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Plant Growth-Promotion and IAA Secretion With *Pseudomonas fluorescens* and *Pseudomonas putida*

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Research Article

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ABSTRACT

The present study was undertaken to investigate the plant growth promoting traits of a PGPR *P. fluorescens* and *P. putida* like production of Indoleacetic Acid (IAA), siderophore and phosphate solubilization. Plant growth-promoting properties of the two isolates were undertaken for their ability to produce siderophore, indole acetic acid and phosphate solubilization activity. The Indole-3-acetic acid and several other tryptophan (Trp) derivatives were separated by HPLC and quantified according to their fluorescence. On the other hand and in function of the two different types of root response to Fe deficiency (strategies), the isolates were tested for their effect on seed germination, seedling growth of *Lens culinaris* (strategy II) and *Hordeum vulgar* (strategy I) under gnotobiotic conditions and for tryptophan (Trp)-dependent IAA production. Both P. fluorescens and P. putida were shown to produce 3-indole-acetic acid (IAA) *in vitro*, at concentrations of 89 $\mu g.ml^{-1}$ and 116 $\mu g.ml^{-1}$, respectively. Furthermore, high levels of IAA excreted by *P. putida* were most consistent in enhancing plant growth across the calculation of Vigor index. On a comparative basis *P. fluorescens* was most promising in promoting plant growth in the case of the plant of strategy I compared to plant of strategy II..

INTRODUCTION

Many indigenous species and specific strains of *Pseudomonas* have been considered important in sustainable agriculture due to their plant growth promotional ability as well as biocontrol potential. These ubiquitous microorganisms can be a significant Plant growth promoting rhizobacteria (PGPR) that actively colonize plant roots and increase plant growth and yield. These fluorescent *Pseudomonas* are important plant growth-promoting (PGP) and biocontrol (BC) agents due to their production of antibiotics and anti-fungal metabolites.

Directly or indirectly the PGPR agents can exhibit a variety of characteristics responsible for influencing plant growth [1]. The common traits include production of plant growth regulators (auxin, gibberellin, ethylene etc.), siderophores, HCN and antibiotics and solubilization of PO_4. One of the most important mechanisms involved in the plant promotion is through the production of siderophore for sequestering iron and the auxin indole-3-acetic acid (IAA) which has been associated with plant growth promotion, especially root initiation and elongation [2,3].

Barley (*Hordeum vulgare* L.) is a fast growing, cool season, annual grain crop that could be used as forage as well as cover crop to improve soil fertility [4]. However, the growing of lentil providing opportunities to return reduced nitrogen to the soil (symbiotic nitrogen fixation) [5]. The inoculation of seeds or roots with fluorescent *Pseudomonads* to increase plant vigor and productivity has been a worldwide studied practice. The present study was undertaken to screen the PGPR strains and to investigate their effect on seed germination seedling growth of *Lens* sp. and *Hordeum* sp. seedlings under the same gnobiotics conditions across the detection and characterization of Indole-3-Acetic Acid (IAA) and PO_4 solubilization.

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MATERIAL AND METHODS

Isolation and Biochemical Characterization of Pseudomonas fluorescents (PGPR)

Pseudomonas fluorescents strains were originally isolated from *Triticum* spp. Rhizoplane and rhizosphere of the region of Mascara (agroecosystem) (Northern-Algerian West, 2°, 11' W, 35°, 26' N). Colonies that came up on KB plates were observed under UV light, purified and preserved. The biochemical characterization was determined by means of API 20NE; bio Merieux Vitek strips and on the basis of *Pseudomonas* biochemical tests as described in Bergy's Manual of Determinative Bacteriology.

Each isolate was tested for morphology, motility and Gram stain. The following physiological tests were performed: fluorescent and pigment production, accumulation of levans production, oxidative or fermentative acid production, growth at 4°C and 41°C. The enzymatic activities tested were: lipase production, pectinolytic activity, starch hydrolysis, lecithinase production and proteolytic enzyme production.

Most of the tests conducted for their identification have been based on physiological, nutritional tests and by the use of the Analytical Profile Index (API 20NE; Bio Merieux Vitek), strains were maintained in LB at -80°C with 50% glycerol [6]. Plant growth-promoting properties of the two strains were confirmed with their ability to produce siderophore, indole acetic acid and phosphate solubilization.

On the basis of Meliani data, and possessing traits of stress tolerance, survivability in soils, these bacteria may exhibit good performance in the rhizosphere of crop plants, and thereby compete for any ecological niche [7].

