

## Enhancement of Maize Growth Using Some Plant Growth Promoting Rhizobacteria (PGPR) Under Laboratory Conditions

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**Abstract:** Thirty one bacterial isolates were isolated and identified in nine genera, with twelve taxa as *Pseudomonas putida*, *P. fluorescens*, *P. areuginosa*, *Serratia marcescens*, *Xanthomonas* sp., *Bacillus cereus*, *Micrococcus* sp., *B. subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Pseudomonas* sp., *Staphylococcus* sp. The highest percentage distribution was *B. subtilis*, followed by *P. putida*, *P. areuginosa* and *S. marcescens*. Eight isolates had the ability for production of IAA and siderophores. *S. marcescens* then *P. putida* followed by *P. fluorescens* were the highest in IAA production. The eight isolates of PGPR were increased seed germination by 7 to 13% over control. The highest seed germination was recorded when seeds were pretreated with *S. marcescens*, then *P. putida* followed by *B. subtilis*. The highest seedling height and shoot dry weight were observed in seeds treated with *S. marcescens* then *P. putida* followed by *B. cereus* and *B. subtilis*. The use of *S. marcescens* produced the highest root length and weight, and also increased the chlorophyll contents. The MDG was the highest in case of seed soaking and inoculums added with irrigation water treatments, while was the lowest at spray the inoculums on the soil surface. Also, the best results for MGT and GI was found in seed soaking and inoculums added with irrigation water treatments. The best PGPR bacteria for MDG, MGT and GI in seed soaking treatment were *B. megaterium*, *P. putida*, *S. marcescens* and *P. areuginosa*, while in adding inoculums with irrigation water were *B. subtilis*, *B. cereus*, *S. marcescens* and *P. fluorescens*. When cultured the high efficacy isolates of *Serratia marcescens* with *Pseudomonas putida* and *Bacillus subtilis* without any antagonistic effect between them.

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### 1. Introduction

Maize can thrive in diverse climates as high mountain plains or arid desert plains. Maize plant is a major source of food for both humans and animals, and is grown in more countries than any other crop. Maize can only be produced in areas that do not have extreme cold temperature. The majority of the crop is used as livestock feed; the remainder is processed into a range of food and industrial products including starch, ethanol for use as a fuel, sweeteners such as high fructose maize syrup and maize oil (UNCTAD, 2012). Intensive agriculture relies on the use of chemical fertilizers to provide high fruit quality and yield. On the other hand, excessive use of chemical fertilizers causes problems not only in terms of financial cost but also in terms of the cost to the environment. The interest in sustainable agriculture recently has increased. The development and application of sustainable agricultural techniques and biofertilization are vital to alleviating environmental pollution (Rodriguez and Fraga, 1999; Esitken *et al.*, 2003; Vessey, 2003). Many bacterial species, mostly associated with plant rhizosphere, have been tested

and found to be beneficial for plant growth, yield, and crop quality. They have been called plant growth promoting rhizobacteria (PGPR). These bacterial species include the strains in the genera *Serratia*, *Pseudomonas*, *Burkholderia*, *Agrobacterium*, *Erwinia*, *Xanthomonas*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Alcanigenes*, *Arthrobacter*, *Acetobacter*, *Acinetobacter*, *Achromobacter*, *Aerobacter*, *Artrobacter*, *Azotobacter*, *Clostridium*, *Klebsiella*, *Micrococcus*, *Rhodobacter*, *Rhodospirillum* and *Flavobacterium* (Rodriguez and Fraga, 1999; Bloemberg and Lugtenberg, 2001; Esitken *et al.*, 2003). The mechanisms of PGPR action are not fully understood but are thought to include: a- the ability to produce plant hormones, such as auxins (Jeon *et al.*, 2003; Egamberdiyeva, 2005), cytokinins (García de Salamone *et al.*, 2001), and gibberellins (Gutiérrez-Manero *et al.*, 2001); b- asymbiotic N<sub>2</sub> fixation (Sahin *et al.*, 2004; Canbolat *et al.*, 2006); c- solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients (Jeon *et al.*, 2003) and d- antagonism against phytopathogenic microorganisms by production of

