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Activity screening of plant growth promoting rhizobacteria isolated from alfalfa rhizosphere

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Abstract

Introduction: Some rhizobacteria by various mechanisms influence plant growth as they are called plant growth promoting rhizobacteria (PGPR). Scientists identified some PGPR characters involved in promoting plant growth, while all these characters are not able to study. The aim of this study was to evaluate PGP activities of bacterial isolates, (45 isolates belonged to *rhizobium* and 2 bacterial isolates belonged to *Pseudomonas fluorescens*), which were isolated from alfalfa (*Medicago sativa*) rhizosphere and root nodules grown around Zanjan.

Materials and methods**:** These bacteria were isolated from alfalfa roots grown around Zinc industries in Zanjan province. After bacterial isolation and purification from root and soil samples, isolates were screened in vitro for plant growth promoting traits such as IAA (Indole Acetic Acid), ACC- deaminase (Amino Cyclopropan Carboxylate), HCN (Hydrogen Cyanide), siderophore, chitinase production and mineral and organic phosphate solubilization activities.

Results: The results indicated that 43 bacterial isolates produced IAA (4.04- 4.95 µg/ml) and 15 isolates produced ACC- deaminase (0.23- 1.05 µg/ml). Only one isolate (Rm66) produced high amount of HCN. Qualitative siderophore production was observed in 9 isolates. None of the isolates produced chitinase. Solubilization of mineral phosphate was commonly detected in 19 isolates (4.33- 5.86 µg/ml), and 15 isolates solubilized organic phosphate (1.66- 144.28 µg/ml).

Discussion and conclusion: This study shows that most of the bacterial strains which isolated from alfalfa cultivated lands had PGP activities and also a good potential to increase plant growth after inoculation with to seeds as eco- friendly fertilizers.

Key words: ACC- deaminase, Chitinase, HCN, IAA, PGPR, Siderophore

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Introduction

Many microorganisms live in 'rhizosphere' as the part of soil modified or influenced by plant roots (1). Some of them such as *Azospirillum*, *Azotobacter Pseudomonas* and several gram positive *Bacilli* have positive effects on plants and therefore, are called plant growth promoting rhizobacteria (PGPR) (1). In various cropping systems, PGPR are directly or indirectly involved in increasing plant growth, as well as inducing resistance to pathogens and pests (2). The direct promotion of plant growth by PGPR can be either by providing the bacterial synthesized compound or by facilitating the uptake of certain nutrients from the environment. Some of direct effects include providing the host plant with i) fixed nitrogen, ii) soil phosphorus and iron solubilization iii) bacterial synthesized phytohormones such as auxins, cytokinins and gibberelins (3). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. It was suggested that soil bacteria that indirectly stimulate plant growth be referred to as biocontrol- PGPB or biocontrol plant growth- promoting bacteria versus directly plant growth promoting bacteria as PGPB (4). In fact, there is a complex interaction between plant roots and soil microbes (5). So a part of photosynthesis products were released from root to soil, which were defined as rhizodeposition (6). It provides nutrients for soil microorganisms and is the main factor of rhizosphere creation and also plays a vital role in activity, population and the relative composition of species (7).

Detection of bacteria other than *rhizobia* in root nodules was first reported by Beijerinck and Van Delden in the clover plants to be as *Agrobacterium radiobacter* (8). Manninger and Antal also detected *rhizobia* and other bacteria in the root nodules of legumes (9). Many studies have shown that simultaneous infection with *rhizobia* and the other rhizospheric bacteria increase nodulation and growth in a wide variety of legumes (10- 12). Co-inoculation of chickpea (*Cicer arietinum)* with some *Pseudomonas* and *Bacillus* strains along with effective *Rhizobium* spp. stimulated plantgrowth, nodulation and nitrogen fixation (13).

