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EVALUATION AND CHARACTERIZATION OF SOME PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ISOLATED FROM SHARKIA GOVERNORATE

Safaa M.B. El-Said*, V. S. Bedrous, Howaida M. Labib and M.I. Hegazy

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

ABSTRACT

Ninety one bacterial strains isolated from the rhizosphere of maize, wheat, clover and rice plants were screened for their plant growth promoting activities. The results of *in vitro* assays showed that all rhizobacterial isolates had the ability to produce indole acetic acid (IAA) in presence or absence of tryptophan (L-TRP) with a wide variation observed among them. Auxin production, however, by some isolates was enhanced when culture media were supplemented with L-TRP. The results also showed that 11 rhizobacterial isolates were able to solubilize calcium super phostphate $Ca_3 (PO_4)_2$ on Bunt and Rovira medium. These isolates were tested for nitrogenase activity and cyanide production. Consequently, the more efficient isolates, namely Wh5 and Rh6 were identified to the species level in Cairo MIRCEN (ASU) as *Micrococcus luteus* and *Bacillus licheniformis* and isolate Mh4 was identified by 16S rRNA analysis *as Pseudomonas aeruginosa*. The antimicrobial activity of the four selected plant growth promoting rhizobacteria (PGPR) was assayed against six phytopathogenic fungi and two phytopathogenic bacteria. *P. aeruginosa* showed the most powerful effect against all the tested fungi and *Erwinia carotovora* pathovar *carotovora*. Also, these four selected PGPRs were tested as bio-inoculants for maize plants in pot experiments. The results showed that the application of bio-inoculants alone or combined with half of the recommended dose, significantly increased the dry weight of both shoots and roots of maize plants.

Key words: Indole acetic acid, plant growth promoting rhizobacteria, phosphate solubilization.

INTRODUCTION

Biofertilizer is a substance containing living microorganisms, which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the availability of primary nutrients to the host plant (Vessey, 2003). These bacterial genera stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediation of the polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds (like pesticides) (Ahemad, 2012). Bacterial genera like *Azotobacter, Azospirillum, Pseudomonas, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Serratia, Rhizobium, Bradyrhizobium, Mesorhizobium, Streptomyces and micromonospora* are reported as the most significant PGPRs in soil (Ahemad and Kibret, 2014). Microbial synthesis of IAA has been known for a long time (Patten and Glick, 1996). It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites. Spaepen and Vanderleyden (2011) reported that IAA acts as a reciprocal signaling molecule affecting gene expression in several microorganisms. Consequently, IAA plays a very important role in rhizobacteria-plant interactions. Glick (2012) reported that IAA affects plant cell division, extension, and differentiation, stimulates seed and tuber germination, controls processes of

* **Corresponding author: Tel. :** +201142891168
E-mail address: safaa_95@yahoo.com

vegetative growth, initiates lateral and adventitious root formation, affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. N_2-fixing and P solubilizing bacteria are important for plant nutrition by increasing N and P uptake by the plants, and playing a significant role as PGPR in the biofertilization of crops. Trials with rhizosphere associated PGPR, indicated significant increases in plant growth of maize plants under field conditions (Gholami *et al.*, 2009) and promoted plant growth, facilitated soil metal mobilization and enhanced Cr and Pb uptake on pots experiment (Braud *et al.*, 2009). The application of microorganisms to control diseases is an environment-friendly approach. It's suggested that competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of bio-control activity in PGPR (Lugtenberg and Kamilova, 2009). Nowadays, inoculation with PGPR is a major asset for biological agriculture. This approach is receiving much attention as a way to reduce chemical fertilizer doses without affecting crop yield. Furthermore, the use of indigenous PGPR can be an added advantage since it can easily acclimatize to the natural conditions. Thus the present study was carried out to isolate and identify some PGPR from the rhizosphere of legume and non legume plants growing in Sharkia Governorate, Egypt and investigate their impact on maize plant growth parameters.

MATERIALS AND METHODS

Rhizosphere Soil Samples

Soil samples were collected from the rhizosphere of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), clover (*Trifolium alexandrinum* L.) and rice (*Oriza sativa* L.) plants grown in Sharkia Governorate, Egypt.

