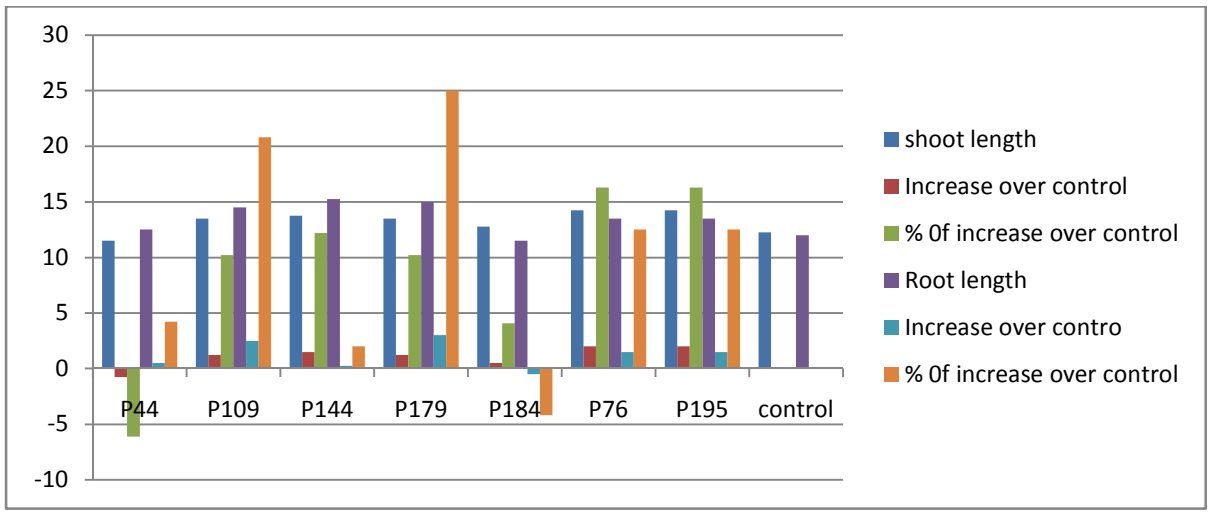

Fig. 2: Efficacy of isolates of fluorescent *Pseudomonas* for plant growth promoting response in terms of shoot and root length in Tomato

Pea

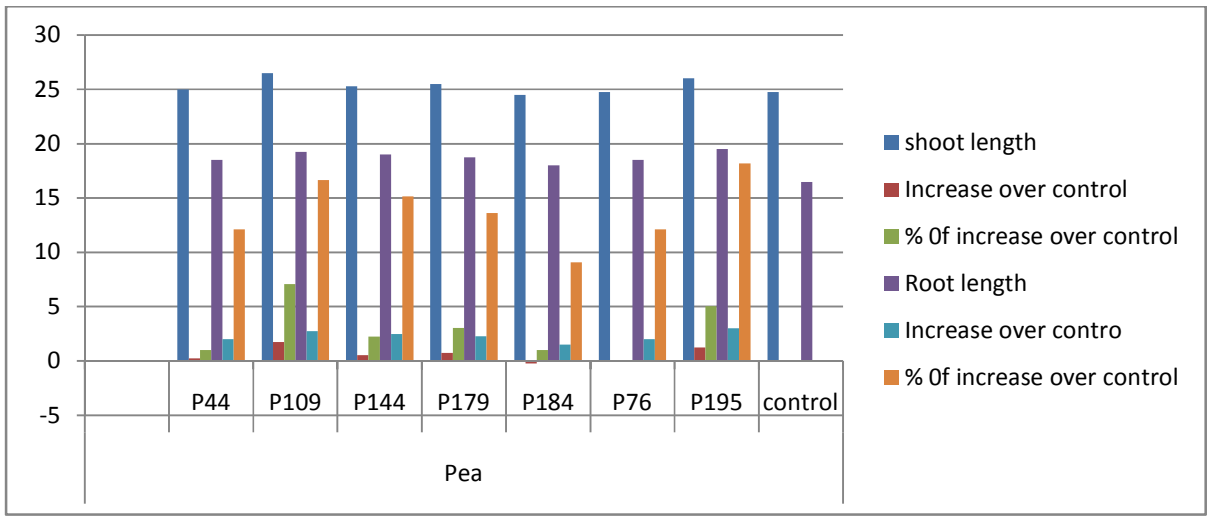

Fig. 3: Efficacy of isolates of fluorescent *Pseudomonas* for plant growth promoting response in terms of shoot and root length in Pea

Okra

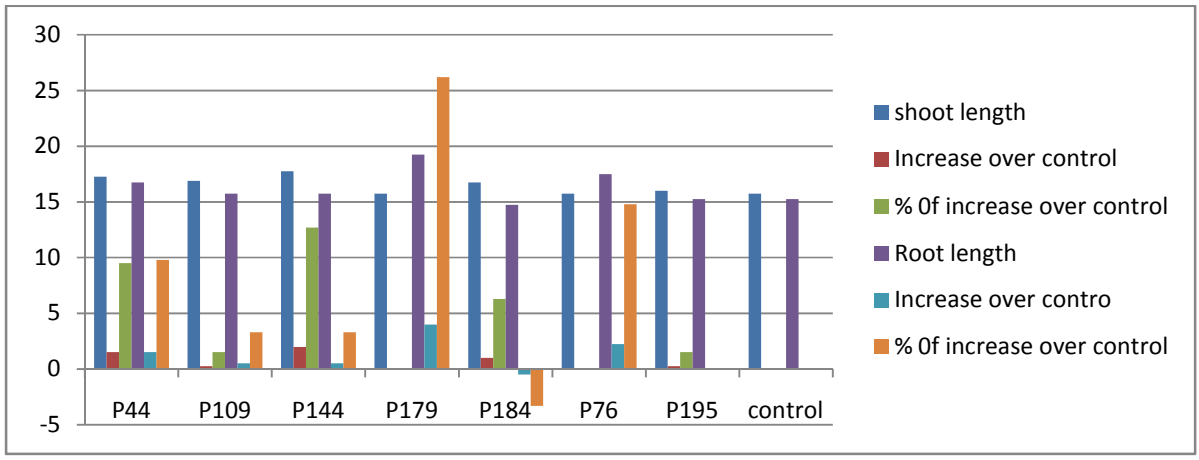

Fig 4: Efficacy of isolates of fluorescent *Pseudomonas* for plant growth promoting response in terms of shoot and root length in Okra

The PGPR concept has been indicated by the isolation of many bacterial strains that fulfill at least two of the three criteria like aggressive colonization, plant growth stimulation, and biocontrol. Seed bacterization significantly influenced root and shoot growth in tested plants. Rhizobacteria belonging to the *P. fluorescens* and *P. putida* species are able to promote plant growth[6,12].

In agriculture inoculation of soil *with Pseudomonas putida* which produces pseudobactin, increase plant growth and yield of various plants[4]. Powell *et al*.,[7] suggested that hydroxamate siderophores are present in soil at concentrations high enough (10^7 to 10^8) to be taken up by plant roots. In the present investigation, significantly higher increase in root and shoot length over control was observed in the tested plants. Similar results were reported by many workers like Weller *et al*,[11], Lucoy *et al*,[5], Kloepper *et al*,[4]. However, with few exceptions, PGPR initially establish high population densities and decline with time and distance from the inoculum source, comprising a progressively smaller proportion of the total rhizosphere microflora[1].

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