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Microbiology

Isolation and Assessment of Plant Growth Promoting Activity of Siderophore Producing *Pseudomonas fluorescens* in Crops

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Abstract

The *Pseudomonas fluorescens* a major Rhizobacteria encourage the plant growth through producing yellowish green fluroscent siderophore involve in high affinity transport of iron into the cell. The *P. fluorescens* isolates grow in iron deficient media because of production capacity of siderophore depends on iron content. Fifty nine *Pseudomonas fluorescens* were isolated in King's B media under 260 nm wavelengths from the rhizosphere and non-rhizospheric soil of cave, forest, fellow land and agriculture field in Chhattisgarh region. The amounts of siderophore produce by *P. fluorescens* isolates were screen in iron deficient succinate media and most of them were found positive for the production of much siderophores. One of the isolate from Pakhanjore area "P3" produce highest siderophore, which further assessed for plant growth promotion activities of crops rice, urd, mung and arhar. The study showed significantly higher increase in root length over control plants was observed in Rice (68.45%) followed by Urd (40.1%), Mung (33.71%) and Arhar (29.53%). Similarly, significantly higher increase in shoot length over control plants was observed in Rice (68.925%) followed by Urd (43.42%), Mung (39.96%) and Arhar (19.45%). It has been shown that these bacteria competively colonize plant roots and cause the plant statistically significant root and shoot increases by stimulating plant growth and reduce the incidence of plant disease of above mention crops under green house of tray culture conditions.

Highlights

- There were 59 isolates of *pseudomonas fluorescens* isolated from rhizosphere and non-rhizospheric soil.
- *P. fluorescens* isolates were screen in iron deficient succinate media for amount of siderophore production.
- One of isolate highest siderophore producing further assessed for PGPA.
- The study showed significantly higher increase in shoot length and root length by *P. fluorescens*

Keywords: *Pseudomonas fluroscent*, Siderophore, PGPA

Introduction

The *Pseudomonas fluorescens* recognized as a plant growth-promoting rhizobacteria (PGPR), potentially useful for stimulating plant growth and increasing crop yields. It has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. The *P. fluorescens* produce yellow-green fluorescent water-soluble pigments commonly found on plant surfaces as well as in decaying vegetation, soil and water (Bradbury, 1986). Out of 115 isolates twenty isolates

shown multifarious PGP traits. Metabolic characterization of representative strains revealed a large versatility with respect to carbon utilization and reported systematic, genetic and functional diversity of PGPR radish cv. Jaunpuri Giant Newar rhizosphere (Srivastava *et al*., 2013). The PGPA activity of *P. fluorescens in* the rhizosphere of plants has been widely reported to enhance the plant productivity (Egamberdiyeva, 2007). The *P. fluorescens* improves plant growth in two different ways, directly or indirectly. The direct promotion of plant growth by PGPR is through production of plant growth-promoting substances or facilitation of uptake of certain nutrients from the soil (Glick and Ibid, 1995). Indirectly, it has the potential to suppress fungal plant pathogens (Vidhyasekaran and Muthamilan, 1995) and there by improve plant growth. The assessment of PGPR offers an attractive way to replace chemical fertilizer, pesticides, and supplements; most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. PGPR as a component in integrated management systems in which reduced rates of agrochemicals and cultural control practices are used as biocontrol agents. Selected strains of beneficial PGPR trigger a plant mediated induced systemic resistance (ISR) response that is effective against a broad spectrum of plant pathogens. The present study focused on the application of *P. fluorescens* in rice, urd, mung and arhar to assess the PGPR activity.

Materials and Methods

Isolation and identification of *P. fluorescent* isolates

Naturally occurring population of *Pseudomonas fluorescens* were isolated from the various area of Chhattisgarh soil samples. One gram soil samples were subjected to serial dilutions at 10^{-3} times was used for plating on the King's medium "B" (King's *et al*., 1954). The bacterial colonies were examined under UV light and colonies with yellow-green and blue-white colour pigmentation were marked and recorded. Individual florescent colony was picked up with the help of sterilized loop and inoculated on solidified King's medium 'B' (KMB) by zig-zag streaking. The plates were incubated at 28°C for 24 hr. The colony growing at the last tip of the zig-zag line was transferred to KMB slants.

Screening for siderophore production

Screening of the isolates was carried out on standard iron deficient succinate medium (SM) (Meyer and Abdullah, 1978). 20 ml of SM broth was poured in conical flasks of 100 ml capacity. After sterilization, the flasks were inoculated with young cultures of individual isolate of *P. fluorescent* growing on KMB. The flasks were incubated for 48 hr. at 28°C on rotating shaker running at 120 rpm.

