Zagazig Journal of Agricultural Research
www.zu.edu.eg/agr/journals

ISOLATION AND CHARACTERIZATION OF HIGHLY EFFICIENT PLANT GROWTH-PROMOTING RHIZOBACTERIA ISOLATED FROM WHEAT AND FABA BEAN RHIZOSPHERE

Sally M.H. Attia*, S.H. Salem, V.S. Bedrous and M.I. Hegazy

Agric. Microbiology Dept., Fac. Agric., Zagazig, Univ., Egypt

ABSTRACT

Forty two rhizobacterial isolates were isolated from the rhizosphere soil of faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) in different locations at Sharkia governorate, Egypt. These isolates were screened for indole acetic acid (IAA) equivalents and siderophore production, phosphate solubilization, cellulase enzymes production and nitrogenase enzyme activity. All isolates were able to produce IAA in presence or absence of tryptophan (L-TRP) although much variation was observed between them. The values of IAA in the absence of L-TRP ranged between 7.913 and 19.953 mg L^{-1} for the wheat isolates and 8.519 to 20.559 mg L^{-1} for the faba bean isolates, while the values of IAA in the presence of L-TRP ranged from 10.171 to 43.273 mg L^{-1} for the wheat isolates and from 9.779 to 47.445 mg L^{-1} for the faba bean isolates. Thirty nine isolates were able to solubilize $Ca_3 (PO_4)_2$ on Modified Bunt and Rovira Medium, 35 isolates resulted in positive reactions with the chrome azurol S (CAS) agar medium, 26 isolates showed positive cellulase reactions with carboxymethyl cellulose medium (CMC) and 28 isolates gave a positive reactions with nitrogenase enzyme activities. Out of the forty two isolates, 10 were unidentified and 32 were identified at the genus level, six of them proved to be efficient PGPR were identified as *Azospirillum brasilense, Serratia marcescens, Pseudomonas fluorescens, Pseudomonas putida* and *Bacillus brevis* as well as *Azotobacter* sp. all of which will be used as bio-fertilizers in further studies.

Keywords: Plant growth promoting rhizobacterial (PGPR), wheat and faba bean plants, *Pseudomonas fluorescens, Serratia marcescens , Bacillus brevis*.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide various indirect and direct mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements (Kloepper and Schroth, 1978; Saharan and Nehra, 2011 and Myresiotis *et al.*, 2012). PGPR can be divided into two groups according to their relationship with the plants; a) symbiotic bacteria, b) free-living rhizobacteria. Although the direct and indirect mechanisms by which PGPR promote plant growth are not yet fully understood, many different traits of these bacteria are responsible for growth promotion activities (Cattelan *et al.*, 1999). The direct mechanisms involve improved nutrient acquisition (Bio-fertilizers) by nitrogen fixation and phosphorus solubilization, production of phytohormones (Bio-stimulants) such as auxins, cytokinins and gibberellins, lowering of ethylene concentration, while enhancing Hydrogen Cyanide (HCN), siderophores (FeIII chelating agent) or/ and antibiotics production. Indirect mechanisms of PGPR include competition with detrimental microorganisms for sites on plant roots, antibiotic protection against pathogenic bacteria (Bio-protectants), reduction of iron available to phytopathogens in the rhizosphere, and synthesis of fungal cell wall lysing enzymes (Kloepper *et al.*, 1989; Glick, 1995 and Glick *et al.*, 1999).

* **Corresponding author: Tel. :** +2001121263731
E-mail address: sallyelaidy@ymail.com.

Numerous plant growth promoting rhizobacteria (PGPR) of the genera *Pseudomonas, Bacillus, Arthrobacter, Azospirillum, Azotobacter, Klebsiella,* and *Enterobacter* have been isolated from the rhizosphere of various crops and noted for their synergistic effects on plant growth (Kloepper and Beauchamp, 1992; Egamberdiyeva and Hoflich, 2001). Many species of PGPR are able to synthesize and release IAA as secondary metabolites *in vitro* in the presence or absence of physiological precursors, mainly tryptophan (Muller *et al.*, 1989; Caron *et al.*, 1995 and Davies, 1995). It's noteworthy that tryptophan is one of the main compounds present in several plant exudates. Different bacteria are capable of producing IAA include *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Azospirillum* sp., *Enterobacter* sp. and *Serratia* sp. (Martens and Frankenberger, 1991; Frankenberger and Arshad, 1995).

