Available online at www.jpsscientificpublications.com
Volume – 1; Issue - 3; Year – 2015; Page: 244– 251

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

SCREENING OF PHYTOHORMONES BY *Pseudomonas fluorescens* FROM RHIZOSPHERE SOIL OF PADDY IN AND AROUND TIRUVANNAMALAI

S. Shahira[1*], B. Vedha[1], T. Panneerselvam[1] and K. Anandaraj[2]

[1]*PG and Research Department of Microbiology, Adhiparasakthi College of Arts and Science (Autonomous), G.B Nagar, Kalavai – 632 506, Tamil Nadu, India.*

[2]*PG Department of Microbiology, Shanmuga Industries Arts and Science College, Tiruvannamalai - 606 601, Tamil Nadu, India.*

Abstract

This present study shows isolates of *Pseudomonas fluorescens* from various rhizosphere samples were made on different locations of Tiruvannamalai district. The rhizobacterial isolates of *Pseudomonas fluorescens* characterize and analyses the ability of screening Phytohormones, estimate Indole acetic acid, Gibberellic acid, Siderophore productions and screening the antagonistic activity of *Pseudomonas fluorescens.* The rhizobacterial isolate PF 8 (38 mm in dm) showed maximum zone of inhibition followed by PF 2 (35 mm in dm).

Key words: Antagonistic activity, Indole acetic acid, Gibberellic acid and Siderophore.

1. Introduction

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner (1904) and describes the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria known as plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangajaran *et al*., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001). *Pseudomonas* sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* spp.

Considerable research is underway globally to exploit the potential of one group of bacteria that belong to fluorescent *Pseudomonas* (FLPs). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata *et al*., 2002). Mahmoud Reza Ramezanpour *et al*. (2011) revealed that *Pseudomonas* have plant growth promoting properties. Isolated strains showed high ability of IAA production, phosphate solubilization and siderophore production, while genotyping analysis showed that *Pseudomonas* isolated from the rhizosphere of rice are genetically diverse. Nevertheless, the strains were distributed into 11 genotypes, including five groups of fluorescent *Pseudomonas*.

Corresponding author*: **S. Shahira
Received: 08.05.2015; *Revised*: 23.06.2015;
Accepted: 20.07.2015.

2. Materials and Methods

2.1. Collection of paddy rhizosphere soil from different locations

The survey was conducted at ten locations in Tiruvannamalai district of Tamil Nadu comprising Kilpennathur, Somasipadi, Vettavalam, Polur, Chengam, Avalurpet, Avoor, Kattumalaiyanur, Veraiur and Mangalam. In each and every locations of the survey area a field which has been under long behind mono culture practice was selected. The locations of rhizosphere samples were made at different location of the paddy field. The collected soil samples were brought to laboratory for further analysis.

2.2. Isolation, enumeration and identification of *Pseudomonas fluorescens* from rhizosphere of Paddy

2.2.1. Isolation and enumeration of *Pseudomonas fluorescens* population

The paddy rhizosphere soil samples collected from ten paddy field of a particular location were pooled and one ml of paddy rhizosphere soil sample was transferred to 100 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotator shaker (100 rpm) for 30 minutes at ambient temperature. The well mixed suspension was then diluted appropriately upto 10^{-6} dilution. 1 ml of suspension from 10^{-4} and 10^{-5} dilution was aseptically transferred to sterile petriplates and10 - 20ml of selective King's B medium was added and incubated at 37ºC for 24 hours. Three replications were maintained for each dilution. The colonies were counted by using Colony counter. The total number of colonies in the original samples was expressed as cfu g^{-1}.

2.2.2. Purification of *Pseudomonas fluorescens* isolates

All the ten *Pseudomonas fluorescens* isolates were purified by Streak plate method using King's B medium frequently.

2.3. Characterization of *Pseudomonas fluorescens*

Identification of the *Pseudomonas fluorescens* were carried out by the routine bacteriological methods as followed by Bergey's manual.

2.4. Screening of *Pseudomonas fluorescens* isolates based on production of phytohormones, siderophore production and antagonistic activity

2.4.1. Production of phytohormones by *Pseudomonas fluorescens*

The *in vitro* production of phytohormones such as indole acetic acid (IAA) and gibberellic acid (GA_3) by plant growth promoting rhizobacterial isolates were estimated.