Production of plant growth regulators such as auxin, cytokinin and gibberellin by PGPR has also been suggested as a possible mechanism to promote plant growth and development (14). As some bacteria, such as *Pseudomonas* can stimulate plant growth by the production of indole- 3- acetic acid (IAA) (15- 17). In this regard, Patten and Glick showed that the production of IAA by *Pseudomonas. putida* was closely linked to optimal development of the root system in canola (*Brassica napus* L.) and mung bean (*Vigna radiata*) (18). As previous studies showed, rice is on of the most important stable crops in the developing world, and needs nitrogen as the most important essential nutrition (19). To make rice production sustainable and less dependent on nitrogen fertilizer, it is extremely important to know how to use PGPR fixing nitrogen and producing substances, as indole- 3- acetic acid and siderophores to improve rice growth (17).

To evaluate the efficacy of plant growth promoting bacteria (*Azospirillum sp*.) on canola crop, an experiment was conducted. Results indicated that *Azospirillum sp*. had positive effects on yield and yield components of oilseed rapes (20).

Azotobacter is a plant growth promoting rhizobacterium that can stimulate the growth of plants by different mechanisms. 10 isolates of *Azotobacter chroococcum* native to Iranian soils, were used for field experiments. In Azerbaijan Sharghi, 9 isolates increased the total wheat yield, in Khorasan Razavi only one isolate (T11) increased yield of straw and stubble. In Fars, T8 isolate had the highest seed yield and shoot dry weight. There was not significant effect of inoculation on growth indices of wheat in Kordestan and Azerbaijan gharbi provinces (21). *Pseudomonas fluorescens* is the most important plant growth promoting rhizobacteria. Thirty four *Pseudomonas fluorescens* isolates were isolated from pistachio orchards located in Rafsanjan. The PGP of all isolates were evaluated. Inoculation of pistachio seedlings with superior isolates increased the seedlings height, leaf area, shoot and root dry weights and concentrations of P, Fe, Zn, Mn and Cu in shoot significantly (22).

Enhancement of legume nitrogen-fixation by co- inoculation of *rhizobia* and plant growth promoting bacteria (PGPB) is important for improving nitrogen availability in sustainable agricultural systems. The aim of this study was to evaluate PGP activities of bacteria isolated from rhizospheric soil and plant root nodules of alfalfa (*M. sativa*) in Zanjan province.

Materials and methods

In order to isolate bacterial strains, rhizospheric soil and plant root nodules, samples were firstly collected from alfalfa (*M. sativa*) cultivated around Zanjan province. Then serial dilutions of samples were prepared. Dilutions were inoculated on plates which contain nutrient agar media. Inoculated plates were incubated at 28^{o}C for one week. Grown colonies were transfered to new plates containing Nutrient Agar (NA) media. After several subculturing, finally pure isolates were obtained. Isolates tested in the present study were grown on Nutrient Agar medium for their viability and maintained in Nutrient Broth (NB) medium with 15% glycerol at -80ºC for long- term storage. From the isolated bacterial strains, Bv1 and Bv2 were *Pseudomonas* and the other bacterial strains from Rm1 to Rm45 were *Rhizobium*. It should be noted that all measurements had 3 replications.

Assay for IAA production: IAA production was detected by the method described by Pattern and Glick (18). Each bacterial isolate was first grown in TSB (Trypton Soy Broth) medium (dextrose, 2.5 g; K_2HPO_4, 5 g; NaCl, 2.5 g; soybean peptone, 3 g; tryptone, 17 g; distilled water, 1 L; pH=7.2) for 48 h. Bacteria were then grown for 72 h on TSB medium amended with 25 µg/ml of L- tryptophane at about 28ºC. Fully grown cultures were centrifuged at 10000 g for 2 min. The culture supernatant (1 ml) was mixed with 4 ml of Salkowski reagent (concentrated sulfuric acid 150 g; distilled water 250 ml; 0.5 M $FeCl_3.6H_2O$ 7.5 ml). Tubes

containing the mixture were standed for 20 min, for color development. Optical density was read at 535 nm using spectrophotometer (Beckman DU 530). Concentration of IAA was measured with the help of standard graph of IAA obtained in the range of 0- 6 mg/ml. (18).

