J. Agr. Sci. Tech. (2012) Vol. 14: 985-994

Enhanced Auxin Production by *Azospirillum* Pure Cultures from Plant Root Exudates

M. J. Mehdipour Moghaddam[1*], G. Emtiazi [1], Z. Salehi [2]

ABSTRACT

Bacteria of the genus *Azospirillum* are well known as plant growth promoting rhizobacteria. The ability to synthesize phytohormones is considered one of the most important mechanisms to promote plant growth and is widely distributed among plant-associated rhizobacteria. The most important phytohormone produced by *Azospirillum* is the auxin indole-3-acetic acid, with the L-tryptophan as the precursor. In the present study, we evaluate the capacity of eight *Azospirillum* strains isolated from rice and wheat, to produce *in vitro* auxins using plant exudates. Our results show that isolates produced auxins in tryptophan free media, but, generally, the amount produced increased when the tryptophan concentration increased. Some plants root exudates had a similar effect to tryptophan for the auxin production. In this sense, bean, rice and canola root extracts produced, respectively, 93.3%, 96.2%, and 88.31% more auxin than L-tryptophan. *Azospirillum* sp. isolate A_3 had the maximal capacity to produce auxin. Therefore, the effect of cell free supernatant was studied on rice root development. Statistical analysis did not show any significant difference between root number and dry weight of the treated and control seedlings. However, significant differences were observed in root length and wet weight at α=0.01 and α=0.05, respectively.

Keywords: *Azospirillum*; Phytohormones; Auxins; Indole-3-acetic acid; Rice; Wheat; Root exudates.

INTRODUCTION

Bacteria of the genus *Azospirillum* (α-subclass of proteobacteria) have been known for many years as a plant growth promoting rhizobacteria (PGPR) and have been isolated from the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates (15). One of the alternative explanations for the observed plant growth stimulation by *Azospirillum* inoculation involves the production of plant growth regulating substances such as phytohormones (15). Under certain environmental and soil conditions, *Azospirillum* could positively influence the plant growth, development, and even the crop yield. This stimulatory effect has been attributed to several mechanisms, including the extensively studied biological nitrogen fixation or the phytohormone production. In this sense, three kinds of plant growth promoting substances have been detected in the supernatant of *Azospirillum* cultures media: auxins, cytokinins and gibberellins (14). However, the quantitatively most important phytohormone produced by these bacteria is the auxin, indole-3-acetic acid (IAA) (15). It has been found that *Azospirillum* sp. synthesizes IAA by way of several pathways (2, 16). L-tryptophan (Trp) is generally considered as the IAA precursor, because its addition to IAA-producing bacteria increases the IAA biosynthesis.

[1] Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Islamic Republic of Iran.
[2] Department of Biology, Faculty of Science, University of Guilan, Rasht, Islamic Republic of Iran.
*Corresponding author; mehdipourmoghaddam@yahoo.com

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

Bacterial phytohormone production is assumed to cause the detected changes in root morphology after *Azospirillum* inoculation, which in turn may be related to enhanced mineral uptake (13). Additionally, inoculation could result in a significant change in various growth parameters such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size, tiller numbers, and root length and volume in many plant species (7, 13). An increased number of lateral roots and root hairs enlarge the root surface available for nutrients. This results in a higher nutrient uptake by inoculated roots and an improved water status of the plant, which in turn could be the main factor enhancing plant growth (15). Among auxins, IAA is the most common and is known to stimulate both rapid responses (e.g. increases in cell elongation) and long-term responses (e.g. cell division and differentiation) in plants (6).

Some plants exudates include the amino acid L-tryptophan in their composition and it is possible for some microorganisms to produce auxin using this substrate in the rhizosphere. For example, it was demonstrated that aseptic tomato and radish roots exude between 2.8–5.3 and 290–390 ng L-tryptophan per seedling per day, respectively (11). Considering this, the aim of the present work was to identify and quantify IAA in liquid culture medium of *Azospirillum*'*s* strains isolated as endophytes of rice and wheat plants, and to investigate their effects on rice roots development. Another objective of the study was to compare the *in vitro* bacterial production of IAA in presence of L-tryptophan or root exudates collected from different plant species.