Siderophore Production

PGPR isolates were assayed for siderophore production on the chrome azurole S agar (CAS) described by Alexander and Zuberer [8]. Chrome azurole S agar plates were prepared and spot inoculated with test organism and incubated at 30°C for 5 days. Development of yellow orange halo around the colony was considered as positive for siderophore production.

Indole-3-Acetic Acid (IAA) Quantification

PGPR strains were inoculated into 10 ml of DF salts minimal media supplemented with 0.2 $g.l^{-1}$ of tryptophan and incubated at 30°C under shaking for 48 h. Broth cultures were centrifuged at 7500 rpm for 10 minutes. To one ml of aliquot of the supernatant of the cultures 2 ml of Salkowskis reagent was added and incubated at 30°C for 25 minutes [9]. Absorption was read at 530 nm and the concentration of IAA in each in each culture medium was determined by comparison to a standard curve generated from known concentrations of IAA.

P. fluorescens (P9) *and P. putida* (P10) were propagated overnight in 5 ml of DF salts minimal media, 20 µl aliquots were transferred into 5 ml of DF salts minimal media supplemented with 0.2 $g.l^{-1}$ of tryptophan. After incubation under orbital shaking for 48 h at 30°C, the density of each culture was measured spectrophotometrically at 600 nm, and then the bacterial cells were removed from the culture medium by centrifugation (5,500×*g*, 10 min). A 1-ml aliquot of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled H_2O, 7.5 ml of 0.5 M $FeCl_3$·$6H_2O$) and allowed to stand at room temperature for 20 min before the absorbance at 535 nm was measured [10]. The concentration of IAA in each culture medium was determined by comparison with a standard curve.

Eventually, bacterial cells were separated from the supernatant by centrifugation for 10 min at 13,000 rpm, and supernatants were acidified to pH 2.5-3.0 with 1 N HCl. The supernatant (30 ml) was extracted twice with 60 ml ethyl acetate, and subsequently ethyl acetate was evaporated to dryness in a rotaevaporator. The extracts were dissolved in 300 µL methanol and kept at -20°C. The ethyl acetate extracts (10-20 Ml*)* were plated on TLC plates (20 × 20 F254, 0.2 mm, Merck) and developed in either ethyl acetate-isopropanol-ammonium hydroxide (45:35:20) or in ethyl acetate-chloroform-formic acid (55:35:10). Spots with *Rf* values identical to authentic IAA were identified under UV light (254 nm) [11]. The A_{280} was monitored, and UV absorption profiles were obtained. IAA was quantified by reference to a standard curve of peak area versus concentration generated for standard AIA.

HPLC Detection of IAA

Ethyl-acetate extraction and evaporation were applied to minimize losses of IAA in the samples [3]. Extracts from the two isolates were analyzed by HPLC (KNAUER) in a column 4.6 × 250 mm reverse phase C18 (Eurospher II 100-5) under isocratic conditions with 40% methanol and 60% of a 1% acetic acid solution as the separation solvent. An injection volume of 20 µl was utilized at a flow rate of 1 ml min^{-1}. Compounds were detected at 280 nm. IAA was quantified using a standard curve created by analysis of IAA standards of known concentrations (Sigma Chemical Co).

Solubilization of Phosphate

The bacterial culture was grown in modified Pikovskayas medium with 0.5% of tricalcium phosphate at 27°C for 8 days at 175 rpm. The culture supernatants were collected by centrifugation at 10,000 rpm for 15 minutes [12].

The halo size (h) was determined by subtracting the diameter of the colony from the diameter of the halo. According to Azziz isolates were grouped into 5 classes according to their h score; class 0: lack of halo, class 1: h= 0 mm to 2 mm, class 2: h = 2 mm

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to 4 mm, class 3: h > 4 and class 4: h > 4 and a perfectly transparent halo. Class 4 includes a qualitative aspect of the halo, as halos of class 3 were as large as class 4 but rather diffuse [13].

Plant Growth Promotion

The bacterial isolates were grown on KB with constant shaking at 150 rpm for 48 h at room temperature (28°C ± 2°C). The bacterial cells were harvested and centrifuged at 6000 rpm for 15 min and resuspended in phosphate buffer (0.01 M, pH 7.0). The concentration was adjusted using a spectrophotometer to approximately 10^8 cfu ml^{-1} (OD_{595}= 0.3) and used as bacterial inoculum. This one was produced by transferring one loopful from each culture to 100 ml of KB broth in a 250 ml Erlenmeyer flask and incubating at room temperature (28°C ± 2°C) on a shaker at 150 rpm for 48 h. The bacterial suspension of the *P. fluorescens* and *P. putida* strains prepared were tested for their plant growth-promoting activity, which was carried out by the standard roll towel method [14].