Screening for plant growth promoting activity

The isolates of rhizobacteria having shown reasonably good producing siderophore, were screened to assess their PGPA. The isolates were grown at 28°C for 48 hrs on KMB medium (broth). The seeds were then dipped into bacterial cell suspension to ensure uniform coating. These coated seeds were air dried for 24 hrs on plastic sheets. Seeds were deep in sterilized distilled water served as control. Treated seeds were placed in sterilized moist soil in a pot, with six seeds per pot and were replicated thrice. The pots were then placed in the sunlight and treated seed were allowed to grow. The plants were uprooted at 45 DAS. While uprooting, care was taken to avoid damage to root system. All the seedlings per bag/treatment were taken a one replication. Root and shoot length observation was taken from fifteen randomly selected plants. The plant were washed, fully stretched and fixed on clean transparent surface. The length of root system was measured from ground level to longest root, while shoot length was measured from the base of the shoot to the upper most leaf.

Results and Discussion

Screening of putative *P. fluorescens* for siderophore production

Screening of *P. fluorescens* isolates for siderophore production was done on the iron deficient succinate medium. The isolates produced variation in yellow green pigment production. Only one isolate P3 (from Pakhanjore) produced very high yellow green pigment (Table-1). In the present investigation all the isolates were round to irregular colonies with yellowish, dull yellowish and greenish yellowish water-soluble pigment producers. These characteristic features confirmed them as the *P. fluorescens*. The growth on succinate medium for siderophore production confirmed them to be the *P. fluorescens* isolates as referred in the standard reports (Leong, 1986).

Assessment of plant growth promoting activity

Isolate P3 from Pakhanjore was observed to produced bright yellow green colour on succinate medium which fluoresced very bright in UV light, and confirmed through catalase, ammonification and gelatin hydrolysis biochemical

Table 1: Screening of putative *Pseudomonas fluorescens* for siderophore production on iron deficient succinate medium collected from different geographical locations of Chhattisgarh

S. No.	+++	S. No.	++	S. No.	++	S. No.	+	S. No.	+	S. No.	-	S. No.	-
1	P3	1	P-1	9	M-6	1	KM-3	12	RF-3	1	P-2	9	RF-7
		2	KM-1	10	KCO-1	2	M-7	13	RF-5	2	P-4	10	RF-8
		3	KM-2	11	KCO-2	3	KCO-3	14	RF-6	3	KCO-4	11	RF-14
		4	M-1	12	K-1	4	K-1	15	RF-9	4	K-4	12	RF-15
		5	M-2	13	K-8	5	K-2	16	RF-11	5	NK-3	13	RF17
		6	M-3	14	TG-4	6	K-3	17	RF-13	6	NK7	14	TG-3
		7	M-4	15	TG-7	7	K-5	18	TG-1	7	RF-1	15	TG-2
		8	M-5	16	RF-10	8	K-6	29	TG-6	8	RF-4	16	TG-5
				17	RF-12	9	K-7	20	NK-1				
				18	RF-16	10	K-10	21	NK-2				
						11	RF-2	22	NK-4				
								23	NK-5				
								24	NK-6				

(-) = No change in colour of medium, no siderophore production; (+) = Development of yellow pigment in medium; (++) = Development of yellow green pigment in medium; (+++) = Development of green pigment in medium. P- Pakhanjore, TG- Tirathgarh Ghat, M – Mango plantation of Kumrawand farm, NK- Negaraai Kamaraai, KM – Jagdalpur area, K – Kutumsar cave, KCO –Field area of Kumrawand farm, RF-Research farm of Raipur

Table 2: : Effect of seed bacterization with candidate *Pseudomonas fluorescens* on root length of rice, mung, urid and arhar