MATERIALS AND METHODS

Azospirillum sp. Isolation

Fresh root samples were obtained from the tillering stage of three cultivars of wheat (Golestan, Shirazi, Sefied) and rice (Tarom, Khazar, Hashemi) in Guilan province, Iran. Root samples were washed in fresh water for 20 min to remove the soil particles adhering to the root surface. The washed roots were surface-sterilized with a 0.5% NaClO solution for 30 min. The roots were rinsed in sterile water at least 4 times, then, cut into pieces (5–8 mm), macerated and introduced into semisolid NFb media according to Gunarto *et al.* (9). The NFB composition was: 5.0 g malic acid, 0.50 g K_2HPO_4, 0.20 g $MgSO_4.7H_2O$, 0.10 g NaCl, 20.0 mg $CaCl_2$, 2 ml micronutrients solution, 2 ml (5%) Bromothymol Blue solution, 4 ml FeEDTA, 1 ml vitamin solution, 4.0 g KOH, 1.75 g agar and 1000 ml distilled H_2O to obtain a pH of 6.8. Micronutrients solution contained 200.0 mg $Na_2MoO_4.2H_2O$, 235.0 mg $MnSO_4.7H_2O$, 280.0 mg H_3BO_3, 8.0 mg $CuSO_4.5H_2O$, 24.0 mg $ZnSO_4.7H_2O$ and 200.0 ml distilled H_2O. The vitamin solution contained 10.0 mg biotin, 20.0 mg pyridoxine and 100.0 ml distilled H_2O (9). According to Krieg and Döbereiner (12), azospirilla multiply in semisolid NFB media forming a white pellicle at 0.5-1.0 cm under the surface due to the microaerophyllic conditions to fix atmospheric nitrogen. In this sense, the isolates that presented this typical growth were considered positive.

Biochemical and Molecular Characterization of Isolates

Isolates were identified through biochemical tests (12) and amplification of 16s rDNA gene by PCR. Genomic DNA was obtained from pure cultures by proteinase K-sodium dodecyl sulfate (SDS) treatment followed by phenol-chloroform extraction and subsequent ethanol precipitation (8). The bacterial DNA was prepared for PCR amplification of the 16S rDNA using forward primer (5'-AGA GGG GCC CGC GTC CGA TTA GGT AGT T-3' location 37-64 in *Azospirillum*) and reverse primer (5'-CCC GAC AGT ATC AAA TGC AGT TCC CAG GTT-3', location 436-407 in *Azospirillum*), which were designed by Primer Premier 5 software. *Azospirillum brasilense* Sp7 and *E. coli* K-12 were used

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

as positive and negative controls, respectively. Each 25 µl of PCR reaction solution contained 2 µl (10 µM) of each primer, 2 µl of template DNA (80 ng), 0.5 µl of 10 mM dNTPs (dATP, dCTP, dGTP and dTTP), 0.5 µl (0.25 unit) of *Taq* DNA polymerase (Gen Fanavaran, Iran), 2.5 µl of 10X PCR buffer, 0.5 µl of 50 mM $MgCl_2$ and 15 µl sterile distilled water. PCR amplification was performed in an automated thermal cycler (BIO RAD, USA). The program includes an initial denaturation at 95 °C for 4 min. Thermal cycling then proceeded with 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 4 min. An aliquot of 5 µl of each PCR reaction solution was analyzed by 6% polyacrylamide gel electrophoresis. DNA fragments sizes were estimated by comparison with the standard marker 100 bp DNA ladder.

Culture Media for Auxin Production

For qualitative analysis, *Azospirillum* sp. isolates grew on Luria-Bertani (LB) or Luria-Bertani supplemented with L-tryptophan (LBT) agar media with 10.0 g Bacto-tryptone, 5.0 g yeast extract, 5.0 g NaCl, 20.0 g Bacto-agar and 1000 ml distilled water at pH 7.5. LBT was supplemented with 5 mM L-tryptophan (4). For quantitative analysis, *Azospirillum* sp. isolates were grown on liquid Trypticase soybean broth (TSB) or supplemented with different concentrations of L-tryptophan (TSBT) (3).

IAA Qualitative Determination

Two plates (9 cm diameter) containing LB and LBT media were divided into a grid pattern and inoculated individually with isolates of *Azospirillum* sp. in aseptic conditions. Each inoculated plate was overlaid with an 82 mm diameter nitrocellulose membrane immediately after inoculation, and then incubated until formation of 0.5 to 2 mm colonies. After that, membranes were removed from the plates and treated with Salkowski's reagent (2% 0.5M $FeCl_3$ in 35% perchloric acid) according to Bric *et al.* (4). Reaction was allowed at room temperature (30°C) until color appearance. Bacteria producers of IAA were identified by the formation of a characteristic pink to red halo surrounding the colony. Color and diameter of each hallo was recorded after 30 min and 2 h (4).