Subsequently, to investigate the effects of PGPR isolates on the growth of *Lens* sp, *Hordeum* and tomato and prior to seeds grown in plastic pots (data not shown), seeds were surface sterilized with 1% sodium hypochlorite for 30 seconds rinsed in sterile distilled water and dried overnight under sterile stream of air in a laminar air flow. *Lens* and *Hordeum* seeds soaked in 10 ml of the bacterial suspension (10^8 cfu. ml^{-1}) for 2 h were blot dried, were placed in wet blotters and incubated in growth chamber for 10 d. The seeds soaked in sterile water served as control. The germination percentage of seeds was recorded and the vigor index was calculated using the following formula: Vigor index= Percent germination × seedling length (shoot length + root length) [15].

Statistical analysis

The field trial data was analyzed in triplicate, data were subjected to analysis of variance (ANOVA) using the STATISTICA 7.0 software version.

RESULTS

Isolation and Screening of PGPR

A total of 27 isolates of fluorescent *Pseudomonas* sp. were identified on the basis of biochemical tests and sugar fermentation as described in Bergy's Manual of Determinative Bacteriology. The largest number of confirmed fluorescent *Pseudomonads* (38.57%) were determined to be either *P. fluorescens* (P9) (dominance of Biovar I (80%) or *P. putida* (P10)), P9 and P10 were chosen for further study and were found better in fluorescence intensity, biofilm formation and growth kinetics.

Indole-3-Acetic Acid (IAA) Production

HPLC is a useful tool to identify and confirm metabolites in bacterial supernatant; it permits the fast screening of crude biological extracts for detailed information about metabolic profiles, with a minimum amount of material. Retention time information is obtained from the HPLC and UV detector generates UV chromatogram based on UV responses, compound purity and information on the chromophore can be assessed as well. Both the strains showed a peak comparable with the peak of standard IAA on HPLC system **(Table 1)**. That indicated the bacterial ability to synthesize the IAA, 116 $\mu g.ml^{-1}$ and 89 $\mu g.ml^{-1}$ for *P. putida* and *P. fluorescens,* respectively. This production was further confirmed by subsequent TLC analysis of crude extract of IAA. Spots from the extracted IAA preparation were found corresponding to the spots of standard IAA with same Rf value.

Table 1a and 1b. The retention time and peak area of *P. fluorescensa* and *P. putidab* Indole-3-acetic acid (IAA).

a	Reten.Time (min)	Area (mA U.s)	Height (mAU)	Area (%)	Height (%)	W05 (min)	Peak Purity (-)
1	2.467	1009.341	105.266	9.5	12.9	0.15	898
2	2.833	2846.899	185.377	23.7	38.6	0.23	873
3	3.467	2350.018	95.181	3.8	6.9	0.27	798
4	4.150	7092.267	391.529	6.7	15.1	0.28	944
5	5.783	3026.739	130.824	1.8	8.1	0.30	940
6	7.433	470.419	12.916	2.9	8.3	0.65	940
7	8.900	1466.376	61.274	11.0	15.4	0.30	946
8	10.533	377.757	20.478	6.3	7.1	0.30	985
9	11.733	21.736	1.006	39.2	39.5	0.30	978
10	12.450	668.162	27.641	15.7	13.2	0.40	962
11	14.483	693.287	27.064	3.0	1.4	0.38	970
12	16.450	97.851	2.994	9.2	6.4	0.38	961
13	17.950	46.289	1.377	2.4	2.2	0.55	978
14	19.567	87.688	2.027	0.1	0.1	0.53	982
15	22.467	23.911	0.404	4.2	2.9	0.93	984