siderophores, the synthesis of antibiotics, enzymes or fungicidal compounds and competition with detrimental microorganisms (Döbbelaere *et al.*, 2002; Dey *et al.*, 2004; Lucy *et al.*, 2004). It was reported by different researchers that due to N fixing or P solubilization bacterial applications increased growth rate and yield of apricot (Esitken *et al.*, 2003), peanut (Dey *et al.*, 2004), and apple (Aslantas *et al.*, 2007) in long term conditions. The PGPR and their interactions with plants are exploited commercially (Podile and Kishore, 2006) and hold great promise for sustainable agriculture. Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils, radicchio and cucumber (Gray and Smith, 2005). The aim of this study to investigate some locally isolated PGPR strains for production of IAA and siderophores, also enhancement of the maize growth, and finally choose the best way for application in the field.

## 2. Materials and Methods

### -Isolation and screening of PGPR from soil samples

Bacteria were isolated from the rhizosphere and rhizoplane soils of different plants, which growing in province Khulais according to Wollum-II (1982). Morphological characteristics of the colony of each isolate were examined on Luria-Bertani (LB) agar plates in pure culture and then identified by Biology system (Biolog Gen III).

### -Detection and measurement of indole acetic acids (IAA)

Auxin production by the PGPR isolates was tested in the presence and absence of L-tryptophan (L-TRP), and determined by colorimetric method. For this purpose, 20 ml of glucose peptone medium broth were added in 100-ml Erlenmeyer flasks, autoclaved and cooled. Five milliliter of filter sterilized (0.2  $\mu\text{m}$  membrane filter, Whatmann) L-TRP solution (5%) were added to the liquid medium to achieve a final concentration of 1.0 g l<sup>-1</sup>. The flask contents were inoculated by adding 1.0 ml of 4-day-old bacterial broth adjusted to optical density of 0.5 (10<sup>7</sup>-10<sup>8</sup> CFU ml<sup>-1</sup>) measured at 550 nm by spectrophotometer. The flasks were plugged and incubated at 28±1°C for 48 hrs with shaking at 100 rpm. Non-inoculated/untreated control was kept for comparison. After incubation, the contents were filtered through Whatmann filter paper no. 2, then the filter paper was transferred to another Petri dish filed with Salkowski's reagent (4.5 g of FeCl<sub>3</sub> per liter in 10.8 M H<sub>2</sub>SO<sub>4</sub>), let paper saturated, and incubated at room temperature in the dark for 30 min. Organisms which producing IAA gave pink to red color after incubation period as described by Sarwar *et al.* (1992).

The isolates which give a positive result were cultured on auxin production medium (K<sub>2</sub>HPO<sub>4</sub>, 0.4 g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.2 g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.5 g; NaCl, 0.1 g;

CaCl<sub>2</sub>, 0.1 g; Malic acid, 2.5 g; Yeast extract, 0.5 g; L-Trp, 10 g; Distilled water, 1000 ml) and incubated for 48 hrs. 2 ml of bacterial broth transferred to 2 ml-Eppendorf tubes and centrifuged for 10 min at 15000 rpm under cooling. One ml of above mentioned solution mixed with 4 ml of Salkowsky indicator. The mixture was kept for 20 min in dark at room temperature and then measured at 535 nm using spectrophotometer. The auxin amount calculated using IAA standard curve according to Khakipour *et al.* (2008).

### -Siderophores detection

The bacterial isolates were tested for their production of siderophores, as described by Alexander and Zuberer (1991). Chrome Azurol S (CAS) agar plats were inoculated and incubated at 28°C for five days. Siderophore was detected by the formation of orange halos surrounding bacterial colonies on CAS.

### -Effect of PGPR on maize germination rate and seedling growth *in vitro*

To study the effect of PGPR on plant germination rate, 100 seeds of maize (*Zea mays* L.) were prepared for each treatment. For sterilization, seeds were soaked in 2% sodium hypochlorite for 3min and then they were washed by sterile distilled water for 5 times. Sterilized seeds were incubated in 10 ml from LB broth for the eight bacterial isolates separately with rate 1×10<sup>8</sup> CFU/ml at room temperature for 24h and one treatment incubated with LB media free from bacterial cells as control. After 24h incubation, the soaked seeds were placed in sterilized cup containing wet peat moos (2 seeds/cup and 50 cup/each treatment), and they were incubated in growth room at 25°C for 15 days to calculate the percentage of germination and some seedling parameter as seedling height (cm), shoot dry weight (mg/plant), root length (cm), root dry weight (mg/plant) and the chlorophyll content in leaves were measured by Chlorophyll Content Meter (model CL-01 Co. Hana Tech Instruments).