ACC- deaminase production: Production of ACC deaminase was determined as described by Amico et al. (23). Bacterial strains producing ACC- deaminase, use amino cyclopropane-1 carboxylic acid (ACC) as a nitrogen source. In this method 50 ml of fresh bacterial suspension was added to 20 ml DF (glucose, 2g; gluconic acid, 2g; citric acid, 2g; Na_2HPO_4, 6g; KH_2PO_4, 4g; $ZnSO_4.7H_2O$, 0.124mg; $MnSO_4.7H_2O$, 0.10mg; H_3BO_3, 0.10mg; $FeSO_4.7H_2O$, 1 mg; $MgSO_4.7H_2O$, 0.01mg; ; $CuSO_4.7H_2O$, 0.078mg; MoO_3, 0.01mg) medium containing 3 mM ACC, DF medium containing ammonium sulfate and DF medium without ACC and ammonium sulfate. After 48h, light absorbance was measured at 405 nm wave length by spectrophotometer in three mentioned media. The ratio of absorbance in ACC containing DF medium to absorbance of ammonium sulfate containing DF medium considered as ACC- deaminase production index.

HCN production: All bacterial strains were screened for the production of hydrogen cyanide (HCN) using the method described by Donate- correa et al. (24). At first each bacterial strain was cultured in TSA medium (peptone from casein 15 g; peptone from soymeal 5 g; sodium chloride 5 g; agar 15 g; distilled water 1000 ml) and glycine was added as precursor of hydrogen cyanide. Whatman filter papers no.1 soaked in sodium picrate solution (picric acid 0.5%; sodium carbonate 2%) was placed in the top of the plate and then plates were sealed with Parafilm and incubated at 28°C for 120 h. Development of light brown (low HCN production) to dark brown color (high HCN production) indicated production of hydrogen cyanide.

Siderophore production: Bacterial isolates were assayed for siderophore production on the Chrome azurol S agar medium as described by Alexander and Zuberer (15). For this medium, four distinct solutions (Fe- CAS [Chrome Azurol S] indicator solution; buffer solution; nutrient solution.

Casaamino acid solution: Casamino acids are a mixture of amino acids and some very small peptides obtained from acid hydrolysis of casein) were prepared separately, sterilized and then mixed with each other. Fresh cultures were plated onto blue- agar CAS plates, buffered with 0.1 M piperazin-1, 4-bisethanesulfonic acid (PIPES). In this media 10 mM $FeCl_3$ is complexed by 10 Mm chrome azurol S in the presence of 0.2 mM hexadecyltrimethylammonium bromide (HDTMA). Chrome azurol S agar plates were prepared and divided into equal sectors and spot inoculated with isolates and incubated at 28°C for 48 h. Development of orange halos around colonies was determined and considered as an ability of siderophore production.

Chitinase production: Chitinase production was determined as described by Boller and Mauch (25). Bacterial suspension was spot inoculated on nutrient agar plates which amended with 0.1% colloidal chitin and zone of clearance was recorded after incubation at 28°C for 120 h.

Mineral phosphate solublization: Bacterial isolates previously grown in TSB medium were dropped into the basal Sperber (26) medium (glucose, 10 g; yeast extract, 0.5 g; $MgSO_4.7H_2O$, 0.32 g; $CaCl_2$, 0.14 g; $Ca_3(PO_4)_2$, 2.5 g; Agar, 15 g; distilled water, 1000 ml; pH=7.2) and incubated for 7 days at 28°C. Those isolates that formed visible clearing halos zone around their colonies were considered phosphate solubilizers, so their ratio of halo zone diameter to colony diameter was recorded. Quantitative analysis of mineral phosphate solubilization, in Sperber broth medium was made as described by Bano and Musarrat (3). The isolates were inoculated in 50 ml Sperber broth medium and shacked (125 rpm) for 120 h at 28°C. After this period, pH was measured and also bacterial suspensions were centrifuged at 10000 g for 10 min. 1ml of culture supernatant was placed into test tubes containing 3ml of distilled water, 1 ml ammonium molibdate- vanadate indicator and 1 ml of 1.5% ascorbic acid solution. The tubes containing the mixture were allowed to stand for 20min, for color development. The absorbance of yellow color was read at 470 nm using spectrophotometer (Beckman DU 530). The amount of soluble phosphate was measured from the standard curve of KH_2PO_4.