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16	24.683	13.362	0.361	4.4	2.8	0.62	978
17	31.500	40.030	0.710	0.6	0.3	0.82	994
18	32.883	4.811	0.146	0.3	0.1	0.60	998
19	41.517	18428.154	53.972	0.6	0.2	4.85	997
20	43.033	3738.321	43.216	0.2	0.0	1.53	1000
21	54.033	12276.188	5.317	0.1	0.0	1.03	1000
	Total	0.000	0.000	100.0	100.0		
b	**Reten. Time (min)**	**Area (mA U.s)**	**Height (mAU)**	**Area (%)**	**Height (%)**	**W05 (min)**	**Peak Purity (-)**
1	2.467	668.441	48.898	4.0	5.8	0.22	876
2	2.833	1893.432	167.997	11.4	20.0	0.20	802
3	3.433	1092.376	78.438	6.6	9.3	0.18	855
4	4.133	5452.062	305.436	32.8	36.3	0.30	947
5	4.750	43.725	5.067	0.3	0.6	0.17	997
6	5.033	44.608	5.678	0.3	0.7	0.17	990
7	5.333	9.109	1.555	0.1	0.2	0.12	997
8	5.783	513.043	24.568	3.1	2.9	0.33	960
9	6.333	32.471	2.440	0.2	0.3	0.25	995
10	7.083	50.876	2.556	0.3	0.3	0.43	995
11	7.617	142.646	7.443	0.9	0.9	0.37	979
12	8.900	1519.091	73.663	9.1	8.8	0.28	908
13	10.000	30.676	1.404	0.2	0.2	0.45	998
14	10.567	287.687	14.671	1.7	1.7	0.30	983
15	11.717	29.805	1.334	0.2	0.2	0.33	986
16	12.517	599.763	27.737	3.6	3.3	0.23	973
17	14.017	9.971	0.724	0.1	0.1	0.35	1000
18	14.533	364.801	15.767	2.2	1.9	0.35	959
19	16.500	68.009	3.213	0.4	0.4	0.45	989
20	30.717	19.767	0.786	0.1	0.1	0.62	1000
21	31.717	65.646	1.857	0.4	0.2	0.95	1000
22	32.517	164.096	2.876	1.0	0.3	0.97	1000
23	34.450	169.748	2.994	1.0	0.4	1.20	999
24	36.433	209.967	3.178	1.3	0.4	1.58	1000
25	38.200	383.313	4.032	2.3	0.5	1.15	1000
26	40.000	181.210	3.108	1.1	0.4	1.12	1000
27	41.817	451.602	5.920	2.7	0.7	1.12	1000
28	43.783	128.885	2.229	0.8	0.3	1.60	1000
29	45.300	293.592	3.487	1.8	0.4	1.90	999
30	47.550	405.935	4.198	2.4	0.5	1.40	1000
31	49.517	196.509	3.013	1.2	0.4	1.35	1000
32	50.983	179.212	2.289	1.1	0.3	1.07	1000
33	52.900	244.666	3.732	1.5	0.4	1.60	1000
34	54.967	347.157	4.005	2.1	0.5	0.87	1000
35	56.433	140.393	2.896	0.8	0.3	1.05	1000
36	58.333	171.216	2.521	1.0	0.3	1.05	1000
	Total	16605.476	841.709	100.0	100.0		

Phosphates Solubilization

The solubilization levels of tricalcium phosphate (TCP) varied with other isolates. All isolates were tested for semi-quantitative phosphate solubilization activity. The P4 (*P. fluorescens*) strain was classified as class 5, whereas P9 (*P. fluorescens*), *B. subtilis, Rhizobium leguminosarum, Azotobacter vinelandii* and P10 (*P. putida*) belonged to class 4, 3, 2 and 1 respectively **(Figure 1)**. In a study conducted by Rodríguez and Fraga and in this same field experiment, the authors found that *Pseudomonas* strains were the most active in the P solubilization, our result corroborate with [16-18].

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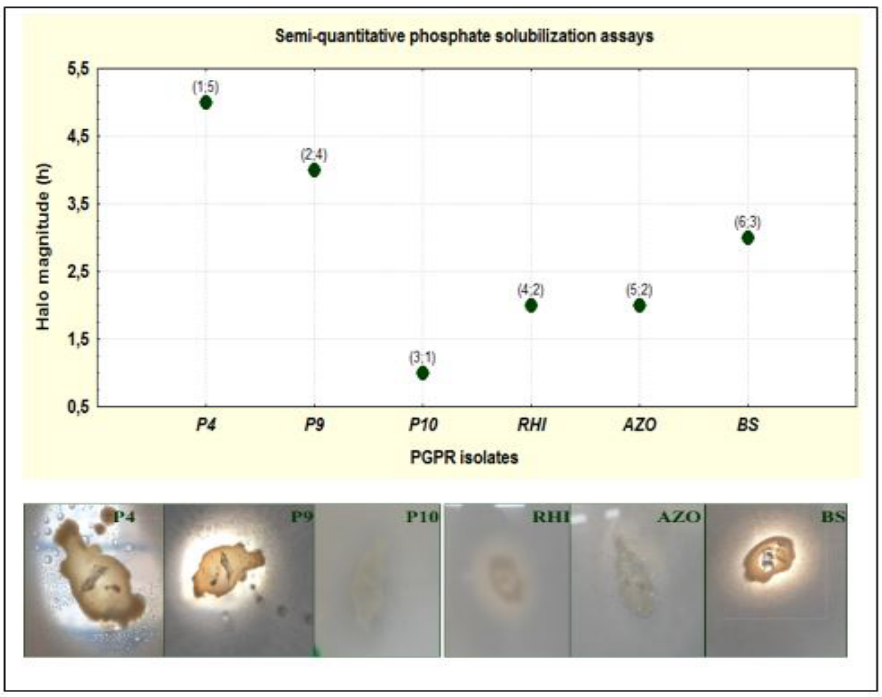