IAA Quantitative Determination

Two colonies per LBT plates were used to inoculate 50-ml flasks containing 10 ml 50% TSB media by duplicate (pre-inocule). The flasks were incubated for 18 h at 27°C with 100 rpm orbital agitation. Then, 125-ml flasks containing 40 ml 50% TSBT with 0, 0.1, 25, or 200 mg tryptophan to obtain a final concentration of 0, 2.5, 625 and 5000 ppm, were inoculated with 1 ml pre-inocule of each isolate. Uninoculated flasks were used as the controls. All flasks were incubated for 72 h in the dark at 27°C with 100 rpm orbital agitation. An aliquot of 1 ml of each flask was collected 24, 48, and 72 h after inoculation and centrifuged at 7160 g for 5 min at 4°C. A solution of the sample and Salkowski's reagent (1 ml $FeCl_3$ + 50 ml 35% $HClO_4$) at the ratio of 1:2 was incubated at 24°C for 25 min. Each reaction solution was centrifuged at 19700 g for 5 min to remove precipitates and/or the remaining cells. Color development was measured spectrophotometrically at 530 nm. To generate a standard curve, different solutions of pure IAA were prepared and analyzed in TSB media with final concentration of 0, 5, 10, 15, 20, 25, 30, 40, 60, 80, 100, 150, 200 and 300 ppm (3).

Biological Test on Rice Seeds

Seeds of rice (cv. Hashemi) were surface-sterilized and germinated under aseptic conditions in the dark at 20°C. The seedlings

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

were grown in Yoshida hydroponic culture using Pyrex glass desiccator flasks (6). Seedlings were placed in plastic tubes (eppendorff) of 1.5 ml capacity (perforated in the bottom to allow the root immersion in the solution). Twenty-four eppendorff tubes were fixed in the desiccator plate. The plate was placed on a desiccator base containing 1000 ml Yoshida nutrient solution. Different concentrations (0, 2%, 4%, and 9 %) of filter-sterilized culture of strain A_3 were added to the media and their effect on the development of roots was investigated (6). Data were collected for 21-day-old seedlings and subjected to ANOVA analysis and Duncan's multiple range tests to compare the growth responses of each supernatant treatment versus the control (6).

IAA Production with L-tryptophan, Plant Exudates, or Extracts

Root exudates of bean (*Phaseolus vulgaris* L.), lentil (Lens culinaris medic L.), radish (two cultivars of *Raphanus sativus* L.), rice (Oryza sativa L.), tomato (*Lycopersicum esculentum* L.), canola (Brassica napus L.) and clover (Trifolium alexandrinum L.) seedlings were used to evaluate the IAA production by Azospirillum A_3 isolate (highly IAA producer) and to compare its capacity with the principal precursor L-tryptophan (3). To this end, seeds of the different plants with the same weight (5 g) were germinated in the Petri dishes containing 8 ml sterile distilled water for 4 days. Then, 300 µl of exudates were transferred to A_3 culture flasks containing 40 ml TSB media. Besides, A_3 free flasks without exudates addition were used as blanks. In the case of root extracts, 0.01 g of plant material was macerated in 1 ml sterile distilled water and then 300 µl was transferred to the flasks. In all cases, auxin production was determined as mentioned above during three days. The experiment was repeated using 0.04 g root weight.

RESULTS

Six strains were isolated from rice and wheat roots and one was obtained from a commercial biofertilizer (*Azospirillum lipoferum*, Greenbiotech Co, Iran). *Azospirillum brasilense* Sp7 was also used. As shown in Table 1, all eight isolates were able to reduce NO_3^- and had catalase. All isolates fixed nitrogen under microaerophilic condition when malate, citrate and L-rhamnnose were used as the only sources of carbon, and formed pellicle near the semisolid NFb medium surface, too. As to the molecular characterization through 16s rDNA amplification, the expected PCR product of 400 bp was obtained in all isolates (Figure 1). The result verified that all bacteria used in this experiment were *Azospirillum* sp. according to 16s rDNA patterns. All isolates were analyzed for the auxin production capacity through quantitative and qualitative methods, both based on Salkowski reaction. In quantitative analysis 24 hours after culturing in TSB, most of the isolates produced auxin at tryptophan free flasks. Maximal auxin production was obtained for isolates A_7 and A_8 in the 625 ppm tryptophan solution, but, for the other isolates, increase was based on the tryptophan concentration increment (Figures 2 and 3). The largest amount of IAA in this period was for A_3 and A_5, respectively, and the isolate A_1 did not produce auxin in any tryptophan concentrations. This condition was repeated in 48 and 72 hours after culturing, with one exception. Amount of auxin produced by A_5 in 5000 ppm tryptophan solution decreased after 72 hours, at which time the maximal amount was obtained for A_3 and A_6, respectively. Additionally, isolate A_1 produced small amount of auxin in 625 ppm tryptophan solution after 72 hours. Qualitative analysis of auxin production on LB and LBT media in both 0.5 and 2 hours after culture showed that the maximal amount on LB belonged to A_6 followed by A_7, A_8, A_3, A_2 and A_4, respectively. Isolates

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

Table 1. Biochemical tests for identification of bacteria isolated from wheat and rice cultivars.

