Egyptian J. Desert Res., **66**, No. 2, 305-326 (2016)

ROLE OF ENDOPHYTIC *PSEUDOMONAS* AS PLANT GROWTH PROMOTERS UNDER DESERT CONDITION

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Plant growth promoting endophytes enhance the plant growth using multiple metabolic activities directed and contribute to the growth, health and development of plant. A total of 46 strains isolated from the rhizosphere of maize, sorghum, barley and wheat plants were screened for their efficiency as plant growth promoting endophytes. Bacterial isolates induced the highest length and weight of maize seedlings on Hoagland media were selected for further investigations. All selected endophytic bacteria possess HCN and indole acetic acid production, while only four isolates showed phosphate dissolving activities. Using Chrome Azurol S plates revealed that all selected isolates were positive to siderophore production. For characterization of siderophores, ferrate hydroxamate type were detected in five bacterial isolates, ferrate catecholate type detected in only three isolates, while no carboxylate siderophores were recorded for any isolates. HPLC analysis of the highest active two isolates revealed the presence of indole acetic acid and indole butyric acid. Phylogenetic analysis of 16S rRNA sequence of the two isolates showed maximum sequence similarity with *Pseudomonas aeruginosa* strain PAO1 and *Pseudomonas geniculata* strain ATCC 19374.

A field experiment was conducted at El-Qantara Sharq experimental station, Desert Research Center (DRC) for evaluation of *Ps. aeruginosa* and *Ps. geniculata* as plant growth promoting endophytes on maize. Bacterial inoculation recorded an increase in biological yield reaching 55.2 and 33% over control for *Ps. geniculata* and *Ps. aeruginosa*, respectively. Concerning to chemical constituent of maize grains, inoculation process significantly increased the carbohydrate and oil % with about 4.6 and 4.2% for both bacterial strains. While no change in endophytic *Pseudomonads* count were detected inside the root of treated plants comparing to control , the inoculation

process have a positive effect on the activity and abundance of microbial community in the rhizospheric zone.

Keywords: endophytes, *Ps .geniculata , Ps. aeruginosa*, siderophores, plant hormones, maize

Endophytic bacteria is known as a group of beneficial free-living soil bacteria that live in plant tissues without doing substantive harm or gaining benefit other than residency without showing any external sign of infection on their host (Babalola, 2010; Ahemad and Kibret, 2014 and Arora et al., 2014). Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants, such as flowers, stems, and cotyledons, may also be used for entry (Kobayashi and Palumbo, 2000).

The major two types of endophytes are obligate and facultative ones. The first one are unable to proliferate outside of plants and are likely transmitted via seed rather than originating from the rhizosphere, while facultative endophytes are free living in soil but will colonize plants when the opportunity arises, through coordinated infection (Hardoim et al., 2008). This study will deal with endophytes relating to plant growth promotion belong to facultative one.

Once endophytes establish themselves inside a plant, some of them can stimulate plant growth and/or protect plants against phytopathogens. These endophytic bacteria with diverse properties will have much effective role to manipulate and improve plant growth (Jasim et al., 2014). The common trait for endophytic bacteria is production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics (Arshad et al., 1992).

Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism produced by several microorganisms (Lynch, 1985). The production of siderophore by biocontrol agents (BGA) and plant growth promoting microbes (PGPM) is one of the important mechanisms for plant growth promotion and disease suppression gradually (Sayyed et al., 2005). The microbial ability to produce siderophores not only improve rhizosphere colonization of producer strain but also play an important role in iron nutrition of plant and its growth (Lemanceau et al., 2007). Typically, microbial sideophores are classified as catecholates, hydroxamates and α-carboxylates, depending on chemical nature of their coordination sites with iron. *Pseudomonas spp.* have been known for their siderophore production for many years and therefore many reports on the isolation and characterization of their siderophores have been published (Decheng et al., 2005).

Pseudomonas aeruginosa was employed as a model system considering its frequent isolation as an endophyte, wide antagonistic effects was reported against different phytopathogens and soil pests (Sekhar and Thomas, 2015). *Pseudomonas geniculata*, isolated from nodules of chickpea

grown has the potential for plant growth-promoting (PGP) that it produced indole acetic acid, siderophore, hydrocyanic acid, protease, and β-1,3-glucanase and it significantly enhanced nodule number and weight, shoot, and root weight, stover and grain yield and total dry matter as well as it significantly enhanced the total nitrogen, available phosphorus and organic carbon% (Subramaniam et al., 2015).

The potential of plant growth-promoting bacteria endophytes (PGPBEs) to improve plant health has led to a great number of studies examining their applied use as inoculants, primarily in agricultural crops (Kuklinsky-Sobral et al., 2004). The potential for microbial inoculants to reduce the need for chemicals such as pesticides and fertilizers makes them important in the development of sustainable agricultural practices (Horrigan et al., 2002). This study focused on the potential of *Pseudomonas spp.* endophytes as plant inoculants for plant growth promotion.

MATERIALS AND METHODS

1. Isolation of Endophytic *Pseudomonas spp.*

Different plants with economic importance and from five different geographical locations were randomly collected, put into plastic bags and kept on ice for the isolation of potential endophytic bacteria. Maize, sorghum, barley and wheat plants collected from Baloza, Ras-Sudr, El-Quantra, Sahl-Eltina and El-Maghara were screened. Plant roots were surface sterilized using methodology described by Petrini et al. (1992) and Werner et al. (1997) in which samples were immersed two times in 70% ethanol for three minutes and immersed twice in 2-4% aqueous solution of sodium hypochlorite for five minutes and again immersed for one minute in70% ethanol. Finally, washed two times in sterile distilled water for five minutes for removing surface sterilization agents with further drying in sterilized paper in a laminar flow hood. Subsequently, sterilized plant samples were crushed under sterile conditions and the resulting juices were plated on King's agar medium (About 1 ml of the macerated tissue was serially diluted up to 10^{-3} using sterile10 mM potassium phosphate buffer, pH 7). About 1 ml from each dilution of intercellular fluid of its tissue was plated on King plates in triplicate and kept in an incubator at 28°C for 48 hours. After incubation, shiny, mucous and transparent colonies originating from plant juice were subjected to microscopic investigation to select rod shape Gram –ve colonies for further studies. About 46 bacterial colonies were selected.