Phosphate-solubilizing microbes play fundamental roles in biogeochemical phosphorus cycling in natural and agricultural ecosystems. Phosphate solubilizing microbes can transform the insoluble phosphorus to soluble forms $(H_2PO_4)^{1-}$ and $(HPO_4)^{2-}$ by acidification, chelating, exchange reactions, and polymeric substances formation (Delvasto *et al.*, 2006). Many bacterial species are phosphate solubilizing bacteria (PSB) capable to solubilize phosphates *in vitro* and most of them live in the plant rhizosphere. At present, bacilli, rhizobia and pseudomonads are the most studied P-solubilizers (Kim *et al.*, 1998 and Rodriguez and Fraga, 1999). Strains from *Bacillus megaterium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Pseudomonas striata, Rhizobium and Enterobacter of the bacteria along with Penicillium and Aspergillus* of the fungi could be referred to as the most powerful P solubilizers (Subba Rao 1988; Kucey *et al.* 1989 and Whitelaw, 2000).The principal mechanism for mineral phosphate solubilization is the production of organic acids, however, acid phosphatases play a major role in the mineralization of organic phosphorus in soil. Under iron-limiting conditions PGPR produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion (Whipps, 2001). *Pseudomonas* spp. have the capacity to utilize siderophores produced by diverse species of bacteria and fungi, and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper and Henkels, 1997). Nitrogen fixation can be carried out by several associative and free-living microorganisms in the rhizosphere of plants and it is recognized to play an important role in nitrogen nutrition of plants (Boddey *et al.*, 1996).

The aim of this study was to assess the potential of 42 rhizobacterial isolates isolated from wheat and faba bean rhizosphere from different locations in Sharkia governorate to act as PGPR. The study included the auxin biosynthesis, phosphate solubilization, siderophores production as well as cellulase enzyme production and nitrogenase enzyme activity, in order to select effective PGPR to be used as bio-fertilizers.

MATERIALS AND METHODS

Isolation and Purification of Plant growth Promoting Rhizobacteria

Rhizosphere soil samples were collected from the rhizosphere of faba bean and wheat grown in fields in Sharkia governorate, Egypt. Forty two rhizobacterial cultures were isolated on plates by dilution plate technique. Ten grams of each rhizosphere soil were taken into 250 ml conical flask and 90 ml of sterile distilled water was added to it. After serial dilution up to 10^{7-} 0.1 ml aliquots were spread on plates of five different media namely; Nutrient Agar (NA) (Difco, 1985), Burks-N-Free Medium (NFM) (Subba Rao, 1999), King's B Agar Medium (KBA) (Starr *et al.*, 1981), Semi Solid Malate Medium (Dobereiner *et al.*, 1976), and Modified Ashby's Medium (Abd El-Malik and Ishac, 1968). After incubation at 28°C; morphologically different colonies on the different media were isolated and streaked for purification. These isolates were designated as shown in Table 1.

Screening for IAA- Producing Activity

A modified colorimetric method was used for the determination of IAA (Asghar *et al.*, 2000).

Table 1. Description of the PGPR isolates

Plants	Wheat *(Triticum aestivum L.)*	Faba bean *(Vicia fabe L.)*
Locations	Isolates codes	Isolates codes
Menia El-Kamh M)	MW3, MW4, MW7, MW8, MW9.	MB1, MB2, MB6, MB8.
Zagazig (Z)	ZW1, ZW2, ZW3, ZW4, ZW5, ZW6, ZW10.	ZB3, ZB4, ZB5, ZB7.
Belbies (B)	BW4, BW5, BW6, BW8, BW9.	BB1, BB3, BB4, BB5, BB11.
Abo-Hammad (A)	AW5.	AB2, AB6.
Hahia (H)	HW2, HW4.	HB4, HB5.
El-Hassenia (E)	EW4.	EB3, EB4, EB5, EB6.

Pure colonies of 42 isolates were obtained from wheat and faba bean rhizosphere and grown in 100 ml Erlenmeyer flasks containing 25 mL nutrient broth with or without 5 ml (0.5%) of tryptophan (L-TRP) solution and incubated at 28 ± 2 °C for 24 h on a rotary shaker at 150 rpm. The cultures were then centrifuged at 4000 rpm for 20 min. Non-inoculated flasks were kept for comparison as control. One ml solutions of the supernatant were placed in test-tubes and mixed each with 2 mL Salkowski reagent (2% of 0.5M FeCl3 in 35% perchloric acid). After 25-30 min incubation in the dark, the color of supernatant containing IAA turned red; the color absorbance was read using a spectrophotometer Model JENWAY No. 6405 UV/ Vis at 540 nm. Pure IAA was used for preparing the standards of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 mg mL^{-1}.

Screening for Phosphate Solubilizing Activity

The ability of isolates to solubilize phosphate was assessed qualitatively using medium of Bunt and Rovira (1955), modified by Abdel-Hafez (1966) containing freshly precipitated calcium phosphate as the following: 50 mL of sterile 10% (wt. vol^{-1}) K_2HPO_4 and 100 mL of sterile 10% (wt.vol^{-1}) $CaCl_2$ were added per liter of sterile medium to produce a precipitate of $CaHPO_4$. The pH of the medium was again readjusted to pH 7.2 by sterile standard NaOH. Phosphate solubilization was assessed by measuring the halo zone diameter.

Screening for Siderophore Producing Activity

Siderophores production was tested qualitatively using chrome azurol S (CAS agar medium) as described by Schwyn and Neilands, (1987). The rhizobacterial inoculum was placed on the surface of CAS medium and incubated in the dark at 30 °C for 7 days. The production of siderophores was indicated by orange halos around the colonies.