Organic phosphate solubilization: Quantitative and qualitative methods which were used to evaluate bacterial isolates capability to solubilize organic phosphate were same as methods described for mineral phosphate, except that Inositol hexaphosphoric acid was used instead of $Ca_3(PO_4)_2$ (26).

Results

In this study, 47 bacterial isolates were studied for their PGP activities. On the basis of morphological and biochemical characteristics, two bacterial isolates were grouped into *Pseudomonas fluorescent* and they were shown as Bv1 and Bv2. A total of 45 nodule isolates of alfalfa were characterized as *Sinorhizobium* and they were shown as Rm1 to Rm45 (Table 1).

Quantitative assay of IAA production: The results of this study showed that 91% of bacterial isolates (43 isolates) had the ability of IAA production. Mean amount of auxin production was 4.29 µg/ml and the range of production was between 4.04 µg/ml and 4.95 µg/ml. From the bacterial isolates, Rm4 produced the highest amount of IAA (4.95µg/ml) and Rm33, Rm41, Rm42, Bv2 and, Rm3 produced lowest amount (4.04 µg/ml), while Rm16, Rm20, Rm39 and Rm44 didn't produce any IAA (Table 1).

Assay of siderophore production: Only 9 bacterial isolates of total 47 isolates (19%) produced visible orange halo zones around the colonies, showing that they were able to produce siderophore. The ratio of halo zone diameter to colony diameter ranged between 1.28 and 2. The lowest and highest

ability of siderophore production (ratio of halo zone diameter to colony diameter) belonged to isolates Rm7and Rm43 respectively (Table 1).

Assay of ACC- deaminase production: ACC- deaminase production was detected in 15 isolates and 32% out of total bacterial isolates were able to produce this enzyme. The range of production was 0.16- 1.05 µg/ml. Isolate Rm42 produced the lowest amount, while Rm40 produced the highest amount of ACC- deaminase (Table 1).

HCN production: The results of this study showed that HCN production ability in 24 isolates (51%) was weak, in 20 isolates (42%) was medium, in 2 isolates (4%) was high and in only one isolate (2%) (Rm42) was very high and the color filter paper changed to dark brown (Table 1).

Chitinase activity: The results of investigation showed that none of the bacterial isolates were able to produce chitinase, so all of them were known as chitinase negative isolates (Table 1).

Phosphate solubilization: The results of this study showed that 19 isolates (40%), were able to solubilize mineral phosphate. The highest amount of this ability belonged to Rm42 (96.41 µg/ml) and the lowest amount belonged to Rm15 (21.86 µg/ml). When a strain was active in solubilizing mineral phosphate, as a consequence of this effect, pH medium decreased more. Isolate Bv1 decreased the pH value of medium from 7.2 to 4.33 and was the most active strain in this way. Among the total bacterial isolates, 15 strains (32%) were able to solubilize organic phosphate (Table 2).

Thus it is possible that decreasing media pH might be due to mineral phosphate solublization activities (organic acids production) of bacterial isolates, and the relationship between $Ca_3(PO_4)_2$ solublization (µg/ml) and pH can be shown as Fig. 1. Most of the isolates had the ability of auxin production (more than 90%) while none of them produced chitinase enzyme (Fig. 2).