Isolation and Purification of PGPR

A total of ninety one isolates of rhizobacteria were isolated by serial dilution plate technique. Ten grams of each soil sample were placed into 250 ml Erlenmeyer flask containing 90 ml of sterile distilled water. After serial dilutions up to 10^{-7}, 1 ml aliquots of these suspensions were transferred onto petri dishes, and mixed with Nutrient Agar medium (Difco, 1985). To isolate specific PGPRs, other media were used, namely: King's B agar medium (Starr *et al.*, 1981) for IAA production, and Modified Ashby's medium (Abd El-Malik and Ishac, 1968) for *Azotobacter* isolation. After incubation at 28^{o}C for 48 hr., morphologically different colonies on the different media were isolated. These isolates were screened for several traits, *i.e.*, IAA production, phosphate solubilization, nitrogenase activity, antibiotic resistance, antimicrobial activity and cyanide production.

In vitro Screening of Bacterial Isolates for their Plant Growth Promoting Activities

A modified colorimetric method was used for the determination of IAA (Ashgar *et al*., 2000). The ability of the isolates to solubilize phosphate was assessed using Bunt and Rovira medium (1955) modified by Abd El-Hafez (1966). Nitrogen fixation ability was determined by the ability to grow on Burk's nitrogen-free liquid medium (NFM) (Subba, 1999). Nitrogenase enzyme activity was measured by the acetylene reduction assay technique in the Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst., Agric. Res. Cent. (ARC), Egypt, according to the standard procedure of Hardy *et al.* (1973). Cyanide production was detected as described by Bakker and Schippers (1987). Resistance to some antibiotics (Penicillin (10 mg), Streptomycin (10mg), and Tetracycline (30mg) were tested by the conventional disk-diffusion method as described by Grammer (1976).

Identification of the Selected Rhizobacterial Isolates

Four of the most efficient isolates were selected on the basis of sharing 4 to 5 PGPR traits, in order to be identified to the species level. Two isolates were identified in Cairo MIRCEN, Faculty of Agriculture, Ain Shams University and one isolate was identified in Sigma company using the 16s rRNA technique. *Azotobacter chroococcum* was identified in our laboratory based on the microscopic, morphological characters and pigment when growing in Ashby's nitrogen free medium Hofer (1944).

Determination of the Antimicrobial Activity

The antagonistic activity of each PGPR isolate was tested on plates against six different phytopathogenic fungi (*Fusarium* spp., *Rhizoctonia* spp., *Botrytis* spp., *Verticillium* spp., *Sclerotium* spp., and *Helminthosporium* spp.) and two phytopathogenic bacteria (*Erwinia carotovora* p.v. *carotovora* and *Erwinia carotovora* p.v. *atroseptica*) which were kindly provided by the Dept. of Plant Pathology, Faculty of Agriculture, Zagazig University.

Application of the PGPRs as Bio-Fertilizers in Combination with Chemical Fertilizers in an Insect Net-Proof House Experiment

The pot experiment was conducted with 8 treatments (3 replicates each) on May 2014, in the Insect Net-Proof house of the Department of Soil Science. Sandy clay soil collected from organic farm was passed through a 10 mesh sieve and autoclaved at 121^{o}C, 15 lb pressure for 1h (Clay % , 36.1; Silt %, 17.0 ; Sand % , 46.9). Plastic pots of 30 cm diameter were also sterilized. Each pot was filled with 6 Kg soil/ pot. Maize seeds were surface sterilized with 0.1% mercuric chloride for 2 min and rinsed six times with sterile distilled water. Sterilized seeds were soaked in combination of broth cultures of *A. chroococcum*, + either of *B. licheniformis*, or *M. luteus* or *P. aeruginosa* containing about 1×10^{8} CFU/ml for 30 min. Chemical fertilizers were applied at the recommended dose or half dose of each of ammonium sulphate (20.6% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) fertilizers. Five inoculated maize seeds of respective treatments were sown in each pot. After two weeks of sowing, thinning was done to leave 1 seedling per pot. Un-inoculated seeds treated with nutrient broth were sown in pots as controls. Pots were arranged in a completely randomized block design, and each treatment was replicated three times. Pots were irrigated with tap water to maintain 50 % of the water holding capacity, and the experiment was terminated after 45 days of planting.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) and least significant differences (LSD) using Duncan's multiple range test (DMRT) at $P \leq 0.05$. Statistical analysis was performed by using SPSS software (Tedesco *et al.*, 1995).