Nitrogenase enzyme activity

Nitrogen fixation ability of the isolates was determined by growing the isolates in nitrogen free liquid medium. The isolates which showed positive growth in the latter medium were considered as nitrogen fixers. Nitrogenase enzyme activity of these isolates was measured according to Hardy *et al.* (1973).

Cellulase enzyme production

The cellulase enzyme activity of rhizobacterial isolates was tested on M9 medium agar containing CMC as source of carbon as described by (Cattelan *et al*, 1999). Rhizobacterial isolates surrounded by clear halo after incubation at 28 °C for 5 days were considered as positive for cellulase enzyme production.

Identification of Selected Rhizobacterial Isolates

Forty two rhizobacterial isolates which were characterized by their efficiencies in phosphate solubilization, IAA, siderophores and cellulase enzyme production as well as nitrogenase enzyme activity were subjected to morphological, physiological and biochemical studies i.e., the cell size, colony elevation, surface, margin, color, pigmentation, shape, as well as Gram reaction, spores staining, motility, catalase and oxidase test, starch and gelatin hydrolysis, H_2S production and IMViC test in order to recognize the genera of the microbes. Among all the most efficient plant growth

promoting rhizobacteria (PGPR) isolates; only six were selected on the basis of sharing 4 or 5 characters to be identified on the species level according to Mac Faddin, 1976; Holt *et al.*, 1994; Sneath *et al.*, 1986 and Krieg and Holt, 1984 in order to use them in further investigation.

Statistical Analysis

All experimental treatments were done in three replicates and the results were analysed according to Snedecor and Cochran (1982).

RESULTS and DISCUSSION

The results in Tables 2 and 3 demonstrated that all the isolates isolated from wheat and faba bean rhizosphere shared in three or more of the PGPR traits such as: IAA production, phosphate solubilization, siderophores and cellulase enzyme production as well as nitrogenase enzyme activity.

IAA- Producing Activity

From Tables 2 and 3 it can be shown that all isolates isolated from wheat and faba bean rhizosphere had the ability to produce the auxin in the presence and absence of tryptophan (L-TRP), with much variation observed between them. However auxin production by some of the tested rhizobacterial isolates were enhanced when culture media were supplemented with L-TRP, and consequently, considered as IAA producing rhizobacteria. Regarding wheat rhizobacterial isolates, the results in Table 2 show that concentration of IAA in the absence of L-TRP ranged between 7.913 and 19.953mgL^{-1}. There were significant differences in the concentration of IAA produced among the isolates for example isolates *Azospirillum brasilense* (BW9) and *Bacillus* sp. (BW5) were able to produce the highest values of IAA being 19.953 and 15.318 mg L^{-1}, respectively. On the other hand, in the presence of L-TRP. The highest concentration of IAA varied according to the location i.e., ZW2, BW9, HW4 and EW4 were superior among these isolated from Zagazig, Belbies, Hehia and El-Hussein, respectively. However, no significant differences ($P< 0.05$) were found between Menia El-Kamh isolates except MW3 which was inferior among the other isolates. These results support those reported by Frankenberger and Arshad (1995), Patten and Glick (1996) and Chopade *et al.* (2008) who confirmed that IAA biosynthesis by rhizobacteria is greatly influenced by presence of L-TRP precursor and it is considered the primary precursor for formation of IAA in several microorganisms.

Also, Khalid *et al.* (2004) reported that among 30 strains of rhizobacteria isolated from wheat rhizosphere soil at different locations produced variable values of auxin (ranging from 1.1 to 12.1 mg L^{-1}) in the absence of L-TRP, and amendment of the culture medium with L-TRP stimulated auxin biosynthesis (ranging from 1.8 to 24.8 mg L^{-1}). Addition of L-TRP resulted in two folds or more increase in the auxin production, i.e., ZW2 five folds, BW9 and EW4 two folds. These results confirmed those obtained by Yasmin *et al.*, (2009) who reported that most of the isolates required L-TRP precursor for IAA production. Whereas, three of the isolates *Klebsiella* UPMSP9, *Pseudomonas* UPMSP2 and *Pseudomonas* UPMSP13 showed 9, 7 and 6-folds increases, respectively in IAA production when grown in media with L-TRP. Not all isolates responded to the precursor application such as the isolates BW4, BW5 and HW2 which showed no significant change in IAA with L-TRP addition. Such discrepancy of the non-responsive isolates to the addition of L-TRP can be explained by the existence of five pathways in bacteria for the biosynthesis of IAA, some of which is not L-TRP-dependent (Verma *et al.*, 2010).

The results in Table 3 show that in the absence of L-TRP there was significant differences ($P < 0.05$) in the concentration of IAA produced among the faba bean rhizobacterial isolates. *Azotobacter* sp. (HB5) and *Bacillus* sp. (MB8) produced the highest values of IAA being 20.559 and 20.084 mg L^{-1}, respectively. L-TRP supplementation resulted in several folds increase in auxin production, i.e., more than five folds in the case of MB2 of *Serratia marcescens* and almost 4 folds in the case of BB3 of *Bacillus* sp.. However, HB5 of *Azotobacter* sp. produced the highest level among Hahia isolates being 37.211mgl^{-1}.

