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ORIGINAL ARTICLE

Plant growth-promoting activities of fluorescent pseudomonads, isolated from the Iranian soils

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Abstract Fluorescent pseudomonads are among the most influencing plant growth-promoting rhizobacteria in plants rhizosphere. In this research work the plant growth-promoting activities of 40 different strains of *Pseudomonas fluorescens* and *Pseudomonas putida*, previously isolated from the rhizosphere of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) and maintained in the microbial collection of Soil and Water Research Institute, Tehran, Iran, were evaluated. The ability of bacteria to produce auxin and siderophores and utilizing P sources with little solubility was determined. Four strains of Wp1 (*P. putida*), Cfp10 (*Pseudomonas* sp.), Wp150 (*P. putida*), and Wp159 (*P. putida*) were able to grow in the DF medium with ACC. Thirty percent of bacterial isolates from canola rhizosphere and 33% of bacterial isolates from wheat rhizosphere were able to produce HCN. The results indicate that most of the bacteria, tested in the experiment, have plant growth-promoting activities. This is the first time that such PGPR species are isolated from the Iranian soils. With respect to their great biological capacities they can be used for wheat and canola inoculation in different parts of the world, which is of very important agricultural implications.

Keywords ACC deaminase · Auxin · Plant growth-promoting rhizobacteria (PGPR) · *Pseudomonas fluorescens* · *Pseudomonas putida* · P solubilizing activities · Siderophore

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Introduction

Plant–microbe interactions are among the most important mutual and in the most cases beneficial behavior, determining soil potential productivity and health (Jeffries et al. 2003; Miransari et al. 2007, 2008). Hence, the functioning of soil microbes, indicated in the following, can very much affect the establishment and maintenance of sustainable agricultural strategies: (1) their physiological and biochemical activities to increase nutrient availability and inhibit soil pathogens, and (2) their alleviating effects on adverse soil conditions such as suboptimal root zone temperature and unfavorable soil pH and salinity (Dey et al. 2004; Miransari and Smith 2007, 2008, 2009).

Plant growth-promoting bacteria (PGPR) are rhizosphere bacteria, with the ability to stimulate and enhance plant growth through different mechanisms (Kloepper et al. 1989; Glick et al. 1999). These mechanisms include the production of plant growth hormone, auxin (Khakipour

et al. 2008) and 1-aminocyclopropane-1-carboxyilic acid (ACC) deaminase, increased solubility of immobile nutrients such as P and Fe (by producing siderophores), fixation of atmospheric N, and controlling the unfavorable effects of pathogens on plant growth (Jalili et al. 2009). PGPR bacteria include the strains of *Azotobacter*, *Acetobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas,* and *Burkholderia* (Elmerich 1984; Kloepper et al. 1988, 1989).

Plant hormones are very effective on plant growth and development and among them auxin (IAA) is one of the most important growth regulators (Friml et al. 2003; Stepanova et al. 2009), often produced by rhizosphere bacteria (Glick et al. 1998; Mayak et al. 2004). Production of auxin at the rates higher than plant requirement produces extra amounts of ACC, which is a prerequisite for ethylene production and is catalyzed by ACC-oxidase. Such a mechanism is not favorable because excessive ethylene decreases plant growth including root growth. However, in some plants ethylene can increase seed germination and interrupt seed dormancy (Glick et al. 1994). Under soil stresses increased ethylene level decreases plant growth. PGPR are able to alleviate such stresses on plant growth by producing ACC-deaminase enzyme, which turns ACC into ammonium and α-ketobutyric acid (Glick et al. 1998; Penrose and Glick 2003).

Phosphorous (P) solubilizing bacteria are abundant in the rhizosphere and produce organic acids turning immobile P into available P for plant growth and production (Sundara et al. 2002). Some bacterial strains produce chelating products, called siderophores, at molecular weight of just 400–1,000 Da, with high affinity for Fe absorption ($K_d = 10^{-20}$–10^{-50}). Because of specific receptors in their cellular membrane, these bacteria are able to absorb Fe–siderophores complexes. Microbial siderophores can enhance plant growth through increasing Fe solubility in the plant rhizosphere (Kloepper et al. 1980). Such products are also able to alleviate the unfavorable effects of pathogens on plant growth.