RESULTS AND DISCUSSION

The rhizobacterial, isolates were screened *in-vitro* for the basic characters as PGPR, then the most effective isolates were identified and used for the field experiment.

Screening of the Isolated Rhizobacteria for some Plant Growth Traits

Eighteen rizobacterial isolates were selected according to their ability to produce IAA in presence or absence of tryptophan (L-TRP). IAA was determined in colour-based assay (Table 1) using King's B medium and Salkowski reagent in the presence of 5 mM l- tryptophan. Auxin production by the tested rhizobacteria was enhanced with wide variation, when culture media were supplemented with L-TRP, and thus, was considered as IAA-producing rhizobacteria. On the other hand, in the presence of L- TRP, some isolates were superior in their IAA production, *i.e.*, Rh6 (53.70 mg l^{-1}), Mh4 (42.79 mg l^{-1}), Ch7 (24.34 mg l^{-1}), Wm8 (23.60 mg l^{-1}), Wb1 (15.34 mg l^{-1}) and Wh5 (13.56 mg l^{-1}). These results confirmed those obtained by Yasmin *et al.* (2009) who reported that most of the PGPR isolates required L-TRP precursor for IAA production. They mentioned that three of the isolates, showed 6 to 9 folds increases, in IAA production when grown in media with L-TRP. Our results showed also that not all isolates responded to the precursor application, *e.g.*, Mh2, and Ck2 showed no considerable increase in IAA production 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 bio-synthesis of IAA, some of which are not L-TRP-dependent (Verma *et al.*, 2010). As for phosphate solubilization, the largest clear zones on modified Bunt and Rovira medium were observed in Wm2, Mb3, Ch7, Rh6, and Cb5, being 13, 11, 11, 10, and 10 mm, respectively. Conversely, seven isolates were unable to solubilize the phosphate. The clear zones formed by the tested bacteria may indicate the excretion of organic acids, which have high

Table 1. Plant growth promoting activities of different PGPR strains *In vitro*

Code of isolate	IAA equivalent (mg l^{-1})		P-solubilization Diameter (cm)	Grown on N free media	Nitrogenase enzyme activity µ moles C_2H_4 ml/hr.	Cyanogenesis	Sensitivity to antibiotics diameter in cm		
	Without L-TRP	With L-TRP					Streptomycin (10mg)	Tetracycline (30mg)	Penicillin (10mg)
Mm3	0.688	3.31	0.00	ND	ND	-	-	1.2	-
Mb3	1.84	6.94	1.10	ND	ND	-	-	2.2	-
Mb4	3.42	4.91	0.37	ND	ND	-	2.0	3.0	3.0
Mh1	5.26	6.54	0.97	ND	ND	+	-	-	-
Mh2	1.32	1.43	0.47	ND	ND	+	2.0	3.0	1.5
[1]Mh4	2.53	42.79	0.63	ND	ND	+	-	-	-
Wm2	1.63	2.13	1.30	ND	ND	-	2.0	3.0	1.0
[1]Wm8	2.25	23.60	0.71	+++	18.6	++	[2]ND	ND	ND
Wb1	1.33	15.34	0.00	+	1.43	-	-	2.5	-
Wh1	3.16	11.12	0.00	+	1.41	-	1.5	2.0	-
[1]Wh5	1.63	13.56	0.00	+	0.97	+++	-	2.0	-
Wk4	0.30	4.86	0.00	ND	ND	-	1.0	2.8	-
Cm1	4.25	4.98	0.00	+	0.08	+	1.5	2.0	-
Cb2	2.06	8.38	0.00	ND	ND	-	2.4	3.0	1.2
Cb5	3.64	7.90	1.00	ND	ND	-	2.5	3.0	3.0
Ch7	3.83	24.34	1.10	ND	ND	+	1.0	3.5	2.2
Ck2	4.25	4.55	0.87	ND	ND	-	-	1.8	-
[1]Rh6	2.02	53.70	1.03	ND	ND	-	1.5	1.6	-

