ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 81-85: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

CHARACTERIZATION OF FLUORESCENT *PSEUDOMONAS* WITH CROP SPECIFIC PLANT GROWTH PROMOTING RESPONSES ON WHEAT AND COW PEA

Vandana Shukla *et al.,*

KEYWORDS

Biological control
Fluorescent *Pseudomonas*
PGPR
Seed bacterization
siderophores

Proceedings of National Conference on Harmony with Nature in Context of Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

VANDANA SHUKLA*[1], NITIN KUMAR TORRAY[1], TOSHY AGRAWAL[2] AND ANIL S. KOTASTHANE[2]
[1]Department of Plant Pathology, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur - 492 012, Chhattisgarh, INDIA
[2]Department of Plant Molecular Biology and Biotechnology,
Indira Gandhi Krishi Vishwa Vidyalaya, Raipur - 492 012, Chhattisgarh, INDIA
e-mail: vandana2089shukla@gmail.com

ABSTRACT

Siderophores producing and Cynogenic (HCN producers) fluorescent pseudomonads exerted strong inhibitory against *Sclerotium rolfsii*. Descriptive method was used with the analysis of IAA production, gelatine hydrolysis, starch hydrolysis, lipolytic activity, casein hydrolysis, carbohydrate fermentation, litmus milk reaction, Arginine dihydrolase test, nitrate reduction, egg yolk test, phosphate solubilisation, siderophore production and antibiotic sensitivity. Plant growth promoting response of wheat, and cow pea was observed following seed bacterization with fluorescent *Pseudomonas* (expressing different levels siderophore) isolates. Fluorescent *pseudomonas* isolates P66 (Wheat (*Triticum aestivum*) and P229 (Cowpea (*Vigna unguiculata* var. AS-77) induced crop specific growth promoting response.

***Corresponding author**

INTRODUCTION

The plant rhizosphere is an important soil ecological environment for plant microbe interactions. It involves colonization by a variety of micro-organisms in and around the roots which may result in symbiotic, associative, neutralistic or parasitic relations within the plant, depending on the type of microorganism, soil nutrient status, and plant defense system and soil environment. A substantial number of bacterial species, mostly those associated with rhizosphere, may exert a beneficial effect on plant growth and development. This group of bacteria has been termed "plant growth promoting rhizobacteria" or PGPR. Plant growth promoting rhizobacteria (PGPR) can stimulate growth by one or more different mechanisms. The direct mechanisms include production of growth hormones like IAA, phosphate-solubilization and uptake of iron, whereas indirect mechanisms include check on phytopathogens by the release of HCN, antibiotics and siderophores (O'Sullivan and O'Gara, 1992). Among PGPR, fluorescent pseudomonads (FLPs) possess several properties best suited for survival and colonization in the rhizosphere environment. Antagonistic rhizobacteria, more specifically, fluorescent pseudomonads, are well known to suppress fungal root diseases of agronomic crops, such as seedling disease of cow pea and wheat caused by *S. rolfsii*. Secondary metabolites produced by fluorescent Pseudomonads have been reported antifungal activity inhibiting *R. solani* (Mina *et al.,* 2013). Considerable progress has been made over the past two decades to elucidate the mechanisms by which fluorescent pseudomonads suppress disease. It is apparent that a large variety of antifungal metabolites are produced by fluorescent pseudomonads. Hydrogen cyanide, phloroglucinol, pyoluteorin and pyrrolnitrin are produced by many of these bacteria, and attempts have been made to quantify the importance of each in disease suppression. PGPR have been considered as an alternative to agrochemicals for controlling plant diseases as plant pathogens developed resistance against pesticides (Kamei *et al*. (2014).