Hydrogen cyanide (HCN) production by different bacterial species including *P. aeruginosa*, *P. fluorescens* and *Chromobacterium violaceum* has also been observed (Siddiqui et al. 2003). Glycine is the prerequisite for CN production in fungi and bacteria (Casteric 1977). Production of HCN by *Pseudomonas* bacteria can enhance plant growth through inhibiting pathogens activities (Schippers et al. 1990). This is the first time that such PGPR species are isolated from the Iranian soils. Hence, with respect to their great biological capacities, their plant growth-promoting activities were measured to determine the most efficient isolates. These isolates can be used for the inoculation of wheat and canola in different parts of the world and hence are of very important agricultural implications.

Materials and methods

Different strains of fluorescent pseudomonads including 15 strains isolated from wheat (W) rhizosphere, 23 strains, isolated from canola (C) rhizosphere, and two exotic strains, all belonging to the microbial collection of Soil and Water Research Institute, Tehran, Iran, were tested for their plant growth-promoting activities. The isolates of *Pseudomonas putida* including Wp1, Wp30, Wp41, Wp69, Wp112, Wp143, Wp150, Wp159, Wp168, and the isolates of *P. fluorescens* including Wf26, Wf36, Wf93, Wf99, Wf173, Wf187 were isolated from wheat rhizosphere. The two exotic strains including GRP3 and MPFM were *P. aeruginosa*. The isolates of *P. putida* including Cp1, Cp2, Cp3, Cp4, Cp7, Cp13, Cp14, Cp15, Cp17, Cp18 and Cp19, *P. fluorescens* including Cf5, Cf6, Cf12, Cf16, Cf20, Cf21, Cf22 and Cf23, and *P. fluorescens* or *P. putida* including Cfp8, Cfp9, Cfp10 and Cfp11 were isolated from canola rhizosphere. A total of 40 species were tested.

Measuring auxin production in the Dworkin and Foster (DF) medium

The production of auxin by different isolates and the effects of L-tryptophan on auxin production were determined using the following method. First, the isolates were grown on a tryptic soy broth (TSB) medium including 2.5 g/L dextrose, 2.5 g/L di-potassium hydrogen phosphate, 5 g/L sodium chloride, 3 g/L peptone soya and 17 g/L triptone. Then 50 µl of bacterial inoculum was inoculated into 25 ml of DF medium (Glick et al. 1994) (including 4 g/L KH_2PO_4, 6 g/L Na_2HPO_4, 0.2 g/L $MgSO_4$, 2 g/L glucose, 2 g/L gluconic acid, 2 g/L citric acid, and micronutrients including 1 mg/L $FeSO_4{\cdot}7H_2O$, 10 µg/L H_3BO_3, 10 µg/L $MnSO_4{\cdot}7H_2O$, 124.6 µg/L $ZnSO_4.H_2O$, 78.22 µg/L, $CuSO_4{\cdot}7H_2O$ and 10 µg/L MoO_3 at pH = 7.2) at the L-tryptophan concentrations of 0, 50, 100 and 200 mg/L. Hence, the experiment was a factorial including different bacterial strains and different L-tryptophan concentrations.

After 48 h the bacterial solution was centrifuged and 1 ml of supernatant was mixed with 4 ml of Salkowski indicator (150 ml of concentrated sulfuric acid, 250 ml of distilled water and 7.5 ml of $FeCl_3{\cdot}6H_2O$ 0.5 M). The solution was kept at room temperature for 20 min and light absorbance was immediately measured at 535 nm using spectrophotometer (Patten and Glick 2002). The amount of auxin was calculated using the standard graph of IAA.

ACC-deaminase measurement

Using the method of Amico et al. (2005) with some modifications, the amount of ACC-deaminase was

determined (Jalili et al. 2009). The bacteria were grown on a TSB medium for 48 h to determine the utilization of ACC as the sole N source. Then different DF media with (1) 3 mM of ACC, (2) with 2 g/L ammonium sulfate, and (3) control (ACC and ammonium sulfate was not used) were inoculated with 50 µl of fresh bacterial inoculum. After 48 h using light spectrophotometer, absorbance at 405 nM was determined.