Plant No.	Rice			Mung			Urd			Arhar		
	Treat.	Control	% increase over control	Treat.	Control	% increase over control	Treat.	Control	% increase over control	Treat.	Control	% increase over control
1	16.5	12.5	32	23	20	15	27	16	68.75	21.5	12.5	72
2	15	13	15.3846	22	15	46.6667	25	22	13.6364	20	17	17.6471
3	21	17	23.5294	23.5	16.5	42.4242	25.5	16	59.375	25	18	38.8889
4	17.5	14.5	20.6897	24	20	20	26.5	18.5	43.2432	17	14	21.4286
5	14	10	40	30	22	36.3636	28	20	40	23	15	53.3333
6	20	13	53.8462	27	22	22.7273	13.5	12	12.5	21	13	61.5385
7	15.5	12.5	24	17	16	6.25	18	13	38.4615	17	16.2	4.93827
8	26	6.5	300	17	12	41.6667	18	16	12.5	22	19	15.7895
9	26	12	116.667	20	8.5	135.294	25	22	13.6364	20	18.7	6.95187
10	20.5	9	127.778	23	11.4	101.754	18	9	100	18.2	15	21.3333
11	15.4	10.6	45.283	19	17.2	10.4651	20.3	10.2	99.0196	22.8	14	62.8571
12	22.2	8.2	170.732	22.6	20	13	19	11	72.7273	18	16	12.5
13	24	12.8	87.5	28	14.2	97.1831	17.5	16	9.375	20.8	17	22.3529
14	16	7.5	113.333	25	20.8	20.1923	25.5	20.2	26.2376	20	15.5	29.0323
15	20.3	13	56.1539	22	21	4.76191	27	16	68.75	22.5	17.5	28.5714
Min	14	6.5		16	8.5		13	9		16.2	12.5	
Max	26	21		30	27		28	22		25	20.8	
Average	19.326	11.473	68.4486	22.87	17.11	33.71	22.25	15.86	40.31	20.59	15.89	29.53

tests. Isolate P3 was found most siderophore producing isolate therefore further tested to assess its efficacy for plant growth promoting activity on rice, mung, urd and arhar following seed bacterization. Seed of rice (*oryza sativa*), mung, urd and arhar (*Cajanus cajan*) were treated with the culture and shoot and root growth were recorded after 45 days of showing. The result showed that seed bacterization significantly influenced the root and shoot growth in different crops (Fig. 1). The minimum, maximum and average root length in treated and control plants indicated significant differences and are shown in table - 2. The root length ranges from 14-26 cm with average 19.326 cm in

Table 3: Effect of seed bacterization with candidate *Pseudomonas fluorescens* on shoot length of rice, mung, urid and arhar

Plant No.	Rice			Mung			Urd			Arhar		
	Treat.	Control	% increase over control	Treat.	Control	% increase over control	Treat.	Control	% increase over control	Treat.	Control	% increase over control
1	19.5	17	14.7059	18	15.5	16.129	21	13	61.5385	24	20.5	17.0732
2	21.5	19	13.1579	14	13.3	5.26316	18.7	13.2	41.6667	22.7	20.2	12.3762
3	26.5	18	47.2222	14.2	13.6	4.41177	16	11	45.4546	23.8	20	19
4	24	13	84.6154	14.4	10.5	37.1429	15.8	11.4	38.5965	22.5	19.5	15.3846
5	23.5	18.5	27.027	13.5	10.2	32.3529	17.9	13	37.6923	25	17.7	41.2429
6	29.5	16.5	78.7879	20.5	18	13.8889	22	10.5	109.524	23	10	130
7	27	11	145.455	19.5	15	30	25	12	108.333	22.7	20.1	12.9353
8	32	15.3	109.15	19	14.5	31.0345	15.5	11	40.9091	21	20.3	3.44828
9	31.5	13.5	133.333	18.2	9.5	91.579	13	13	0	22.5	21	7.14286
10	29	14	107.143	21	8.6	144.186	16	12.5	28	20.2	20	1
11	26.2	13.5	94.0741	19.5	11.5	69.5652	20	14	42.8571	22.5	18	25
12	30	17.2	74.4186	18.6	14	32.8571	15	12.5	20	24	17.5	37.1429
13	18.6	18	3.33333	15.2	10	52	17.2	11.2	53.5714	20.5	20.5	0
14	20	12	66.6667	19	13.8	37.6812	18	13.5	33.3333	18.6	17	9.41177
15	26	11.3	130.089	17	13	30.7692	16.8	15	12	23	19	21.0526
Min	18.6	11		13.5	8.6		13	10.5		18.6	10	
Max	32	19		21	18		25	15		25	21	
Average	25.65	15.19	68.92	17.44	12.73	36.96	17.86	12.45	43.42	22.4	18.75	19.45