Comparing production of IAA by wild types and engineered trp mutants (Idris *et al.*, 2007)

Table 2. Characteristics and activities of Rhizobacterial isolates isolated from wheat rhizosphere

*Isolates number	**Characteristics of isolates	Name of isolates	IAA equivalent in (mg/L^{-1})		Phosphate solubilization (diameter-cm)	Siderophores production (diameter-cm)	Enzymes activities	
			without L-TRP	with L-TRP			Cellulase production (diameter-cm)	Nitrogenase activity (moles C_2H_4/ml/h)
MW3	Straight long rods, M+, G+ and S+	*Bacillus* sp.	10.516 c	13.083 b	1.03 a	+	1.8 a b	0
MW4	Straight short rods M- G- and S-	Un-identified.	10.979 a	15.306 a	1.03 a	+	1.9 a b	0
MW7	Straight or slightly curved rods M+, G- and S-.	*Pseudomonas putida*	8.757 e	14.355 a	0.97 a	+	2.03 a	0
MW8	Straight short rods M-G- and S-	Un-identified.	11.193 a	14.450 a	0.93 a	+	1.97 a	1.52
MW9	Straight or slightly curved rods M+, G- and S-	*Pseudomonas fluorescens*	9.517 d	15.211 a	1.10 a	+	2.00 a	0
L.S.D. at 5%			0.42	1.093	0.20		0.13	
ZW1	Spherical M-, G+ and S-.	Un-identified.	8.269 c	13.440 d	0.97 b	+	3.3 a	0
ZW2	Straight long rods M+, G+ and S+	*Bacillus brevis*	8.483 c	43.273 a	0.87 b	+	2.2 b	0
ZW3	Straight short rods M-, G- and S-	Un-identified.	10.231 a b	18.194 b	0.80 b	+	1.1 c	0
ZW4	Straight short rods M-, G- and S-	Un-identified.	9.624 b	12.465 de	1.07 a b	+	0	0.97
ZW5	Straight long rods M+, G+ and S+	*Bacillus* sp.	7.913 c	15.817 c	1.17 a	-	0	0
ZW6	Straight long rods M+, G+ and S+	*Bacillus* sp.	9.957 a b	12.988 de	1.07 a b	+	0	0
ZW10	Straight long rods M+, G+ and S+	*Bacillus* sp.	10.754 a	12.025 e	0.97 b	+	0	0.1
L.S.D at 5%			0.83	1.21	0.18		0.11	
BW4	Straight long rods M+, G+ and S+	*Bacillus* sp.	9.815 c	10.171 e	0.70 b	+	1.3 b	0
BW5	Straight long rods M+, G+ and S+	*Bacillus* sp.	15.318 b	16.197 c	1.00 b	+	0	0.97
BW6	Straight long rods M+, G+ and S+	*Bacillus* sp.	9.684 c	18.812 b	1.07 a b	-	1.3 b	0
BW8	Straight long rods M+, G+ and S+	*Bacillus* sp.	9.696 c	12.406 d	1.13 a b	+	2.8 a	0
BW9	Viroid or helical M+, G- and S-	*Azospirillum brasilense*	19.953 a	41.847 a	1.4 a	+	1.2 b	0.88
L.S.D. at 5%			1.30	1.15	0.39		0.19	
HW2	Straight long rods M+, G+ and S+	*Bacillus* sp.	10.052 a	11.538 b	0.8 a	+	2.1 a	0
HW4	Straight long rods M+, G+ and S+	*Bacillus* sp.	9.351 a	13.321 a	0.9 a	+	1.3 a	0
L.S.D. at 5%			1.22	1.72	0.19		1.03	0
AW5	Spherical M-, G+ and S-	Un-identified.	9.292 b	13.321 b	1.5	-	0	0
EW4	Straight long rods M+, G+ and S+	*Bacillus* sp.	11.122 a	23.127 a	0	+	1.7	0.09

* Areas of isolation are coded as: M, Menia El-Kamh; Z, Zagazig; B, Belbies; H, Hehia; A, Abo-Hammad; E, El-Huseineya. ** M: Motility, G: Gram reaction and S: spores forming. Numbers having the same letter within the same area are not significantly different at $P < 0.05$

Table 3. Characteristics and activities of Rhizobacterial isolates isolated from faba bean rhizosphere