Characteristics, especially biochemical characteristics, of fluorescent *Pseudomonas* has an important role to determine its potential level in inhibition of plant pathogens but the characterization never been done. The characteristics should be tested in order to know the field application of antagonist. The aim of this research was to know biochemical characteristics of fluorescent *Pseudomonas* isolates and assessment of potential PGPR agents. The present study evaluated local isolates of phosphate solubilising bacteria (*Pseudomonas spp*.) from different geographical locations of Chhattisgarh on growth parameters of wheat and cow pea.

MATERIALS AND METHODS

The present investigation was carried out at Microbial Biotechnology Laboratory at the Department of Plant Molecular Biology and Biotechnology, R. H. Ricchharia Research Laboratory, College of Agriculture, IGKV, Raipur characterise siderophore producing fluorescent *Pseudomonas* and to screen the isolates for phosphate solubilization, siderophore and at Research cum Instructional farm, College of Agriculture, IGKV to carry out pot experiments. The experimental material consisted

of purified five isolates of fluorescent *Pseudomonas* species kindly provided by Dr. A. S. Kotasthane, Professor of Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur which were used for biochemical characterization and to screen the isolates for phosphate solubilization, siderophore producing and plant growth promoting ability. These isolates were isolated from soil samples of different geographical locations of Chhattisgarh. During the course of study, King'B medium was used for maintaining the cultures of *Pseudomonas*.

Biochemical characterization of bacterial isolates

The biochemical characterization of fluorescent *Pseudomonas* isolates was carried out to characterize all the isolates of fluorescent *Pseudomonas*. A rapid antibiotic sensitivity test was used to distinguish different species of fluorescent *Pseudomonas*. Isolates was then characterized based on biochemical tests as per the procedures outlined in Bergey's Manual of Systematic Bacteriology (Sneath *et al*. 1984). The siderophore production was determined qualitatively by CAS agar plate method and specific tests were carried out for identification of hydroxamate and Catecholate types of siderophores following the standard methods (Arnow 1937). Qualitative screening of phosphate solubilizing fluorescent *Pseudomonas* was performed on Pikovskaya's agar medium (Hi media) containing tricalcium phosphate as a phosphate source and Bromo Cresol Purple (0.1 g/l) as a pH indicator for acidification (Vazquez *et al*. 2000). Based on the production of siderophores, IAA and phosphate solubilisation candidate isolates were selected for experiments and therefore was also characterized for other plant growth promoting potentials.

***In vitro* screening for antagonistic activity against soil borne phytopathogens**

The five isolates were also tested for their efficacy as bio control agent against the phytopathogens *Sclerotium rolfsii*. Equal volume of sterilized potato dextrose agar (PDA) and King's B medium was mixed and poured in sterilized petridishes. Heavy inoculums from an actively growing fluorescent *Pseudomonas* was streaked at 1 cm away from the edges of the plate and the mycelia disc of the pathogens were placed at the centre of petriplates. Control plates were inoculated only with phytopathogens but not with *Pseudomonas* isolates. Percent inhibition of pathogens by *Pseudomonas* isolates over control was calculated by using the formula:

[(Growth of pathogen in control - Growth of pathogen with *Pseudomonas* isolate)/ Growth of pathogen in control] x 100

Host seeds

Seeds of Wheat (*Triticum aestivum* var. MP 1203) and Cowpea (*Vigna unguiculata* var. AS-77) was obtained from Research cum Instructional farm, IGKV, Raipur, India.

Seed bacterization

Five isolates of fluorescent *Pseudomonas* (P66, P141, P200, P229 and P260) were tested for their ability to promote plant growth in pot experiments. The bacterial cultures were inoculated in 25ml King's B broth and incubated at $28 \pm 2°C$ for 48 hrs. For seed soaking inoculums of all the isolates were diluted to final concentration of 10^8-10^9 CFU mL^{-1}(600 nm). Seeds of chick pea variety were soaked in the diluted suspension for 1 hour. Seeds soaked in water served as control.

