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Occurrence and Role of Fluorescent Pseudomonads as PGPR on the Growth of Maize (*Zea mays L.*)

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Distribution and occurrence of fluorescent pseudomonads (FLPs) in the rhizosphere soil of maize grown in different agro-edaphic conditions were evaluated. Out of 250, 20 FLP isolates were found to be promising strains. These strains were further screened for plant growth promoting traits like production of IAA, GA, HCN, siderophores, phosphate solubilization. Based on these characteristics three isolates viz, MPF-1, MPF-7 and MPF-30 were selected for further evaluation. These strains have also shown resistance towards heavy metals, high salt concentration and p^H and have also exhibited significant antifungal activity and enhanced the seed germination and efficient root colonization. Further, artificial inoculations have also clearly shown to enhance the growth in terms of height, dry weight of shoot and root. Hence, it is concluded that these strains could be used as bioinoculants for maize crop.

Key words: Fluorescent pseudomonads, rhizosphere, maize, plant growth promotion.

In recent years, a considerable attention has been paid to fluorescent pseudomonads as plant growth promoting rhizo bacteria (PGPR) due to their effective seed bacterization, aggressive root colonization[1] and their ability to control soil borne pathogens. They are equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion through production of a variety of antibiotics, chitinilytic enzymes, siderophores, HCN and growth promoting hormones like IAA, GA. Inorganic phosphate solubilization by fluorescent pseudomonads was also reported[2,3]. Recently, Ali *et al.* [4] reported that fluorescent pseudomonad also confer thermotolerance to the crop plants. The present investigations were aimed at to assess the plant growth promoting abilities of FLPs isolated from rhizosphere of maize, their role in enhanced plant growth and productivity of maize and further to project the possibility of using FLPs as bioinoculants for maize crop.

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MATERIALS AND METHODS

Samples collection

Five locations (Mulugu, Hasanparthy, ARC, KU Campus, Paluvalpula) cultivating maize and representing most of the agro-edaphic conditions of Warangal districts of A.P. were selected. Plants in the age group of 30 to 90 days, grown in these soils were selected for collecting rhizosphere and non rhizosphere soil samples. Samples of soil from the field were carried to laboratory in an ice-box and stored in a refrigerator and isolation and enumeration of FLPs and non-FLPs were made within 12 hrs of sample collection.

Isolation of fluorescent pseudomonads

Soil serial dilution technique followed by

spread plate technique was adopted. Dilutions ranging from 10^{-2} to 10^{-6} were standardized and used for isolations. Number of viable colonies were counted and recorded as number per gram of sample taken. King's B medium was employed for fluorescein detection of FLPs. The colonies without fluorescein were treated as non-FLPs.

Studies on PGPR traits

Indole acetic acid (IAA) production

IAA production was tested by Salkowski colorimetric technique.[5]

Gibberellic acid (GA) production

GA production was determined by Cho *et al*.[6]

Siderophore production

Deferrated glassware and iron deficient MM9 medium was used in these experiments. Production of siderophores was tested and confirmed by $FeCl_3$ test[7] and the assay was performed by spectrophotometry [8].

Phosphate solubilization

Molybdophosphoric acid blue method [9] was adopted for the estimation of phosphate solubilization.

Hydrogen cyanide (HCN) production

Production of HCN by an isolate was identified by the method [10].

Ammonia production

Bacterial isolates were grown in test tubes containing peptone water (10.0 g peptone, 5.0 g NaCl, 1 l DW, pH 7.0) and the production of ammonia was estimated [11].

β-1, 3-glucanase assay

b-1, 3-glucanase activity was determined as per the method outlined by Lim *et al.* [12] and the enzyme activity was expressed as 1 nmol of glucose released per minute per mg of protein.

Protease production

Production of protease was tested by spot inoculation method as suggested by Maurhofer *et al.* [13]

Chitinase production

Chitinase production by the FLPs was determined by the method suggested by Lim *et al*.[12]. Tolerance of isolates against environmental stress conditions

Heavy metals

Tolerance of selected isolates of FLPs to different heavy metals was tested by agar dilution method[14].

