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RESEARCH ARTICLE

Production of Plant growth promoting substance by Pseudomonads

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Abstract

In our study, 55 *Pseudomonas aeruginosa*, 22 *P. putida*, 26 *P. cepacia* and 37 *P. fluorescens* strains were screened for their plant growth promoting activity i.e. indole acetic acid (IAA), hydrogen cyanide (HCN), siderophore production and P-solubilization. Plant growth promoting activity was carried by standard methods. Most *P. aeruginosa* strains showed positive PGPR activity as compared to other species of *Pseudomonas*. *Pseudomonas aeruginosa* showed positive PGPR activity i.e. 44 (IAA), 34 (HCN), 35 (siderophore production), 43 (P-solubilization). Similarly *P. putida* confirmed positive PGPR activity i.e. 14 (IAA), 12 (HCN), 14 (Siderophore production), 14 (P-solubilization). *Pseudomonas cepacia* also produced positive results i.e. 19 (IAA), 16 (HCN), 15 (siderophore production), 15 (P-solubilization) and *P. fluorescens* showed 22 (IAA), 19 (HCN), 21 (siderophore production), 23 (P-solubilization) positive PGPR activity. The study showed that *Pseudomonas* as an effective plant growth promoting bacterium.

Keywords: *Pseudomonas*, plant growth promoting activity, indole acetic acid, hydrogen cyanide, siderophore.

Introduction

When natural fossils fuels finished up, ultimately the present practices of industrial production of fertilizer will suffer. Further inorganic chemical fertilizer is immobilized rapidly and become unavailable to plants (Goldstein, 1986). Further use of chemical fertilizer causes soil erosion and lower crop yield (Kumar and Kumar, 2000). Chemical farming disturbs environment, subvert ecology, degrade soil productivity, mismanage water resources (Ayala and Rao, 2002; Deshwal *et al.*, 2011a). All above facts force to search alternative of chemical fertilizer. Microorganisms have capability to improve plant growth promoting activity. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize 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.*, 2003, 2010, 2011a). Few strains from genera such as *Pseudomonas*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are well known PGPRs (Rodriguez and Fraga, 1999; Misko and Germida, 2002).

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*. Recently Pandey *et al.* (2013) reported that *Pseudomonas* strains were plant growth promoting Endorhizospheric bacteria inhabiting sunflower (*Helianthus annus*).

Competition for iron is another mechanism by which fluorescent pseudomonads may inhibit the growth of pathogens. Siderophores of PGPR in the rhizosphere under iron deficient environment could efficiently chelate environment iron and inhibit the growth of native microflora including root pathogen (Lim and Kim, 1990). The fluorescent pseudomonads are characterized by their production of yellow green pigments, termed pyoverdines or pseudobactins, that fluorescence under UV irradiation and function as siderophore (Abdallah, 1991). Pseudomonads produce HCN which control the growth of root-rot pathogens. Thomshow and Weller (1995) observed same observation that pseudomonads exert beneficial effect on plants by the production of diverse microbial metabolites like HCN. Deshwal *et al.* (2011a, b) mentioned that *Pseudomonas* strains isolated from Mucuna produced HCN. Gupta *et al.* (2002) isolated the IAA producing fluorescent pseudomonads in the potato rhizosphere. Glick *et al.* (1999) reported that IAA producing rhizobacteria enhanced the root length which is one of the plant growth promoting activity rhizobacteria. Rhizobacteria also produce Gibberellic acid (Mahmoud *et al.*, 1984), cytokinins (Tien *et al.*, 1979) and ethylene (Glick *et al.*, 1995). Deshwal *et al.* (2011c) reported that *Pseudomonas* strains improve plant growth in soybean crop. Unsolubilized phosphate is not taken up by plant but some rhizobacteria solubilize phosphate that is readily taken up by plant. Whitelaw *et al.* (1997) reported that some P-solubilizing organism have been reported as plant growth promoters. Chabot *et al.* (1996) observed that P-solubilizing rhizobacteria increased plant growth, productivity in maize, lettuce.

Pseudomonad is also P-solubilizer (Gupta *et al.*, 2002). Deshwal *et al.* (2011c) observed that *Pseudomonas* strains solubilized phosphorous. Again, Deshwal *et al.* (2011d) reported phosphorus solubilizing *Pseudomonas aeruginosa* PMV-14 enhanced productivity in rice crop. Dehradun is well known for cultivation of rice crop and literature suggests that few investigation on *Pseudomonas* as PGPR has been conducted at Dehardun. Therefore, this study was carried out to screen the PGPR activity of previously characterized *Pseudomonas* strains.