[1]Mh4: *Pseudomonas aeruginosa.* Wm8: *Azotobacter chroococcum.* Wh5: *Micrococcus luteus.*

Rh6: *Bacillus licheniformis.* ND: Not Detected

affinity to chelate calcium ions (Yasmin *et al.*, 2009). Out of the eighteen selected isolate, only 5 isolates were able to grow on N-free broth medium, indicating the N_2 fixing activity of these isolates. The Table also shows that nitrogenase enzyme activity of the tested wheat isolates ranged between 0.97 and 18.6 µ mole C_2H_4/ml/hr., with isolate Wm8 showing the highest nitrogenase activity reaching up to 18.6 µ mole C_2H_4/ml/hr. This isolate was proven to be *A. chroococcum*, asymbiotic diazotroph, based on the microscopic, morphological, and pigmentation characters. Cyanides are biocides that have a poisoning effect against the hytopathogenic agents. Table 1 shows a positive cyanide production by seven of the tested isolates, which differed in production level. This was expressed by a remarkable variation in color from yellow to orange-red. Isolate Wh5 recorded the highest positive reaction revealed by a strong orange-red pigmentation, followed by isolate Wm8. Over the last decades many studies have reported the natural activity of some fungi and bacteria against pathogens, and this is considered as a very appealing alternative to the use of chemicals (Bhattacharyya and Jha, 2012).

Regarding the sensitivity to antibiotics, isolates Mh1 and Mh4 showed high resistance (no inhibition zone) to the three antibiotics used during the preliminary screening test. All other isolates were sensitive to tetracycline and formed inhibition zone ranging between 12 and 35 mm in diameter. Ten isolates were sensitive to streptomycin, and six were sensitive to penicillin. Antibiotic resistance of the candidate PGPRs confer them the ability to grow and compete with other microorganisms producing antibiotics in their microhabitats, and such competition capability by itself is one of the modes of activity in PGPRs. These results are in agreement with Lugtenberg and Kamilova (2009) who reported that many rhizobacteria can survive in soil along with microorganisms producing antibiotics.

Determination of Antimicrobial Activity of the Selected PGPR

One of our goals was to obtain indigenous isolates that have the ability to produce antimicrobial agents against pathogenic microorganisms. Therefore, the antifungal and antibacterial activities of the selected PGPR isolates were assayed against six different phytopathogenic fungi and two phytopathogenic bacteria and presented in Table 2. It can be shown that *P. aeruginosa* is the most powerful against all fungi while, *B. licheniformis* and *M. luteus* showed moderate effect on the tested pathogens. All the tested PGPRs were effective against *E. carotovora* p.v. *carotovora* (the causative of soft rot) Czajkowski *et al.* (2009). Verma *et al.* (2013) have reported that *P. aeruginosa* was capable of IAA, siderophores and HCN production and also showed mycelial growth inhibition against *Fusarium oxysporium* and *Rhizoctonia solani*.

Effect of Inoculation with Selected PGPR Isolates and/or Chemical Fertilization on the Growth of Maize

The effect on maize dry weight

Data in Table 3 show that the dry weight of maize plants increased in response to inoculation with *A. chroococcum* combined with either of *P. aeruginosa*, *B. licheniformis* and *M. luteus* with or without the two doses of chemical fertilizers. Without chemical fertilization, *A. chroococcum* + *M. luteus* was the most efficient combination for roots dry weight which amounted 9.36 g/plant. While *A. chroococcum* + *P. aeruginosa* gave the highest shoot dry weight, *i.e.* 13.82 g/plant. Similar observations were obtained by Gholami *et al.* (2009) who reported that seeds inoculated with PGPR increased dry matter accumulation and yield of maize plants under field conditions.

Regarding the means of the two-microbe inoculations (*A. chroococcum* plus either of *M. luteus*, *P. aeruginosa or B. licheniformis*), they showed the highest shoots dry weight, being 23.06, 21.13, and 18.58 g/plant, respectively without significant difference ($P \leq 0.05$) between them. As for the roots means of the two-microbe inoculations, they were similarly better than the mixture of inocula and the control, with the treatment containing *M. luteus* being significantly higher than the rest of them. Jiang *et al.* (2008) reported significant increases ($P \leq 0.05$) of root and shoot dry weight of maize plants (75%) and tomato plants (30% to 54%) when the soil was inoculated with *Burkholderia* J62, compared to the uninoculated soil. Such increase in the plant shoot and root is most likely due to the increased nitrogen fixation caused by *A. chroococcum* (Table 1) and the availability of other nutrients caused by the three other bacterial species, as well be shown below.

