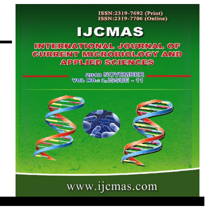

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Original Research Article

Plant growth promoting activity of Pseudomonads in Rice crop

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A B S T R A C T

Keywords

PGPR; *Pseudomonas;* IAA; Siderophore; HCN; P-solubilization; Rice plant.

Effect of Plant Growth Promoting (PGP) activity of *Pseudomonas aeruginosa*, *P. putida*, *P. cepacia* and *P. fluorescens* in rice crop was observed in this study. Selected strains *P. aeruginosa* PW-99, *P. aeruginosa* PW-136, *P. putida* PW-2, *P. putida* PW-56, *P. cepacia* PW-18, *P. cepacia* PW-43, *P. fluorescens* PW-5, *P. fluorescens* PW-104 were resistant against heavy metal such as Cu, Cr, Ni, Cd. These strains effectively showed four major PGPR activities such as Indole Acetic Acid (IAA), Hydrogen cyanide (HCN), Siderophore and Phosphorous solubilisation. In present study, nine treatments were prepared i.e. *P. aeruginosa* PW-99 + Rice, *P. aeruginosa* PW-136 + Rice, *P. putida* PW-2 + Rice, *P. putida* PW-56 + Rice, *P. cepacia* PW-18 + Rice, *P. cepacia* PW-43 + Rice, *P. fluorescens* PW-5 + Rice, *P. fluorescens* PW-104 + Rice and Control. Each seed coated with 10^8 cfu of *Pseudomonas* culture. Four seed in each pot were sown. Plant growth data such as shoot length, root length and dry weight of rice plant were analyzed after 45 days. Our results suggested that all *Pseudomonas* strains enhanced plant growth in rice plant. *P. fluorescens* PW-5 produced maximum shoot, root and dry weight by 157.72, 408.06 and 233.84% respectively as compared to control.

Introduction

Excess use of chemical fertilizer is undesirable because (i) production of chemical fertilizer is a costly process, (ii) most of the energy is provided by consumption of non-renewable fossil fuel, (iii) considerable pollution is caused through both the production and use of mineral N- fertilizer (Mai *et al.*, 2010; Deshwal *et al.*, 2011a). Therefore, now a days, scientist are searching new alternative of chemical fertilizer.

Biofertilizer promote plant growth and productivity has internationally been accepted as an alternative source of chemical fertilizer. Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which actively colonize in plant root and increase plant growth by production of various plant growth hormones, P-solubilizing activity, N_2 fixation and Biological activity (Deshwal *et al.*, 2003a; 2003b;

2010, 2011b). Similarly, Noori and Saud (2012) mentioned that *Pseudomonas fluorescens* bacteria, a major constituent of Rhizobacteria, encourage the plant growth through their diverse mechanisms.

Indole acetic acid is essential for growth of root and shoots development. Many microbes including PGPR produce IAA. Available literature suggested that *Pseudomonas* strains produced plant growth hormones. Deshwal *et al.* (2011a) observed that 75% *Pseudomonas* strains produced indole acetic acid (IAA) and increased plant growth in soybean. Bharucha *et al.* (2013) Isolated nine Indole-3-acetic acid (IAA) producing rhizobacteria from the rhizospheric soil of alfalfa (*Medicago sativa*). Deshwal *et al.* (2011c) observed that IAA producing *Pseudomonas aeruginosa* MR-9 enhanced maximum plant dry weight, plant height, nodule per plant, nodule fresh weight per *Mucuna pruriens* by 184, 124, 139, 180% respectively as compared to control.

Phosphorous (P) is major essential macronutrient for plant growth and development. Consequently to achieve optimum crop yields, soluble phosphate fertilizers have to be applied at high rates (Brady, 1990). Such soluble inorganic fertilizer in soil is immobilized rapidly and become unavailable to plants (Goldstein, 1986). Use of chemical fertilizer causes soil erosion and lower crop yield (Kumar and Kumar, 2000). The ability of a few soil microorganisms to convert insoluble form of phosphorus to an accessible form is an important trait in plant growth promoting bacteria for increasing plant yields (Chen *et al.*, 2006; Deshwal *et al.*, 2011d). Peix *et al.* (2004) isolated a novel phosphate-solubilizing *Pseudomonas lutea* sp. nov. isolated from the rhizosphere of grasses.