Figure 1. Phosphate solubilization activity *in vitro* of the PGPR isolates P4, P9, P10 and RHI (*Rhizobiums leguminosarum*), Azo (*Azotobacter vinelandii*) and BS (*Bacillus subtilis*).

Growth Stimulation

Results in **Figures 2 and 3** revealed that inoculation with certain of these selected PGPR significantly improved root and shoot growth as well as germination as compared to control. Seeds treated with the different bacterial suspensions showed improvement in plant growth parameters over untreated seeds. In the present investigation, PGPR strain P9 (*P. fluorescens* biovar IV), P4 (*P. fluorescens* biovar I) and P10 (*P. putida*) were found to increase remarkably the vigor index of *Lens* culinaris and *Hordeum Vulgare*. For *Lens culinaris*, the increase in mean root length (1.9 cm) and shoot length (1.8 cm) due to P9 and P4 respectively was significantly higher compared to the seedlings from the untreated control. The maximum vigor index of 65.89 was attributed to P4 but the seeds germination (71.66%) was attributed to P9; however, the least vigor index of 24.22 was recorded from the untreated control. In the case of *Hordeum Vulgare*, treated seedlings showed a higher vigor index (21.93) for P10; although the maximum seeds germination was signaled for the P9 (Data are expressed as 100%).

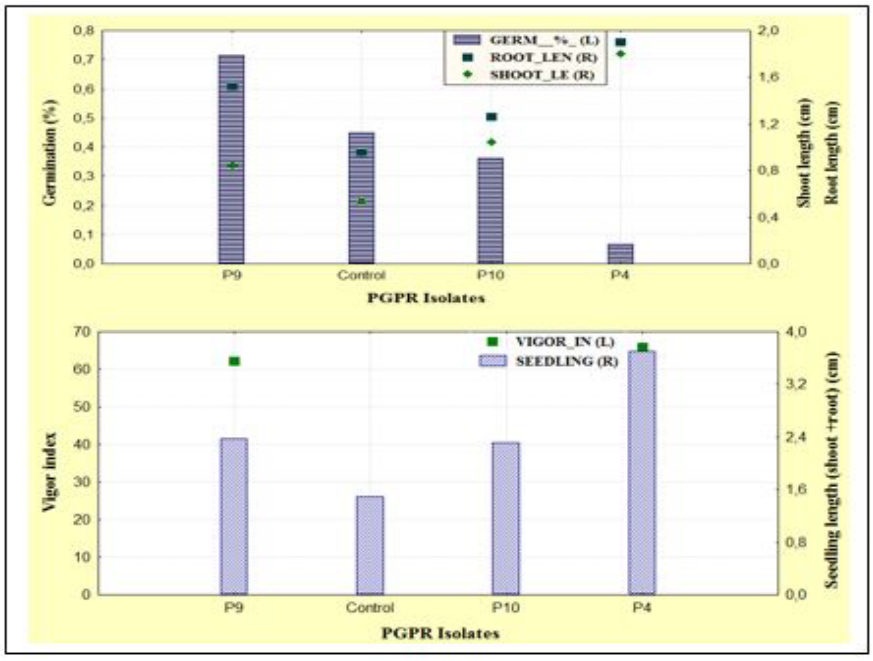

Figure 2. Inoculation of *Lens culinaris* with fluorescents PGPR.