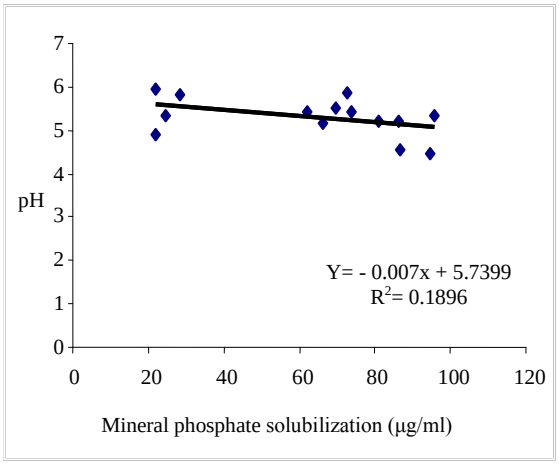

Fig. 1- Correlation between phosphate solublization (µg/ml) and pH decreasing

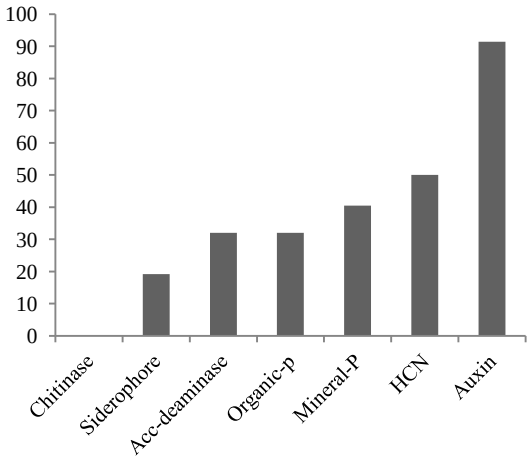

Fig. 2- Percentage (%) of tested isolates that had different PGP activities

Table 1- Production of IAA, siderophore, HCN, ACC- deaminase and chitinase by selected bacterial isolates*

Isolates	IAA production (µg/ml)	siderophore production**	ACC- deaminase (µg/ml)	HCN production***	chitinase production
Rm1	4.73	1.5	0.52	2	-
Rm2	4.48	-	-	2	-
Rm3	4.04	-	-	2	-
Rm4	4.95	-	-	1	-
Rm5	4.48	-	-	1	-
Rm6	4.40	1.66	-	3	-
Rm7	4.62	1.28	0.35	1	-
Rm8	4.18	-	-	1	-
Rm9	4.62	1.71	0.45	2	-
Rm10	4.48	-	0.57	2	-
Rm11	4.22	-	-	1	-
Rm12	4.91	-	-	1	-
Rm13	4.55	-	0.41	2	-
Rm14	4.26	-	-	1	-
Rm15	4.00	-	-	2	-
Rm16	-	-	-	1	-
Rm17	4.15	1.4	-	1	-
Rm18	4.18	-	0.40	2	-
Rm19	4.26	-	-	1	-
Rm20	-	-	-	2	-
Rm21	4.40	-	0.69	2	-
Rm22	4.15	-	-	1	-
Rm23	4.33	-	-	1	-
Rm24	4.73	-	0.50	1	-
Rm25	4.33	-	-	2	-
Rm26	4.73	-	-	3	-
Rm27	4.62	-	-	1	-
Rm28	4.84	1.5	-	1	-
Rm29	4.73	-	0.30	1	-
Rm30	4.08	-	-	1	-
Rm31	4.48	-	-	1	-
Rm32	4.44	-	-	1	-
Rm33	4.04	-	-	2	-
Rm34	4.15	1.75	-	2	-
Rm35	4.88	-	-	1	-
Rm36	4.18	-	-	2	-
Rm37	4.77	-	-	1	-
Rm38	4.37	-	-	2	-
Rm39	-	-	0.76	2	-
Bv1	4.15	-	-	2	-
Bv2	4.04	-	0.64	2	-
Rm40	-	-	1.05	1	-
Rm41	4.04	-	-	1	-
Rm42	4.04	1.8	0.16	4	-
Rm43	4.74	2	0.23	2	-
Rm44	-	-	0.39	1	-
Rm45	4.11	-	-	2	-
Rnage	4.04 - 4.95	1.28 - 2	0.16 - 0.76	1 - 4	-
Mean	4.29	1.62	0.49	1.72	-

* It should be noted that all measurements had 3 replications

** The ratio of orange halo zones diameter to colony diameter in CAS- agar medium

*** The color of filter paper in the test for 1, 2, 3, and 4 was yellow, cream, light brown, and dark brown respectively.