### -Study the best method for treated plant with PGPR

Eight isolates of bacteria were added to maize plants using different treatments (seed soaking, soil drench, spray on the surface of soil and adding with irrigation water) for selection the best treatment, which can be used in the field experiment. Eight-hundred seeds of maize (*Zea mays* L.) were sterilized by soaking in 2% sodium hypochlorite for 3 min and then they were washed by sterile distilled water for 5 times. Sterilized seeds were treated in four forms as seed soaking (30 min prior to planting), soil drench (5ml ×10<sup>8</sup> CFU/cup), spray on the soil surface after planting and adding with irrigation water. Finally, seeds were planted free from any treatment with

antagonists and/or fungal pathogen (non-infested control).

All plants were incubated in growth room at temperature for 25°C for 10 day to calculate the final germination percent (FGP) (ISTA, 1993; 1999) based on the following equation:-

$$FGP = \frac{\text{number of germinating seeds}}{\text{Total number of seeds}} \times 100$$

Mean daily germination (MDG) was calculated according to the following equation:-

$$MDG = \frac{FGP}{d}$$

Where "d" are the days to the maximum of final germination.

Mean germination time (MGT) was calculated according to the following equation (Moradi *et al.*, 2008):-

$$MGT = \frac{\sum Dn}{\sum n}$$

Where "n" is the number of seeds, which were germinated on day "D", and "D" is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described in AOSA (1983) by following formula:-

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

### -The interaction between eight isolates of PGPR

The isolates were tested against each other on LB medium by streak method. The tested isolate on the surface of 8 plates with antagonistic isolates ( $10\mu\text{l} \times 10^8$  CFU/plate). All treatments (64 treatments) were incubated for 48 hrs at  $30 \pm 2^\circ\text{C}$  and checked by calculation the antagonistic effect: (-) = No suppression; (+/-) = weak suppression; (+) = medium suppression; (++) = good suppression and (+++) = high suppression.

### 3. Results

Morphological characteristics of the bacterial colonies were used to differentiate between different isolates on plate agar and they purified into 31 isolates. The bacterial isolates were identified in nine genera, with twelve taxa as *Pseudomonas putida*, *P. fluorescens*, *P. areuginosa*, *Serratia marcescens*, *Xanthomonas* sp., *Bacillus cereus*, *Micrococcus* sp., *B. subtilis*, *B. megaterium*, *B. amyloliquefaciens*, *Pseudomonas* sp., *Staphylococcus* sp. The highest percentage distribution was *B. subtilis*, followed by *P. putida*, *P. areuginosa* and *S. marcescens* where the distribution percentages were 25.8, 9.6, 9.6 and 9.6, respectively, while the lowest distribution were *B. cereus*, *B. amyloliquefaciens*, *Pseudomonas* sp. and *Staphylococcus* sp. (all were 3.2%) as shown in Figure (1).

Bacterial isolates were screened for plant growth promoting traits such as production of indole 3-acetic acid (IAA) and siderophore components. Eight isolates had the ability for production of IAA (Figure 2) and

siderophores (Figure 3). The best isolates for production of IAA were *S. marcescens* (2.76 mg/l) then *P. putida* (1.26 mg/l) followed by *P. fluorescens* (0.87 mg/l). On the other hand, four bacterial isolates were not produced IAA and siderophores like *Xanthomonas* sp.; *Micrococcus* sp.; *Pseudomonas* sp. and *Staphylococcus* sp.

The effect of PGPR isolates on maize seed germination and seedling growth were presented with different isolates in Table (1). The eight isolates of PGPR were increased seed germination by 7.0 to 13.0% over control. The highest seed germination was recorded when seeds were pretreated with *S. marcescens*, then *P. putida* followed by *B. subtilis*. The other isolates were showed good result in seed germination.