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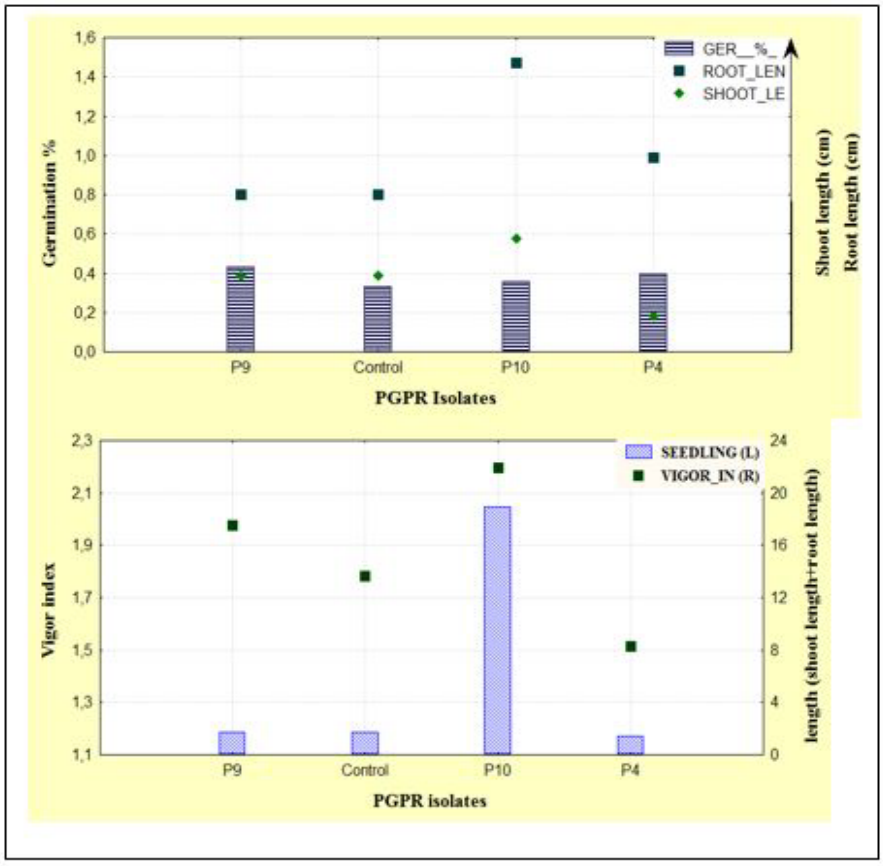

Figure 3. Inoculation of *Hordeum vulgare* with fluorescents PGPR.

DISCUSSION

Similar kind of results were obtained in other experiments, significant increase in growth and yield of agronomically important crops in response to inoculation with PGPR has been reported by many workers [19-22].

Till date, five different IAA biosynthesis routes were determined with tryptophan (Trp) as a precursor: the indole-3-pyruvate (IPyA), indole-3-acetamide (IAM), tryptamine (TAM), indole-3-acetonitrile (IAN) and the Trp side chain oxidase pathways [23]. Furthermore, these authors considerate that the group of indoles acidic (IAA, ILA), amphoteric (Trp), basic (TAM) or essentially neutral (IAN, IAM, TOL) influence retention time and peak shape of ionizable compounds of the mobile phase, they had indicated that the retention times were approximately 3.5, 5.9, 7.7, 9.3, 13.8, 15.5 and 24.1 min for Trp, TAM, ILA, IAM, IAA, TOL and IAN, respectively. Taken together, our data indicate that the presence of different retention time confirmed that several AIA conjugates were produced.

These findings are consistent with other report of focused on the secretion of the AIA by these species [10,24]. *Pseudomonas* bacteria, especially *P. fluorescens* and *P. putida* are the most important kinds of PGPR which produce auxin and promote the yield of AIA in *P. putida* was 41.9% but in *P. fluorescens* it was 27%. In this context it is noteworthy, that these findings suggest that the exact biochemical mechanism and pathway of IAA synthesis has remained elusive and versatile in *Pseudomonas* species [25].