*Isolates number	**Characteristics of isolates	Name of isolates	IAA equivalent in (mg/L^{-1})		Phosphate solubilization (diameter-cm)	Siderophores production (diameter-cm)	Enzymes activities	
			without L-TRP	with L-TRP			Cellulase production	Nitrogenase activity(mole C_2H_4/ml/h)
MB1	Straight long rods M+, G+ and S+	*Bacillus* sp.	13.333b	21.688b	0.9 a b	-	2.00	0
MB8	Straight long rods M+, G+ and S+	*Bacillus* sp.	20.084a	21.415b	1.1a	+	0	0
MB6	Straight long rods M+, G+ and S+	*Bacillus* sp.	8.519d	16.744c	0	-	0	0
MB2	Straight short rods M+, G- and S-	*Serratia marcescans*	9.173c	47.445a	0.8b	-	0	0
L.S.D. at 5%			0.51	1.28	0.17			
ZB3	Straight short rods M-, G- and S-	Un-identified.	14.581a	17.528a	0	+	0	1.24
ZB4	Straight short rods M-, G- and S-	Un-identified.	9.066c	16.269b	1.00b	+	1.1b	0.97
ZB5	Straight short rods M-, G- and S-	Un-identified.	10.801b	13.309c	1.2a	+	0	1.52
ZB7	Straight short rods M-, G- and S-	Un-identified.	9.173c	12.465d	0.97b	+	1.9 a	0
L.S.D. at 5%			0.45	0.55	0.096		0.07	
BB1	Straight long rods M+, G+ and S+	*Bacillus* sp.	13.832a	16.471d	1.3 a b	+	0	0
BB3	Straight long rods M+, G+ and S+	*Bacillus* sp.	11.871 a b	43.297a	1.2 a b	+	0	0
BB4	Straight long rods M+, G+ and S+	*Bacillus* sp.	10.694b	19.038c	1.4a	+	0	0
BB5	Straight long rods M+, G+ and S+	*Bacillus* sp.	12.727 a b	22.556b	1.2 a b	+	0	0
BB11	Straight long rods M+, G+ and S+	*Bacillus* sp.	12.739 a b	15.520d	1.1b	+	0	0
L.S.D. at 5%			1.96	1.43	0.21			
EB3	Straight long rods, G^+, M^+ and S^+	*Bacillus* sp.	10.326 a b	12.358a	1.3a	+	2.8a	0
EB4	Straight long rods, G^+, M^+ and S^+	*Bacillus* sp.	9.363b	9.779b	0.9b	+	1.9c	0
EB5	Straight long rods, G^+, M^+ and S^+	*Bacillus* sp.	11.217a	10.421b	0.7b	-	0.9c	2.35
EB6	Straight short rods, G^-, M^- and S^-	Un-identified.	11.098a	13.963a	0.8b	+	2.3b	0
L.S.D. at 5%			1.45	1.58	0.31		0.38	
HB4	Straight long rods M+, G+ and S+	*Bacillus* sp.	13.214b	17.944b	0.6a	+	2.7a	1.02
HB5	Spherical M-, G+ and S-	*Azotobacter sp.*	20.559a	37.211a	0.6a	+	1.2b	13.64
L.S.D. at 5%			1.31	0.12	0.14		0.14	
AB2	Straight long rods M+, G+ and S+	*Bacillus* sp.	11.704a	12.382b	1.00a	+	2.3	0.02
AB6	Straight long rods M+, G+ and S+	*Bacillus* sp.	11.467a	14.462a	0.5b	+	1.3	1.11
L.S.D. at 5%			0.77	1.19	0.15		0.08	

*Areas of isolation are coded as: M, Menia El-Kamh; Z, Zagazig; B, Belbies; H, Hehia; A, Abo-Hammad; E, El-Huseineya.

** M: Motility, G: Gram reaction and S: spores forming. Numbers having the same letter within the same area are not significantly different at $P < 0.05$.

suggested that the main route of IAA biosynthesis in *Bacillus amyloliquefaciens* is dependent on tryptophan. Stimulation of IAA synthesis by tryptophan was described previously by gram negative plant-associated bacteria (Ernsten *et al.*, 1987 and Koga *et al.*, 1991). Patten and Glick (2002) used a mutant plant beneficial bacterium *Pseudomonas putida* deficient in the ipdc gene product (indole pyruvate decarboxylase) to demonstrate that IAA synthesis in bacteria is dependent on tryptophan concentration.

Phosphate-solubilizing Activity

The results in Tables 2 and 3 show that except isolates EW4, MB6 and ZB3 all the tested rhizobacterial isolates were able to form clear zones around the colonies on agar plates, an indication of calcium phosphate $Ca_3 (PO_4)_2$ solubilization. Regarding wheat rhizobacterial isolates, the diameter of solubilization ranged between 0.8 and 1.5 cm. The highest was observed by *Azospirillum brasilense* (BW9) and AW5 (un-identified) with a diameter of 1.4 and 1.5 cm, respectively (Table 2). Regarding faba bean rhizobacterial isolates, *Bacillus* sp. (BB4) formed the highest clear zone the diameter being 1.4 cm. However, *Bacillus* sp. (HB4) formed the smallest clear zone with diameter of 0.6 cm. These results are in harmony with the findings of Freitas *et al.* (1997) and Yasmin *et al.* (2009). Phosphate solubilization by rhizobacterial isolates has been related to the production of organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. The production of organic acids results in a decrease in soil pH, producing H^+ which replace ions the Ca^{2+} and release HPO_4^{2-} to the soil solution. Besides changes in pH, chelation by organic acids which bind metal ions such as Fe, Al, and Ca bring about phosphate into soil solution (Kucey, 1983 and Rodriguez and Fraga, 1999).