Salt tolerance

Salt tolerance was determined by inoculating the test isolates into tubes containing their respective media added with sodium chloride solution giving final concentrations of 2, 4, 6 and 8%.

pH tolerance

The pH of the media was ranging from 4.5 - 9.5 adjusted using 1N NaOH.

***In vitro* antifungal interaction between bacterial and fungal strains**

Antagonism on agar plates was studied by modified method [15].

Seed germination assay

Selected isolates were grown in nutrient broth and a population approximately 1.3 – 2.6 x 10^7 cell / ml was obtained. Seeds of maize were surface sterilized (70% ethanol, 1% $HgCl_2$) and immersed in liquid cultures of isolates separately for 1 hr. Then the seeds were dried in a laminar air-flow and placed on 0.8% water agar (10 seeds / plate). Plates were incubated at 28°C and seed germination and radicle length was recorded at 72 hrs. A simultaneous control was maintained with seeds without any rhizobacterial coating.

Root colonization

Colonization of roots by bacterial isolates was determined by the method described[16].

Formulation of inoculum

The carrier (soil: lignite: 1:1) based bacterial cultures were prepared as per the method described by Tilak *et al.*[17] The results are presented in tables 1-7.

RESULTS AND DISCUSSION

The results presented in table -1 reveal that, in general, the rhizosphere of maize cultivated under different agro-edaphic conditions harboured more FLPs than the non-rhizosphere soil. The ratios of FLPs and non FLPs in rhizosphere soils ranged from 0.05 to 0.25 that varied with both the type of soil and age of the plant. In case of non-rhizosphere soil, the ratio ranged between 0.02 to 0.09. Thus a wide variation exists in the distribution of pseudomonads in rhizosphere and non-rhizosphere soils. It is also evident from the table that the population of both types of pseudomonads varied with the type of soil. This differential distribution of FLPs can be explained

in terms of different edaphic factors and variation within the practice of cultivation of maize. The results also reveal that in rhizosphere soils of maize grown in all soils, the maximum population of both types of pseudomonads was supported at the age of 30 days of plant growth. It may be due to the vigorous growth and metabolism of plant at this age, during which many metabolites are likely to be released which serve as the nutrients for rhizospheric organisms. Subsequently, the populations decreased slowly and steadily. Interestingly, such type of tendency was not observed in case of non-rhizosphere soil. Several reports indicate that the pseudomonads are dominant in the rhizosphere of maize and their inoculation can increase growth and yield production[18,19]. Distribution of *Pseudomonas* sp. populations in the rhizosphere of maize root and growth stage was reported by Battista *et al.*[20]. Gloria and Leda[21] reported that the fluorescent pseudomonads associated with the rhizosphere of several crops.

A total of 250 isolates obtained with different colony morphology were screened for biochemical traits that are expected to be involved in plant growth promotion (PGP) and plant health promoting activities. Twenty isolates (8.0%) proved positive for at least two traits examined. Out of 20 isolates, ten isolates were able to produce detectable amounts of indole-3-acetic acid (Table-2). Significant amounts of gibberellic acid was also produced by eight isolates. Siderophore production in iron free medium was observed in 10 isolates. Production of HCN and ammonia was observed in four and eleven isolates respectively. Similarly, protease production in six isolates, β-1, 3 glucanase production in eight isolates and production of chitinases in five isolates was observed. Solubilization of inorganic phosphates (tri and di calcium) by fluorescent pseudomonads was observed in six isolates. Among all three isolates viz, MPf – 1, MPf – 7, MPf – 30 were found showing positive for more number of traits tested and are expected to be promising isolates. They have shown the relative efficacy ratios of 0.77. These strains were further evaluated for other attributes. Screening of rhizobacteria for their plant growth promoting activities was reported by Chaiharn *et al*.[22]. The ability of pseudomonads to produce auxin can significantly affect the plant growth[23]. As an arsenal of beneficial effects exerted, FLPs are known to produce IAA[24], GA[25] and other plant growth regulators. Some species of pseudomonads can also produce different levels of HCN that are