Pot experiments

The treated seeds were planted in pots containing soil mixed with sand and compost in the ratio of 3:1:1. Each treatment was replicated twice and the experiment was repeated two times. For taking measurement on growth parameters, three plants were randomly uprooted from each pot at 30 days after sowing (DAS). The following observations were taken - shoot and root length (cm), shoot and root fresh weight (gm) and dry weight (gm). Shoot and root dry mass was recorded after drying the samples in oven at 60ºC.

RESULTS AND DISCUSSION

All the five isolates of fluorescent *Pseudomonas* showed positive response for siderophore production and phosphate solubilization (in Pikovskaya's agar medium with and without BCP indicator). Isolates showed variability for traits such as gelatin liquefaction, casein hydrolysis, lipolytic activity, nitrate reduction and antibiotic sensitivity test. Out of five fluorescent *Pseudomonas* isolates, 3 isolates (P66, P200 and P260) showed proteolytic activity (casein hydrolysis) by inducing clear zones around the cells on skim milk agar medium, all five isolates showed lipolytic activity, one (P66) was positive for gelatin liquefaction, all five isolates gave positive result for nitrate test, of which one (P66) was positive before addition of zinc and all five isolates (P66, P141, P200, P229 and P260) showed positive response after addition of zinc. In antibiotic sensitivity test, all five isolates were resistant to antibiotic carbenicillin while in kanamycin amended medium, three isolates (P66, P200 and P229) were able to grow and two isolates (P141 and P260) could not.

Siderophores are also thought to facilitate bio control by sequestering iron from pathogens, thus limiting their growth and for the solubilization of extracellular ferric iron by most bacteria and fungi. CAS assay is the universal assay for detection of siderophores. The principle of this assay is based on a colour change of CAS from blue to orange resulting from

Table 1: Plant growth promotion activity of different isolates of fluorescent *Pseudomonas*

Bacterial Isolates	Phosphate solubilization	Siderophore Production	IAA production (μg/ml)	Antagonism (*S. rolfsii*)
P66	+ + +	+	16.727	+
P141	+ + +	+	17.636	+
P200	+ +	+	10.909	+
P229	+ +	+	17.818	+
P260	+ +	+	11.091	+

(+) positive, (–) negative test, phosphate solubilization; Antifungal test

Table 2: Plant growth promotion activity of fluorescent *Pseudomonas* on Wheat (*Triticum aestivum* var. MP 1203)

Treatment	Isolate No.	COW PEA (AS 77) Shoot length (cm)	Root Length (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
1	P66	19.475bc±0.7	42.625a±10619	3.065a±0.084	1.58b±0.0199	0.865ab±0.024	0.46a±0.0049
2	P141	16.675c±0.7751	37.425a±0.874	2.265a±0.094	1.31c±0.0799	0.94a±0.249	0.155bc±0.3415
3	P200	22.5b±0.8251	41.05a±2.22	3.275a±0.044	1.68ab±0.0099	0.685c±0.0699	0.26b±0.0124
4	P229	30a±0.65	38.4a±0.799	2.355a±0.294	1.735a±0.0199	0.895ab±0.014	0.225b±0.0249
5	P260	19.875bc±1.75	31.1b±1.796	2.3a±0.28	0.78d±0.0649	0.775bc±0.064	0.16bc±0.0299
6	Control	10.45d±1.3751	27.625b±1.79	0.855b±0.729	0.55e±0.0199	0.355d±0.044	0.025c±0.0512
	Max	30a±0.65	42.625a±10619	3.275a±0.044	1.735a±	0.94a±	0.46a±0.0049
	Min	10.45d±1.3751	27.625b±1.79	0.855b±0.729	0.55e±	0.355d±	0.025c±0.0512
	CV	7.788	6.249	20.812	4.956	8.628	29.506
	CD0.01	5.726	8.418	NS	0.237	0.244	0.237
	CD0.05	3.778	5.559	1.193	0.152	0.157	0.153
	Fcal	35.021	13.224	6.021	124.829	21.972	10.577