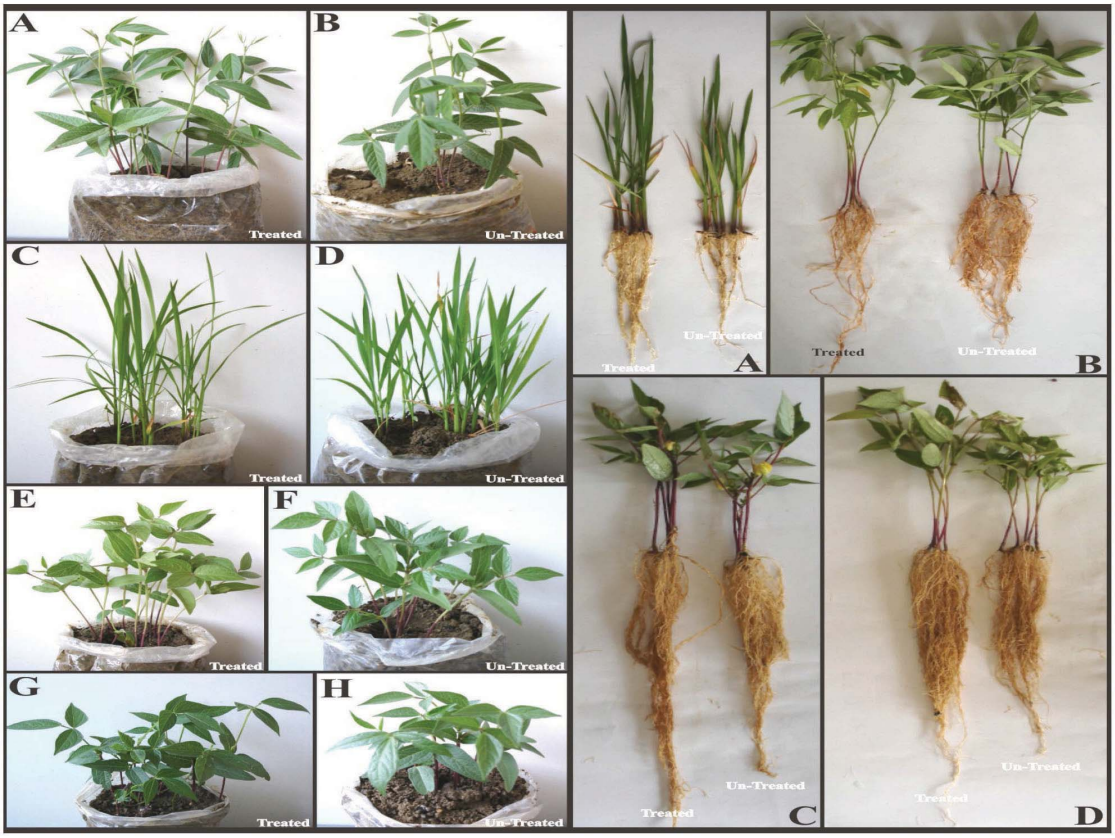

Fig. 1: Growth promoting activity of *Pseudomonas fluorescens*
(A=arhar Treated, B=arhar Non-treated, C= rice Treated, D= rice Non-treated, E=urd Treated, F =urd Non-treated, G = mung Treated, H= mung Non-treated)

rice. Similarly shoot length showed significant differences are shown in (Table-3). The shoot length ranges from 18.6 to 32 with an average 25.65cm in rice.

The study showed significantly higher increase in root length over control plants was observed in Rice (68.45%) followed by Urd (40.1%), Mung (33.71%) and Arhar (29.53%). Similarly significantly higher increase in shoot length over control plants was observed in Rice (68.925%) followed by Urd (43.42%), Mung (39.96%) and Arhar (19.45%). It has been shown that these bacteria rapidly colonize plant roots and cause at the plant level statistically significant root and shoot increases. Plant growth-promoting rhizobacteria (PGPR) competitively colonize plant roots, and stimulate plant growth and reduce the incidence of plant disease (Kloepper and Schroth, 1978). In some PGPR termed biofertilizers, plant growth promotion dominates. The mechanisms that are involved in this process can include nitrogen fixation, phosphate solubilization and the production of phytohormones (such as auxin and cytokinin) and volatile growth stimulants (such as ethylene and 2, 3-butanediol (Vessey, 2003; Ryu *et al.*, 2003). Biocontrol PGPR consisting of certain *P. fluorescens* that protects a range of crop plants from important, mostly fungal, root pathogens and is evidenced by increased seedling emergence, vigour, seedling weight, root system development and yield (Klopper *et al.*, 1980). The mechanism first proposed for induction by PGPR of plant growth increase was the production of siderophores (Klopper *et al.*, 1980 and Leong, 1986). In summery this study showed the significant contribution of *P. fluorescens* on growth of rice, mung, urd, and arhar shoot and root length.

Conclusion

The present study, identified many siderophore producing *P. Fluorescens* bacteria from rhziosphere and non-rhziosphere soil of Chhattisgarh and found most of them were high siderophore producing. The Pseudomonas were shown growth promoting for shoot and root length.

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