The effect on total nitrogen

Results in Table 4 reveal that the total N content of maize plants was significantly influenced by inoculation with PGPR isolates as compared to their respective uninoculated controls. The maximum increase in total N content without fertilizers in shoots was observed in the case of inoculation with *A. chroococcum* + *M. luteus* followed by *A. chroococcum* + *P. aeruginosa* which recorded 145.5 and 131.3 mg/plant, respectively, while it recorded 73.9 and 48.5 mg/plant, respectively in roots. There were significant increases over the control in the nitrogen content in shoots and roots of maize when fertilized with half of the recommended dose showing values of 302.7 mg/plant and 116.1 mg/plant, respectively, for plants inoculated with *A. chroococcum* + *B. licheniformis*. When the same combination was applied along with the

Table 2. *In vitro* antifungal and antibacterial activity of the selected PGPRs strains

Tested organism		PGPR strain		
		B. licheniformis	***M. luteus***	***P. aeruginosa***
Fungi	***Rhizoctonia sp.***	-	-	+
	Fusarium sp.	-	-	+
	Helminthosporium sp.	+	+	+
	Botrytis sp.	+	+	+
	Scelorotium sp.	-	-	+
	Verticillium sp.	+	-	+
Bacteria	***E. carotovora* p.v. *carotovora***	+	+	+
	E. carotovora* p.v. *atroseptica	-	-	-

Table 3. Effect of inoculation with the selected PGPRs combined with chemical fertilizers on maize shoots and roots dry weight

Inoculation	Shoots (g/plant)					Roots (g/plant)				
	0F	**½F**	**1F**	**LSD**	**Mean**	**0F**	**½F**	**1F**	**LSD**	**Mean**
Control	6.80	7.18	7.75	0.67	7.24	4.67	5.55	5.87	0.33	5.36
A. chroococcum +P. aeruginosa	13.82	19.86	29.70	1.80	21.13	5.64	7.93	11.70	0.87	8.42
A. chroococcum +B. licheniformis	9.49	22.59	23.65	1.54	18.58	4.37	10.88	12.86	1.05	9.37
A. chroococcum +M. luteus	12.73	18.86	37.60	1.23	23.06	9.36	9.81	16.40	0.78	11.86
Mixture of inocula	7.64	7.29	8.47	0.42	7.80	5.70	5.63	6.77	0.54	6.03
LSD	1.32	1.06	1.28		6.79	0.68	0.67	0.72		2.90
Mean	9.54	13.76	20.34			5.72	7.38	10.08		
	LSD = 6.14					LSD = 2.81				
LSD interaction	0.56					0.57				

0 F: without chemical fertilization, ½F: half the recommended dose, and 1F : the recommended dose.

Table 4. Effect of inoculation with the selected PGPRs and chemical fertilizers on maize shoots and roots nitrogen content

Inoculation	Total N content (mg / plant)									
	Shoots					Roots				
	0F	½F	1F	LSD	Mean	0F	½F	1F	LSD	Mean
Control	75.9	80.2	101.3	3.7	83.0	38.5	54.4	64.0	1.7	52.3
A. chroococcum* + *P. aeruginosa	131.3	239.6	375.2	8.7	248.7	48.5	73.0	137.3	3.0	86.3
A. chroococcum* + *B. licheniformis	97.7	302.7	322.4	9.1	240.93	39.8	116.1	157.3	3.0	104.4
A. chroococcum* + *M. luteus	145.5	243.3	414.9	8.0	267.9	73.9	109.5	220.3	6.0	134.6
Mixture of inocula	84.5	97.2	101.9	3.3	94.9	53.6	58.0	84.6	1.2	65.4
LSD	5.2	6.7	7.5		67.9	1.2	1.9	4.5		36.6
Mean	102.4	178.6	255.2			50.2	76.8	125.6		
	LSD = 61.4					LSD = 23.5				
LSD interaction	5.6					2.6				