The literature search indicated that Indole-3-acetic acid and several other Trp derivatives were separated by HPLC and quantified according to their fluorescence. The time of analysis for detection of auxine derivatives was <36 min for *P. putida* and <21 min for *P. fluorescens*. The Retention Time and peak area determined upon analysis of the sample are presented in **Table 1** for both the strains. The absorbance spectra were recorded between λ 200 and λ 400 nm intervals and the spectrum data were then processed by using the Clarity Chromatography SW program. **Figures 4 and 5** showed the variation of the absorbance spectra for bacterial IAA, according to Dobrev bacterial hormones were detected at λ 280 nm which is the absorbance maxima of IAA. In terms of this UV scan, the measured values of IAA correspond well to the ones already published, since a maximum absorbance has been recorded at λ 220 nm, 280 nm and a minimum one at λ 240 [26]. The different retention times for AIA proved to be quite versatile. There is no reference for Rt determination of *Pseudomonas* auxines under these isocratic conditions, however earlier publications of other authors are seldom encountered. UV scan exhibited four retention times for *P. fluorescens* (4.150 min, 8.900 min, 10.533 min, 14.483 min) with two retention times (4.133 min and 8.900 min) for *P. putida* [27]. Sergeeva et al. [3] have confirmed that a Rt 7.366 min and 15.720 min corresponds to the indole-3-lactic acid and indole-3-pyruvate, respectively. However, in previous studies Retention Time of indole compounds were 4.8 min for Trp, 8.1 min for indole-acetamide, 10.1 min for IAA, and 10.8 min for indole-acetaldehyde and indole-ethanol, which were not separated under these conditions [28].

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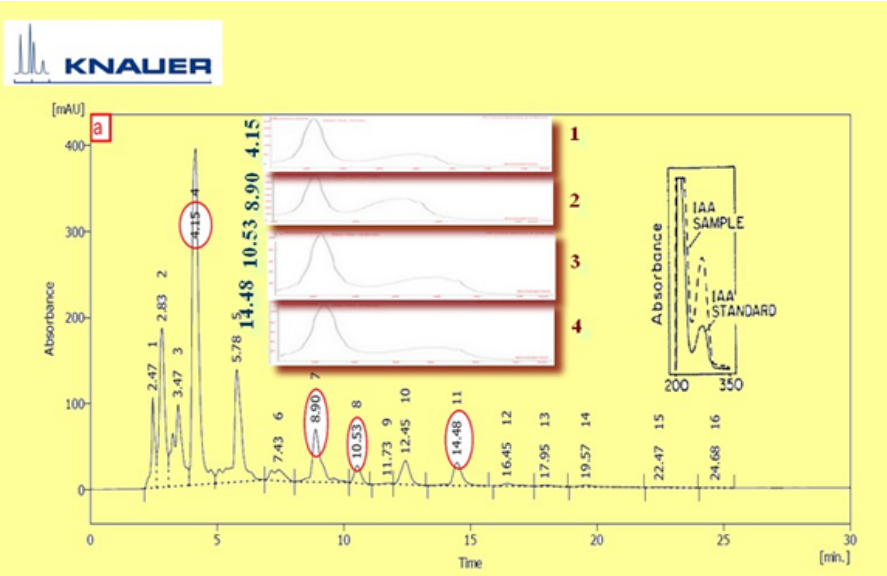

Figure 4. HPLC chromatogram a and UV spectrum (1,2,3,4) of *P. fluorescens* Indole-3-acetic acid (IAA).

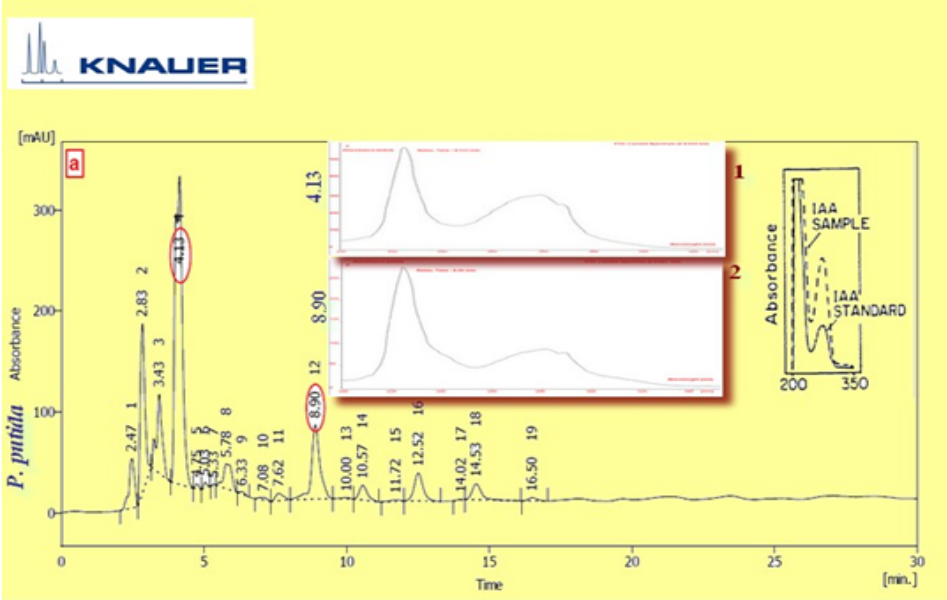

Figure 5. HPLC chromatogram a and UV spectrum (5,6) of *P. putida* Indole-3-acetic acid (IAA).