2. Preliminary Screening of Most Efficient Plant Growth Promoting Endophytes

Hoaglands medium (Hoagland and Arnon, 1950) with all salts and micronutrients was prepared and 60 ml of this medium was used per test tube (100 ml capacity) and solidified with 0.6% agar. Before solidification, one ml of each overnight grown bacterial culture of 46 isolates was added per tube. As un-inoculated control, tubes with 1 ml sterile water added were prepared. After solidification, two germinated maize seeds as target plant were transferred to the test tube and incubated in a growth chamber at 28^0C for 2–3 weeks. After that, length and weight of shoot and root of seedlings were recorded (three replicates). The experiment was performed at least twice. At the end of the experiment, endohytic bacterial counts were be detected in the roots of all treatments. Bacterial isolates which induced the highest length and weight of maize were selected as highly efficient plant growth promoting bacterial endophytes for further investigations.

3. Indole Acetic Acid (IAA) Production

Indole acetic acid (IAA) production was estimated by using spectrophotometer as described by Ehmann (1977). For this purpose, 50 ml of King media supplemented with (1 g/L) of L-Tryptophan as a precursor of IAA was inoculated with selected bacterial isolate, incubated at 30^0C for 48 hours and centrifuged at 6000 rpm for 30 minutes to collect supernatant. Then, supernatant and Salkowski reagent (2.0 ml of 0.5M $FeCl_3$ + 98.0 ml of 35% $HClO_4$) were mixed in test tubes at the ratio of 1:2 and the contents were allowed to stand for half an hour for color development. The intensity of color was measured at 520 nm by using spectrophotometer and compared with standard curve of IAA.

4. Phosphate Solubilization

Pikovskaya medium (glucose 10 g/L, $Ca_3(PO_4)_2$ 5 g/L, $MgSO_4$.7H2O 0.1 g/L, NaCl 0.2 g/L, $(NH_4)_2SO_4$ 0.5 g/L, $FeSO_4.7H_2O$ 0.002 g/L, KCl 0.2 g/L, yeast extract 0.5 g/L, $MnSO_4$ 0.002 g/L, agar 20 g/L, pH7.0) with 2.4 mg/ml bromophenol blue was used for phosphate solubilization. The media inoculated with the isolates were incubated for 48 hours and observed for yellow colour change as positive (Pikovskaya, 1948).

5. HCN Detection

Qualitative cyanide determination were carried out by the method of Alstrom and Burns (1989).

6. Siderophore Detection

Also, for the seven selected endophytes, siderophores were detected by the method described by (Schwyn and Neilands, 1987). Briefly, 24 hours old bacterial culture were inoculated in iron free Succinate medium (Meyer

and Abdallah, 1978). After 24 hours incubation at 28°C with constant shaking at 120 rpm, media were centrifuged at 5000 rpm for 10 minutes, the cell free supernatants were poured in the Chrome Azurol S (CAS) plate and incubated for 24 hours at 28ºC, formation of yellow orange color zone around the colonies in plate assay indicated the siderophore production.

7. Characterization of Siderophores Types

7.1. Hydroxamate type of siderophore

7.1.1. Tetrazolium test

This test is based on the capacity of hydroxamic acids to reduce tetrazolium salt by hydrolysis of hydroxamate groups using a strong alkali. The reduction and the release of alkali show red color. To a pinch of tetrazolium salt, added 1-2 drops of 2 N NaOH and 0.1 ml of the test sample. Instant appearance of a deep red color indicated the presence of hydroxamate siderophore (Dave and Dube, 2000).

7.1.2. Neilands spectrophotometric assay

The hydroxamate nature of siderophore was detected by spectrophotometric assay, where a peak between 420-450 nm on addition of 2% aqueous solution of $FeCl_3$ to 1 ml of supernatant indicated presence of Ferrate hydroxamate (Neilands, 1981).

7.2. Catecholate type of siderophore

7.2.1. Carson method

This method was performed by mixing 4.0 ml of culture supernatant with 0.25 ml 2 molar HCl, then 0.5 ml nitrite- molybdate reagent (sodium nitrite 20 g/100 ml + sodium molybdate 20 g/100 ml) was added. The identification of this type is detected by the formation of a yellow color (Carson et al., 1992).

7.2.2. Spectrophotometric assay

Catecholate nature of siderophore was detected using spectrophotometric assay, where a peak at 490-495 nm on addition of 2% aqueous solution of $FeCl_3$ to 1 ml of supernatant indicated the presence of siderophores of catecholate nature (Jalal and Vander Helm, 1990).

7.3. Carboxylate type of siderophore

It was detected by formation of copper complex which was observed for absorption maximum between 190-280 nm. There is no specific wavelength at which the copper complex gets absorbed. The entire wavelength 190 -280 nm was scanned to observe the peak of absorption of the siderophore (Shenker et al., 1992).

At the end of these experiments, only two highly efficient plant growth promoter isolates (RB and BM) were selected for hormones assay.