2.4.2. Estimation of Indole acetic acid (IAA)

A quantity of 100 ml Nutrient broth for *Bacillus* and King's B broth for *Pseudomonas* isolates were prepared and sterilized. Freshly prepared, filter sterilized solution of L-tryptophan was added to each flask to a final concentration of 100 mg 1^{-1}. One ml of culture broth of plant growth promoting bacterial isolates were inoculated to each flask and incubated at 37°C in dark for seven days.

2.4.3. Estimation of Gibberellic acid (GA_3)

The gibberellic acid production by plant growth promoting rhizobacteria was determined by following the method of Borrow *et al.* (1955).

2.7.4. Estimation of Siderophore Production

Siderophore production by the plant growth promoting rhizobacteria was estimated by the method described by Reeves *et al.* (1983).

2.7.5. Screening the *Pseudomonas fluorescens* isolates for zone of inhibition of *Rhizoctonia solani*

A loopful culture of each *Pseudomonas fluorescens* isolates was transferred aseptically to the centre of PDA plates which have been pre inoculated with *Rhizoctonia solani*. The plates were incubated at 28±2ºC for 72 hrs. After the incubation period the diameter of inhibition zone

was measured. Three replications were maintained for each isolate.

3. RESULTS

The survey was conducted at ten locations in Tiruvannamalai district of Tamil Nadu comprising Kilpennathur, Somasipadi, Vettavalam, Polur, Chengam, Avalurpet, Avoor, Kattumalaiyanur, Veraiur and Mangalam. The locations of rhizosphere samples were made at different location of the paddy field. The collected soil samples were brought to laboratory for further analysis.

3.1 Microbiological analysis of soil samples

The collected soil samples were analyzed for the total population of Bacteria, Fungi and Actinomycetes and the results are presented in Table – 1. The results revealed that the total bacterial population ranged from 15.8 to 24.3 × 10^6 cfu g^{-1} of soil and the highest population of 24.3 × 10^6 cfu $g^{-1}$was observed in soil of Kattumalaiyanur. The total fungal and actinomycetes population were ranged between 8.9 to 13.3 × 10^3 cfu g^{-1} and 10.5 to 19.3 × 10^5 cfu g^{-1} of soil respectively.

3.2 Occurrence and community population of *Pseudomonas fluorescens* from the rhizosphere of paddy grown at Tiruvannamalai district

The occurrence of *Pseudomonas fluorescens* population in the rhizosphere of paddy grown at ten selected locations were designated as PF-1 to PF-10 respectively and numbered randomly. The total bacterial population, *Pseudomonas fluorescens* population and percentage of *Pseudomonas fluorescens* population were estimated and the results are presented in the Table - 2. The location, namely Kattumalaiynur recorded maximum of 7.71cfu × 10^4 g^{-1} community population of *Pseudomonas fluorescens,* from Mangalam recorded least population of 7.21 cfu × 10^4 g^{-1} in the rhizosphere. All other locations recorded the community population of *Pseudomonas fluorescens.*

3.3 Characterization of *Pseudomonas fluorescens*

The *Pseudomonas fluorescens* were characterized by Gram's staining, motility test, plating on King's B medium and biochemical tests. The *Pseudomonas fluorescens* isolates were designated as *Pseudomonas fluorescens* (PF -1) to *Pseudomonas fluorescens* (PF –10). The characteristics of *Pseudomonas fluorescens* was showed in Table - 3. The identified *Pseudomonas fluorescens* was observed as Gram negative slender rods. They were actively motile and aerobic in nature. In King's B agar medium, the *Pseudomonas fluorescens* colonies were observed as individual, small, round, irregular and fluorescent pigmented colonies. The colonies showed positive reaction for catalase, oxidase, citrate and gelatin hydrolysis. Negative results were observed in indole, methyl red test, Voges Proskauer test, urease, starch hydrolysis and nitrate reduction.

3.4 Screening of *pseudomonas fluorescens* isolates for indole acetic acid production

The ten *Pseudomonas fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of Indole acetic acid production and the results were furnished in Table – 2. All the above 10 isolates taken from the study showed positive results producing Indole acetic acid. The amount of indole acetic acid produced expressed in µg/ml of culture filtrate. The maximum indole acetic acid production by *Pseudomonas fluorescens* was recorded by the isolate PF – 8 (28.80 µg 25/ml of broth) followed by the *Pseudomonas fluorescens* isolate PF – 2 (26.80 µg 25/ml of broth). The minimum production of indole acetic acid was observed in PF - 4 (07.36 µg 25/ml of broth) isolates.