The use of phosphate solubilizing bacteria (PSB) as biofertilizers could decrease the environmental problems associated with conventional chemical fertilizers [29]. PSB are well known to promote plant growth because of their ability to convert insoluble form of P to soluble form that can be readily taken up by the plant roots [30]. Usually the soils are supplemented with inorganic P in the form of chemical fertilizers. A large proportion of the applied P gets fixed in the soil as phosphates of iron, aluminum and calcium [31]. According to Browne the fluorescents *Pseudomonas* are known as good phosphate solubilizers against *Azotobacter* and the *Rhizobia* species [32].

Studies have also shown that the growth promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotypes [33]. The results from this study suggest the involvement of two possible mechanisms called strategy I and II for iron acquisition by the plant. In strategy I which occurs in all plant species except grasses, a plasma membrane-bound reductase is induced with enhanced net excretion of protons. Often the release of reductants/chelators is also higher, In Strategy II which is confined to grasses, there is an increase in the biosynthesis and secretion of phytosiderophores which form chelates with Fe^{III}. Uptake of Fe^{III} phytosiderophores is mediated by a specific transporter in the plasma membrane of root cells of grasses [34]. From à practical point of view, this strategy consists to the secretions of bacterio-siderophores and phytosiderophores which characterized leguminous (*Lens culinaris)* and grasses (*Hordeum vulgare*).

The secretion of these siderophores follows a diurnal rhythm which was characterized in several species of grasses: barley, wheat and red festuque (*Festuca rubra* L.) [35-37]. Therefore, Graminaceous plants have evolved a distinct mechanism for iron uptake, known as 'chelation' strategy, which is similar to that of many bacteria and fungi [12]. Root cells secrete phytosiderophores

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to strongly chelate iron and take up the Fe(III)-phytosiderophore complex via transporters called Yellow Stripe 1 (YS1) [38]. Therefore, and for this reason the effect of these fluorescents PGPR was lower in *Hordeum vulgare.*

The species of genus *Pseudomonas* are widely distributed in nature and act as plant growth-promoting rhizobacteria by nitrogen fixation, mineral solubilization, as well as transformation of nutrients, production of phytohormones, siderophores, and 1-Aminocyclopropane-1-Carboxylic Acid (ACC) deaminase [10,39]. In this regard, similar results were reported for the role of IAA and ACC deaminase, inoculation of canola, tomato, and other agriculturally important plants with this strain results in substantial promotion of seedling root growth [40].

Despite the fact that IAA is the most abundant member of the auxin family of phytohormones, has a role in root initiation and elongation and a number of other processes concerned with the differentiation and proliferation of plant tissue, ACC deaminase seems to be important for plant growth. Thus, ACC deaminase- containing plant growth-promoting bacteria facilitate plant growth by (i) decreasing ethylene inhibition of various plant processes and (ii) permitting IAA stimulation of cell proliferation and elongation without the negative effects of increasing ACC synthase and plant ethylene levels [10]. Out of the two PGPR strains used for inoculation, *P. fluorescens* performed relatively better.

CONCLUSION

Very promising is the use of PGPR in plant enhancement, the use these agents as bio inoculants and biofertilizers is an efficient approach to replace chemical fertilizers for sustainable agriculture. The findings of the present investigation highlighted that the use of multi strain inocula of *Pseudomonas* PGPR with known functions is of interest as these formulations may increase consistency in the field. It has been demonstrated that the capacity of two species of the fluorescent *Pseudomonas* (*P. fluorescens* biovar IV and *P. putida*) to promote the reproductive growth may be highly specific to certain plant species. Our results showed that they are able to synthesize IAA from tryptophan as precursor and to promote *Lens* culinaris and *Hordeum vulgare* growth. The plant growth stimulation reported in this study is, most likely, the synergic result of the secretion of IAA and it metabolism in the rhizosphere. The inoculation of this PGPR *Pseudomonas* significantly increased the plant growth (shoot length, root length and the vigor index). More specific works are, however, needed to further study the specific mechanisms involved in the growth stimulation by the *Pseudomonas* species, as well as to better understand the close interaction between the host plant and these two microorganisms.

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