8. Plant Hormones Assay

The production of different hormones by the most efficient two isolates were emphasized using HPLC assay. Bacterial supernatant collected as previously mentioned was acidified to pH 2.5 to 3.0 with 1 N HCl and extracted twice with ethyl acetate at double the volume of the supernatant. Extracted ethyl acetate fraction was evaporated to dryness in a rotatory evaporator at 40^{o}C. The extract was dissolved in 3 ml of methanol and kept at –20 ^{o}C. Samples of microbial Hormone were analyzed by High- Performance Liquid Chromatography using HPLC Ultimate 3000 Thermo dionex. HPLC chromatograms were produced by injecting 10 ml of the filtered extracts onto (C18, 5 m 25 x 0.46 cm) in a chromatograph equipped with a differential ultraviolet detector absorbing at 280 nm. Mobile phase was methanol and water (80:20 [vol/vol]), flow rate was 1.5 ml/min. Retention times for peaks were compared to those of authentic standards added to the medium and extracted by the same procedures used with bacterial cultures. Quantification was done by comparison of peak heights (Hanifi and Elhadramy, 2007).

9. Identification of the Most Efficient Isolates

Two selected isolates were identified to molecular level using partial 16S rRNA gene sequence technique according to (Berg et al., 2002) in Sigma Scientific Services Co. The Bacterial 16S rRNA gene sequences were amplified by PCR using the following universal 16S primers:

F:- AGA GTT TGA TCC TGG CTC AG

R:- GGT TAC CTT GTT ACG ACT T

The PCR was performed by using a total volume of 20 µl containing 1× Taq & Go (MP Biomedicals, Eschwege, Germany), 1.5 mM $MgCl_2$, 0.2 mM of each primer and 1 µl of template DNA (95°C, 5 min; 30 cycles of 95°C, 30 s; 57°C, 30 s; 72°C, 90 s; and elongation at 72°C, 5 min). The PCR product was purified using Gene JET™ PCR Purification Kit (Thermo K0701). The sequencing to the PCR product was performed by using ABI 3730xl DNA sequencer by using forward and reverse primers (Lane, 1991). The sequences obtained from bacterial isolates were analyzed using BLAST tool at the National Center for Biotechnology Information database (NCBI) Gene Bank database using the Basic Local Alignment Search Tool (BLAST) analysis tools (Altschul et al., 1990) to identify the most similar 16S rRNA sequences available in the Gene Bank.

10. Field experiment

A field experiment was conducted at El-Qantara Sharq experimental station of the Desert Research Center (DRC), North Sinai, in a complete randomized design with three replicates. A mechanical and chemical properties of the soil are presented in table (1). A standard plot size of 5 x 4 m^2 was maintained for all treatments. Soil in all treatments was amended

with recommended dose of super phosphate (15.5% P_2O_5) at a rate of 250 kg/feddan, ammonium nitrate (33.3% N) at a rate of 300 kg/feddan and K-sulphate (48% K_2O) at a rate of 200 kg/feddan. Maize seeds (Hybrid 3 Giza 31) were moistened in CMC solution (1%) before application of each bacterial inoculum (*Ps. aeruginosa* or *Ps. geniculata*) to get a thin, uniform coating of inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran et al., 1982), and untreated control was maintained. Maize plants were harvested after 120 days from planting and the following data were recorded: Shoot and root length, straw and grain weights (g/plant) and grain, straw and biological yield (ton/feddan). Seed oil content was determined as recommended by Comstock and Gulbeton (1958).

Chemical analysis of maize grains were carried out after harvest to determine phosphorus, nitrogen and carbohydrate as indicated by Dubois et al. (1956), Watanabe and Olsen (1965) and Bremner and Mulvaney (1982).

For microbiological analysis, both total microbial and *Pseudomonas spp.* counts in the rhizosphere samples were estimated using Nutrient and King media, respectively. Also, endophytic *Pseudomonas spp.* counts were recorded after 48 hours of incubation at 30°C, and the CFU per g of the root system was estimated as described before.

Soil dehydrogenase activity (µg TPF/g dry soil/24 hours) was analyzed by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) and as described by Friedel et al. (1994). Briefly, five grams of fresh soil were incubated for 24 hours at 37°C in 5 ml of a TTC solution (5 g TTC in 0.2 mol/L Tris-HCl buffer, pH 7.4). Two drops of concentrated sulfuric acid were added immediately after the incubation to end the reaction. The sample was then blended with 20 ml of methanol and shaken for one hour at 200 rpm, followed by filtering to extract TPF. The optical density of the filtrate was measured at 485 nm in spectrophotometer.

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Table (1). Mechanical and chemical analysis of soil.

Soil mechanical analysis													
Depth (cm)		**Coarse sand %**		**Fine sand**		**Silt %**		**Clay %**		**Texture**			
0-15		42.26		43.28		13.28		1.18		Sand			
Soil chemical analysis													
Depth (cm)	**pH**	**EC dSm^{-}_{1}**	**$CaCo_3$ %**	**O.M. %**	**Soluble cations (meq/l)**				**Soluble anions (meq/l)**				**N %**
					Na^+	**Ca^{++}**	**Mg^{++}**	**K^+**	**$Co3^-$**	**HCO_3^-**	**Cl^-**	**So_4^{--}**	
0-15	8.31	3.62	6.3	2.6	17.52	8.25	7.14	1.49	-----	2.36	9.04	23.0	0.23
15-30	8.3	3.95	6.5	2.4	20.42	8.92	7.95	1.21	-----	2.43	9.0	27.42	0.17

11. Statistical Analysis

Data were subjected to statistical analysis using the method described by Snedecor and Cochran (1990). The least significant difference (L.S.D.) was used to differentiate means according to (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

1. Isolation and Preliminary Screening of Most Efficient Plant Growth Promoting Endophytes

Adequate number of colonies on King agar plates were obtained, and according to their morphological characteristics, a total of 46 strains similar to that of *Pseudomonas spp.* were obtained and screened for their efficiency as plant growth promoting endophytes. The bacterial endophytes were coded according to their location and plants from, which they were isolated. After 15 days of maize plantation on Hoagland's medium, most of bacterial strains of 47 isolates showed significant increase in shoot and root length, fresh and dry weights comparing to the controls. The most efficient seven isolates were selected on the basis of their plant growth promoting properties of maize seedlings as shown in table (2). At the end of experiments, counting of endophytic Pseudomonads in maize seedling roots detecting remarkable increase in their counts for all treated plants compared to control one and no clear differences in counts were detected among the treatments, which indicated the ability of the isolates to invade the roots as endophytes under controlled condition.