Measuring siderophore using the universal chrome azurol S (CAS) method

To measure the bacterial potential for siderophore production, first the bacteria were grown on a TSB medium. In this method for the semi quantitative recognition of siderophore production, 15 µl of fresh bacterial inoculum were spot-inoculated on plates with CAS medium (Alexander and Zuberer 1991), using the indicator, buffer, nutrient, and CAS amino acid solutions.

After preparing all four solutions, the mixed solutions of buffer and CAS amino acid were mixed with the nutrient solution. Then with a gentle mixing (with no air bubbles) the Fe–CAS solution was also mixed and the final solution was distributed in the plates. The inoculated plates were kept at 28°C. By measuring the diameter of orange halo (color change from blue to orange) around the bacterial colonies at the time intervals of 24, 48, and 72 h the production of siderophore was recognized.

Measurement of solubilized P in solid medium

Measuring P solubility in the liquid medium

The PKV medium with insoluble tricalcium phosphate $Ca_3(PO_4)_2$, was used to determine the potential of bacterial strains in solubilizing mineral phosphates. First, the bacteria were grown on a TSB medium for 48 h and then 50 µL of bacterial suspension was inoculated in 25 ml of PKV medium. The samples were then shaken for 120 h on a shaker at 125 r/m (at 128°C) and their pH was determined. At the same time, the bacterial suspension was centrifuged (at 10,000*g* for 15 min) and 1 ml of supernatant was mixed with 3 ml of distilled water and 1 ml of the indicator molybdate–vanadate ammonium. After 20 min of incubation, using light spectrometry, light absorption by the samples at 470 nm was determined. P solubility was determined using the standard graph of KH_2PO_4 (Jeon et al. 2003).

Production of hydrogen cyanide

For the determination of HCN production, the strains were first grown on a tryptic soy agar (TSA) medium, enriched with glycine (4.4 g/L). The plates were kept at 28°C for 120 h using filter papers, soaked in sodium picrate (5% picric acid and 2% sodium carbonate). The amount of produced HCN was determined according to the color change (Table 1) (Donate et al. 2004).

Table 1 Mean values of auxin, produced by different strains of bacteria at different tryptophan concentrations

Strain	Tryptophan concentrations (mg/L)			
	0	50	100	200
Cp1	0e	2.11j	2.87pq	4.73ml
Cp2	0e	2.41i	3.85lm	6.73j
Cp3	0e	1.78lm	2.89p	4.14mn
Cp4	0e	1.08u	2.60qr	2.8pq
Cf5	0e	1.66mn	4.86j	8.19i
Cf6	0.09d	3.37f	7.12g	8.42i
Cp7	0e	2.4i	4.06kl	5.86k
Cfp8	0.14c	5.57c	7.52f	18.99e
Cfp9	0e	2.04jk	2.82pqr	3.11op
Cfp10	0e	1.49opq	5.75h	8.18i
Cfp11	0e	1.25rst	5.24i	5.36kl
Cf12	0e	0.93v	3.56n	5.63k
Cp13	0e	1.14tu	1.32u	2.26qr
Cp14	0e	2.92h	3.22o	5.88k
Cp15	0e	1.47opq	2.74pqr	4.68lm
Cf16	0e	2.80h	4.73j	7.17j
Cp17	0e	1.34qrs	2.59r	3.7no
Cp18	0e	1.49opq	3.7mn	7.2j
Cp19	0e	1.50op	2.66pqr	5.65k
Cf20	0.14c	3.10g	4.74j	5.48kl
Cf21	0e	2.06jk	2.66pqr	5.64k
Cf22	0e	1.22stu	2.31s	4.67lm
Cf23	0e	1.09u	10.45c	63.7a
Wp1	0e	1.37pqr	1.79t	3.43nop
Wf26	0e	1.08u	1.62t	1.79rs
Wp30	0.13c	4.99e	7.09g	14.1f
Wf36	0e	0.61w	0.84v	0.91t
Wp41	0.17b	6.45b	8.08e	21d
Wp69	0e	0.91v	1.16u	1.71rs
Wf93	0e	0.62w	1.20u	1.85rs
Wf99	0e	1.91kl	5.23i	13.05g
Wp112	0.18b	5.31d	8.42d	14.26f
Wp143	0e	1.22stu	1.77t	2.99opq
Wp150	0e	1.79lm	4.21k	4.72lm
Wp159	0.22a	10.17a	25.19a	39.71b
Wp168	0e	1.53no	11.17b	27.95c
Wf173	0e	2.06jk	10.50c	12.3h
Wf187	0e	1.24rst	1.36u	2.25rs
GRP3	0e	0.59w	0.81v	1.07st
MPFM	0e	1.21stu	1.27u	1.42st