Table 2- Mineral and organic phosphate solubilization activities among selected bacterial isolates*

strains	Mineral phosphate solublization			Organic phosphate solublization		
	Soluble phosphate concentration (µg/ml)	pH of medium	halo diameter/ colony diameter	halo diameter/ colony diameter	Soluble phosphate concentration (µg/ml))	pH of medium
Rm1	-	-	-	1	1.66	6.20
Rm2	24.67	5.33	1.67	2.15	81.42	4.4
Rm3	-	-	-	-	-	-
Rm4	-	-	-	-	-	-
Rm5	28.32	5.82	1.26	1.41	24.28	5.42
Rm6	22.12	5.86	1.34	2.3	94.28	4.97
Rm7	-	-	-	1.10	7.14	5.91
Rm8	66.43	5.15	1.41	-	-	-
Rm9	-	-	-	1.46	41.42	5.36
Rm10	-	-	-	1.20	12.85	5.82
Rm11	92.96	4.48	1.86	-	-	-
Rm12	-	-	-	-	-	-
Rm13	69.77	5.5	1.42	-	-	-
Rm14	-	-	-	-	-	-
Rm15	21.86	5.21	1.86	-	-	-
Rm16	95.67	5.53	1.45	-	-	-
Rm17	-	-	-	-	-	-
Rm18	-	-	-	-	-	-
Rm19	94.7	5.44	1.67	2.13	80	4.60
Rm20	-	-	-	-	-	-
Rm21	-	-	-	-	-	-
Rm22	80.86	5.45	1.39	-	-	-
Rm23	-	-	-	-	-	-
Rm24	-	-	-	-	-	-
Rm25	71.73	5.53	2.56	1.51	42.85	5.40
Rm26	72.52	5.86	1.34	-	-	-
Rm27	-	-	-	-	-	-
Rm28	-	-	-	-	-	-
Rm29	-	-	-	-	-	-
Rm30	-	-	-	1.44	58.57	5.12
Rm31	86.21	5.29	1.26	1.11	8.57	5.82
Rm32	62.24	5.43	1.11	-	-	-
Rm33	-	-	-	-	-	-
Rm34	-	-	-	-	-	-
Rm35	-	-	-	-	-	-
Rm36	-	-	-	-	-	-
Rm37	-	-	-	-	-	-
Rm38	-	-	-	1.97	77.14	4.94
Rm39	-	-	-	-	-	-
Bv1	82.22	4.33	1.9	2.97	144.28	4.15
Bv2	85.72	4.36	2.01	2.71	127.14	4.30
Rm40	-	-	-	-	-	-
Rm41	-	-	-	-	-	-
Rm42	96.41	4.67	1.2	-	-	-
Rm43	84	5.86	1.5	-	-	-
Rm44	82.85	5.04	1.3	2.16	82.85	4.86
Rm45	-	-	-	-	-	-
Range	21.86 - 96.41	4.33 – 5.86	1.11 – 2.56	1-2.97	1.66 – 144.28	4.15-6.20
Mean	69.54	5.21	1.56	1.77	58.96	5.15

* It should be noted that all measurements had 3 replications.

Discussion and conclusion

The rhizosphere is a favored ecological niche for various types of soil microorganisms due to rich nutrient availability (1). There is a complex interaction between plant roots and soil microbes. Plant released some photosynthesis products from root to soil as rhizodeposition. Rhizodeposition provides nutrients for soil microorganisms and microbes through different mechanisms improve plant growth.