Characteristic	A_1 [a]	A_2	A_3	A_4	A_5	A_6	A_7	A_8
Growth with NaCl 3%	-	-	+	-	-	-	-	-
Pigment in BMS agar	Pink	-	-	-	-	-	-	-
NO_3^- reduction	+	+	+	+	+	+	+	+
Sole carbon sources:Citrate	+	+	+	+	+	+	+	+
Glucose	-	+	+	+	-	+	+	+
Mannitol	-	+	+	+	-	+	+	+
Sucrose	-	-	+	+	-	+	+	+
Myo-inositol	+	-	+	+			+	
D-Sorbitol	-	+	+	+	+	+	+	+
L-Rhamnnose	+	+	+	+	+	+	+	+
Maltose	-	+	+	+	+	+	+	+
Lactose	-	-	+	+	+	+	+	-
D-Mannose	-	+	+	+	-	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-
SIM medium: Sulfide	-	-	-	-	-	-	-	-
Indole	+	+	+	+	+	+	+	+
Motility	-	-	-	a	b	b	b	a
Alkalization in Nfb medium	-	+	+	+	+	+	+	+
Acidification in peptone-Glucose	-	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+

BMS agar: PDA+Malic Acid Agar, a: surface layer, b: surface layer+distribution

[a] A_1: *A. brasilense* Sp7 (ATCC), A_2: *A. Lipoferum* (isolate from biofertilizer), A_3: from Hashemi cultivar, A_4: from Khazar cultivar, A_5: from Tarom cultivar, A_6: from Golestan cultivar, A_7: from Shirazi cultivar, A_8: from Sefied cultivar.

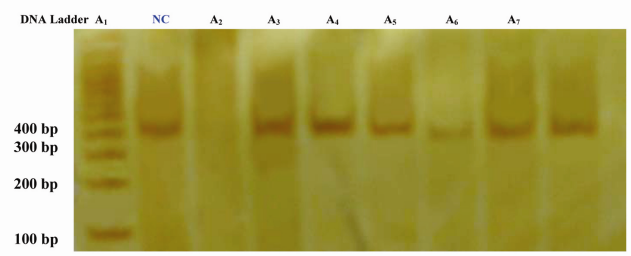

Figure 1. Typical polyacrylamide gel electrophoresis of 16s rDNAs. The amplified DNA shows a band of 400bp in an acrylamide gel. A_1: *A. brasilense* Sp7 (ATCC), NC: negative control (*E. coli* K-12), A_2: *A. lipoferum* (from biofertilizer), A_3: isolate from Hashemi cultivar, A_4: from Khazar cultivar, A_5: from Tarom cultivar, A_6: from Golestan cultivar, A_7: from Shirazi cultivar.

A_1 and A_5 did not form any halo on this media. On LBT and in both times, the maximal amount of auxin was produced by A_3 followed by A_2, A_8, A_7, A_4, A_6 and A_5. Isolate A_1 did not form any hal o on this media (Figure 4). When we studied the impact of different concentrations of A_3 culture filtrate (0, 2%, 4% and 9%) on root growth of rice (cv. Hashemi), statistical analysis did not show any significant difference between treatments with respect to root number and dry weight, although the major concentration solutions i.e.4% and 9%, had a higher root number and dry weight. Significant difference between treatments were observed for root length and wet weight at α=0.01 and α=0.05, respectively (Tables 2, 3).

When tryptophan was used as substrate for auxin production, the maximal amount of auxin was produced in the flasks containing 5000 ppm tryptophan, after 48 h (678 ppm). When plants root extracts (0.01 g) and exudates substituted pure tryptophan, auxin

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

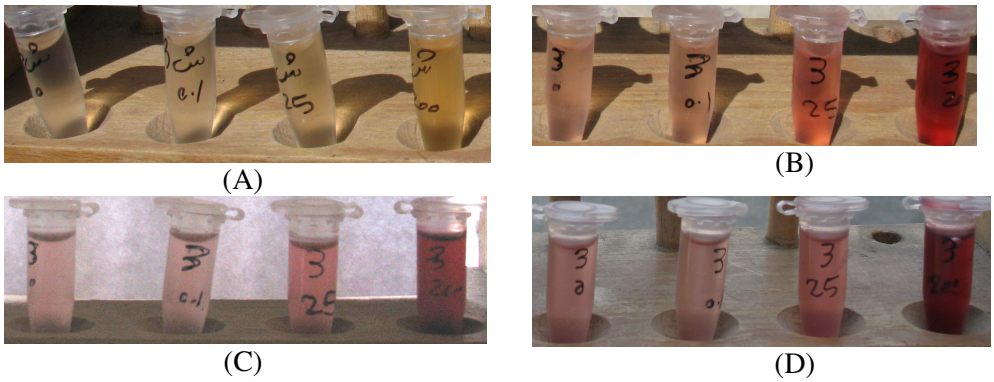

Figure 2. Auxin production by *Azospirillum* sp. isolate A_3 in different concentrations of L-tryptophan. From left to right: 0, 2.5, 625 and 5000 ppm solutions. Treatments: A) Blank, B) 24 h, C) 48 h and D) 72 h after incubation.