Siderophore Production Activity

The results in Tables 2 and 3 show that only thirty five rhizobacterial isolates were able to form orange halos around the colonies on CAS-agar plates. Pseudomonads are known to produce pyoverdines and pseudobactins, which can be detected by their yellow-green color fluorescence under ultraviolet light when grown on iron deficient medium (Buysens *et al.*, 1996). Some of the isolates under investigation (5 bacilli, *Serratia marcescens* and unidentified) could not grow on CAS agar. It was suggested that this could be due to the toxicity of HDTMA present in CAS agar which affects mainly fungi and gram-positive bacteria, where the detergent HDTMA has been reported to play a crucial role in the interference of the CAS assay. Too low concentrations of the detergent led to precipitation of the dye and too high concentrations are toxic to many bacteria (Schwyn and Neilands 1987).

Cellulase Enzyme Production

From data in Tables 2 and 3 it can be shown that the diameter of clear halos the widest diameter was formed by an unidentified isolate ZW1 followed by BW8 and EB3 of the *Bacillus* sp.. Next to them were ZW2 of the *Bacillus brevis*, MW9 of *Psedumonas fluorescens* and MW7 of *Pseudomonas putida*. An indication of the ability of these isolates to degrade fungal cell wall an important mechanism of fungal inhibition. This is one of the indirect mechanisms of PGPR mentioned by Klopper *et al.*, 1989; Glick, 1995; Glick *et al.*, 1999 and Chaiharn, *et al.*, 2008. It can be noticed from the cellulase activities in Tables 2 and 3 that the activity is clearly higher in wheat rhizosphere as compared to its counterpart in faba bean rhizosphere. This result can propably explained by the higher cellulose content in wheat plant than that of faba bean plant (Ververis *et al.*, 2004).

Nitrogenase Enzyme Activities

The results in Table 2 show that nitrogenase enzyme activities of the tested isolates ranged between 0.1 : 1.52 μ moles C_2H_4/ml/h. Three isolates MW8, ZW4 and BW4 show the highest nitrogenase activity being 1.52, 0.97 and 0.97 μ moles C_2H_4/ml/h, respectively. While the data in Table 3 indicate that isolate HB5 of *Azotobacter* sp. recorded the highest nitrogenase activity reaching to 13.64 μ moles C_2H_4/ml/h followed by EB4 of *Bacillus* sp. which recorded 2.35 μ moles C_2H_4/ml/h. Being a symbiotic diazotroph *Azotobacter* showed the highest efficiency since the determination was carried out in liquid culture not in association with plants. Previous

studies have reported that nitrogen fixation carried out by associative and free-living microorganisms in the rhizosphere of plants has been recognized to play an important role in nitrogen nutrition of plants (Boddey *et al.*, 1996; Sylvia *et al.*, 1998).

It can be concluded that there are several beneficial traits obtained in the rhizobacteria isolates under investigation that could improve plant growth. On this basis among the 42 isolates 5 strains namely *Azospirillum brasilense, Serratia marcescens, Pseudomonas fluorescens, Pseudomonas putida* and *Bacillus brevis* as well as *Azotobacter* sp. that characterized by their efficiencies for IAA production, phosphate solubilization, siderophores and cellulase enzyme production as well as nitrogenase enzyme activity could be used in further investigation as bio-fertilizers for application.

REFERENCES

Abd El-Hafez, A.M. (1966). Some studies on acid producing microorganisms in soil and rhizosphere with special reference to phosphate dissolvers. Ph.D. Thesis, Fac. Agric., Ain Shams Uinv., Cairo, Egypt.

Abd EL-Malek, Y. and Y.Z. Ishac (1968). Evaluation methods used in counting *Azotobacter*. J. Appl. Bact., 31: 269-275.

Asghar, H.N., Z.A. Zahir, A. Khaliq and M. Arshad (2000). Assessment of auxin production from rhizobacteria isolated from different varieties of rapeseed. Pakistan J. Agric. Sci., 37 (3-4): 101-104.

Boddey, R.M., B.J.R. Alves, S. Urquiaga and K.A.I. Malik (1996). Evaluation of biological nitrogen fixation associated with non-legumes. In Nitrogen Fixation with Non-legumes eds., Kluwer Academic Publishers, London, pp: 287-306.

Bunt, J.S. and A.D. Rovira (1955). Microbiological studies of some subantractic soils. Soil Sci., 6: 119-128.

Buysens, S., K. Heungens, J. Poppe and M. Hofte (1996). Involvement of pyochelin and pyoverdine in suppression of *Pythium*-induced damping off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl. Environ. Microbiol., 62: 865-871.

Caron, M., C.L. Patten and S. Ghosh (1995). Effects of plant growth promoting rhizobacteria *Pseudomonas putida* GR-122 on the physiology of canola roots. Plant Growth Reg. Soc. Am. 22nd proceeding, Ed. Green D.W., 18-20.