Table (2). Evaluation of endophytic bacteria as plant growth promoting bacteria.

Bacterial source			Parameter measured				Endophytic bacterial counts*10^3 CFU/g root
Localities of isolation	**Plant**	**Bacterial isolates code**	**Stem length (cm)**	**Stem F.W. (g)**	**Root length (cm)**	**Root F.W. (g)**	
Baloza	Barley	BB	19.3 d	0.5 bc	6.06 cd	0.36 c	18.2
Ras-Sudr	Barley	RB	23.1 a	0.57a	6.86 b	0.49a	19.5
El-Quantra	Maize	EC	20.8 c	0.5 bc	6.26 c	0.41 b	19.7
Sahl-Eltina	Maize	SC	19.9cd	0.52b	5.8 de	0.37 bc	16.4
El-Maghara	Wheat	MW	23.5 a	0.61 a	8.33 a	0.47a	18.9
Baloza	Wheat	BW	23.1 a	0.55 ab	6.79 b	0.47a	17.8
Ras-Sudr	Sorghm	RS	21.3 b	0.52 b	5.5 d	0.37 bc	18.2
Control			18.5d	0.44 c	5.1 de	0.3 d	0.22
L.S.D. 0.05			1.3	0.072	0.42	0.046	-

F.W.: Fresh weight

2. IAA Detection

Investigation the IAA production of the seven endophytic isolates indicated that all isolates possess IAA production, which varied among the bacterial isolates and ranged from 33 to 186 µg ml^{-1}. Five isolates produced high amounts of IAA over 100 µg ml^{-1} , while only two isolates produced IAA less than 50 µg ml^{-1} (Fig. 1). The endophytic bacterial isolates (RB, MW and BW) recorded the highest levels reaching 174, 186 and 176 µg ml^{-1}. Both *Azoctobacter spp.* and *Pseudomonas spp.* produced high levels of IAA when they cultured in a nutrient broth amended with 2 to 5 µg ml^{-1} of tryptophan (Ahmad et al., 2005).

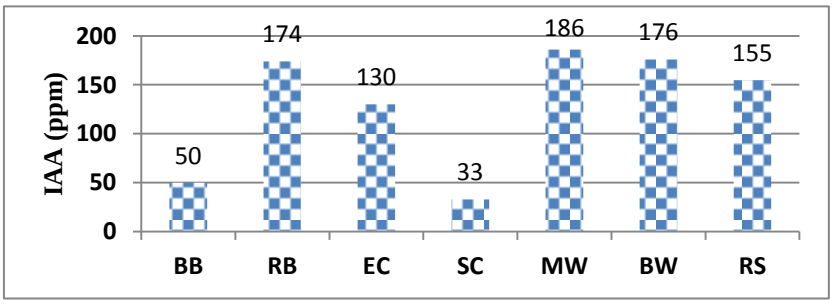

Fig. (1). IAA production by selected bacterial isolates**.**

3. Phosphate Solubilization, HCN and Siderophores Characterization

The seven isolates were subjected for dissolving phosphate in growing medium, only four isolates (RB, SC, MW and RS) showed positive results with different intensity. *Ps. aeruginosa* KUPSB12 was effective in phosphate solubilization with an index of 2.85 in Pikovskaya's agar plates along with very high soluble phosphate production of 219.64 ± 0.330 µg ml^{-1} in liquid medium (Dipak and Sankar, 2016). For HCN, all isolates were positive for the production of HCN with different intensity (Table 3).

Siderophore production using Chrome Azurol S (CAS) plates revealed that six of the seven selected isolates were positive to CAS, which mean that about 85% of isolated *Pseudomonas spp.* have the ability for siderophore production regardless to the type of sidrophores produced.

For characterization of sidrophores analysis of the seven bacterial culture grown in standard succinate medium, appearance of a deep red color using tetrazolium salts were detected for five of the seven studied isolates while formation of a yellow color using molybdate reagent were detected for only three of the seven isolates, which indicated the presence of hydroxamate - type siderophore and catecholate - type siderophore, respectively. None of the studied isolates can produce carboxylate type of siderophore. In general, siderophores are classified as hydroxymates or catecholates and more recently with new group polycarboxylates, which is detected only in bacteria as *Staphylococcus* strain, *Rhizobium* and fungi,

which belongs to Mucorales (Priyanka et al., 2012).

Using spectrophotometerical assay, the presence of ferrate hydroxamate siderophores of the five bacterial isolates were confirmed by detecting peaks between 420 to 450 nm, while the presence of ferrate catecholate siderophores for the three isolates were confirmed by detecting peaks between 490 to 495 nm. Both RB and MW isolates can produce both hydroxamate and catecholate - type siderophores as indicated in the table (3) and fig. (2). Many bacteria produce more than one type of siderophore as shown for *Enterobacter cloacae*, *Mycobacterium smegmatis* and *Pseudomonas spp.* (Adilakshmi et al., 2000 and Mercado-Blanco et al., 2001). In addition to a direct mechanism for growth promotion, plant growth promotion is also thought to be due to the suppression of a deleterious microflora by the introduced endophyte releasing siderophores (Kloepper et al., 1991).