Sarwar and Kremer reported that isolates from the rhizosphere were more efficient auxin producers than isolates from bulk soil (27). Moreover, it has been reported that IAA production by plant growth promoting rhizobacteria (PGPR) can vary among different species and strains, and that it is also influenced by culture condition, growth stage and substrate availability (28). Barazani and Friedman (29) reported some PGPRs were able to secrete up to 13.5 μg IAA ml^{-1}, Strains of *Enterobacter* sp. isolated from the rhizosphere of sugarcane *(Saccharum officinarum)* produced about 2.21 μg IAA ml- 1 in vitro, when tryptophan was added to the medium (28). As IAA production by the isolates was measured in a medium containing tryptophan, it concludes that several of the bacterial isolates in the present study are IAA producers, because the mean amount of IAA production in this study was 4.295 μg IAA ml^{-1}.

The ability of bacteria to solubilize mineral phosphate and produce siderophores has long been of interest to agricultural microbiologists, as it can enhance the availability of phosphorus and iron for microbial and/or plant growth. Therefore, the ability of rhizobacteria to solubilize fixed phosphates and enhance its availability to rice represents a possible mechanism of plant growth promotion under field conditions (17). Only 19 out of 47 isolates (40.42%) were able to solubilize mineral phosphate while 15 isolates (31.91%) were able to solubilize organic phosphate. It has long been known that a considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere, in comparison to non- rhizospheric soil (30). Although several phosphate solubilizing bacteria occur in soil (31), their numbers are not usually high enough to compete with other bacteria commonly established in the rhizosphere (32). Siderophore production is another PGP feature that may influence plant growth by binding to the available iron form (Fe^{3+}) in the rhizosphere. Through this process, iron is made available to plants, but unavailable to phytopathogens. Thus, at the same time, the siderophore protects the plant health (33). Of the 47 baterial isolates tested in the current study, 9 isolates (19.15%) were able to produce siderophores. It has often been assumed that competition for Fe in the rhizosphere is controlled by Fe affinity of the siderophores, whereby the ligands produced by the biocontrol agents have higher formation constants than those of the pathogen. Other important factors include the concentrations of the various

siderophores involved, the kinetics of exchange, and the availability of Fe complexes to microorganisms that are present (34). The kind of siderophores produced by these isolates and their affinities for different Fe complexes will be important to further investigate.

Free- living as well as symbiotic plant growth promoting rhizobacteria (PGPR) can improve plant growth, competitiveness and responses to environmental stresses (25, 35 & 36). Ethylene is important for plant growth, but excessive ethylene promoted by stresses would be a kind of preventer. A prominent mechanism used by many PGPR to facilitate plant growth is to reduce the level of this compound by consuming (ACC), the immediate precursor of ethylene, through the synthesis of ACC deaminase (ACCD) (11). In this study, of 47 isolates, only 15 bacterial isolates (36) were able to produce ACC deaminase. Thus, bacterial isolates which have ACCD activity can partially prevent any reduction in the length of plant roots and shoots, and in biomass caused by high ethylene levels (37- 39). HCN is a potent inhibitor of cytochrome- C oxidase and of several other metalloenzymes. No role is known for HCN in primary bacterial metabolism and it is generally considered as a secondary metabolite (10). HCN producing bacteria can help plants in their defense against fungal pathogens (10 & 12). This property was predominantly described among *Pseudomonas* strains (40). In the present investigation, only one of root nodule isolated *Sinorhizobium* was a potential HCN producer. The physico- chemical conditions prevailing in the rhizosphere could explain this stimulation of HCN producing bacteria. Indeed, Castric showed that the bacterial ability to produce HCN was enhanced under microoxic conditions (41). Oxygen consumption by roots and respiration of rhizosphere microbial populations create a decreasing gradient of oxygen from soil to root (42). Though the isolates irrespective of host rhizospheres produced varying levels of siderophores, only isolates producing detectable levels of chitinases showed antagonism implying the significance of the enzyme in antifungal activity. Interestingly, in either cases, the production dipped with increasing pH beyond 5.2 (soil pH) indicating the significance of soil reaction. Though the possible role of inherent genetic property could not be negated, expression of such genetic properties is greatly influenced by soil environment (18). In this study, none of the bacterial strains were able to produce chitinase.