0 F: without chemical fertilization, ½F: half the recommended dose, and 1F : the recommended dose.

full recommended dose, the values were 414.9 and 220.3 mg / plant in shoots and roots, respectively. Our results contradicted with those obtained by Shaharoona *et al.* (2008) who mentioned that the increases in fertilizers doses reduced the efficacy of inoculation as indicated from a significant negative linear correlation (R^2 = 0.94; P=0.03) between percent increases in nitrogen use efficiency in response to inoculation with *P. fluorescens* and N, P, and K fertilizers. In addition, Attia (2012) found that maximum increase in total N content was observed in case of inoculation with *B. brevis* followed by *P. fluorescens* under unfertilized conditions. Regarding the mean of chemical fertilization for each inoculation treatment, *A. chroococcum* + either of *P. aeruginosa*, *B.licheniformis* or *M.luteus* showed the highest significant N content in shoots / plant, while in roots the combinations of *A. chroococcum* +*M. luteus* and *A. chroococcum* + *B. licheniformis* were much higher than any other treatment. It is noteworthy that Fallik and Okon (1998) reported that *Azospirillum* inoculation induced increasing phosphorus, nitrogen and potassium content by maize and sorghum root through improving root system.

The effect on total phosphorous

In all cases, the mixture of inocula were much less than the two-microbe inoculation, which may be due to the negative interaction (inhibition effect) among all the bacteria when mixed together. Inoculation with the PGPRs increased the total phosphorous content with increasing the levels of calcium superphosphate (along with other fertilizers). This could be due to the efficiencies of these isolates as phosphate dissolving bacteria as shown in Table 1. Similar to the general trend shown before, the mixture of inocula showed much less increase in P content compared with the two-microbe cases, which may be due to the antagonism between the microbial inocula. These results are not in agreement with those of Attia (2012) who indicated that *P. fluorescens* or *Azospirillum brasilense* resulted in significant increase in P content/plant but the efficacy of the inoculation decreased with increasing the levels of phosphate fertilization.

The effect on total potassium

Data in Table 6 show that total potassium content was positively influenced by bacterial inoculations under all levels of fertilization

Table 5. Effect of inoculation with the selected PGPRs and chemical fertilizers on maize shoots and roots phosphorus content

Inoculation	Total P content (mg / plant)									
	Shoots					Roots				
	0F	½F	1F	LSD	Mean	0F	½F	1F	LSD	Mean
Control	28.8	31.3	39.5	2.7	33.2	11.7	21.8	27.2	1.5	20.2
A. chroococcum +P. aeruginosa	46.5	92.0	144.5	9.2	94.3	13.0	34.6	64.0	3.7	37.2
A. chroococcum +B. licheniformis	49.0	128.8	156.9	6.8	111.6	12.2	47.1	80.6	3.9	46.6
A. chroococcum +M. luteus	62.8	101.8	188.0	12.3	117.5	29.3	39.9	105.0	4.5	58.1
Mixture of inocula	37.4	43.6	45.2	2.6	42.1	15.6	26.3	48.1	2.5	30.0
LSD	4.1	5.00	9.2		29.0	1.7	2.6	3.9		21.1
Mean	42.9	73.7	107.9			16.3	32.4	62.5		
	LSD = 28.1					LSD = 10.6				
LSD interaction	5.7					2.6				

0 F: without chemical fertilization, ½F: half the recommended dose, and 1F : the recommended dose.

Table 6. Effect of inoculation with the selected PGPRs and chemical fertilizers on maize shoots and roots potassium content

Inoculation	Total K content (mg / plant)									
	Shoots					Roots				
	0F	½F	1F	LSD	Mean	0F	½F	1F	LSD	Mean
Control	100.6	115.7	157.9	2.9	124.7	60.6	72.5	90.4	2.6	74.5
A. chroococcum +P. aeruginosa	206.4	349.5	530.6	9.2	362.2	91.7	113.9	191.5	4.9	132.4
A. chroococcum +B. licheniformis	162.3	403.6	462.0	7.3	342.6	76.5	165.7	198.9	4.4	147.0
A. chroococcum +M. luteus	207.9	361.5	763.3	10.0	444.2	170.4	157.9	265.1	4.3	197.8
Mixture of inocula	118.4	144.8	166.3	2.8	143.2	111.3	97.6	116.7	1.8	108.5
LSD	3.2	3.8	8.4		116.0	3.0	2.4	3.9		34.1
Mean	151.8	252.7	398.8			100.0	114.8	163.2		
	LSD = 100.2					LSD = 30.6				
LSD interaction	5.3					2.9				