4. Plant Growth Promoter Hormones

The two isolates with high IAA and siderophore production were subjected to HPLC assay for identification and quantification of the plant growth regulator produced. As shown in fig. (3), ethyl acetate extracts from the bacterial culture revealed that two strains secrete different types and quantities of growth hormones. However, results indicated that indole acetic acid and indole butyric acid at the retention time of 7.707 and 8.063 were detected in two isolates while gibberellin and abscisic acid were not detected in any of two isolates. The range of indole acetic acid and indole butyric acid production in two isolates were 231.8, 59.1 and 173.8, 97.8 ppm for RB and MW isolates, respectively. Auxin is quantitatively the most abundant phytohormones secreted by plant associated rhizobacteria, among auxin-like compounds, there are indole-3-propionic acid and indole butyric acid (Lin and Xu, 2013).

Table (3). HCN, Siderophores assay and its characterization for selected isolates.

Biochemical assays	**BB**	**RB**	**EC**	**SC**	**MW**	**BW**	**RS**
HCN	++	+	+	++	+	+	++
Phosphate solubilization	-	++	-	++	+++	-	+++
Siderohores	+	++	+	+	++	+	-
Hydroxymate -Type	+	+	+	+	+	-	-
Catecholate -Type	-	+	-	-	+	+	-
Carboxylate -Type	-	-	-	-	-	-	-

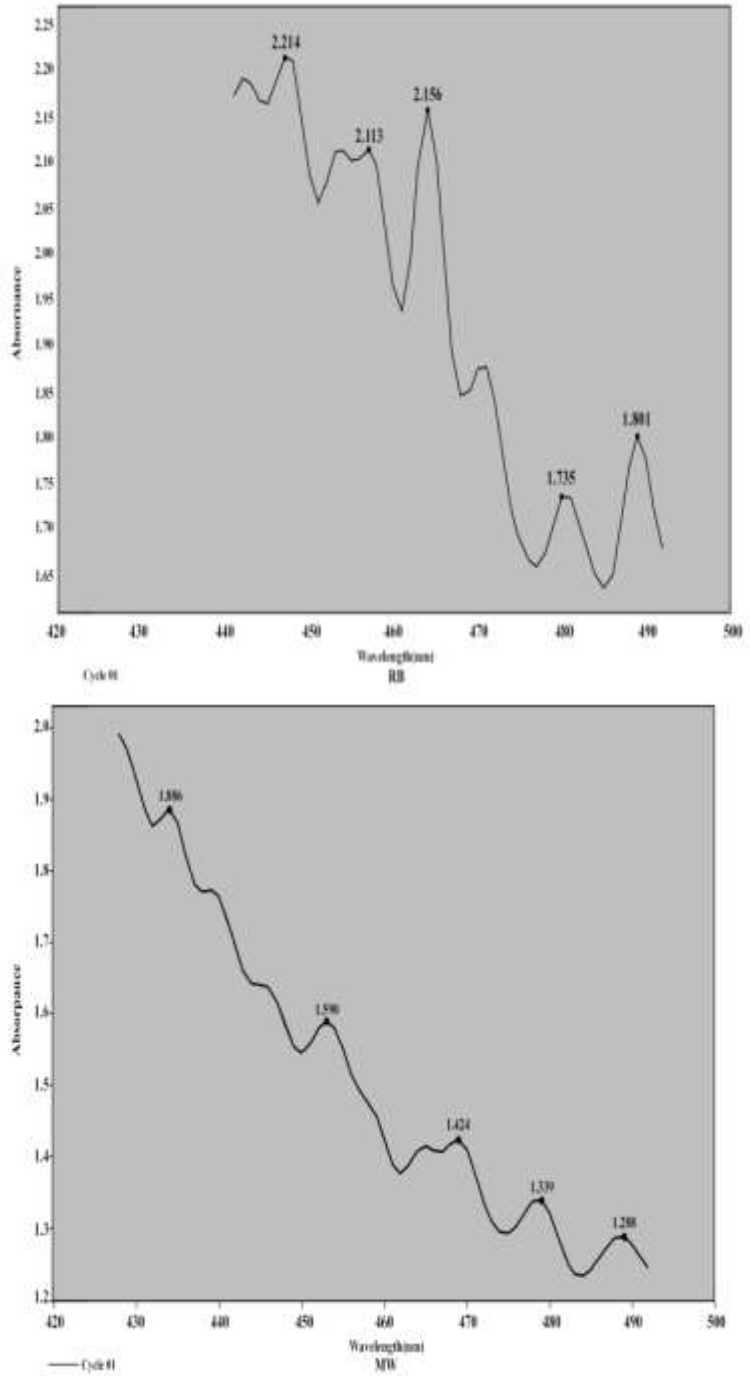

Fig. (2). Characterization of siderophores of RB and MW isolates.