Results in Table (1) revealed that the PGPR isolates were significantly enhance the height of maize seedlings over control. The highest seedling height (14.50 cm) was observed in seeds treated with *S. marcescens* then *P. putida* (13.6 cm) followed by *B. cereus* (13.3 cm) and also illustrated in Figure (4). The root length of seedling was significant increase in response to treatments with PGPR isolates. The isolate *S. marcescens* was produced the highest root length (7.7 cm), which was statistically similar to isolate *B. subtilis* (7.0 cm) Figure (4). The significant increase in shoot dry weight of maize seedlings was observed in all PGPR isolates. The lowest shoot dry weight was observed in untreated (4.47 mg/plant), while the highest shoot dry matter was recorded in seeds treated with *S. marcescens* (10.43 mg/plant) followed by *P. putida* (8.94 mg/plant), *B. cereus* (8.53 mg/plant), *B. subtilis* (8.48 mg/plant) and *P. fluorescens* (8.13 mg/plant) without significantly difference between them. The PGPR isolates significantly increased root dry weight of maize seedlings. The highest value of root dry weight was recorded in seeds treated with *S. marcescens* (5.75 mg/plant), which was statistically similar to isolate *B. subtilis* (5.14 mg/plant). The lowest root dry weight was observed in untreated seeds (1.89 mg/plant).

The highest value in chlorophyll content was recorded in *S. marcescens* (7.70 Unit) followed *P. fluorescens* (6.92 Unit) and *P. areuginosa* (6.84 Unit) without any significant differences between them. The lowest chlorophyll content was observed in untreated seeds (4.63 Unit) (Table 1).

Data presented in Table (2) showed the effects of four treatments in seed soaking, soil drench, spray and adding with irrigation water to select the best method for treated maize seeds with the eight isolates of PGPR on final germination percentage (FGP), mean daily germination (MDG), mean germination time (MGT) and germination index (GI) after 10 day from planting. The MDG was the highest in case of seed soaking and

inoculums added with irrigation water treatments, while was the lowest at spray the inoculums on the soil surface. Also, the best results for MGT and GI was found in seed soaking and inoculums added with irrigation water treatments. The best PGPR bacteria for MDG, MGT and GI in seed soaking treatment were *B. megaterium*, *P. putida*, *S. marcences* and *P. areuginosa*, while in adding inoculums with irrigation water were *B. subtilis*, *B. cereus*, *S. marcences* and *P. fluorescens* (Table 2).

Data presented in Table (3) showed the results of antagonistic tests between the eight isolates of PGPR when cultured together to select the best way for blend more than isolates to increase their efficacy. The best result was found when culture the high efficacy isolates of *Serratia marcences* with *Pseudomonas pitida* and *Bacillus subtilis* without any antagonistic

effect between them. On contrary, the suppressed effect of the high isolates for producing IAA and siderophore components was found between *P. areuginosa* against *S. marcences* and *P. putida*. The highly suppressed effect was found between *B. megaterium* to *B. amyloliquefaciens*. From the previous results, we concluded that the use of the three isolates *S. marcences*, *P. pitida* and *B. subtilis* together will be more efficient in the field experiment.

The best two treatments seed soaking and adding the inoculums with irrigation water. For some reasons related to soil structure, irrigation water scarcity, increasing temperatures and solar radiation in Saudi Arabia environment. So, we concluded that the seed soaking treatment will be more suitable in the field experiment.

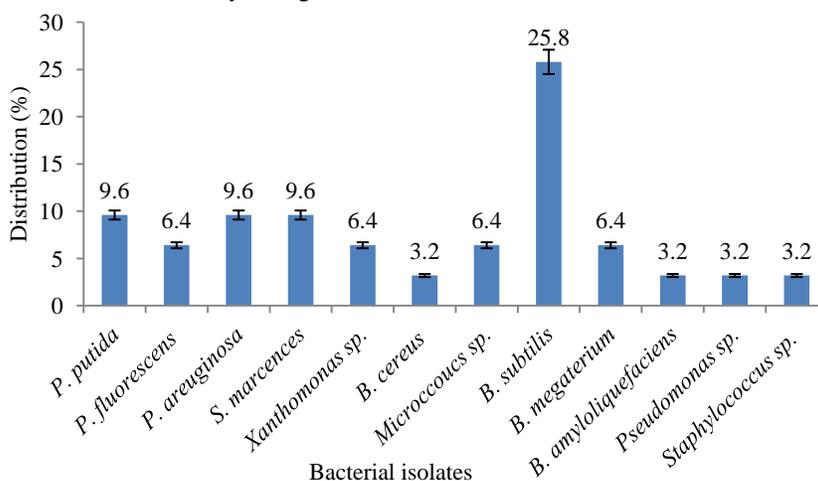


Figure 1. Percentage distribution of bacterial isolates in collected soil samples