Materials and methods

Pseudomonas strains: Previously characterized strains namely 55 *Pseudomonas aeruginosa*, 22 *P. putida*, 26 *P. cepacia* and 37 *P. fluorescens* strains were selected for present study (Deshwal *et al.*, 2013).

Screening of plant growth promoting activity of Pseudomonas strains: These strains were screened on the basis of plant growth promoting activity such as IAA, HCN, siderophore production and P-solubilization.

(i) Indole production test: Tryptone broth was prepared and transferred into test tubes. After sterilization, these test tubes were then inoculated with the culture and one tube was kept uninoculated as control. These inoculated tubes incubated at 28°C for 24 h. After 24 h of incubation, 1 mL of kovac's reagent was added to each tube including control. Shaked the tubes gently after intervals for 10-15 min and allowed tubes in standing position. Development of cherry red color in the top layer of the tube indicated a positive result.

(ii) HCN production: Pseudomonas strains were streaked on TSM medium plates supplemented with 4.4 g per litre glycine with simultaneously supplemented filter paper soaked in a 0.5% picric acid in 1% Na_2CO_3 in the upper lid of petri plate. The plates were sealed with paraffin and control plates did not receive any *Pseudomonas* inoculum. Plates were incubated at 28°C for 1-2 d. Change in color of the filter paper from yellow to brown.

(iii) Siderophore production: Pseudomonas strains were spread over tryptic soya agar medium and incubated at 28±1°C for 24 h. Thereafter, a thin layer of CAS reagent in 0.7% agar was spread over the colonies of *Pseudomonas and* plates were re-incubated at 28±1°C for 24-48 h. Observe formation of yellow-orange halo around the colony showing siderophore production.

(iv) P-solubilization test: Characterized *Pseudomonas* strains were transferred on Pikovskya's agar medium and inoculated at 28±1°C for 3-5 d and clear zone around the colony showed P-solubilization.

Results and discussion

Previously characterized *Pseudomonad* strains namely 55 *Pseudomonas aeruginosa*, 22 *P. putida*, 26 *P. cepacia* and 37 *P. fluorescens* strains were screened for their plant growth promoting activity such as IAA, HCN, siderophore production and P-solubilization. When added 1 mL of kovac's reagent in log phase, *Pseudomonas* culture in tryptone broth and development of cherry red color in the top layer of the tube confirmed that *Pseudomonas* strains produced indole acetic acid. After incubation at 28°C for 1-2 d, there was change in color of the filter paper from yellow (0.5% picric acid in 1% Na_2CO_3) to brown showed that *Pseudomonas* strains produce HCN. Formation of yellow-orange halo around the colony on thin layer of CAS reagent-medium showed siderophore production. Clear zone around the colony on Pikovskya's agar medium showed P-solubilization (Data not shown). *Pseudomonas aeruginosa* strains showed 80% IAA, 61.81% HCN, 63.63% siderophore production and 78.18% P-solubilization. Only *P. aeruginosa* strains PW-9, PW-12, PW-21, PW-29, PW-33, PW-36, PW-52, PW-57, PW-59, PW-63, PW-64, PW-77, PW-79, PW-81, PW-90, PW-98, PW-99, PW-100, PW-123, PW-125, PW-135 and PW-136 confirmed IAA, HCN, siderophore production, P-solubilization test but PW-14, PW-96 failed to show any PGPR activity. Other strains showed more than one but less than three PGPR activities (Table 1). Further, *P. putida* strains showed 63.63% IAA, 54.54% HCN, 63.63% siderophore production and 63.63% P-solubilization. Only *P. putida* strains PW-2, PW-41, PW-56, PW-82 and PW-101 confirmed IAA, HCN, siderophore production, P-solubilization test. Other strains also showed PGPR activity (Table 2).