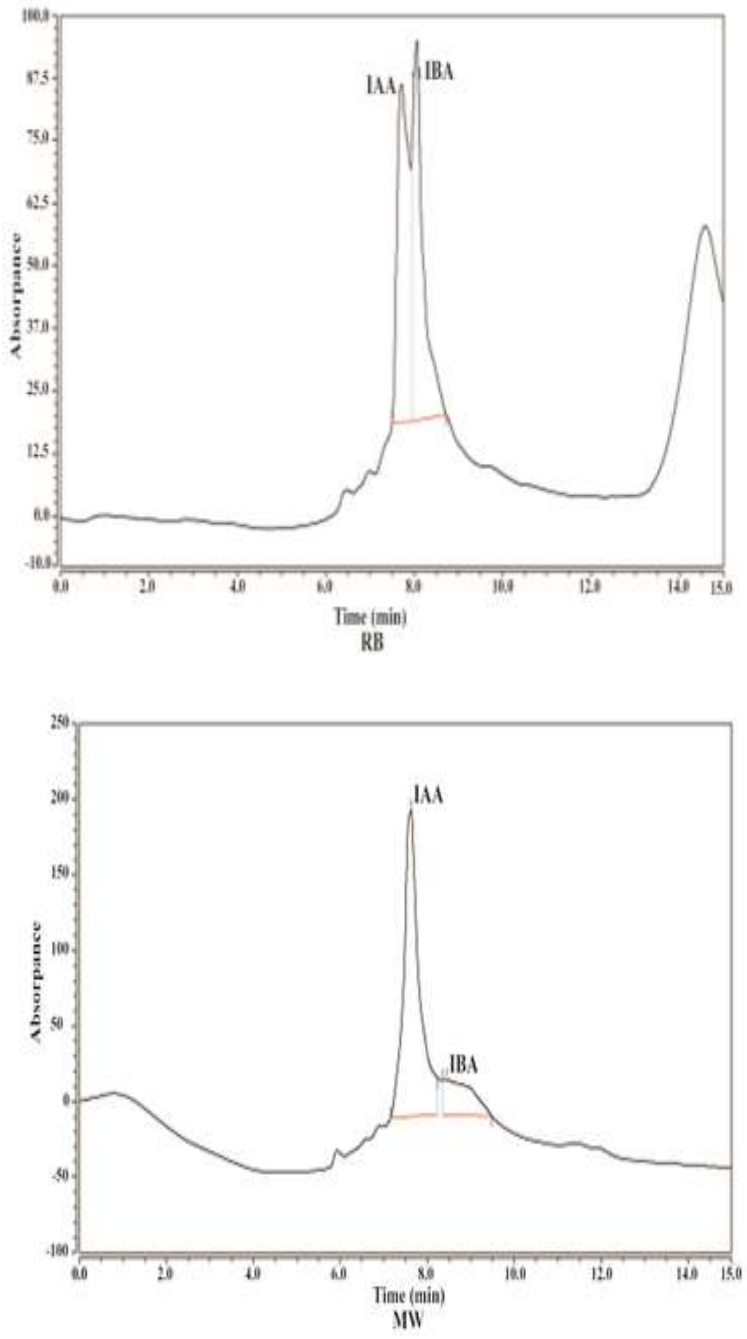

Fig. (3). HPLC profiles of plant growth regulator production.

5. Identification of the Most Efficient Isolates

Phylogenetic analysis of 16S rRNA sequence of the two isolates RB and MW showed maximum sequence similarity (88%) with *Ps. aeruginosa* PAO1 strain PAO1 and (96%) with *Ps. geniculata* strain ATCC 19374 (Fig. 4 and 5). When the nucleotide sequences were submitted, GenBank assigned NCBI accession number as NR024708.1 and NR117678.1 for *Ps. aeruginosa and Ps. geniculata*, respectively.

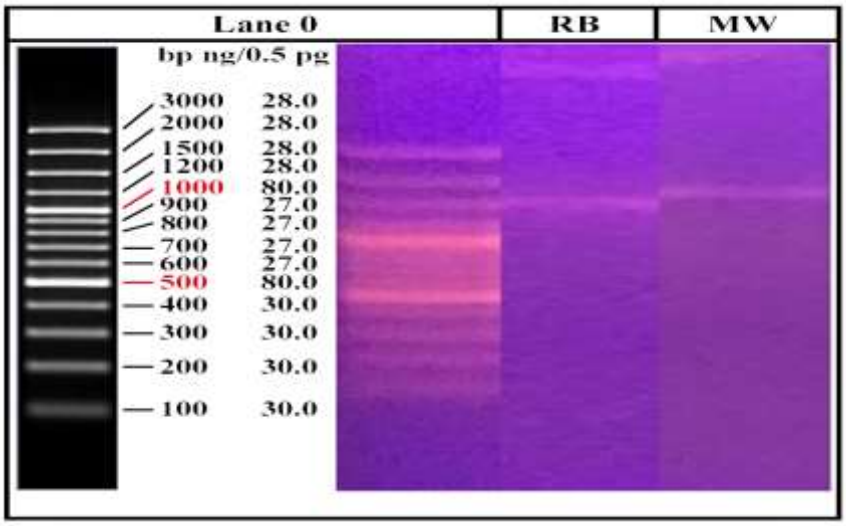

Fig. (4). PCR profiles of 16S rRNA fragments amplification of the isolates (RB and MW), lane 0; 100 pb plus DNA ladder.