Table 3: Plant growth promotion activity of fluorescent *Pseudomonas* on Cowpea (*Vigna unguiculata* var. AS-77)

Treatment	Isolate No.	WHEAT (MP 1203) Shoot length (cm)	Root Length (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
1	P66	36.283a±0.268546	39.4a±1.2501	0.856a±1.2501	1.626a±0.1949	0.143ab±0.002	0.106abc±0.006
2	P141	35.583a±0.5666	38.116a±2.132	0.95a±2.132	1.763a±0.0932	0.155ab±0.035	0.126ab±0.02164
3	P200	30.716b±0.416	31.416b±0.6499	0.466bc±0.6499	0.726bc±0.1866	0.073c±0.0212	0.046bc±0.0366
4	P229	29.133b±2.65	30.233b±1.35	0.555b±1.35	0.886b±0.0932	0.093bc±0.0266	0.036c±0.0183
5	P260	36.25a±3.8	35.866a±0.1666	0.856a±0.1666	1.993a±0.0266	0.19a±0.0066	0.143a±0.0166
6	Control	29.616b±0.1166	29.35b±0.299	0.333c±0.299	0.316c±0.0016	0.03c±0.0066	0.023c±0.0333
	Max	36.283a±0.268546	39.4a±1.2501	0.95a±2.132	1.993a±	0.19a±0.0066	0.143a±0.0166
	Min	29.616b±0.1166	29.35b±0.299	0.333c±0.299	0.316c±	0.03c±0.0066	0.023c±0.0333
	CV	4.984	4.935	11.247	14.282	22.91	43.803
	CD0.01	6.081	6.231	0.278	0.646	0.096	NS
	CD0.05	4.011	4.111	0.18	0.421	0.064	0.085
	Fcal	8.843	13.036	21.832	29.61	10.235	4.425

Figure 1: Plant growth promotion activity of florescent Pseudomonas on whwat

Figure 2: Plant growth promotion activity of florescent Pseudomonas on cowpea

siderophoral removal of iron from the chrome azurol dye. All the isolates showed positive response for siderophore production on CAS agar plate i.e. orange colour halo was observed in all the isolates. Overall, these isolates were found to be more efficient against *S. rolfsii* indicating that these isolates could therefore be exploited as potential candidates for the development of bio pesticides more specifically against these most devastating pathogens of crops.

Plant growth promoting response of *Pseudomonas* on wheat and cow pea

Efficacy of different isolates of *Pseudomonas* for wheat plants

varied to induce root and shoot length ranging from 39.4 to 29.35 cm and 36.283 to 29.35 cm respectively. Maximum root length (39.4 cm) and shoot length (36.283 cm) were recorded when seeds were treated with P66, respectively as compared to control (Fig.1)

Similarly in case of cow pea efficacy of different isolates of *Pseudomonas* varied to induce shoot and root length ranging from 30 to 10.45 cm and 42.62 to 27.625 cm respectively. Maximum root length (42.62 cm) and shoot length (30 cm) were recorded when seeds were treated with P229 as compared to control (Fig.2).

investigations are in the agreement with Patel *et al*. (2011), Sivasakthi *et al*. (2013), Trivedi *et al*. (2013) and Saranya and Sowndaram (2014) who reported that fluorescent *Pseudomonas* is a heterogenous group of growth promoting rhizobacteria that regulate plant growth by releasing secondary metabolic compounds viz., indole acetic acid (IAA), siderophores, ammonia and hydrogen cyanide. Kamei *et al*. (2014), Prashant *et al*. (2014) and Solanki *et al*. (2014) were reported fluorescent *Pseudomonas* exhibited antifungal activity against the plant pathogens.

ACKNOWLEDGMENT

The authors are thankful to the Dr. Anil S. Kotasthane, Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur (C.G.) for his untiring help and assistance during the experiment and preparation of the manuscript.

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