Table 1. Distribution of fluorescent pseudomonads in rhizosphere and non-rhizosphere soils of maize grown in different soils of Warangal

Place	Age of the crop	Rhizosphere			Non-rhizosphere		
		Flourescent (DAS)	Non-	Ratio flourescent	Flourescent	Non-	Ratio flourescent
Mulugu	30	3.4*	24.5	0.13	2.75	36	0.07
	60	2.9	28.55	0.1	2.1	36.55	0.05
	90	2.2	20.55	0.1	1.5	48.75	0.03
Hasanparthy	30	5.25	25.55	0.21	3.3	37.5	0.08
	60	3.05	20.5	0.14	2.3	38.55	0.05
	90	2.3	13.75	0.16	1.5	31.55	0.04
ARC	30	8.16	31.5	0.25	3.64	39.82	0.09
	60	7.25	46.17	0.15	2.64	40.12	0.06
	90	6.1	40.55	0.15	1.54	46.12	0.03
KU Campus	30	8.8	64.25	0.13	2.5	42.5	0.05
	60	4.66	43.5	0.1	1.25	25.55	0.04
	90	5.5	51.5	0.1	1.15	42.35	0.02
Paluvalpula	30	4.55	62.5	0.07	2.5	37.9	0.06
	60	3.5	54.76	0.06	3.7	69.61	0.05
	90	2.85	56.1	0.05	2.4	47.5	0.05

*Values are expressed in colony forming units (10^4 CFU/g)

Table 2. Production of growth promoting substances by fluorescent pseudomonads isolated from rhizotic zones of maize

S. No.	Isolate	IAA like compounds	Gibberellic acid	Siderophores	HCN	Ammonia	β 1,3-glucanase	Protease	Chitinase	P solubilization	Relative efficacy
1	MPf-1	+	+	+	-	+	-	+	+	+	0.77
2	MPf-2	-	+	+	-	+	-	-	-	-	0.33
3	MPf-3	+	-	-	-	-	-	-	-	-	0.11
4	MPf-4	-	-	-	+	-	-	-	-	-	0.11
5	MPf-5	+	-	-	-	+	-	-	-	-	0.22
6	MPf-6	+	-	-	-	-	-	+	+	+	0.44
7	MPf-7	+	+	+	+	+	-	+	-	+	0.77
8	MPf-8	-	-	+	-	+	-	-	-	-	0.22
9	MPf-9	-	-	+	-	-	+	-	+	-	0.22
10	MPf-10	-	+	-	-	+	+	-	-	-	0.33
11	MPf-11	-	-	+	-	+	-	-	-	-	0.22
12	MPf-12	-	+	-	-	-	+	-	-	-	0.22
13	MPf-13	+	-	+	-	+	+	-	+	-	0.55
14	MPf-14	+	-	-	+	+	+	+	-	-	0.55
15	MPf-15	-	+	-	-	+	+	-	-	-	0.33
16	MPf-16	+	-	+	-	-	-	+	-	+	0.44
17	MP-17	+	-	+	-	-	+	-	-	+	0.44
18	MPf-18	-	+	-	+	-	-	-	+	-	0.33
19	MPf-19	-	-	-	-	+	-	-	-	-	0.11
20	MPf-30	+	+	+	0	- -	+	+	-	+	0.77
	% of isolate	50	40	50	25	55	40	30	25	30	

+ = Positive, - = Negative

toxic to certain pathogenic fungi[26]. Under conditions of low iron regime, the pseudomonads isolates studied, produced yellow-green iron binding peptides, the siderophores[27]. Suresh *et al*.[28] reported that plant growth promoting activities of fluorescent pseudomonads associated with some

Table 3. Resistance of fluorescernt pseudomonads to different heavy metals and NaCl concentrations