Pseudomonas cepacia strains showed 73.07% IAA, 61.53% HCN, 57.69% siderophore production and 57.69% P-solubilization. PW-18, PW-34, PW-43, PW-60, PW-112 and PW-121 strains confirmed all PGPR activity. But only three strains PW-23, PW-40, PW-44 failed to show any PGPR activity. Other strains showed few PGPR activities (Table 3). *Pseudomonas fluorescens* strains showed 59.45% IAA, 51.35% HCN, 56.75% siderophore production and 63.16% P-solubilization. *Pseudomonas fluorescens* strains PW-5, PW-7, PW-25, PW-37, PW-46, PW-67, PW-94, PW-104, PW-126 showed PGPR activity but PW-28, PW-87, PW-114 failed to show PGPR activity. Other strains showed few PGPR activities (Table 4). Our results suggest that *Pseudomonas* strains produce IAA. IAA is one of the most physiologically active auxins and these IAA controls cell enlargement and division, tissue differentiation (Teale *et al.*, 2006). IAA is a common product of L-tryptophan metabolism by various PGPR strains. Similar observations have been showed by Ahmad *et al.* (2005) and Sasirekha *et al.* (2012). We observed that major *Pseudomonas* strains effectively produced HCN.

Table 1. PGPR activity of *Pseudomonas aeruginosa* strains.

Strains	PGPR activity			
	IAA	HCN	Siderophore production	P-solubilization
PW-1	+	-	+	-
PW-4	+	+	-	+
PW-6	+	-	+	+
PW-9	+	+	+	+
PW-12	+	+	+	+
PW-13	+	-	-	-
PW-14	-	-	-	-
PW-20	-	+	-	-
PW-21	+	+	+	+
PW-24	+	-	+	-
PW-27	-	+	-	+
PW-29	+	+	+	+
PW-31	-	+	+	+
PW-32	+	-	-	+
PW-33	+	+	+	+
PW-36	+	+	+	+
PW-42	-	-	+	+
PW-47	+	-	-	+
PW-48	+	+	-	-
PW-52	+	+	+	+
PW-54	-	-	+	+
PW-57	+	+	+	+
PW-58	+	-	-	-
PW-59	+	+	+	+
PW-63	+	+	+	+
PW-64	+	+	+	+
PW-70	+	-	-	+
PW-72	+	-	-	+
PW-74	+	+	-	+
PW-77	+	+	+	+
PW-79	+	+	+	+
PW-81	+	+	+	+
PW-84	-	+	+	-
PW-85	+	-	+	+
PW-86	+	+	-	+
PW-90	+	+	+	+
PW-91	+	+	-	+
PW-95	+	-	-	+
PW-96	-	-	-	-
PW-97	+	-	-	-
PW-98	+	+	+	+
PW-99	+	+	+	+
PW-100	+	+	+	+
PW-105	+	-	+	+
PW-108	+	-	-	+
PW-111	-	+	+	-
PW-115	-	+	-	+
PW-118	+	-	+	+
PW-123	+	+	+	+
PW-125	+	+	+	+
PW-128	+	-	-	+
PW-131	-	+	+	+
PW-135	+	+	+	+
PW-136	+	+	+	+
PW-140	+	-	+	-
Total positive	44	34	35	43
Percentage positive	80.00	61.81	63.63	78.18

Table 2. PGPR activity of *Pseudomonas putida* strains.

Strains	PGPR activity			
	IAA	HCN	Siderophore production	P-solubilization
PW-2	+	+	+	+
PW-10	-	-	-	+
PW-11	-	+	+	-
PW-15	+	-	+	+
PW-16	+	-	+	-
PW-26	+	+	-	+
PW-41	+	+	+	+
PW-49	-	+	-	-
PW-50	+	-	-	+
PW-55	+	-	+	+
PW-56	+	+	+	+
PW-62	-	-	+	-
PW-82	+	+	+	+
PW-83	-	-	-	-
PW-93	-	+	+	+
PW-101	+	+	+	+
PW-109	-	+	-	-
PW-110	+	-	+	+
PW-120	+	+	+	-
PW-124	+	-	+	-
PW-134	-	+	-	+
PW-137	+	-	-	+
Total positive	14	12	14	14
Percentage positive	63.63	54.54	63.63	63.63