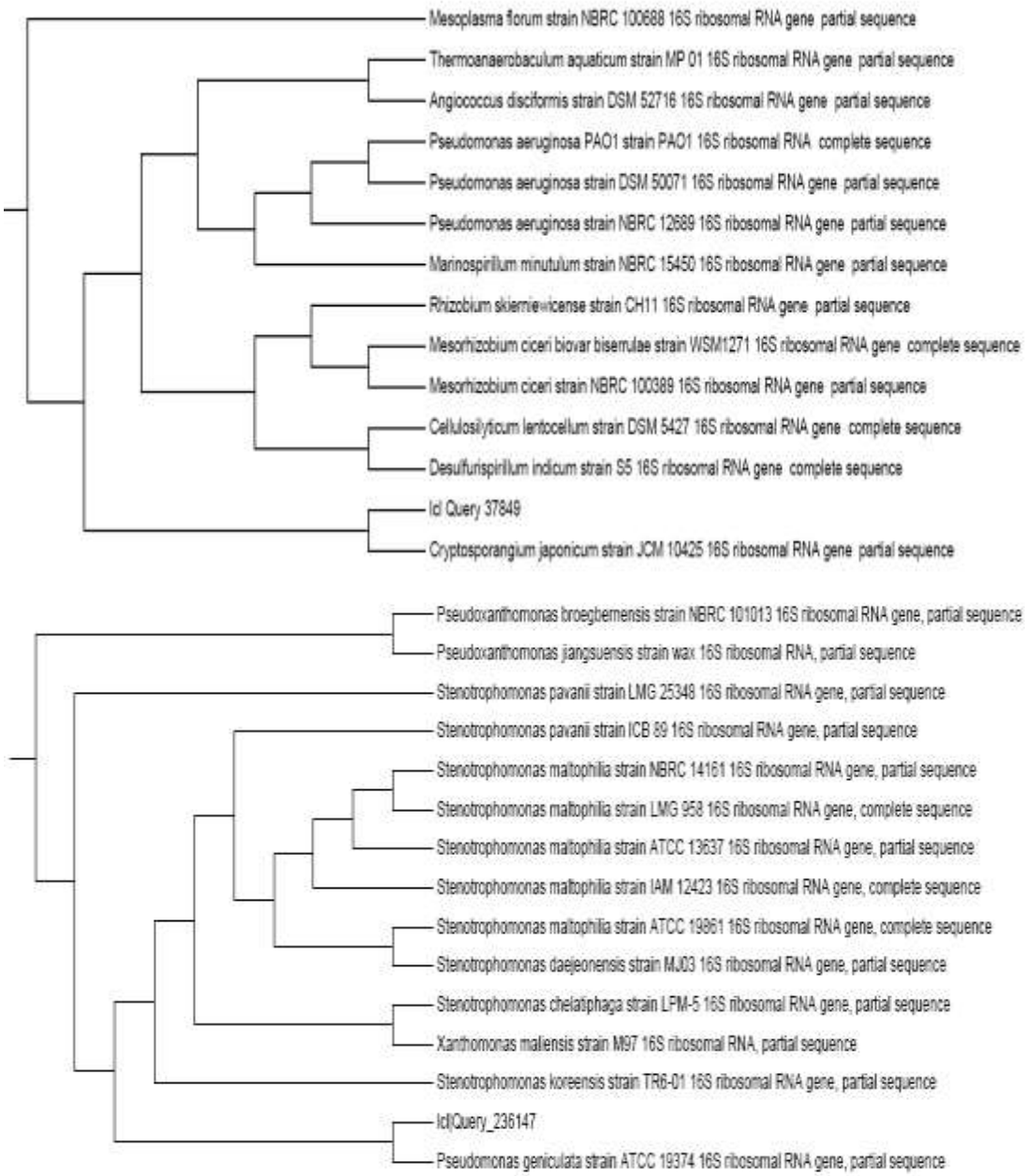

Fig. (5). Evolutionary relationships between the identified isolates and their relatives in the Gene Bank.

6. Field Experiment

6.1. Effect of endophytic bacterial inoculation on growth and yield parameters of maize

Generally, inoculation of maize seeds with strains *Ps .geniculata* or *Ps. aeruginosa* showed significant increase in all plant growth parameters measured (Table 4). At harvest, maximum shoot, root length and plant weight were observed with bacterial inoculation, regardless the type of bacteria used. Plant yields represented as straw yield were also increased in similar pattern, while for seed and biological yield, *Ps .geniculata* recorded the highest result followed by that of *Ps. aeruginosa* compared to control recording increase in biological yield reaching 55.2 and 33% over control for *Ps .geniculata* and *Ps. aeruginosa*, respectively. In Pongamia seedlings treated with a combination of *Ps. aeruginosa* and chemical fertilizer, the dry matter increased by 30.75% (Radhapriya et al., 2015). Inoculation of cowpea seeds with the *Ps. aeruginosa* FP6 enhanced seedling vigor index, plant height, and also fresh and dry weight in comparison with the control, which suggested the multifarious plant growth promoting activities of *Ps. aeruginosa* and suggests its potential use in developing a cost-effective eco-friendly multifunctional biofertilizer (Sasirekha et al., 2013).

Table (4). Effect of endophytic bacterial inoculation on growth and yield parameters of maize plants at harvest.

Treatments	Shoot Length (cm)	Root length (cm)	Straw (g/plant)	Grain (g/plant)	Grain yield (ton/fed)	Straw yield (ton/fed)	Biological yield (ton/fed)
Control	161.3a	18.70 b	193.3 b	154.0b	3.080 c	3.87b	6.950c
Ps. aeruginosa	163.6a	29.00a	293.4 a	173.3a	3.466b	5.78a	9.246b
Ps. geniculata	168.4a	26.60 a	313.3 a	226.7a	4.534a	6.26a	10.790a
L.S.D. 0.05	16.5	6.88	24.4	31.8	0.330	1.76	1.460

6.2. Effect of endophytic bacterial inoculation on chemical constituents of maize

Biofertilization treatments had no effective action on N and protien content in grains relative to the control as indicated in table (5). No significant differences between the two bacterial strains were detected. Concerning to other constituent measured in grains, inoculation process significantly increased the total phosphorus, carbohydrate and oil with about 24, 4.6 and 4.2% for both strains, where no significant differences were recorded between them. Inoculation of *Ps. pinnata* with *Ps. aeruginosa* increased nitrogen, phosphorus and potassium uptake by 34.1, 27.08 and 31.84%, respectively, when compared to control. Significant enhancement of total sugar, amino acids and organic acids content by 23.4, 29.39 and 26.53%, respectively, was seen in the root exudates of *Ps. pinnata* (Radhapriya et al., 2015).

Table (5). Effect of endophytic bacterial inoculation on chemical constituents of maize plants at harvest.

