J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8 (9): 231 – 235, 2017

Efficiency Evaluation of some Rhizobacteria Isolated from Egyptian Soils, *In vitro* as Biofertilizers.

Hauka, F. I. A. ; Aida H. Afify and A. M. El-Sawah
Microbiol. Dept., Fac. Agric., Mansoura Univ., Mansoura Gover., Egypt.

ABSTRACT

Nitrogen (N), Phosphorus (P) and Potassium (K) are the major essential macro-nutrients for plants. The ability of soil bacteria to provide available forms of these nutrients to plants is an important feature in the bacterial strain used as a bio-fertilizer. In this study, fifteen isolates of *Azotobacter* spp were isolated. The efficiency levels of the N_2-fixation were tested, the isolates reduced acetylene at rates of 6.348 - 1381.023 nmoles C_2H_4/ml/day. The most active isolate was No. AZ 8 that identified as *Azotobacter chroococcum* MF135558. Twenty-one phosphate-solubilizing bacteria (PSB) were isolated and solubilization efficiency % (SE%) of each isolate was determined by spot inoculation technique. Five isolates (PSB 3, PSB 7, PSB 8, PSB 12 and PSB 14) were chosen due to their high solubilization efficiency % (SE%) 182.35, 173.33, 222.22, 170.00 and 194.11 %, respectively. The five P-solubilizing bacteria were tested for their ability to solubilize P from tri-calcium phosphate and rock phosphate in liquid Pikovskaya's medium. The highest quantity of released phosphorus in actively growing culture was 36.52 mg/100 ml after 28 days of incubation and 1.095 mg/100 ml after 3 days of incubation on tri-calcium phosphate and rock phosphate, respectively by isolate PSB 14 which was identified as *Klebsiella oxytoca* MF135559. Five isolates of K-releasing bacteria (KRB) were isolated on Alexandroov's medium and were tested to release K from mica-muscovite. All the tested isolates can release potassium from mica but not in equal efficiency. The most efficient isolate namely KRB-2 could release 7.05 ppm after 6 weeks of incubation. It was identified as *Rhizobium pusense* MF135560.

Keywords: Bio-fertilizer, N_2-fixation, P-solubilizing, K-releasing.

INTRODUCTION

Chemical fertilizers provide essential nutrients for plants mainly nitrogen, phosphorus and potassium for increasing the yield, but they cause several health hazards. In recent years, bio- fertilizers have considered one of the best modern tools for providing nutrients to plants (Bhattacharjee and Dey, 2014).

A bio-fertilizer is a substance, which contains living microorganisms that applied to seed, plant surfaces and\or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003).

Bio-fertilizers promote plant growth through nitrogen fixation, phytohormones, phosphate solubilization and potassium releasing (Bashan and de- Bashan, 2005).

Nitrogen-fixing bacteria include symbiotic and free- living genera. The free-living, N-fixing bacteria, such as *Azospirullum*, *Azotobacter* and *Pseudomonas* that fix atmospheric nitrogen do not need a specific host plant. These free-living, N-fixing bacteria thrive within the plant. During the association, the invading bacteria benefit the acquired host with a marked increase in plant growth and yield (Bhattacharjee *et al*., 2008).

The phosphate-solubilizing bacteria (PSB) can convert the insoluble inorganic phosphate compounds, such as tri-calcium phosphate, di-calcium phosphate, hydroxyapatite, and rock phosphate, to plant available forms. Many bacterial genera were reported to have P-solubilization activity such as *Pseudomonas*, *Bacillus, Rhizobium, Burkholderia, Erwinia* and *Klebsiella* (Rodríguez and Fraga, 1999; Yao *et al*., 2006 and Walpola *et al*., 2014).

The Potassium-releasing bacteria (KRB) are able to release unavailable potassium and provide available forms to plant by secretion of organic acid (Han and Lee, 2006). A wide range of bacteria namely *Pseudomonas, Burkholderia*, *Acidothiobacillus ferrooxidans*, *Bacillus mucilaginosus, Bacillus edaphicus*, B. *circulans, Paenibacillus* sp. and *Rhizobium pusense* have been reported to release potassium in accessible form from potassium-bearing minerals in soils. (Sheng, 2005; Liu *et al*., 2012 and Meena *et al*., 2015).

Thus, this study focused on the isolation of efficient bacterial strains, which can fix nitrogen, solubilize phosphorus and release potassium.