Cattelan, A.J., P.G. Hartel and J. Fuhrmann (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J., 63: 1670–1680.

Chaiharn, M., S. Chunhaleuchanon, A. Kozo and S. Lumyong (2008). Screening of rhizobacteria for their plant growth promoting activities. J. KMITL Sci. Tech., 8 (1): 18-23.

Chopade, V.V., A.N. Tankar, R.O. Ganjiwale, and P.G.A. Yeole (2008). Antimicrobial activity of *Capparis zeylanica* Linn. roots., Int. J. Green Pharm., 2: 28-30.

Davies, P.J. (1995). Plant hormones physiology, biochemistry and molecular biology, 2nd eds. Dordrecht : Kluwer Academic Publishers, pp: 112.

Delvasto, P., A. Valverde, A. Ballester, J.M. Igual, J.A. Munoz, M.L. Blazquez, and C. Garcia (2006). Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. Soil Biol. Biochem., 38 : 2645–2654.

Difco Manual (1985). Dehydrated culture media and reagents for microbiology. 10th Ed. Difco Laboratories Detroit Michigan, 48232 USA, pp: 487-623.

Dobereiner, J., L.E. Marrial and M. Nery (1976). Ecologyical distribution of *Azospirillum lipoferum*. Peijerink. Can. J. Microbiol., 22: 1464-1473.

Egamberdiyeva, D. and G. Hoflich (2001). Influence of growth promoting bacteria from Uzbekistan and Germany on the growth and nutrient uptake of cotton and wheat on different soils. In: Plant Nutrition-Food Security and Sustainability of Agro-ecosystems. W.J. Horst *et al.*, (eds), pp: 674-675.

Ernsten, A., G. Sandberg, A. Crozier, and C.T. Wheeler (1987). Endogenous indoles and the biosynthesis and metabolism of indole-3-acetic acid in cultures of *Rhizobium phaseoli*. Planta, 171:422-428.

Frankenberger, W.T. Jr. and M. Arshad (1995). Phytohormones in Soil: Microbial Production and Function, pp: 503. NY, USA: Marcel Dekker Inc.

Freitas, J.R., M.R. Banerjee and J.J. Germida (1997). Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L). Biol. Fert. Soils, 24: 358-364.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109-117.

Glick, B.R., C.L. Patten, G. Holguin and D.M. Penrose (1999). Biochemical and Genetic Mechanisms used by Plant Growth Promoting Bacteria. Imperial College Press, London, UK. Biocontrol. Sci. Technol., 11: 557-574.

Hardy, R.W.F., R.C. Burns and R.D. Holsten (1973). Application of the acetylene – ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem., 5: 47–81.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams (eds.) (1994). Seen in Bergey's Manual of Determinative Bacteriology. 9th Ed. Williams and Williams, Baltimore, U.S.A.

Idris, E., D. Iglesias, M. Talon, R. Borriss (2007). Tryptophan-dependent production of Indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. The American Phytopathological Society, 20: 619–626.

Khalid, A., M. Arshad and Z.A. Zahir (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol., 96: 473-480.

Kim, K.Y., D. Jordan and G.A. McDonald (1998). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fert. Soils, 26: 79-87.

Kloepper, J.W. and C.J. Beauchamp (1992). A review of issues related to measuring of plant roots by bacteria. Can. J. Microbiol., 38: 1219–1232.

Kloepper, J.W. and M.N. Schroth (1978). Plant growth promoting rhizobacteria in radishes. 4th Int. Conf. on Plant Path. Bact. Angers, August 27 - September 2, 2 : 879–882.

Kloepper, J.W., R. Lifshitz and R.M. Zablotowicz (1989). Free-living bacterial inoculate for enhancing crop productivity. Trends in Biotechnology, 7: 39-44.

Koga, J., T. Adachi and H. Hidaka (1991). Molecular cloning of the gene for indolepyruvate decarboxylase from *Enterobacter cloacae*. Mol. Gen. Genet., 226: 10-16.

Krieg, N.R. and J.G. Holt (1984). Seen in Bergey's Manual of Systematic Bacteriology, (1) Williams &Wilkins, Baltimore, U.S.A.

Kucey, R.M.N. (1983). Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can. J. Soil Sci., 63: 671-678.

Kucey, R.M.N., H.H. Janzen and M.E. Legget (1989). Microbial mediated increases in plant available phosphorus. Adv., Agron., 42: 199 - 228.

Loper, J.E. and M.D. Henkels (1997). Availability of iron to *Pseudomonas fluorescens* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Appl. Environ. Microbiol., 63 (1): 99-105.

Mac Faddin (1976). Biochemical tests for Identification of Medical Bacteria. Waverly Press, INC. Mt. Royal and Guilford Aves. Baltimore, Md. 21202, U.S.A.

Martens, D.A. and J.R.W.T. Frankenberger (1991). On-line solid phase extraction of soil auxins produced from exogenous applied tryptophan with Ion-suppression reserve phase HPLC analysis. Chromatographia, 32: 417 - 422.