Other report suggests that HCN has antimicrobial activity and effectively control the growth of plant pathogenic fungus. Genus *Pseudomonas* is one of the leading bacteria which inhibit growth of pathogenic fungus in agriculture fields. Lanteigne *et al.* (2012) isolated HCN producing *Pseudomonas* and observed biological control activity of *Pseudomonas.* A similar observation has been reported by DeCoste *et al.* (2010) and Ramyasmruthi *et al.* (2012). Iron is an essential trace element for all living organisms. Siderophore is a low molecular weight (500-1000 da), high affinity ferric iron chelating compound secreted by organisms (Bholay *et al.*, 2012). Our *Pseudomonas* strains produced siderophore. The production of siderophore that sequester iron in the root environment, making it less available to competing deleterious microflora (Bagnasco *et al.*, 1998; Deshwal, 2012; Bholay *et al.*, 2012). Chlorosis is a condition in which leaves produce insufficient chlorophyll. Siderophore producing microorganisms significantly increase chlorophyll concentration in leaf. Jurkevitch *et al.* (1986) observed that siderophore producing *Pseudomonas* improves chlorophyll content and concluded that siderophores producing bacteria may have a potential role in controlling lime-induced iron deficiency in plants. Our *Pseudomonas* strains have capability to solubilize phosphorous. Phosphorus is an essential macronutrient often limiting the plant growth due to its low solubility and fixation in the soil.

Table 3. PGPR activity of *Pseudomonas cepacia* strains.

Strains	PGPR activity			
	IAA	HCN	Siderophore production	P-solubilization
PW-3	-	+	+	-
PW-8	+	+	-	+
PW-18	+	+	+	+
PW-23	-	-	-	-
PW-34	+	+	+	+
PW-38	+	-	-	+
PW-39	+	+	+	+
PW-40	-	-	-	-
PW-43	+	+	+	+
PW-44	-	-	-	-
PW-51	+	-	+	-
PW-60	+	+	+	+
PW-66	+	+	-	+
PW-69	-	-	+	-
PW-76	+	-	-	+
PW-80	+	+	+	+
PW-89	-	+	-	+
PW-103	+	-	+	+
PW-106	+	+	+	-
PW-112	+	+	+	+
PW-113	+	+	+	-
PW-119	+	-	-	-
PW-121	+	+	+	+
PW-122	-	+	-	-
PW-129	+	+	-	-
PW-133	+	-	+	+
Total positive	19	16	15	15
Percentage positive	73.07	61.53	57.69	57.69

Table 4. PGPR activity of *Pseudomonas fluorescens* strains.

Strains	PGPR activity			
	IAA	HCN	Siderophore production	P-solubilization
PW-5	+	+	+	+
PW-7	+	+	+	+
PW-17	+	+	+	-
PW-19	-	-	+	+
PW-22	-	+	-	+
PW-25	+	+	+	+
PW-28	-	-	-	-
PW-30	+	-	+	+
PW-35	+	-	+	-
PW-37	+	+	+	+
PW-45	-	-	-	-
PW-46	+	+	+	+
PW-53	-	-	-	+
PW-61	+	+	+	-
PW-65	-	+	-	+
PW-67	+	+	+	+
PW-68	-	+	-	+
PW-71	+	-	-	-
PW-73	-	-	+	+
PW-75	+	-	-	+
PW-78	+	+	-	-
PW-87	-	-	-	-
PW-88	-	-	+	+
PW-92	+	+	-	-
PW-94	+	+	+	+
PW-102	-	-	-	+
PW-104	+	+	+	+
PW-107	+	-	+	-
PW-114	-	-	-	-
PW-116	+	-	+	-
PW-117	-	+	+	+
PW-126	+	+	+	+
PW-127	-	+	-	+
PW-130	+	-	+	+
PW-132	+	-	-	-
PW-138	+	-	+	-
PW-139	-	+	-	+
Total positive	22	19	21	23
Percentage positive	59.45	51.35	56.75	63.16

Microorganism has the ability to solubilize insoluble phosphorous by production of organic acids or enzymes (Park *et al.*, 2009; Parani and Saha, 2012). Plant growth promoting *Pseudomonas* strains produced IAA, HCN and siderophore activity (Bhakthavatchalu *et al.*, 2013).

Conclusion

Pseudomonas aeruginosa, *P. putida*, *P. cepacia* and *P. fluorescens* strains significantly produced plant growth promoting substance. *Pseudomonas aeruginosa* strains produced highest percentage of indole acetic acid, hydrogen cyanide, siderophore production and P-solubilization as compared to *P. putida*, *P. cepacia* and *P. fluorescens*. All these qualities confirm that selected *Pseudomonas* strains have PGPR activity.

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