S. No	Isolate	Heavy metals (100 ppm)						NaCl conc(%)				
		Control	Hg	Co	Zn	Cu	Mo	Control	2	4	6	8
1	MPf1	1.72	NG	NG	0.75	0.64	1.36	1.4	1.23	1.36	1.14	NG
2	MPf7	1.62	NG	NG	0.85	0.75	1.42	1.61	1.32	0.95	0.84	NG
3	MPf30	1.24	NG	NG	0.2	0.28	1.1	1.76	1.39	0.62	0.96	NG
4	Control	1.18	NG	NG	0.1	0.21	1	1.13	1.1	0.14	0.24	NG

Optical density measured at 680nm, NG = No Growth

Table 4. Antifungal activity of fluorescent pseudomonads against four pathogenic fungi

S. No	Isolate	*Fusarium* oxysporum	*Curvularia* lunata	*Colletotricum* falcatum	*Macrophomina* phaseolina
1	MPf1	0.5	1.4	1.7	1.5
2	MPf7	0.1	1.5	1.7	0.8
3	MPf30	0.6	1.2	1.9	0.5
4	Control	0	0.89	1.2	0.2
	LSD	0.042	0.041	0.011	0.001

Inhibition zone measured in cm
Values are mean of three replicates and significancant at $p<0.05$

Table 5. Influence of selected pseudomonad isolates on seed germination and root colonization of maize

S. No	Isolate	Seed germination bioassay				Root colonization (CFU x 10^4)					
		No. of seeds	No. of seeds	% of	Root length (cm)	Rhizosphere		Rhizoplane		Endorhizosphere	
						30	60	30	60	30	60
1	MPf1	40	30	90	3.2	13.1	13.6	2.9	0.8	1.2	0.6
2	MPf7	40	32	80	2.5	6.6	7.8	4.5	2.6	0.9	0.3
3	MPf30	40	35	87.5	3.5	9.6	12.8	5.2	4.6	0.5	0.2
4	Control	40	20.1	50.25	1.1	-	-	-	-	-	-

Table 6. Influence of inoculations of fluorescent pseudomonads on growth of maize under green house conditions (45 DAS)

S. No	Isolate	Plant height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)
1	MPf-1	10.1*	2.01	11.6	1.26
2	MPf-7	14.2	5.86	18.8	1.25
3	MPf-30	27.4	8.28	2.4	2.1
4	Control	7.13	2.86	4.8	1
	LSD 0.05	0.010	0.040	0.003	0.037

* Values are mean of three replicates and significant at $p<0.05$

crop plants. Similarly plant growth promoting activites of fluorescent pseudomonads isolated from the Iranian soil was reported by Abbas *et al.*[29].

Many abiotic factors such as pH, temperature, salinity, antibiotics, heavy metals, moisture content and pollutants interact with the organisms affecting their characters adversely[30,31]. A successful organism withstands these stress conditions and imparts the beneficial effects. In the present investigations, resistance of selected FLPs towards different pH, heavy metals and sodium chloride concentration was studied and the results are presented in the Fig. 1 and Table 3.The present isolates did not show any growth at pH 4.5 and 9.5. For all the three isolates, pH 7.0 appears to be ideal for growth. A critical perusal of the table-3 reveals that the resistance of FLPs under investigation varied towards different metals and sodium chloride. All the three isolates were completely inhibited in presence of mercury and cobalt. However, these isolates exhibited a significant resistance to molybdenum. With regard to salt concentration, the resistance power of the present FLP isolates decreased with the increase in concentration (upto 6%) of NaCl and all the three isolates were completely inhibited at 8% concentration. Djuric *et al.*[32] reported that the selection of indigenous fluorescent pseudomonad isolates from maize rhizospheric soil in Vojodina as possible PGPR.