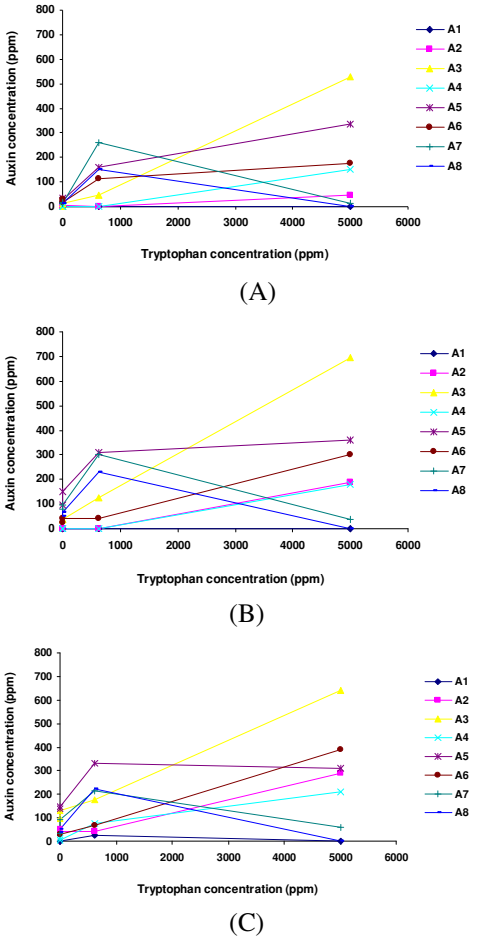

Figure 3. Auxin production by *Azospirillum* sp. isolates in TSBT media containing 0, 2.5, 625 and 5000 ppm tryptophan at (A) 24, (B) 48 and (C) 72 h after incubation. A_1: *A. brasilense* Sp7 (ATCC), A_2: *A. lipoferum* (from biofertilizer), A_3: isolate from Hashemi cultivar, A_4: from Khazar cultivar, A_5: from Tarom cultivar, A_6: from Golestan cultivar, A_7: from Shirazi cultivar, A_8: from Sefied cultivar.

produced in clover exudates was more than the other extracts or exudates at 24 h. However, in 48 h, bean root extract was at higher level. At 72 h, rice root extract promoted auxin production more than the other treatments (Figure 5). Increase in auxin production by *Azospirillum* isolate A_3 from bean and rice root extracts were 93.3% and 96.2%, respectively, in comparison with pure tryptophan. When the extracts of 0.04 g of root were used for auxin production, only canola root extract had additive effect at 72 h (88.31% increase in comparison with tryptophan control), while in other plants

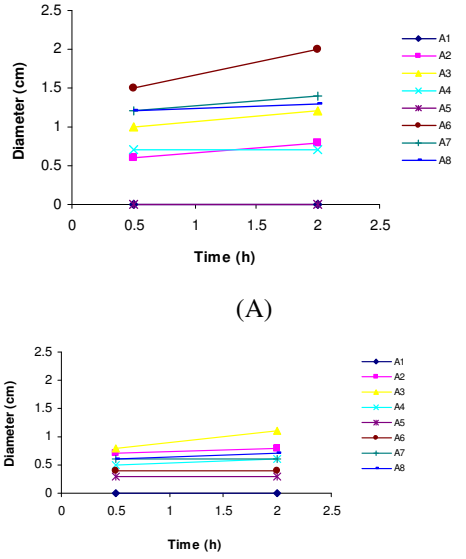

Figure 4. Auxin production by *Azospirillum* sp. isolates on LB (A) and LBT (B) media.

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

Table 2. Root growth parameters in rice (cv. Hashemi) evaluated after treatment with different concentrations of *Azospirillum* sp. strain A_3 culture filtrate.