3.5 Estimation of gibberellic acid from *Pseudomonas fluorescens*

The Gibberellic acid produced by ten different isolates of *Pseudomonas fluorescens* was estimated and the results were showed in Table – 4. The isolate *Pseudomonas fluorescens* (PF – 8) showed maximum Gibberellic acid production

(5.96 µg 25/ml of broth) followed by the *Pseudomonas fluorescens* PF – 2 (5.50 µg 25/ml of broth) and least Gibberellic acid production was showed by PF - 4 (2.89 µg 25/ml of broth).

3.6 Siderophore production by *Pseudomonas fluorescens*

The Siderophore production by *Pseudomonas fluorescens* was estimated and the results were showed in Table – 4. The isolate *Pseudomonas fluorescens* (PF – 8) showed maximum Siderophore production (Catechol type - 9.70 µg/ml and Salicylate type - 10.81 µg/ml) followed by *Pseudomonas fluorescens* PF – 2 (Catechol type - 9.32 µg/ml and Salicylate type - 10.03 µg/ml) and the least Siderophore production was showed by the *Pseudomonas fluorescens* isolate PF - 4 (Catechol type - 6.10 µg/ml and Salicylate type - 7.21 µg/ml).

3.7 Antagonistic activity of *pseudomonas fluorescens* against *Rhizoctonia solani*

The antagonistic activity of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* was investigated and the results were showed in Table – 4. All the *Pseudomonas fluorescens* strains showed zone of inhibition against Rhizoctonia *solani*. Among the ten strains, *Pseudomonas fluorescens* PF – 8 (38 mm in dm) showed maximum zone of inhibition followed by PF – 2 (35 mm in dm). Least zone of inhibition was observed in PF – 4 (28 mm in dm).

Table - 1: Microbiological analysis of soil samples (* cfu x 10^4 g^{-1})

Rhizosphere soil sample	Bacteria*	Actinomycete *	Fung *
Kilpennathur	19.4	18.0	8.9
Somasipadi	22.3	16.0	10.8
Vettavalam	17.6	12.6	12.3
Polur	18.6	14.2	10.5
Chengam	17.6	12.6	12.3
Avalurpet	21.2	14.3	11.8
Avoor	18.0	16.3	11.8
Kattumalaiyanur	24.3	13.0	14.0
Veraiur	16.0	19.3	13.3
Mangalam	15.8	10.5	11.9

Table - 2: Occurrence of community *Pseudomonas fluorescens* population from rhizosphere soil of paddy and Indole Acetic Acid production of isolates

Rhizosphere soil sample	*Pseudomonas fluorescens**	IAA production**
Kilpennathur	7.65	19.21
Somasipadi	7.66	26.8
Vettavalam	7.55	25.8
Polur	7.60	7.36
Chengam	7.36	9.75
Avalurpet	7.63	13.2
Avoor	7.66	16.6
Kattumalaiyanur	7.71	28.8
Veraiur	7.31	24.77
Mangalam	7.21	21.07

(* cfu x 10^4 g^{-1}, ** Quantity of IAA production (µg 25/ml of broth)

Table - 3: Characterization of *Pseudomonas fluorescens* from the rhizosphere paddy soil

Microscopy : Gram negative slender rods
Motility test : Active motile
Cultural characteristics : Aerobic
Colony morphology : Individual, small, round, irregular and fluorescent pigmented colonies (In King's B medium).

Characters studied	***Pseudomonas fluorescens* (PF) isolates**									
	PF1	**PF2**	**PF3**	**PF4**	**PF5**	**PF6**	**PF7**	**PF8**	**PF9**	**PF10**
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-
Organism isolated	*Pseudomonas fluorescens*									

Table – 4 : Gibberellic acid production by, Siderophore production and screening of antagonistic activity of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani*

Isolates (*Pseudomonas fluorescens*)	Quantity of Gibberellic acid (µg 25/ml of broth)	Siderophore content (µg ml^{-1})		Zone of inhibition (mm) against *Rhizoctonia solani*
		Catechol Type	Salicylate Type	
PF1	4.51	7.40	8.53	33
PF2	5.50	9.32	10.03	35
PF3	5.01	8.72	9.83	32
PF4	2.89	6.10	7.21	28
PF5	3.11	6.50	6.61	34
PF6	3.94	6.75	7.86	32
PF7	4.63	7.90	9.01	33
PF8	5.96	9.70	10.81	38
PF9	4.26	7.11	8.22	31
PF10	4.80	8.30	9.45	32

4. DISCUSSION

Agriculture is heavily dependent on the use of chemical fertilizers and pesticides to achieve higher yields. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that otherwise support crop production. Hence, crop production and pest and disease management have to be achieved in shorter intervals of time with fewer detrimental inputs. The use of bioresources to replace chemical fertilizers and pesticides is growing. In this context, plant growth promoting microorganisms are often novel and

potential tools to provide substantial benefits to agriculture.