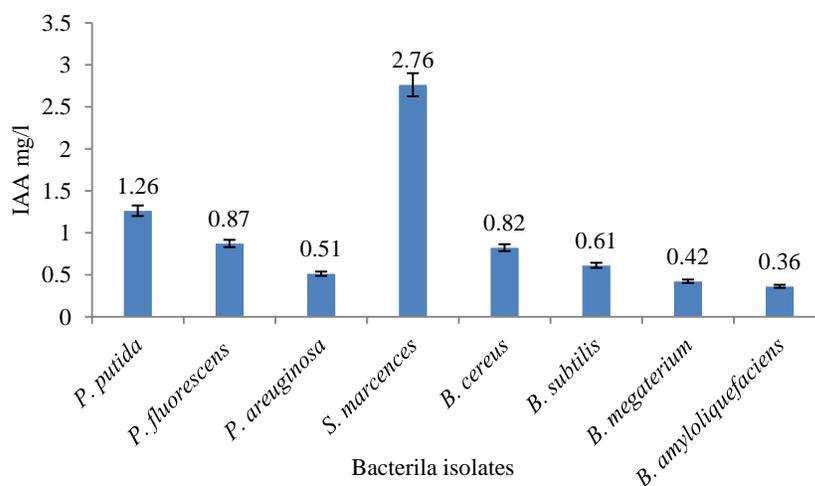


Figure 2. The auxin (IAA mg/l) production by some PGPR isolates that give a positive result in the detection test



Figure 3. Siderophore production by some bacterial isolates on CAS agar medium



Figure 4. The effect of treatments with some isolates of PGPR on plant growth parameters after 30 days from planting: Control (c), *Bacillus subtilis* (BS), *Pseudomonas putida* (PP) and *Serratia marcescens* (S)

Table 1. The effect of PGPR on seed germination and growth of maize seedlings after 15 day

Isolate	Germination (%)	Seedling height (cm)	Root length (cm)	Dry wt. (mg/plant)		Chlorophyll content (Unit)
				Shoot	Root	
Control	83	9.50 <sup>e</sup>	3.3 <sup>e</sup>	4.47 <sup>e</sup>	1.89 <sup>e</sup>	4.63 <sup>d</sup>
<b><i>Bacillus</i></b>						
- <i>B. amyloliquefaciens</i>	92	12.5 <sup>c</sup>	5.3 <sup>c</sup>	7.18 <sup>c</sup>	2.64 <sup>c</sup>	5.00 <sup>cd</sup>
- <i>B. cereus</i>	93	13.3 <sup>b</sup>	6.3 <sup>b</sup>	8.53 <sup>b</sup>	4.52 <sup>b</sup>	6.11 <sup>bc</sup>
- <i>B. megaterium</i>	91	11.6 <sup>c</sup>	5.6 <sup>c</sup>	7.58 <sup>c</sup>	3.91 <sup>c</sup>	5.50 <sup>c</sup>
- <i>B. subtilis</i>	94	13.0 <sup>bc</sup>	7.0 <sup>ab</sup>	8.48 <sup>b</sup>	5.14 <sup>ab</sup>	5.84 <sup>c</sup>
<b><i>Pseudomonas</i></b>						
- <i>P. areuginosa</i>	90	12.0 <sup>c</sup>	5.5 <sup>c</sup>	7.45 <sup>c</sup>	3.82 <sup>bc</sup>	6.84 <sup>a</sup>
- <i>P. fluorescens</i>	92	12.0 <sup>c</sup>	6.0 <sup>b</sup>	8.13 <sup>bc</sup>	4.26 <sup>b</sup>	6.92 <sup>ab</sup>
- <i>P. putida</i>	95	13.6 <sup>b</sup>	6.6 <sup>b</sup>	8.94 <sup>b</sup>	4.78 <sup>b</sup>	7.30 <sup>a</sup>
<b><i>Serratia</i></b>						
- <i>S. marcescens</i>	96	14.5 <sup>a</sup>	7.7 <sup>a</sup>	10.43 <sup>a</sup>	5.75 <sup>a</sup>	7.70 <sup>a</sup>

Means in each column followed by the same letter are not significantly different ( $P < 0.05$ ) as determined by Duncan's multiple range test.