FLPs have been widely tested for biocontrol activity against fungal pathogens because of their rapid growth rate and their ability to colonize rhizosphere to a large extent besides their ability to suppress the soil borne pathogens. They also produce highly potent broad-spectrum antifungal molecules against a variety of

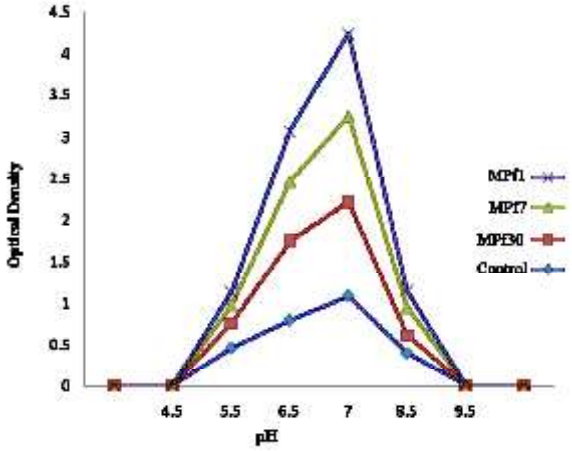

Fig. 1. Resistance of three isolates of fluorescent pseudomonads to different pH

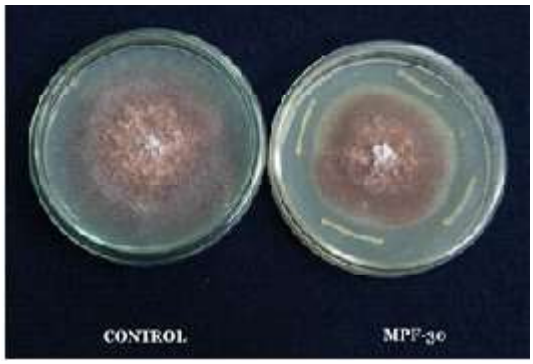

Fig. 2. Anti fungal activity shown by MPF-30 isolate against *Colletotricum falcatum*

Fig. 3. Seed germination assay with MPF-30 isolate in *Zea mays L*

phytopathogens, thus acting as effective biocontrol agents[33]. FLPs colonizing roots of a wide range of crop plants are reported to be antagonistic to soil borne plant pathogens[34]. In the present study, an attempt was made to evaluate the three strains of FLPs for antifungal activity against selected four phytopathogenic fungi viz *Fusarium oxysporum, Curvularia lunata, Colletotrichum falcatum, Macrophomina phaseolina* and the results are presented in table-4. It is evident from the results that these strains caused inhibition zones ranging from 0.1 to 1.9 cm (diameter) for different phytopathogenic fungi (Fig. 2). However, the antifungal activity varied both with the FLP strain as well as test fungi. MPF-30 showed more inhibitory activity against all the four pathogenic fungi. In case of fungi, the inhibitory effect of all the three isolates was more pronounced on *C. falcatum*. Pal *et al*.[35] reported that *P. fluorescens* and *Bacillus* sp. have reduced the root rot, collar rot and stem rot of maize caused by *Rhizoctonia solani* and *Fusarium wilt* of cotton caused by *F. oxysporum* sub sp. *vasinfectum.* Kaur *et al.*[36] reported that antagonistic activity of selected isolates of fluorescent *pseudomonas* against *Fusaium oxysporum* f sp. cicri. Supraja *et al.*[37] studied that Plant growth promotion and biocontrol properties of local isolates of fluorescent pseudomonads.

Inoculation with three isolates of FLPs enhanced the seed germination and root elongation over the control (Table 5, Fig. 3). The highest enhancement was observed with MPf-30 isolate. Significant numbers of FLPs in all the three zones of root indicate a successful colonization and perhaps with a significant physiological functions. Interestingly, the population of FLPs was more in rhizosphere followed by rhizoplane and least in endorhizosphere. The PGP ability of FLPs is a function of good root colonization and production of growth hormones[38]. In one study, seed treatment with rhizosphere pseudomonads resulted in 90% seed germination in contrast to 50% obtained in untreated control in pigeon pea. *In vitro* seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control[39]. Similar improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum[40] and pearl millet[41].