Treatments	Chemical constituents of grains				
	Total P %	**Total N %**	**Protein %**	**Carbohydrate %**	**Oil %**
Control	0.53 b	1.52 a	9.5 a	79.1 b	19.1 b
Ps. aeruginosa	0.66 a	1.651 a	10.36 a	82.7 a	19.8 a
Ps .geniculata	0.66 a	1.656 a	10.33 a	82.84 a	19.9 a
L.S.D. 0.05	0.034	0.16	1.04	2.3	0.52

6.3. Effect of endophytic bacterial inoculation on the microbial densities

As indicated from table (6), the change in total microbial and *Pseudomonads* counts in the rhizosphere tend to increase in treated plants compared to the control regardless the type of bacterial strain used. For endophytic *Pseudomonads* count, no change were detected in the treated plants comparing to control one, which indicated that inoculation process did not mean the invasion of the roots by endophytic bacteria. Facultative endophytes are free living in soil but will colonize plants when the opportunity arises, through coordinated infection (Hardoim et al., 2008). The inoculation process have a positive effect on the activity and abundance of microbial community in the rhizosphere, which appeared as remarkable increase in the dehydrogenase activity of treated plant compared to control. Measurement of dehydrogenase activity by indigenous microorganisms in soil has the potential to serve as a useful indicator of the microbial activity to determine the relative effectiveness of the plant rhizosphere in soils (Mathew and Obbard, 2001).

Table (6). Effect of endophytic bacterial inoculation on the microbial densities at harvest.

Treatments	**Rhizospheric total microbial count*10^5 CFU/g dry soil**	**Rhizospheric *Pseudomonas* count *10^5CFU/g dry soil**	**Endophytic *Pseudomonas* count *10^3CFU/g root**	**Dehydrogenase (µg TPF/g dry soil/24 h)**
Control	108	5.6	4.2	246
Ps. aeruginosa	116	8.2	4.1	320
Ps .geniculata	154	9.7	4.5	398

-Initial total microbial counts was 18 ×10^5 cfu/g dry soil

-Initial *Pseudomonas* counts was ×10^4 cfu/g dry soil

CONCLUSION

Two endophytic rhizobacteria namely *Ps. geniculata and Ps. aeruginosa* are highly efficient plant growth promoters that possess HCN, indole acetic acid, siderophore production and phosphate dissolving activities, and they can be used as plant inoculants for enhancing plant growth and yields.

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دور بكتيريا السيدوموناس الداخلية كمحفز لنمو النبات تحت الظروف الصحراوية

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البكتيريا الداخلية في النبات والمحفزة للنمو تعمل على زيادة النمو بإستخدام العديد من الأنشطة الأيضية التي لها علاقة بنمو وصحة وتطور النبات، ويعد تأثيرها أقوى من تأثير بكتيريا الريزوسفير. تم عزل عدد ٤٦ عزلة بكتيرية من داخل جذور نباتات القمح، الشعير، الذرة والذرة السكرية والمزروعة في مناطق القنطرة، بالوظة، رأس سدر، سهل الطينة والمغارة. وقد تم إختبار قدرتها على تحفيز نمو النبات في المعمل وإختيار أفضل سبعة عزلات والتي أعطت أفضل النتائج في زيادة نمو أطوال وأوزان الجذور والسيقان لبادرات الذرة على بيئة Hoagland وإخضاعها للعديد من الإختبارات المعملية والتي أكدت على قدرة جميع هذه العزلات على إنتاج غاز الـ HCN وهرمون الـ IAA والذي يتراوح بين٣٣ و١٨٦ميكروجرام/مللتر كما أن أربعة من هذه العزلات أعطت نتيجة إيجابية لإذابة الفوسفور.

كما أظهر إختبار Chrome Azurol S أن جميع العزلات البكتيرية السبعة لها القدرة على إنتاج السيدروفور وأن توصيف نوع السيدروفور بإستخدام التحليل الطيفي قد أظهر أن خمسة من العزلات البكتيرية تفرز السيدروفور من نوع الهيدروكسيمات وثلاثة من نوع الكاتيكولات. وقد تم إختيار أفضل العزلات المحفزة لنمو النبات والقادرة على إفراز السيدروفور من نوع الهيدروكسيمات والكاتيكولات وأعلى نسبة من هرمون الـ IAA وغاز الـ HCN وإذابة الفوسفات. وبدراسة قدرة العزلتين على إفراز الأنواع المختلفة من الهرمونات النباتية بواسطة جهاز الكروماتوجرافي السائل، فقد وجد أن هذه العزلات تفرز هرموني الـ IAA و الـ IBA فقط. و قد تم تعريف العزلتين بإستخدام تقنية 16SrRNA على أنهم *Pseudomonas aeruginos* PAO1 strain PAO و *Pseudomonas geniculata* strain ATCC19374

وقد تم إجراء تجربة حقلية في محطة بحوث القنطرة والتابعة لمركز بحوث الصحراء وذلك بهدف تقييم بكتيريا الـ*Pseudomonas aeruginosa* و *Pseudomonas geniculata* على نمو وانتاجية نبات الذرة. وقد وجد أن التلقيح بهذه البكتيريا يزيد من المحصول بنسبه تتراوح من ٥٥.٢ و٣٣٪ عند التلقيح ببكتيريا *Pseudomonas aeruginosa* و *Pseudomonas geniculata*، على التوالي. وبالنسبة للمحتوى الكيميائي للبذور فإن التلقيح البكتيري أوجد زيادة في المحتوى من الكربوهيدرات والزيت بنسبة ٤.٦ و٤.٢٪ مقارنة بالكنترول. وقد أظهرت التحاليل الميكروبيولوجية أنه لا يوجد تأثير للتلقيح البكتيري على أعداد السيدوموناس الداخلية في جذور نبات الذرة في الوقت الذي أحدث التلقيح زيادة في أعداد ونشاط البكتيريا في منطقة الريزوسفير.