0 F: without chemical fertilization, ½F: half the recommended dose, and 1F : the recommended dose.

compared to the un-inoculated controls the positive effect was significant ($P \leq 0.05$) in all cases, except for the full dose of mixture of inocula in shoots. Under chemical fertilization the three combinations *i.e., A. chroococcum* + either of *P. aeruginosa, B. licheniformis* or *M. luteus* showed highest K content in shoots as well as in roots. However, the mixtures of inocula was much less effective than the two-microbe inoculation, which may reflect antagonistic effect among each other.

Many studies showed that bacterial inoculation significantly increased nutrient contents of plants. Increased nutrient uptake by plants inoculated with PGPR has been attributed to the production of plant growth regulators at the root-soil interface, which stimulate root development and results in better absorption of water and nutrients from the soil (Zimmer *et al.*, 1995).

From the above results, it can be concluded that rhizobacteria isolated from the rhizosphere of maize, rice, clover, or wheat plants, *i.e., A. chroococcum, B. licheniformis, P. aeruginosa,* and *M. luteus* are promising plant growth promoters that improved the growth and chemical properties of maize plants. Further field studies are needed to investigate the role of PGPR in improving the yield of non-legume plants.

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 Univ., Cairo, Egypt.

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

Ahemad, M. (2012). Implications of bacterial resistance against heavy metals in bioremediation: a review. IIOABJ, 3: 39–46.

Ahemad, M. and M. Kibert (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Univ. Sci., 26: 1–20.

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

Attia, S.M.H. (2012). Microbiological studies on plant growth promoting rhizobacteria for improving the productivity of some plants. M. Sc. Thesis, Fac. Agric., Zagazig Univ., Egypt.

Bakker, A.W. and B. Schippers (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. Soil Biol. Biochem., 4: 451-457.

Bhattacharyya, P.N. and D.K. Jha (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol., 28: 1327–1350.

Braud, A., K. Je´ze´quel, S. Bazot and T. Lebeau (2009). Enhanced phytoextraction of an agricultural Cr-, Hg- and Pb-contaminated soil by bioaugmentation with siderophoreproducing bacteria. Chemosphere, 74: 280–286.

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

Czajkowski, R., G. Grezegorz and J.M. Van Der Wolf (2009). Distribution of Dickeya spp. and *Pectobacterium caratovorum* subsp. *Caratovorum* in naturally infected seed potatoes. Eur. J. Plant. Pathol., 125: 263-275.

Difco, M. (1985). Dehydrate Culture Media and Reagents for Microbiology. 10th Ed. Difco Laboratories Detroit Michigan, 48232 USA, 487-623.

Fallik, E. and Y. Okon (1998). Growth response of maize roots to *Azospirillum* inoculation: Effect of soil organic matter content, number of rhizosphere bacteria and timing of inoculation. Soil Biol. Biolchem., 20: 45-49.

Glick, B.R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, Scientifica.

Gholami, A., S. Shahsavani and S. Nezarat (2009). The effect of plant growth promoting

rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int. J. Biol. Life Sci., 1 (1): 35-40.

Grammer, A. (1976). Antibiotic sensitivity and assay test. In: Microbiological methods (Collins CH and Lyme PM eds). Butterworth, London, 235.

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

Hofer, A.W. (1944). Bergy's Manual of Determination Bacteriology. 6th Ed., 47: 415.

Jiang, C., X. Sheng, M. Qian and Q. Wang (2008). Isolation and characterization of a heavy metal-resistant Burkholderia sp. From heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal polluted soil. Chemosphere, 72: 157–164.

Lugtenberg, B. and F. Kamilova (2009). Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol., 63: 541–556.

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

Shaharoona, B., M. Naveed, M. Arshad and Z.A. Zahir (2008). Fertilizer-dependant efficiency of Pseudomonads for improving growth, yield, and nutrient, use efficiency of wheat (*Tritcum aestivum* L.) Appl. Microbiol. Biotechnol., 79: 147-155.