The beneficial response of different crops to inoculation with PGPR in general and *Pseudomonas* sp. under green house and field conditions have been extensively studied and reviewed by different workers from India as well as abroad[42,43,44,45,46,47]. In the present investigations, an effort was made to evaluate the influence of inoculations of FLPs on the growth parameters of maize and the results revealed that there is a pronounced enhancement in all the four parameter of maize plants inoculated with FLPs. However, the effect varied with FLPs isolate and also with the parameter. In general, enhancement effect is more on plant height and shoot dry weight than the root parameters (Table 6).

It can be concluded from the present investigations that FLPs owing to their growth promoting traits contribute at least partially, towards enhanced growth and yield of maize cultivars.

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REFERENCES

1. Johri, J.K., Nautiyal, C.S., Singh, H.B Survival of rhizosphere competent *Pseudomonas fluorescens* biocontrol strain NBRI 2650 in the soil and phytosphere. *Can. J. Microbiol*., 2002; **48:** 588.
2. Arti, D., Patel, H.H Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens. Ind. J. Microbiol*., **2003**; 43: 33-36.
3. Srivastava, S., Yadav, K.S., Kundu, B.S Prospects of using phosphate solubilizing *Psuedomonas* as biofungicide. *Ind. J. Microbiol.,* 2004; **44(2)**: 91-94.
4. Ali S K Z, Sandhya V, Grover M, Kishore N, Rao L.V.,Venkateswarlu, B *Pseudomonas* sp. Strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. *Biol. Fert. Soil.*2009; **46**: 45 – 55.
5. Glickman, E., Dessaux, Y A critical examination of the specificity of the salkowski reagent for

indolic compounds produced by phytopathogenic bacteria. *Applied & Environmental Microbiol.,* 1995; **61**: 793-796.