Treatment	Specimen									
	1	2	3	4	5	6	7	8	9	10
Control										
Root No.	5	6	5	1	4	4	4	5	4	6
Root length (mm)	32	29	17	6	19	27	21	31	23	24
Wet Weight (g)	0.0080	0.0052	0.0109	0.0054	0.0023	0.0062	0.0083	0.0107	0.0128	0.0121
Dry weight (g)	0.0015	0.0012	0.0016	0.0011	0.0008	0.0011	0.0015	0.0019	0.0013	0.0012
2% concentration										
Root No.	4	6	5	7	4	7	6	9	3	5
Root length (mm)	34	56	49	74	36	54	87	73	42	39
Wet Weight (g)	0.0125	0.0089	0.0187	0.0099	0.0078	0.0076	0.0042	0.0089	0.0029	0.0014
Dry weight (g)	0.0018	0.0013	0.0019	0.0007	0.0010	0.0006	0.0015	0.0022	0.0003	0.0005
4% concentration										
Root No.	6	4	7	7	3	9	8	5	3	4
Root length (mm)	76	58	108	54	26	125	132	72	35	28
Wet Weight (g)	0.0163	0.0176	0.0169	0.0112	0.0013	0.0103	0.0306	0.0114	0.0127	0.0072
Dry weight (g)	0.0022	0.0019	0.0022	0.0011	0.0002	0.0016	0.0024	0.0011	0.0010	0.0007
9% concentration										
Root No.	5	7	5	6	4	11	9	7	11	4
Root length (mm)	31	52	64	51	69	128	88	108	97	45
Wet Weight (g)	0.0269	0.0223	0.0158	0.0073	0.0154	0.0197	0.0117	0.0080	0.0112	0.0048
Dry weight (g)	0.0024	0.0019	0.0019	0.0006	0.0013	0.0026	0.0020	0.0010	0.0013	0.0004

Table 3. Variance analysis obtained for root growth parameters in rice evaluated after treatment with filter-sterilized culture of *Azospirillum* sp. strain A_3.

Source of variation	df	Mean square			
		Root number	Root length	Root wet weight	Root dry weight
Treatment	3	0.414	5445.4**	0.000*	0.000
Error	36	0.172	716.439	0.000	0.000
Total	39				

*Significant at α=0.05, ** Significant at α=0.01.

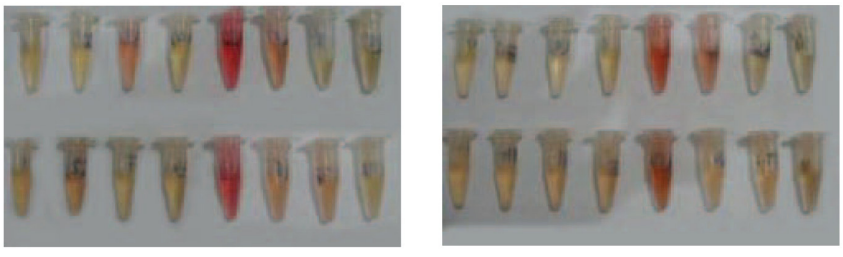

Figure 5. Colorimetric assay of auxin production by isolate A3 induced by plants root extract (0.01 g) (left) or plant exudate (right) after 48 h incubation. a) *Lensculinaris medic* L. b) *Phaseolus vulgaris* L. c) *Trifolium alexandrinum* L. d) *Brassica napus* L. e) *Oryzasativa* L. f) *Lycopersicum esculentum* L. g and h) two cultivars of *Raphanus sativus* L.

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

species, we did not observe higher auxin production.

DISCUSSION

In this study, we identified *Azospirillum* isolates with different auxin production ability and effect on rice root growth. The results in both qualitative and quantitative analysis were similar. Most of the isolates produced auxin in tryptophan free flasks. The amount of auxin produced by the isolates generally increased based on increment of tryptophan concentration, but in some isolates, auxin production decreased in high tryptophan concentrations. Harari *et al*. (10) working with two strains of *Azospirillum brasilense* Cd and IAA-overproducing mutant (FT-326) showed that both bacterial strains produced IAA in culture in the absence of tryptophan and the hormone was excreted to the growth media. IAA production by FT-326 was much higher compared to Cd, *e.g.* 36.6 *vs* 2.9 µg ml $^{-1}$, on the 4th day, respectively. In FT-326 IAA was mainly excreted during the stationary phase and its concentration decreased gradually after the onset of the decline phase. Crozier *et al*. (5) investigated the production of IAA by *A. brasilense* 703 Ebc through analyzing centrifuged culture media with the Salkowski's reagent and indicated that, after 20 h incubation, the liquid media contained more than 20 µg of IAA ml^{-1}. El-Khawas and Adachi (6) reported that *Azospirillum brasilense* produced the maximum of 46 ppm auxin in 100 µg ml^{-1} tryptophan at 72 h. Akbari *et al*. (1) showed which *Azospirillum brasilense* produced a maximum of 46 ppm auxin in 1.2115 µg l^{-1} tryptophan at 24 h. It has been found that bacteria synthesize IAA by way of several pathways and the operation of more than one pathway in certain species has been proposed. Tryptophan (Trp) is generally considered as the IAA precursor, because its addition to IAA-producing bacterial cultures promotes an increase in IAA synthesis.