Mean values followed by the same letters are not statistically different

Statistical analysis

For auxin measurement, as previously mentioned, the experiment was a factorial and hence the related statistical analyses including the analyses of main and interaction effects were performed. The other experiments were conducted on the basis of completely randomized design and hence the related analyses including the comparison of means were conducted (Steel and Torrie 1980). All analyses were performed using SAS Institute Inc. (1988) and SPSS.

Results

Auxin production in a minimum salty DF medium

Regarding auxin production there were significant differences among different strains (Table 1). Accordingly, the effects of strain, different L-tryptophan concentrations, and their interactions were significant at $P = 0.01$ (Table 2). Auxin production by the strains ranged from 0 to 63.70 mg/L, with the highest amount belonging to strain Cf_{23} at tryptophan concentration of 200 mg/L. Strains Cf6, Cfp8, Cf20, Wp30, Wp41, Wp112, and Wp159 produced little amounts of auxin in the medium with no tryptophan (Table 1).

Table 2 Analyses of variance for the effects of different bacterial strains on P solubility in solid (CV = 8.7) and liquid (CV = 9.4) medium, amount of auxin (CV = 10.5), produced at different concentrations of tryptophan and produced siderophore (CV = 10.2)

Source of variation	*df*	Sum of squares
P solubility, solid medium		
Strain	39	1.224**
Error	80	0.007
P solubility, liquid medium		
Strain	39	14391.81**
Error	80	812.32
Auxin production		
Concentration	3	1800.31**
Strain	39	205.24**
Concentration X strain	117	92.66**
Error	320	0.058
Siderophore production		
Strain	39	0.62**
Error	80	0.0055

**Significant at $P = 0.01$

Bacterial P solubilization

All strains were able to solubilize $Ca_3(PO_4)_2$ in the PKV solid medium, although differently. Analysis of variance indicated that the effect of strain on P solubility was significant at $P = 0.01$ (Table 2). The halo/colony diameter ratio ranged from 1.25 to 3.58. The maximum and minimum ratio values were related to strains Wf93 (3.58) and Cfp10 (1.25), respectively. The average ratio of total diameter to the colony was 2.17 and 37.5% of the strains produced higher ratios, comparatively (Table 4).

In addition, these strains were capable of solubilizing $Ca_3(PO4)_2$ in a PKV liquid medium. The amounts of P, solubilized by the bacterial strains ranged between 158.34 (Wp69) and 438.38 mg/L (Wf187). According to the analysis of variance the effect of bacterial strain on P solubility in the liquid medium was significant at $P = 0.01$ (Table 2). The average amount of P, solubilized by the bacterial strains was 333.02 mg/L and 55% of the strains produced higher amounts. The comparison of pH among different inoculated medium with control (not inoculated) indicated that pH values decreased in all inoculated medium. The medium pH for strain Wp69, with the minimum solubility, decreased from 5.6 to 4.57 and for strain Wf187 with the highest P solubility decreased to 3.45 (Table 4).

The coefficients of correlation between the amounts of solubilized P and pH values indicated that there is a negative and significant correlation between pH and solubilized P ($P = 0.01$, $r = -0.852$). There were significant correlations between P solubility in liquid and solid medium at $P = 0.01$ and the highest correlation was related to the fourth day ($r = 0.585$, $P = 0.01$) (Table 3).