6. Cho, K.Y., Sakurai, A., Kamiya, Y., Takahashi, N., Tamuro, S Effects of the new plant growth retardants of quartenary Azmmonium iodides on gibberellin biosynthesis in *Gibberella fujikuroi*. *Plant Cell Physiol*., 1979; **20**: 25-81.
7. Jalal, M.A.F., Vander H.D Isolation and spectroscopic identification of fungal siderophores. In : Handbook of Microbial Iron Chelates (Winkelmann G. ed.), CRC Press, Boca Raton., (1991); **p**. 235.
8. Meyer, J.M., Aballah, M.A The fluorescent pigment of *Pseudomonas fluorescens* : biosynthesis, purification and physicochemical properties. *J. Gen. Microbiol.,* 1978; **107**: 319-328.
9. Koening, H.A Johnson, C.R Method for estimating phosphorus. *Indust. Eng. Chem*. 1942; **14**: 155. (Anal Ed),
10. Castric, P Glycine metabolism of *Pseudomonas aeruginosa*: Hydrogen cyanide biosynthesis. *J. Bacteriol*., 1977; **130**: 826-831.
11. Dye, D.W The inadequacy of the usual determination tests for the identification of Xanthomonas spp. *Nzt.Sci.,* 1962; **5**: 393-416.
12. Lim, H.S., Kim, Y.S., Kim, S.D *Pseudomonas stutzeri* YPL-1genetic transformation and antifungal mechanism against *Fusarium solani* an agent of plant root rot. *Appl. Environ. Microbiol.,* 1991; **57**: 510 – 516**.**
13. Maurhofer, M., Keel, C., Haas, D., Defago, G Influence of plant species on disease suppression by *Psuedomonas fluorescens* strains CHAO with enhanced production. *Plant Pathol*., 1995;**44**: 40-50.
14. Ahmed, S., Yadava, J.N. S Infectious mecury resistance and its co-transfer with R-plasmid among E.coli strains. *Ind. J. Exp. Biol.,* 1998; **26**: 601-605.
15. Fokkema, N.J The role of saprophytic fungi in antagonism against Dreschlera sorokianiana (*Helminthosporium sativum*) on agar plates and rye leaves with pollen. *Physiological Plant Pathol.,* 1973; **3**: 195 – 205.
16. Misaghi, I.J., Donndelinger, C.R Endophytic bacteria in symptom-free cotton plants. *Phytopathol.* 1990; **9**: 808 – 811.
17. Tilak, K.V.B.R., Pal, K.K., Dye, R Microbes for Sustainable Agriculture. 2010; pp: 200 I.K International publishing House Pvt. Ltd, New Delhi.
18. Kloepper, J.W., Lifshitz, R., Zablotovicz, R.M Free-living bacterial inoculation for enhancing crop productivity. *Trends in Biotechnol.,* 1989; **7**: 39-44.
19. Raimam, M.P., Albino, U., Cruz, M.F., Lovato, G.M., Spago, F., Ferracin, T.P., Lima D.S., Goulart, T., Bernardi, C.M., Miyauchi, M., Nogueira, M.A., Andrade, G Interaction among free-living N-fixing bacteria isolated from *Drosera villosa* var. *villosa* and AM fungi in rice. *Applied Soil Ecol.,* 2007; **35**: 25 – 34**.**
20. Battista, G.D., Benziri, E., Corbl, G., Piutti, S., Guckert, A Distribution of Pseudomonads sp. Populations in relation to maize root location and growth stage. *J. Agonomy*., 2003; **6**: 441-446.
21. Gloria, R.B., Leda, C.M.H Fluorescent pseudomonads associated with the rhizosphere of crops-an overview. *Brazilian J. Microbiol.,* 2006; **37**: 401 – 416.
22. Chaiharn, M., Chunhaleuchanon, S., Kozo, A., Lumyong, S Screening of rhizobacteria for their plant growth promoting activities. *King Mongkut's Institute of Technology Ladkrabang Sci. and Technol. J.,* 2008; **8**: p 1**.**
23. Khakipour, N., Khavazi, K., Mojallali, H., Pazira, E., Asadirahmani, H Production of auxin hormone by fluorescent pseudomonads. *American-Eurasian J.Agri. & Environ.Sci.,* 2008; **4** : 687–692.
24. Ahmad, F., Ahmad, I., Khan, M.S Indole acetic acid production by the indigenous isolates of *Azotobacter* and Fluorescent pseudomonads in the presence and absence of tryptophan. *Turkish J. Biolo.,* 2005; **29**: 29-34.
25. Basiacik, S.K., Nilufer, A Optimization of carbon-nitrogen ratio for production of gibberillic acid by *Pseudomonas* sp. *Polish J. Microbiol.,* 2004; **53(2)**: 117-120.
26. David, N.D., Gara, F.O Metabolites of *Pseudomonas* involved in the biocontrol of plant diseases. *Trends in Biotechnol.,* 1994; **12**: 133 – 141.
27. Rached, Ahmed Effect of iron and growth inhibitors on siderophores production by *Pseudomonads fluorescens*. *African J. Biol,.* 2005; **4(7)**: 697-702**.**
28. Suresh, A., Pallavi, P., Srinivas, P., Praveen Kumar, V., Jeevan Chandra, S., Ram Reddy S Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. *African J. Microbiol.,* 2010; **4(14)**: 1491-1494.
29. Abbas, Z.P., Rastin, S.N., Rahmani, A.H., Khavazi, K., Soltani, A., Shoary, N.A.R., Mohammad, M Plant growth promoting activites of fluorescent pseudomonads isolated from the Iranian soil. *Acta Physiol., Plant*., 2009; 1738-009-0405-1.