Plant growth promoting rhizobacteria produce hydrogen cyanide (HCN) which inhibits the growth of microorganisms. HCN producing *Pseudomonas* strains effectively reduced or kill the plant pathogenic microbes. Deshwal *et al.* (2013) reported about HCN producing *Pseudomonas*. These HCN producing rhizobacteria significantly reduced plant pathogen. Only DAPG and HCN *Pseudomonas* sp. LBUM300 was capable of significantly reducing cancer of tomato disease development and *C. michiganensis* subsp. *michiganensis* rhizospheric population (Lanteigne *et al.*, 2012). HCN and Siderophore producing *Pseudomonas* inhibited the growth of the growth of plant pathogenic fungus *Sclerotina sclerotiorum* (Deshwal, 2012).

Iron is essential metal for microorganism for reduction of oxygen for synthesis of ATP, reduction of ribotide precursors of DNA and for other essential purposes (Neilands,1995). Siderophores are low molecular weight, non-ribosomal peptides, secreted under low iron stress conditions and absorb iron from soil (Budzikkiewicz, 1993). Pseudomonas is a potentent bio-control agent and produces siderophore that sequester iron in the root environment, making it less available to competing deleterious microflora (Kloepper *et al.*, 1980; Bagnasco *et al.*, 1998; Deshwal *et al.*, 2012). Rice is a dietary staple for at least 62.8% of the inhabitants on the planet and accounts for 20% of the caloric intake for the world population (Giraud, 2013). Dehradun in well known for cultivation of Rice. Against these backdrops, this study was carried out to evaluate the effect of Plant growth promoting activity of previously characterized *Pseudomonas* strains on the growth of Rice plant.

Materials and Methods

Pseudomonas strains

Previously characterized strains namely *Pseudomonas aeruginosa* PW-99 and PW-136; *P. putida* PW-2 and PW-56; *P. cepacia* PW-18, PW-43 and *P. fluorescens* PW-5, PW-104 strains were selected for the present study. These strains produced IAA, HCN, Siderophore, P-solubilization (Deshwal *et al.*, 2013). These strains tolerate various concentration of heavy metal such as Cu, Cr, Ni and Cd (Deshwal and Kumar, 2013b).

Pot experiment

8 *Pseudomonas* strains were selected for pot experiment.

(i) Seed bacterization

Rice seeds were surface-sterilized with 0.5% NaOCl solution for 1-2 min, rinsed in sterilized distilled water and dried under a sterile air stream. Cells of *Pseudomonas* strains were grown under continuous shaking condition (150 rpm) on King'B broth at 28 ± 1°C for 24h. Each culture was separately centrifuged at 7000 rpm for 15 min at 4°C. The culture supernatant was discarded and the pellets were washed with sterile distilled water (SDW) and resuspended in SDW to obtain a population density of 10^8 cfu ml^{-1}. The cell suspension was mixed with 1% carboxymethylcellulose (CMC) solution. The slurry was coated separately on the surface of rice seeds and allowed to air-dry overnight in aseptic condition. The seeds coated with 1% CMC slurry without bacterial strains served as control.

(ii) Pot size and soil

Sterile earthen pots (24 cm × 12 cm × 12 cm) were filled with sterilized sandy loam soil (0.25% total organic matter, 0.096% total organic C, 38% water-holding capacity, pH 6.8).

(iii)Treatments

Total 09 treatments were prepared and these were *P. aeruginosa* PW-99 + Rice, *P. aeruginosa* PW-136 + Rice, *P. putida* PW-2 + Rice, *P. putida* PW-56 + Rice, *P. cepacia* PW-18 + Rice, *P. cepacia* PW-43 + Rice, *P. fluorescens* PW-5 + Rice, *P. fluorescens* PW-104 + Rice and uninoculated seed (control). Four bacterized seeds per pot were sown except control (non-bacterized seeds). After 15 days, thinning was done to raise only single healthy plant in each pot. The plants were irrigated with sterilized water whenever required. Plant data such as plant dry weight, plant height per plant were recoded after 45 days of sowing. The data were statistically analyzed by standard deviation, percentile and analysis of variance (ANOVA).

Results and Discussion

All *Pseudomonas* strains enhanced plant growth in rice plant. *P. fluorescens* PW-5 produced maximum shoot, root and dry weight of rice plant by 157.72, 408.06 and 233.84% respectively as compared to control. All *Pseudomonas* strains increased shoot, root and dry weight of rice plant by 52.80 to 157.72, 172.04 to 408.06 and 93.15 to 233.84% respectively as compared to control. *P. aeruginosa*

Table.1 Effect of *Pseudomonads* on growth of Rice plant after 60 days after sowing