Müller, M., C. Deigele and H. Ziegler (1989). Hormonal interactions in the rhizospheres of maize (*Zea mays* L.) and their effect on plant

development. Z. P. flanzenernahar. Bodenkd, 152: 247-254.

Myresiotis, K.C., G.S. Karaoglanidis, Z. Vryzas and E. Papadopoulou-Mourkidou (2012). Evaluation of plant growth promoting rhizobacteria, acibenzolar-*S*-methyl and hymexazol for integrated control of *Fusarium* crown and root rot on tomato. Pest Management Sci., 68 (3): 404–411.

Patten, C.L. and B.R. Glick (1996). Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol., 42: 207-220.

Patten, C.L. and B.R. Glick (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl. Environ. Microbiol., 68: 3795-3801.

Rodriguez, H. and R. Fraga (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17 (4-5): 319-339.

Saharan, B.S., and V. Nehra (2011). Plant growth promoting rhizobacteria: A Critical Review. Life Sci. and Medicine Res., pp: 21.

Schwyn, B. and J.B. Neilands (1987). Universal assay for the detection and determination of siderophores. Anal. Biochem., 160: 47–56.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (1986). Seen in Bergey's Manual of Systematic Bacteriology, (2), Williams & Wilkins, Baltimore, U.S.A.

Snedecor, G.W. and W.G. Cochran (1982). Statistical method. 7th Ed, Iowa State Univ., Press Ames., Iowa, USA, pp: 325-330.

Starr, D.L., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (1981). The Prokaryotes, a handbook on habitats, isolation and identification of bacteria. Springer-Verlag, Berlin, Germany.

Subba Rao, N.S. (1988). Phosphate solubilizing micro-organism. In: Bio-fertilizer in agriculture and forestry. Regional Biofert. Dev. Centre, Hissar, India, pp: 133-142.

Subba Rao, N.S. (1999). Soil Microbiology (4th ed. Soil Microorganisms and Plant Growth). Sci. Publ., Inc. USA.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, D.A. Zuberer (1998). Biological dinitrogen fixation : Introduction and non-symbiotic. In: Principles and applications of soil microbiology. New Jersey, pp: 295- 321.

Verma, J.P., J. Yadaw, K.N. Tiwari, and L.V. Singh (2010). Impact of plant growth promoting rhizobacteria on crop production. Int. J. Agric. Res., 5 (11): 954-983.

Ververis, C., K. Georghiou, N. Christodoulakis, P. Santas and R. Santas (2004). Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production. Industrial Crops and Products, 19 (3): 245–254.

Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot., 52: 487–511.

Whitelaw, M.A. (2000). Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agron., 69: 99-151.

Yasmin, F., R. Othman, K. Sijam and M.S. Saad (2009). Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. African J. Microbiol. Res., 3 (11): 815-821.

عـزل وتوصيف العـزلات عاليـة الكفـاءة من الريزوبكتريا المشجعـــة لنمو النبـات المعزولـة من ريزوسفير القمح والفـــول

سالى محمد حسن عطيه – سمير حماد سالم – فيكتور صمونيل بدروس – محمد إبراهيم حجازى

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق - مصر

تهدف هذه الدراسة لعزل وتوصيف العزلات الأكثر كفاءة من بكتريا الجذر المحفزة لنمو النباتات (الريزوبكتيريا) المعزولة من ريزوسفير نباتات القمح والفول المزروعه في مواقع مختلفة من محافظة الشرقية وذلك لأهمية هذه البكتيريا كأسمدة حيوية. ومن أهم نقاط هذه الدراسة عزل ٤٢عزلة من البكتيريا المحيطة بجذورنباتات القمح والفول، اختبار قدرة هذه العزلات على إنتاج الإندول أستيك أسيد فى البيئات السائلة فى وغياب أو وجود التربتوفان كمادة أولية لبناء التربتوفان، اختبار قدرة العزلات على إنتاج مخلبيات الحديد (السيدروفورس)، كذلك اختبار قدرة العزلات على إذابة الفوسفات الثلاثي، علاوة على اختبار نشاط إنزيم النيتروجينيز والسليوليز. تم تعريف ٣٢ عزلة على مستوى الجنس من بينها ٥ عزلات تم تعريفها على مستوى الجنس والنوع. يمكن إيجاز أهم النتائج فيما يلى: أظهرت كل العزلات كفاءة عالية فى إنتاج الإندول أستيك أسيد سواء فى غياب أو وجود التربتوفان كمادة أولية كذلك أعطت ٣٥ عزلة نتائج إيجابية فى إنتاج السيدروفورس بينما أعطت ٣٩ عزلة كفاءات متباينة فى إذابة الفوسفات الثلاثي وكذلك أظهرت ٢٨عزلة نتائج إيجابية مع اختبار كفاءة نشاط إنزيم النيتروجينيز بينما ٢٦ عزلة كانت متباينة في ايجابيتها لنشاط إنزيم السليوليز.