Siderophores production

According to the results of this experiment, production of the orange halo indicates that the strains are able to produce siderophores. The effect of bacterial strain on siderophore production was significant at $P = 0.01$. The ratio of

Table 3 Coefficients of correlation between P solubility in the liquid medium with pH and P solubility in the solid medium

	pH	Total ratio/colony diameter (2nd day)	Total ratio/colony diameter (4th day)	Total ratio/colony diameter (6th day)	Total ratio/colony diameter (8th day)
P solubility in liquid medium (mg/L)	−0.852**	0.525**	0.585**	0.514**	0.484**

**Significant at $P = 0.01$

Table 4 Plant growth-promoting activities of different bacterial strains

Strain #	Strain	P solubility (halo/colony ratio)	pH of bacterial suspension	P solubility (μg/mL)	Siderophore (halo/colony diameter)	HCN
Cp1	*putida*	1.79jik	4.18	289.05jklmno	1.73lmnop	1
Cp2	*putida*	2.57e	3.66	355.62defgh	1.56qrs	1
Cp3	*putida*	1.72klm	4.23	318.09fghijk	1.79jklm	1
Cp4	*putida*	1.27o	4.27	279.59klmnop	2.05 fg	1
Cf5	*fluorescens*	2.58e	3.77	412.88abc	2.17ef	4
Cf6	*fluorescens*	3.04c	3.61	384.67abcde	1.87ijk	5
Cp7	*putida*	1.67klmn	3.84	362.71cdefg	1.28u	1
Cfp8	*fluorescens/putida*	2.54e	3.63	345.78efghi	1.85ijkl	1
Cfp9	*fluorescens/putida*	1.62lmn	4.38	304.13hijklm	1.89hijk	1
Cfp10	*fluorescens/putida*	1.25o	4.11	295.00ijklmn	2.01gh	1
Cfp11	*fluorescens/putida*	2.14 fg	3.84	361.34cdefg	1.33tu	1
Cf12	*fluorescens*	2.08gh	3.72	419.13ab	1.77klmn	4
Cp13	*putida*	1.79ijk	4.27	282.48jklmno	1.59qr	1
Cp14	*putida*	1.75jkl	4.16	261.79lmnop	1.86ijkl	1
Cp15	*putida*	1.91i	4.29	287.77jklmno	1.76klmno	1
Cf16	*fluorescens*	2.72d	3.63	419.13ab	1.56qrs	4
Cp17	*putida*	1.59mn	4.16	284.02jklmno	2.33cd	1
Cp18	*putida*	1.92i	3.83	370.94bcdef	1.43st	1
Cp19	*putida*	1.65klmn	4.30	314.72ghijkl	1.68mnopq	1
Cf20	*fluorescens*	2.44e	3.76	418.65ab	1.28u	4
Cf21	*fluorescens*	2.56e	3.76	422.98ab	1.65nopq	4
Cf22	*fluorescens*	1.79ijk	4.57	207.61q	1.34tu	1
Cf23	*fluorescens*	1.52n	4.01	338.30efghij	2.73a	2
Wp1	*putida*	1.95hi	3.72	423.94ab	1.46rst	5
Wf26	*fluorescens*	3.06c	3.93	349.36efghi	2.17ef	1
Wp30	*putida*	1.55n	4.35	235.98opq	1.30u	1
Wf36	*fluorescens*	3.44ab	3.94	316.96fghijkl	2.53b	1
Wp41	*putida*	1.28o	4.23	228.20pq	1.98ghi	1
Wp69	*putida*	2.20fg	4.56	158.34r	1.88hijk	1
Wf93	*fluorescens*	3.58a	3.87	335.26efghijk	2.43bc	1
Wf99	*fluorescens*	2.13fg	3.55	407.09abcd	1.67mnopq	5
Wp112	*putida*	1.28o	4.42	246.39nopq	1.92ghij	1
Wp143	*putida*	1.92i	3.94	290.18jklmno	2.18e	1
Wp150	*putida*	2.07gh	3.73	374.27bcde	1.62opq	1
Wp159	*putida*	3.11c	3.90	374.86bcde	2.21de	3
Wp168	*putida*	3.13c	3.57	404.34abcd	2.25de	4
Wf173	*fluorescens*	1.92i	3.52	420.09ab	1.61pq	5
Wf187	*fluorescens*	3.42b	3.45	438.38a	1.55qrs	1
GRP3	*aeroginosa*	2.24f	4.13	330.12efghijk	0.71v	5
MPFM	*aeroginosa*	1.89ij	4.26	250.73mnopq	0.37w	1
Range		1.25–3.58	3.45–4.57	158.34–438.38	0.37–2.73	1–5
Average		12.17	3.97	333.02	1.76	3