30. Chaudary, A.M., McGrath, S.P., Knight, B.P., Jhonson, D.L., Jones, K.C Toxicity of organic compounds to indigenous population of *R. leguminosarum. Soil Biol. & Biochem.,* 1996; **25**: 301-309.
31. Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A Principles and applications of soil microbiology. 1998; Prentice Hall, Upper Saddle River, NJ. USA.
32. Djuric, S., Pavic, A., Jarak, M., Pavlovic, S., Starovic, M., Pivic, R., Josic, D Selection of indigenous fluorescent pseudomonad isolates from maize rhizospheric soil in Vojodina aas possible PGPR. *Romanian Biotechnolo. Lett.,* 2011; **16**: 6580-6590.
33. Srivastava, R., Shalini Antifungal activity of *Pseudomonas fluorescens* against different plant pathogenic fungi. *Electronic J. Env. Agric. & Food Chem.,* 2008; **7(4)**: 2789 – 2796.
34. Siddiqui, I.A., Shaukat S.S., Hussain Sheikh, I., Khan, A Role of cyanide production by *Pseudomoas fluorescens CHAO* in the suppression of root- knot nematode, *Meloidogyne javanica* in tomato. *World J. Microbiol. & Biotechnol.,* 2006; **22**: 641-650.
35. Pal, K.K, Tilak, K.V.B.R., Saxena, A.K., Dey, R., Singh, C.S Suppression of maize root disease caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol. Research.,* 2001; **156**: 209-223.
36. Kaur, R., Singh, R.S., Alabouvette, C Antagonistic activity of selected isolates of fluorescent Pseudomonas against *Fusarium oxysporum* f.sp. *ciceri*. *Asian J. Plant Sciences.,* 2007; **6**: 446-454.
37. Supraja, Y., Subhash Reddy, R., Sokka Reddy, S., Durga Rani, V Plant growth promotion and biocontrol properties of local isolates of fluorescent pseudomonads. *Res J. ANGRAU.,* 2011; **39**(3):1-5**.**
38. Sullivan, O., Gara, O. F Traits of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. *Microbial. Rev.* 1992; **56**: 662-676.
39. Gholami, A., Shahsavani, S., Nezaral, S The effect of Plant Growth Pramoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. *World Academy of Science. Engineerning & Technol.,* 2009; **49**: 1-24.
40. Raju, N.S., Niranjana, S.R., Janardhana, G.R., Prakash, H.S., Shetty, H.S., Mathur, S. B Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents. *J. Sci. Food & Agric.,* 1999; **79**: 206-212.
41. Niranjan, S.R., Deepak, S.A., Basavaraju, P., Shetty, H.S, Reddy, M.S Kloeppr, J.W Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in peral millet. *Crop Protection*. 2003 ; **72**: 579-588.
42. Pandey, A., Sharma, E., Plni, L.K.S Influence of bacterial inoculation on maize in upland farming systems of Sikkim Himalaya. *Soil. Biol. Biochem.,* 1998; **30(3)**: 379-384.
43. Shaharoona, A.A., Muhammad, A.B., Azeem, K.A Performance of *Pseudomonas spp*. containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of nitrogenous fertilizer. *Soil Biol. & Biochem*., 2006; **38**: 2971-2975.
44. Angaw, T., Saxena, A.K Tilak, K.V.B.R Response of mustard, sunflower and teff to inoculation with plant growth promoting rhizobacteria. *J. Indian Bot. Soci.,* 2011; **90 (1&2):** 103-108.
45. Tilak, K.V.B.R., Manoharachary, C Diversity and bioprospecting of plant growth promoting soil supporting microorganisms (PGPSM)-Aspects and prospects. In:Advances in Botany Indian Botanical Society Commemoration Volume (eds. Y Vimala PC Trivedi and CM Govil). 2012; pp: 1-16. Pointer Publishers, Jodhpur, India.
46. Sangeetha, D., Stella, D Survival of plant growth promoting bacterial inoculants in different carrier materials. *Inter. J. Pharma & Biological Archives*., 2012; **3(1)**: 170-178.
47. Mirjana, J., Nastasija, M., Dragana, B., Dragana, J., Timea, H.J., Dragana, S Effects of plant growth promoting rhizobacteria on maize in green house and field trail. *African J. Microbiol. Research.* 2012; **6**(27): 5683 – 5690.