Table 2. Effect of the four different treatments on maize seeds treated with the eight isolates of PGPR after 10 day from planting

Isolate	No. of germinated seeds	FGP	MDG	MGT	GI
<b>Seed soaking</b>					
<i>B. amyloliquefaciens</i>	21	84	8.4	2.8	15.5
<i>B. megaterium</i>	20	80	8.0	1.8	13
<i>B. cereus</i>	21	84	8.4	2.6	12.8
<i>B. subtilis</i>	22	88	8.8	2.7	12.5
<i>P. areuginosa</i>	21	84	8.4	2.4	13.8
<i>P. fluorescens</i>	21	84	8.4	2.6	12.8
<i>P. putida</i>	22	88	8.8	2.3	15.0
<i>S. marcences</i>	23	92	9.2	2.3	15.8
<b>Soil drench</b>					
<i>B. amyloliquefaciens</i>	17	68	6.8	3.2	10.8
<i>B. cereus</i>	20	80	8.0	2.7	15.1
<i>B. megaterium</i>	21	84	8.4	2.9	11.3
<i>B. subtilis</i>	18	72	7.2	2.9	7.0
<i>P. areuginosa</i>	22	88	8.8	2.8	16.2
<i>P. fluorescens</i>	18	72	7.2	2.8	13.3
<i>P. putida</i>	20	80	8.0	3.0	13.6
<i>S. marcences</i>	20	80	8.0	2.6	12.0
<b>Spray on the surface of soil</b>					
<i>B. amyloliquefaciens</i>	19	76	7.6	3.8	10.4
<i>B. cereus</i>	13	52	5.2	3.4	10.1
<i>B. megaterium</i>	15	60	6.0	3.2	9.8
<i>B. subtilis</i>	16	64	6.4	2.9	8.5
<i>P. areuginosa</i>	11	44	4.4	2.7	6.3
<i>P. fluorescens</i>	17	68	6.8	2.9	8.8
<i>P. putida</i>	15	60	6.0	3.2	9.8
<i>S. marcences</i>	18	72	7.2	3.2	11.8
<b>With irrigation water</b>					
<i>B. amyloliquefaciens</i>	20	80	8.0	2.4	13.0
<i>B. cereus</i>	20	80	8.0	2.2	14.0
<i>B. megaterium</i>	20	80	8.0	2.6	18.7
<i>B. subtilis</i>	19	76	7.6	2.0	9.5
<i>P. areuginosa</i>	22	88	8.8	2.7	16.4
<i>P. fluorescens</i>	21	84	8.4	2.3	14.3
<i>P. putida</i>	21	84	8.4	2.7	16.0
<i>S. marcences</i>	21	84	8.4	2.2	14.8

FGP = Final Germination Percent, MDG = Mean Daily Germination, MGT = Mean Germination Time, GI = Germination Index.

Table 3. The interaction between the eight isolates of PGPR *in vitro* blended together

Isolate	<i>P. putida</i>	<i>P. fluorescens</i>	<i>P. areuginosa</i>	<i>S. marcences</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>B. megaterium</i>	<i>B. amyloliquefaciens</i>
<i>Pseudomonas putida</i>	-	++	+	-	+	-	-	++
<i>P. fluorescens</i>	++	-	-	+/-	+/-	++	+	+
<i>P. areuginosa</i>	+	-	-	+	+/-	+/-	-	+/-
<i>Serratia marcences</i>	-	+/-	+	-	+	-	+/-	+/-
<i>Bacillus cereus</i>	++	+/-	+/-	+	-	+	+/-	-
<i>B. subtilis</i>	-	++	+/-	-	+	-	+/-	+
<i>B. megaterium</i>	-	+	-	+/-	+/-	+/-	-	+++
<i>B. amyloliquefaciens</i>	++	+	+/-	+/-	-	+	+++	-

Antagonistic effects: (-) = No suppression; (+/-) = weak suppression; (+) = medium suppression; (++) = good suppression and (+++) = high suppression.