Plant growth promoting rhizobacteria (PGPR) may promote growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus and potassium, production of siderophore that solublize and sequester iron, or production of plant growth regulators (Kloepper, 1997). Some bacteria support plant growth indirectly, by improving growth restricting conditions either *via* production of antagonistic substances or by inducing host resistance towards plant pathogens. Since associative interactions of plant and microorganisms must have come into existence as a result of co-evaluation, the use of either former or latter groups of bioinoculants form one of the vital components for a long term sustainable agriculture system (Tilak *et al*., 2005).

The occurrence of *Pseudomonas fluorescens* population in the rhizosphere of paddy grown at ten selected locations were designated as PF-1 to PF-10 respectively and numbered randomly. The locations, namely Kattumalaiyanur recorded maximum of 7.71cfu x 10^6 g^{-1} community population of *Pseudomonas fluorescens*, from Mangalam recorded least population of 7.21 cfu x 10^6 g^{-1} in the rhizosphere. All other locations recorded the community population of *Pseudomonas fluorescens.* The *Pseudomonas fluorescens* were characterized by Gram's staining, motility test, plating on King's B medium and biochemical tests. The *Pseudomonas fluorescens* isolates were designated as *Pseudomonas fluorescens* (PF -1) to *Pseudomonas fluorescens* (PF –10).

The identified *Pseudomonas fluorescens* was observed as Gram negative slender rods. They are actively motile and aerobic in nature. In King's B agar medium, the *Pseudomonas fluorescens* colonies were observed as individual, small, round, irregular and fluorescent pigmented colonies. The colonies showed positive reaction for catalase, oxidase, citrate and gelatin hydrolysis. Negative results were observed in indole, methyl red test, Voges Proskauer test, urease, starch hydrolysis and nitrate reduction.

Lindow and Brandl (2003) reported the ubiquitous occurrence of rhizosphere of many crop plants and also revealed the marked variations in the community populations of *Pseudomonas fluorescens* at phyllosphere and rhizosphere level of IR-50 rice, among the five locations studied. Occurrence of *Pseudomonas fluorescens* the rhizosphere of rice has been reported by (Mew and Rosales, 1986; Rabindran and Vidhyasekaran, 1996). In the present study, all ten isolates were identified characterized as *Pseudomonas fluorescens*.

The ten *Pseudomonas fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of Indole acetic acid production. All the above 10 isolates taken from the study showed positive results producing Indole acetic acid. The amount of indole acetic acid produced expressed in µg/ml of culture filtrate. The maximum indole acetic acid production by *Pseudomonas fluorescens* was recorded by the isolate PF - 8(28.80 µg/ml) followed by the *Pseudomonas fluorescens* isolate PF – 2 (26.80 µg/ml). The minimum production of indole acetic acid was observed in PF - 4 (07.36 µg/ml) isolates.

Prassana Reddy Battu and Reddy (2009) isolated twenty *Pseudomonas fluorescens* strains from rice growing soil samples and characterized. One of the *Pseudomonas fluorescens* isolated and identified from the dual culture test. It was fermented for secondary metabolite in a small scale and extracted with ethyl acetate. The isolated metabolite tested against rice fungal pathogens. The structure of the compound was elucidated by high-resolution NMR spectroscopy.

Mandira Kochar *et al*. (2011) analyzed the biocontrol strain *Pseudomonas fluorescens* Psd for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant-growth-promoting (PGP) potential. While the indole pyruvic acid (IPyA) pathway commonly associated with PGP bacteria was lacking, the indole acetamide (IAM) pathway generally observed in phytopathogens was expressed in strain Psd. Overexpression of IAM pathway genes *iaaM-iaaH*, from *Pseudomonas*