S.No	Treatment	Seed germination (%)	Length (cm)		Dry weight (g)*
			Shoot*	Root*	
1	*P. aeruginosa* PW-99 + Rice	100	72.54±0.58	12.56±0.23	8.16±0.17
2	*P. aeruginosa* PW-136 + Rice	100	71.62±0.39	12.32±0.25	7.88±0.06
3	*P. putida* PW-2 + Rice	100	43.52±0.37	10.12±0.14	5.08±0.11
4	*P. putida* PW-56 + Rice	100	47.76±0.49	9.88±0.54	5.27±0.11
5	*P. cepacia* PW-18 + Rice	100	43.18±0.65	16.38±0.20	5.54±0.32
6	*P. cepacia* PW-43 + Rice	100	49.56±0.47	16.94±0.15	6.26±0.22
7	*P. fluorescens* PW-5 + Rice	100	73.4±0.81	18.9±0.25	8.78±0.07
8	*P. fluorescens* PW-104 + Rice	100	71.14±0.34	18.86±0.20	8.5±0.37
9	Control	90	28.48±0.20	3.72±0.13	2.63±0.14

Values are mean of five replicate ±SD; * significant at 1% level

Table.2 Statistical date of effect of *Pseudomonads* on growth of Rice plant after 60 days after sowing.

S. No	Treatment	Percentile of Shoot length		Percentile of Root length		Percentile of Plant dry weight	
		5	95	5	95	5	95
1	*P. aeruginosa* PW-99 + Rice	72.04	73.32	12.26	12.78	7.94	8.32
2	*P. aeruginosa* PW-136 + Rice	71.10	71.98	12.04	12.64	7.82	7.96
3	*P. putida* PW-2 + Rice	43.02	43.80	09.94	10.28	4.94	5.21
4	*P. putida* PW-56 + Rice	47.16	48.20	09.28	10.52	5.16	5.42
5	*P. cepacia* PW-18 + Rice	42.72	44.08	16.20	16.64	5.30	5.98
6	*P. cepacia* PW-43 + Rice	48.98	50.06	16.80	17.10	6.06	6.54
7	*P. fluorescens* PW-5 + Rice	72.80	74.46	18.62	19.18	8.72	8.88
8	*P. fluorescens* PW-104 + Rice	70.82	71.56	18.62	19.08	8.09	8.89
9	Control	28.30	28.70	03.60	03.88	2.47	2.80

PW-99 increased shoot, root and dry weight of rice plant by 154.70, 237.63 and 210.26 % as compared to control. *P. aeruginosa* PW-136 enhanced shoot, root and dry weight of rice plant by 151.47, 231.18, and 199.61 % as compared to control. *P. putida* PW-2 improved shoot, root and dry weight of rice plant by 52.80, 172.04, and 93.15 % as compared to control. *P. putida* PW-56 enhanced shoot, root and dry weight of rice plant by 67.69, 165.59, and 100.38 % as compared to control. *P. cepacia* PW-18 enhanced shoot, root and dry weight of rice plant by 51.61, 340.32, and 110.64 % as compared to control. *P. cepacia* PW-43 increased shoot, root and dry weight of plant by 74.01, 355.37, and 138.02 % as compared to control. *P. fluorescens* PW-104 enhanced shoot, root and dry weight of plant by 149.78, 406.98, and 223.19 % as compared to control. Biostatical analysis of data showed that each treatment increase the plant root, shoot and dry weight of plant at 1% level of ANOVA (Table-1). Further, another statistical analysis in term of percentile was also analysed (Table- 2). *P. fluorescens* PW-5 increased maximum shoot, root and dry weight of rice plant as compared to control. Further *P. aeruginosa* PW-99, *P. aeruginosa* PW-136, *P. putida* PW-2, *P. putida* PW-56, *P. cepacia* PW-18, *P. cepacia* PW-43, *P. fluorescens* PW-104 were also enhanced plant growth parameter. Previously, Fenton *et al.* (1992) also reported *Pseudomonas fluorescence* strain F113 produced antibiotics and increased plant growth. Cattelan *et al.* (1999) concluded that *Pseudomonas* GN 1201 increased significantly the dry shoot weight, root length and root dry weight in soybean crop as compared to control. Similarly, Mishra *et al.* (2010) reported that Plant growth promoting *Pseudomonas* strains increased 27.6% productivity in *Pelargonium graveolens* L. *herit*. *Pseudomonas* MR-18 increased dry weight, height of *Mucuna pruriens* by 84 and 24% respectively (Deshwal *et al.*, 2011a). All above literature supported that plant growth promoting *Pseudomonas* increased plant growth.

Present study revealed that IAA, Siderophore, HCN and P-solubilizing bacteria *P. fluorescens* PW-5 increased maximum shoot, root and dry weight in rice plant. Other *Pseudomonas* strains were also improved plant growth in Rice. All these results showed that PGP *Pseudomonas* strains increased plant growth in rice plant

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