About the effect of auxin on the rice root growth, we studied the impact of different concentrations of A_3 (isolate with high auxin production ability) culture filtrate on root growth of rice (cultivar Hashemi). Statistical analysis did not show any significant difference between treatments with respect to root number and dry weight, but significant difference was observed between treatments in the case of root length and wet weight at α=0.01 and α=0.05, respectively. In all cases, the higher average was for 4 and 9 percent concentrations. El-Khawas and Adachi (6) studied the effect of different concentrations of filter-sterilized culture supernatant of *Azospirillum brasilense* on the development of rice roots grown in hydroponic culture media. Addition of the optimum concentrations (6–8%) of bacterial supernatant to such hydroponic cultures increased root elongation, root surface area, root dry matter, and development of lateral roots and root hairs compared to the untreated roots. On the other hand, addition of high concentrations of the supernatant (more than 10%) strongly inhibited root elongation, lateral root development, and caused root outgrowths, i.e. round nodule-like tumors. In *Panicum* as well as in other grasses, inhibition of root elongation by *A. brasilense* is usually associated with root hair proliferation. In sorghum, increasing concentrations of *A. brasilense* increased the density and the length of root-hairs (6). At higher ($\geq 10^8$ CFU ml $^{-1}$) bacterial concentrations, root-hairs covered all the elongation region of the root, including the meristematic region near the root cap.

Aseptic tomato and radish roots were found to exude, respectively, 2.8–5.3 and 290–390 ng tryptophan per seedling per day (11). The inoculation of radish plants with rhizosphere pseudomonads increased the root biomass by 1.4 times. The inoculation of tomato plants with the same pseudomonads was ineffective. The beneficial effect of bacterial inoculation on the radish plants was explained by the fact that the introduced rhizobacteria produced

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

the plant growth–stimulating hormone indole-3-acetic acid. In pot experiments, the addition of this phytohormone to the soil increased the mass of radish roots by 36% (11).

In this study, some plants root extracts or exudates were used as substrate for auxin production by A_3 isolate as bean, rice and clover roots that promoted auxin production more than other plants root. These substrates had about 95% additive effect on auxin production in comparison with pure tryptophan. It was demonstrated that rice and bean root extracts were suitable substitutions for tryptophan. When extracts of 0.04 g root weights were used for auxin production, only canola root extract had additive effect on auxin production (in 72 h) while for other plants, we did not observe increment of auxin production. Seemingly, this was because of the suppression effect of auxin or other root products on *Azospirillum* isolates or auxin biosynthesis enzymes. We concluded that it was not necessary to use higher amount of roots (0.04 g); even 0.01 g can be effective and can substitute tryptophan, an expensive substrate.

CONCLUSION

By identifying suitable local isolates containing high auxin production and other important characteristics needed for their commercial use, it is possible to apply these selected isolates in the plant production systems such as hydroponic cultures, greenhouse, and the fields for achieving better plant growth and, consequently, higher yield. We also identified that some plants root extracts or exudates can be good and cheap substitutions for tryptophan in order to produce auxin by *Azospirillum* isolates.

ACKNOWLEDGEMENTS

This work was supported by grants from University of Isfahan. The authors are grateful for this support.

REFERENCES

1. Akbari, G. A., Arab, S. M, Alikhani, H. A, Allahdadi, I. and Arzanesh, M. H. 2007. Isolation and Selection of Indigenous *Azospirillum* Spp. And the IAA of Superior Strains Effects on Wheat Roots. World *J. Agr. Sci.*, **3**: 523-529.
2. Bashan, Y., Holguin G. and Bashan, L. D. 2004. *Azospirillum*–plant Relationships: Physiological, Molecular, Agricultural, and Environmental Advances. *Can. J. Microbiol.*, **50**: 521–577.
3. Bent, E., Tuzun, S., Chanway, C. and Enebak, S. 2001. Alternation in Plant Growth and in Root Hormone Levels of Lodge Pole Pines Inoculated with Rhizobacteria. *Can. J. Microbiol.*, **47**: 793-800.
4. Bric, J. M., Bostok, R. M. and Silverstone, S.A. 1991. Rapid in Situ Assay for Indoleacetic Acid Production by Bacteria Immobilized on a Nitrocellulose Membrane. *Appl. Environ. Microbiol.*, **57**: 535-538.
5. Crozier, A., Arruda, P., Jasmim, J. M., Monteiro, M. and Sandberg, G. 1988. Analysis of Indole-3-Acetic Acid and Related Indoles in Culture Media from *Azospirillum lipoferum* and *Azospirillum brasilense*. *Appl. Environ. Microbiol.*, **54**: 2833-2837.
6. El-Khawas, H. and Adachi, K. 1999. Identification and Quantification Of Auxins in Culture Media of *Azospirillum* and *Klebsiella* and Their Effect on Rice Roots. *Biol. Fertil. Soils*, **28**: 377–381.
7. Gadagi, R. S., Krishnaraj, P. U., Kulkarni, J. H. and Tongmin, S. 2003. The Effect of Combined *Azospirillum* Inoculation and Nitrogen Fertilizer on Plant Growth Promotion and Yield Response of the Blanket Flower *Gaillardia pulchella*. Sci. Hortic., **100**: 323–332.
8. Gliesche, C.G., Menzel, M. and Fesefeldt, A. 1997. A Rapid Method for Creating Species-Specific Gene Probes for Methylotrophic Bacteria. *J. Microbiol. Meth.*, **28**: 25-34.
9. Gunarto, L., Adachi, K. and Senboku, T. 1999. Isolation and Selection of Indigenous *Azospirillum* Spp. from a Subtropical Island and Effect of Inoculation on Growth of Lowland Rice under Several Levels of N Application. *Biol. Fertil. Soils*, **28**: 129–135.