Values followed by the same letters are not significantly different

HCN scale	1	2	3	4	5
Indicator color	Yellow	Cream	Orange	Light brown	Brick like color
HCN production	–	Minimum	Relatively little	Relatively high	Maximum

halo/colony diameter was different in various strains ranging from 0.37 to 2.73 (Table 4).

The highest ratio of halo to colony diameter belonged to the strain Cf23 (2.73). In this experiment strain MPFM was tested as the Sid^{-}. This strain created a halo with a little diameter and the ratio of halo to colony diameter was 0.37. In 55% of the strains the ratio of halo to colony diameter was higher than the average (1.76).

Using ACC as the sole N source

The bacterial strains of Cfp10, Wp1, Wp159, and Wp150, grown in the DF medium, were able to utilize ACC as the sole N source and hence grew in the medium. In the present research for the evaluation of the bacterial strains to utilize ACC as the sole N source, the turbidity ratio of ACC medium to ammonium sulfate medium was determined. Accordingly, strain WP1 with the ratio of 0.68 had the highest ability of utilizing ACC. The strains, which were not able to utilize ACC, produced little turbidity similar to the control treatment (data not shown).

HCN production

Bacterial strains were different regarding HCN production. Among exotic strains just strain GRP3 was able to produce HCN. Strains Cf5, Cf6, Cf12, Cf16, Cf20, Cf21, Wp1, Wf99, Wp168, Wf173, and GRP3 produced high amounts of HCN, while strains Cf23 and Wp159 produced little amounts. The other strains were not able of producing HCN from glycine (Table 4).

Discussion

The ability of *Pseudomonas* sp. to produce auxin can very much affect plant growth (Khakipour et al. 2008), as it has some very important functioning in plant such as hormonal adjustment, plant cell division and development, and nodule formation. Since tryptophan is necessary for auxin production, its production at the control medium can be related to bacterial cell degradation (Frankenberger and Brunner 1983). According to Benizri et al. (1998), although there was not any tryptophan in corn root exudates, however, *P. fluorescens* strain M31 was able to produce auxin, attributed to the similar reason.

The results of mean comparison related to different concentrations of L-tryptophan indicated that with increasing L-tryptophan concentrations in the DF medium the amounts of auxin produced by bacterial strains was also increased, which is in agreement with the results of Patten and Glick (2002). The significant interaction between bacterial strain and tryptophan concentration indicates that different *Pseudomonas* strains can perform differently under different tryptophan concentrations. Hence, this interesting result can be applicable for the selection of strains, which are more efficient in utilizing tryptophan for auxin production.

According to Jeon et al. (2003) the three strains of *P. fluorescens* including MCO7, M45, and B16, grown in a PKV medium in a 5-day period produced solubilized P at 458.3, 447.6, and 427.7 mg/L, respectively. The corresponding pH values after 5 days decreased from 7 to 4.1, 4 and 4.4, respectively. The ability of *Pseudomonas* sp. to produce organic acid and hence, solubilizing immobile P sources such as tricalcium phosphate in the soil is very advantageous as it can enhance the availability of P to the plant and hence increase plant growth (Dey et al. 2004). Production of organic acid by *Pseudomonas* strains decreases soil pH. This is particularly of great importance in dry area, where calcareous soils and hence high pH are very common. Regarding the correlation coefficients between medium pH and P solubility ($r = -0.852$, $P = 0.01$), Rashid et al. (2004) also found similar results ($r = -0.4$, $P = 0.1$).