It should be noted that superior strains were evaluated in greenhouse condition on corn and soybean plants (data not presented), so that the tested isolates had good potentiality to increase plant growth so can be used as eco- friendly biofertilizers

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غربال‌گری باکتری‌های ریزوسفر یونجه از لحاظ صفات محرک رشدی گیاه

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چکیده

مقدمه: باکتری‌های محرک رشد با مکانیسم‌های مختلف رشد گیاه را تحت تاثیر قرار می‌دهند. بسیاری از پژوهشگران ویژگی‌های خاصی را که این باکتری‌های از طریق آن‌ها رشد گیاه را تحریک می‌کنند را مشخص کرده‌اند، در بیشتر مطالعه‌های انجام شده فقط چند تا از این صفات بررسی شده اند. هدف از پژوهش حاضر ارزیابی فعالیت‌های محرک رشدی جدایه‌های باکتری‌ها است (۴۵ جدایه متعلق به ریزوبیوم‌ها و ۲ جدایه متعلق به *سودوموناس فلورسنس* *Pseudomonas fluorescence*) که از خاک ریزوسفری و گرهک‌های ریشه یونجه در استان زنجان جداسازی شدند.

مواد و روش‌ها: جدایه‌های مورد مطالعه از اراضی زیر کشت یونجه در اطراف کارخانجات روی در استان زنجان جداسازی شدند. پس از جداسازی و خالص‌سازی باکتری‌ها از نمونه‌های ریشه و خاک ریزوسفری، باکتری‌ها در آزمایشگاه از نظر صفات محرک رشدی گیاه از قبیل توانایی تولید ایندول استیک اسید، آنزیم ای-سی سی دآمیناز (آمینو سیکلوپروپان کربوکسیلیک اسید)، سیانید هیدروژن، سیدروفور، آنزیم کیتیناز و توانایی انحلال فسفات‌های معدنی و آلی ارزیابی و غربال‌گری شدند.

نتایج: نتایج نشان داد که ۴۳ جدایه باکتری ایندول استیک اسید (۴/۰۴ تا ۴/۹۵ میکروگرم در میلی‌لیتر) و ۱۵ باکتری آنزیم ای-سی سی دآمیناز (۰/۲۳ تا ۱/۰۵ میکروگرم بر میلی‌لیتر) تولید کردند. فقط یک جدایه بنام Rm66 مقدار بالای سیانیدهیدروژن تولید کرد. تولید سیدروفور در ۹ جدایه مشاهد شد. هیچ یک از باکتری‌ها آنزیم کیتیناز تولید نکردند. انحلال فسفات‌های معدنی در ۱۹ جدایه باکتری تشخیص داده شد (۴/۳۳ تا ۵/۸۶ میکروگرم در میلی‌لیتر) و ۱۵ جدایه باکتری فسفات‌های آلی را حل کردند (۱/۶۶ تا ۱۴۴/۲۸ میکروگرم در میلی‌لیتر).

بحث و نتیجه‌گیری: نتایج این پژوهش نشان داد بیشتر جدایه‌های باکتری به اراضی زیر کشت یونجه دارای خاصیت محرک رشدی گیاه متعلق بودند. این باکتری‌ها در صورت تلقیح به گیاه به‌عنوان کود زیستی پتانسیل خوبی برای افزایش رشد گیاه خواهند داشت.

واژه‌های کلیدی: آنزیم ACC- دآمیناز، کیتیناز، سیانید هیدروژن، ایندول استیک اسید، باکتری‌های ریزوسفری محرک رشد گیاه، سیدروفور

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