#### 4. Discussion

The rhizosphere is the narrow zone of soil specifically influenced by the root system (**Dobbelaere et al., 2003**). This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria (**Gray and Smith, 2005**). This situation is reflected by the number of bacteria that are found around the roots of plants, generally 10 to 100 times higher than that in the bulk soil (**Weller and Thomashow, 1994**). The rhizosphere is populated by a diverse range of microorganisms and the bacteria colonizing this habitat are called rhizobacteria (**Schroth and Hancock, 1982**).

The increasing importance of beneficial bacteria in agriculture has resulted in many efforts to isolate and identify bacteria associated with the rhizosphere of plants in order to trace their roles in plant growth promotion. The benefit isolates of PGPR, which were isolated and identified in this study are *Pseudomonas putida*, *P. fluorescens*, *P. areuginosa*, *Serratia marcescens*, *Bacillus subtilis*, *B. cereus*, *B. amyloliquefaciens* and *B. megaterium*.

Rhizosphere is a rich site by microorganisms and should be explored for obtaining potential plant growth promoting rhizobacteria (PGPR), which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants (**Joshi and Bhatt, 2011**). In last few decades a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia* have reported to enhance plant growth (**Kloepper et al., 1989**; **Gutierrez-Manero et al., 2001**; **Kim and Kim 2008**; **Phi et al., 2010**; **Joshi and Bhatt, 2011**).

The results showed that 25% isolates have highest ability to produce siderophore on CAS agar medium and 41.66% produce IAA. In a mineral limited soil environment, bacterial species produce siderophores using an iron-transport system, resulting in reduction of plant pathogens through inhibition of iron absorption. Some researchers believe that the contribution of siderophores to the overall iron requirements of plants is small (**Glick, 1995**), the vast majority of research on bacterial siderophores in the rhizosphere has found strong biocontrol activities due to competitive effects with plant pathogens (**Hiifte et al., 1994**). The production of phytohormones by bacteria is one of the most important factors of plant growth promotion. Previous research has revealed the existence of bacteria producing phytohormones such as indole-3-acetic acid (IAA), cytokinin, and gibberellins (**Belimov et al., 1995**; **Barazani and Friedman, 1999**; **Timmusk and Wagner, 1999**;

**Gutierrez-Manero et al., 2001**; **Mehnaz et al., 2001**; **Compant et al., 2005**).

In our study, *Bacillus* (33.3%) was dominant group. *Bacillus* genus is the major component of the microbial flora, which live in close association with various types of agricultural crops followed by *Pseudomonas* (25%) as the second dominant genera in the rhizosphere, probably because under favorable environmental conditions, its growth rate is higher than that of *Bacillus* (Bowen and Foster, 1978). Bacteria of diverse genera have been identified as PGPR, of which *Bacillus* and *Pseudomonas* spp. are predominant (**Podile and Kishore, 2006**). Predominance of *Bacillus* sp. is due to its ability to efficiently use the nutrients provided by the plant through exudates. In additions, *Bacillus* has the ability to inhibit the growth of other strains. Many strains of *Bacillus* have been reported to produce substances that act as growth inhibitors for other microorganisms (**Lilinares, et al., 1994**). The increase soil moisture content and optimum temperature enhance the development of microflora.

The isolated bacteria were used to enhance maize plant growth and protection against nutrition deficiency. The results showed that the bacterial isolates have the ability to increase the growth of maize plants clearly through statistical analysis of the data obtained from the experiments comparing the results with plants untreated bacteria. These results due to the mechanisms of PGPR in promote the plant growth, thought to include the ability to produce auxins (**Shaharoon et al., 2006**; **Egamberdiyeva, 2007**; **Gholami et al., 2009**; **Son et al., 2014**). It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere (**Antoun and Kloepper, 2001**). PGPR and their interactions with plants are exploited commercially (**Podile and Kishore, 2006**) and hold great promise for sustainable agriculture. Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils, radicchio and cucumber (**Gray and Smith, 2005**).

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and or indirectly (**Gray and Smith, 2005**). The direct promotion by PGPR entails either providing the plant with a plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic microorganisms.

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