[DOR: 20.1001.1.16807073.2012.14.5.16.4]

10. Harari, A., Kigel, J. and Okon, Y. 1988. Involvement of IAA in the Interaction Between *Azospirillum Brasilense* and *Panicum Miliaceum* Roots. *Plant Soil*, **110**: 275-282.
11. Kravchenko, L. V., Azarova, T. S., Makarova N. M. and Tikhonovich, I. A. 2004. The Effect of Tryptophan Present in Plant Root Exudates on the Phytostimulating Activity of Rhizobacteria. *Microbiology*, **73**: 156-158.
12. Krieg, N. R. and Döbereiner, J. 1984. Genus *Azospirillum.* In: Bergey's Manual of Systematic Bacteriology. N.R. Krieg (ed.), Vol. **1**: 94-104. Williams & Wilkins, Baltimore.
13. Okon, J. 1985. *Azospirillum* as a Potential Inoculant for Agriculture. *Trends Biotech.*, **3**: 223-228.
14. Perrig, D., Boiero, L., Masciarelli, O., Penna, C., Ruíz, O., Cassán F. and Luna V. 2007. Plant Growth Promoting Compounds Produced by two Agronomically Important Strains of *Azospirillum Brasilense*, and Their Implications for Inoculant Formulation. *Appl. Microbial. Biotech.*, **75**:1143–1150.
15. Steenhoudt, O. and Vanderleyden, J. 2000. *Azospirillum*, a Free-Living Nitrogen-Fixing Bacterium Closely Associated with Grasses: Genetic, Biochemical and Ecological Aspects. *FEMS Microbiol. Rev.*, **24**: 487-506.
16. Zakharova, E. A., Shcherbakov, A. A., Brudnik, V. V., Skripko, N. G., Bulkhin, N. S. and Ignatov, V. V. 1999. Biosynthesis of Indole-3-Acetic Acid in *Azospirillum Brasilense*. Insights from Quantum Chemistry. *Eur. J. Biochem.*, **259**: 572-576.

تحریک تولید اکسین از کشت‌های خالص آزوسپیریلوم توسط ترشحات ریشه گیاه

م. ج. مهدی‌پورمقدم، گ. امتیازی و ز. صالحی

چکیده

باکتریهای جنس آزوسپیریلوم به عنوان ریزوباکتری‌های محرک رشد گیاهان شناخته شده‌اند. توانایی تولید هورمون‌های گیاهی به عنوان یکی از مهمترین مکانیسم‌های تحریک رشد گیاهی شناخته شده است و به طور وسیعی در میان باکتریهای همیار ریشه وجود دارد. مهمترین هورمون گیاهی تولیدی توسط آزوسپیریلوم، ایندول-۳- استیک اسید است که از پیش‌ساز ال-تریپتوفان تولید می‌گردد. لذا در این تحقیق توانایی تولید اکسین از اگزودای گیاهی توسط هشت جدایه آزوسپیریلوم از برنج و گندم مورد بررسی قرار گرفت. نتایج نشان داد که اغلب جدایه‌ها در محیط‌های فاقد تریپتوفان تولید اکسین نمودند، اما مقدار اکسین تولیدی توسط جدایه‌ها اغلب با افزایش غلظت تریپتوفان افزایش یافت. ترشحات ریشه بعضی از گیاهان از اثرات مشابه تریپتوفان برای تولید اکسین برخوردار بودند. نتایج نشان داد که ترشحات ریشه لوبیا، برنج و کلزا به ترتیب ۹۳/۳، ۹۶/۲ و ۸۸/۳۱ درصد اکسین بیشتری را در مقایسه با تریپتوفان خالص تولید نمودند. سویه آزوسپیریلوم A_3 توانایی تولید اکسین بالایی داشت. تأثیر سوپرناتانت بدون سلول این جدایه روی توسعه ریشه بررسی گردید. تجزیه آماری هیچ تفاوت معنی‌داری را بین تیمارها برای تعداد ریشه و وزن خشک نشان نداد، اما تفاوت معنی‌دار بین تیمارها برای طول ریشه و وزن تر به ترتیب در سطوح آماری $\alpha=0.01$ و $\alpha=0.05$ مشاهده گردید.

[DOR: 20.1001.1.16807073.2012.14.5.16.4]