Spaepen, S. and J. Vanderleyden (2011). Auxin and plant-microbe interactions. Cold Spring Harb. Perspect. Biol. http://dx.doi.org/ 10.1101 / cshperspect.a001438.

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, R.N.S. (1999). Soil Microbiology (4th Ed. of Soil Microorganisms and Plant Growth). Science Publishers, Inc. USA, 278.

Tedesco, J.M., C. Gianelo, C.A. Bissani, H. Bohnen and S.J. Volkweiss (1995). Análise desolo, plantas e outros materiais, 2nd Ed. UFRGS, Porto Alegre.

Verma, J.P., J. Yadav, K.N. Tiwari and S.V. Lavakush (2010). Impact of plant growth promoting rhizobacteria on crop production. Int. J. Agric. Res., 5: 954–983.

Verma, J.P., J. Yadav, K.N. Tiwaric and A. Kumar (2013). Effect of indigenous Mesorhizobium spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol. Eng., 51: 282– 286.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571–586.

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

Zimmer, D.B., E.H. Cornwall, A. Landar and W. Song (1995). The S100 protein family: History, function, and expression. Brain Res. Bull., 37: 417-429.

تقييم وتوصيف بعض ميكروبات الريزوسفير المشجعة لنمو النبات والمعزولة من محافظة الشرقية

صفاء محمود بكر السيد - فيكتور صموئيل بدروس - هويدا محمد لبيب – محمد إبراهيم حجازى

قسم الميكروبيولوجيا الزراعية - كلية الزراعة - جامعة الزقازيق - مصر

أجريت هذه الدراسة بهدف توصيف عزلات محلية من الريزوبكتيريا المشجعة لنمو النبات والمعزولة من ريزوسفير نباتات الذرة والقمح والبرسيم والأرز، أوضحت النتائج (في المعمل) أن جميع عزلات الريزوبكتيريا لها القدرة على إنتاج إندول حمض الخليك في غياب أو وجود التريبتوفان مع وجود فروق معنوية كبيرة بينهم، ولوحظ إن إنتاج الأكسين بواسطة بعض العزلات زاد عند إضافة التريبتوفان إلى البيئة، وأوضحت النتائج إن ١١ عزله ريزوبكتيرية لها القدرة على إذابة الفوسفات الثلاثي على بيئة modified Bunt and Rovira، هذه العزلات تم اختبارها لدراسة كفاءتها على إنتاج إنزيم النيتروجينيز وإنتاج السيانيد، تم اختيار أكفأ العزلات من بينهم وهي Wh5 , Rh6 وتم تعريفهما في MIRCEN كلية الزراعة جامعة عين شمس باسم *Bacillus licheniformis* and *Micrococcus luteus* والعزلة Mh4 تم تعريفها في شركة Sigma باستخدام تقنية 16S rRNA وهى *Pseudomonas aeruginosa*، تم تقييم التأثير المضاد للفطريات والبكتيريا بواسطة عزلات الريزوبكتيريا المنتقاة باستخدام ٦ فطريات ممرضة للنبات، ٢ من البكتيريا الممرضة للنبات أيضا في التربة ووجد إن *Pseudomonas aeruginosa* أكثرهم قدرة على مقاومة الفطريات، وبكتيريا *Erwinia carotovora* p.v. *carotovora* أكثر من العزلتين الأخريين. تم استخدام هذه السلالات الفعالة والتي تم تعريفها كمخصبات حيوية لدراسة تأثيرها على نمو نبات الذرة منفردة أو مختلطة معا أو في وجود نسب مختلفة من التسميد الكيميائي تحت ظروف الصوبة، وأوضحت النتائج إن استخدام هذه الميكروبات كمخصبات حيوية منفردة أو مختلطة في وجود نصف الجرعة من السماد الكيميائي أدت لحدوث زيادة معنوية في الوزن الجاف للجذر والساق.

المحكمون :

١- أ.د. محمد عبدالعليم علي أستاذ الميكروبيولوجي – كلية الزراعة – جامعة القاهرة.

٢- أ.د. ســـمير حمـاد ســالم أستاذ الميكروبيولجي المتفرغ – كلية الزراعة – جامعة الزقازيق.