syringae subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

The Gibberellic acid produced by ten different isolates of *Pseudomonas fluorescens* was estimated. The isolate *Pseudomonas fluorescens* (PF – 8) showed maximum Gibberellic acid production (5.96 µg/ml) followed by the *Pseudomonas* fluorescens PF – 2 (5.50 µg/ml) and least Gibberellic acid production was showed byPF - 4 (2.89 µg/ml). The Siderophore production by *Pseudomonas fluorescens* was estimated. The isolate *Pseudomonas fluorescens* (PF – 8) showed maximum Siderophore production (Catechol type - 9.70 µg/ml and Salicylate type - 10.81 µg/ml) followed by *Pseudomonas fluorescens* PF – 2 (Catechol type - 9.32 µg/ml and Salicylate type - 10.03 µg/ml) and the least Siderophore production was showed by the *Pseudomonas fluorescens* isolate PF - 4 (Catechol type - 6.10 µg/ml and Salicylate type - 7.21 µg/ml). The antagonistic activity of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* was investigated. All the *Pseudomonas fluorescens* strains showed zone of inhibition against *Rhizoctonia solani*. Among the ten strains, *Pseudomonas fluorescens* PF - 8(38 mm in dm) showed maximum zone of inhibition followed by PF – 2 (35 mm in dm). Least zone of inhibition was observed in PF – 4 (28 mm in dm).

5. CONCLUSION

From this present study, it was concluded that the Plant growth promoting rhizobacterial isolates *Pseudomonas fluorescens* have the capacity to produce plant growth promoting substances. The isolate obtained from the location Kattumalaiyanur, Tiruvannamalai District was highly effective in the production of plant growth promoting substances. It also showed maximum zone of inhibition against *Rhizoctonia solani*. Further work on the enhancement of this strain's antagonistic activity and characterization of the mechanism of action is currently underway.

6. REFERENCES

1) Bloemberg, G. V and B. J. J. Lugtenberg.(2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology*, 4 (4): 343 - 350.
2) Borrow, A., P.W. Brain, U.E. Chester, P.J. Curtis, H.G.Hemming, E.C. Jeffereys, R.B. Lloyd, I.S., Nixon, G.L.F. Norris and N. Radley. 1955. Gibberellic acids a metabolic product of the fungus *Gibberella fujikuroi* some observations on its production and isolation. *Journal of Food Science and Agriculture*, 6: 340-348.
3) Hiltner. L (1904)., Uber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Beru¨cksichtigung der Gru¨ndu¨ngung und Brache. Arb DLG 98:59–78,
4) Kloepper, J.W. 1997. Plant growth promoting rhizobacteria (other system). In *Azospirillum Plant Associations.* Okon, Y. (Ed.), CRC Press, Boca Raton, pp. 137-166.
5) Lata, A., K. Saxena and K. V. Tilak.(2002). Biofertilizers to augment soil fertility and crop production. In Soil Fertility and Crop Production Science Publishers, USA. Edited by Krishna KR, 279 – 312.
6) Lindow, S.E. and M.T. Brandl. 2003. Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* **69:**1875–1883.
7) Mahmoud Reza Ramezanpour, Yuri Popov, Kazem Khavazi and Hadi Asadi Rahmani. (2011). Molecular genosystematic and physiological characters of fluorescent *Pseudomonas* isolated from rice rhizosphere of Iranian paddy fields. *African Journal of Agricultural Research*, 6(1): 145 - 151.
8) Mandira Kochar, Ashudhosh Upadhaya and Sheela Srivastava. (2011). Indole – 3 - acetic acid biosynthesis in the Biocontrol strain Psd and plant growth regulation by hormone over expression. *Research in Microbiology*, 28 (7): 111 - 117.
9) Mew, T. W and A. M Rosales. (1986). Bacterization of rice plants for control of

sheath blight caused by *Rhizoctonia solani*. *Phytopathology*, 76: 1260 - 1264.

10) Prasanna Reddy, B and R. S. Rao. (2009). Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens *in vitro*. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 8 (10): 1062 - 1067.
11) Rangarajan, S., P. Loganathan, L. M. Saleena and S. Nair.(2003). Diversity of *Pseudomonas* isolated from three different plant rhizospheres. *Journal of Applied Microbiology*, 91 (4): 742 –749.
12) Reeves, M., P.L. Neilands and A. Ballows. 1983. Absence of siderophore activity in *Leginella* sp. Grown in iron deficient media. *Journal of Bacteriology*, 154: 324-329.
13) Tilak, K.V., N. Ranganayaki, K.K. Pal, R. De, A.K. Saxena, C.S. Nautiyal, S. Mittal, A.K. Tripathi and B.N. Johri. 2005. Diversity of plant growth and soil health supporting bacteria. *Current Science*, 89 (1): 136-150.