With respect to the specifications of fluorescent pseudomonads, their most common character is the production of fluorescents pigments, when exposed to the short wavelength ultra violet (254 nM) under Fe deficiency. These fluorescent pigments and water-soluble pigments are among the important siderophores (Leoni et al. 2002). Meyer (2000) stated that different *Pseudomonas* strains have the ability to produce high amounts of siderophore. Rasuli et al. (2006) found that 201 strains of *P. fluorescens* isolated from wheat rhizosphere were able to produce siderophore and the ratio of halo/colony diameter was between 2.21 and 3.96. The Fe-chelating property of siderophore can greatly enhance Fe absorption by plant and hence plant growth, especially in calcareous soils where Fe deficiency is very common.

P. fluorescens RS9 was able to utilize ACC as the sole N source (Amico et al. 2005). In addition, Pal et al. (2001) also found that among the 230 strains with ACC deaminase activity, nine strains (six of them *P. fluorescens* stains) significantly increased groundnut root growth. Using ACC as the sole N source is one of the most important properties of *Pseudomonas* strains, based on which the plant growth-promoting attribute of rhizobacteria is determined. ACC deaminase, produced by PGPR is able to hydrolyse ACC, which is a prerequisite for ethylene production. Under soils stresses high amounts of ethylene are produced, adversely affecting plant growth. Production of ACC deaminase by PGPR can enhance plant growth including root growth (Glick et al. 1994, 1999).

The suppressing effects of PGPR on soil pathogens can provide a healthy soil for optimum plant growth. Among

such effects production of HCN by most PGPR such as *P. fluorescens* is of special importance. Almost 4% of *Pseudomonas* strains isolated from the potato and wheat rhizosphere in soils, with different rotation strategies, produced HCN. At least 40% of *Pseudomonas* strains from potato rhizosphere were able to produce HCN under in vitro conditions (Schippers et al. 1990).

Interestingly, the results (Tables 1 and 4) indicate that there are some sort of positive correlations between different bacterial products. In other words, the more efficient bacterial strains (for example, Cf5 Cf6 Cf12 Cf16 Cf20 Cf21 Wp1) produced higher amounts of auxin, organic acids (higher P solubility), siderophores, and HCN. It may be speculated that there are genes that commonly control such activities in the bacterial strains. Identification of such genes can be very useful for the production of efficient inoculum. Hence, the more efficient bacteria indicate higher plant growth-promoting activities as previously mentioned; according to the results, the production of all their beneficial products seemed to be positively correlated to each other. This is very interesting, because it indicates that PGPR usually comprise a collection of advantageous traits, which can be very useful for plant growth (Glick et al. 1994, 1998, 1999; Khan et al. 2009).

Parameters affecting the ability of PGPR to express different attributes include soil and environmental conditions, microbes–plant host interactions, and microbes–microbes interactions (Dey et al. 2004). The more the plant growth-promoting activities of the *Pseudomonas* strains, the higher the chance of enhanced plant growth and yield. This formula can be very useful for selecting the strains, which can be used as bio-inoculants and can increase the probability of a more efficient agricultural strategy, particularly under soil stresses, as such strains can be more competitive. Thus, using precise methods, which can exactly determine the enhancing abilities of bacterial strains on plant growth, is of great significance.

Conclusion

By analysing the multiple growth-promoting activities of different *P. fluorescens* and *P. putida* in this experiment, the strains with superior plant growth-promoting abilities were identified. The higher the plant growth-promoting attributes of PGPR the higher the chance of more efficient plant growth, especially under soil stresses. This is a very important characteristic for the production of right inoculants under different conditions. In addition, among their other very important effects on plant growth, the bacterial strains tested in this research work can also perform greatly in dry areas with limitations such as high pH and hence the less availability of soil nutrients including P. The results of this research work indicate the great importance of recognizing soil biological potentials under different conditions to address appropriate agricultural strategies (Miransari and Smith 2007, 2008; Miransari et al. 2007, 2008; Jalili et al. 2009).

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