MATERIALS AND METHODS

Soil samples

Representative rhizosphere soil samples were collected from fields planted with Corn, Rice, Wheat, Broad bean and Clover plants. For each crop, a mixed rhizosphere soil sample was pooled ready for microbiological analysis.

Isolation of *Azotobacter* spp.

Azotobacter spp. were isolated from Most Probable Number (MPN) tubes on modified Ashby's medium (Abd-El-Malek and Ishac, 1968). For purification a loopful of the culture was transferred to 20 ml sterile tap water in a small bottle containing glass beads, mechanically shaken for 30 min to fragment the mucous around the cells, which usually carries contaminations to be used for streaking the agarized plates. Developed colonies were picked and re-purified at least five times, till proving no contamination. Cultures were considered pure when microscopical examination revealed only typical *Azotobacter* and without contaminating organisms. Single colonies were transferred and kept in Ashby's agar slants.

Isolation of phosphate-solubilizing bacteria:

The phosphate-solubilizing bacteria were isolated using pouring plate technique (Pikovskaya's, 1948). Plates were incubated at 30°C for 7 days. Phosphate-solubilizing bacteria were detected by clear zones around the colonies. Single colonies were picked up, purified and kept on Pikovskaya's agar slants.

Isolation of potassium-releasing bacteria:

The potassium-releasing bacteria were isolated using pouring plate technique (Zahra, 1969). Plates were incubated at 30°C for 5 days. Transport, protuberant and mucoid colonies of potassium-releasing bacteria, having

the shape of a tear were picked up, purified and kept on Alexandroov's agar slants.

Determination of Nitrogenase activity:

Nitrogenase activity was assayed using acetylene reduction technique (Hardy *et al*., 1973).

Assay of water soluble phosphorus:

Solubilization efficiency (SE%) of phosphate-solubilizing bacteria was determined on Pikovskaya's agar medium. The solubilization of phosphate was observed as a zone of clearance with a diameter that was measured in mm according to the following formula (Srinivas and Hemalata, 2014):

$$\text{Solubilization Efficiency Percent (SE \%)} = \frac{\text{Solubilization Diameter (SD)}}{\text{Growth Diameter (GD)}} \times 100$$

The chosen efficient bacteria were grown in 250 ml conical flasks each containing 100 ml sterile Pikovskaya's medium, tri-calcium phosphate and rock phosphate was added as a source of insoluble phosphorus at rate of 50 mg P/100 ml culture. The determination of water soluble phosphorus and pH values were carried out initially and after 3, 7, 14, 21 and 28 days of incubation. Water soluble phosphorus in culture media was determined colorimetrically by using spectrophotometer by the method of Boltz and Mellon (1948) modified by Hemalatha *et al*. (2013).

Assay of water soluble potassium:

Hundred grams portions of acid washed sand were put in conical flasks of 250 ml capacities. Five grams of mica-muscovite as a K- source were then thoroughly mixed with the sand. Two ml of 48 hours old liquid culture of the tested bacterial isolate were transferred aseptically, and moisture content was raised to attain 60 % of the water holding capacity through adding aseptically the mineral solution, which consists of $CaCl_2$, 0.1 g; $MgSO_4$. $7H_2O$, 0.5 g; (NH_4) H_2PO_4, 1.0 g; $H_2MoO_4.H_2O$, 0.001g; glucose, 5.0 g; $FeCl_3.H_2O$ (1%), 10 drops: distilled water. The moisture was adjusted whenever needed throughout the course of incubation period. Potassium released from mica was determined initially and after 2, 4, 6, 8 and 10 weeks of incubation (Afify, 1982). Water soluble potassium was determined in the water extract by shaking 10 g of mineral-sand mixture with 100 ml of distilled water for 30 minutes. The suspension was filtered in which soluble potassium was determined by flame photometry (Jackson, 1967).

Identification of the most efficient isolates:

Some morphological observations, staining properties and biochemical characteristics were applied to identify the bacterial isolates according to (Thompson and Skerman, 1979, Woodward *et al*. 1979 and Panday *et al*., 2011), also the partial sequencing of 16S rRNA for the bacterial isolates was done, the online program BLAST (http://www.ncbi.nlm.nih.gov/BLAST) was used in identifying the related sequences with known taxonomic information available at the databank of NCBI (Altschul *et al*.,1997 and Liang *et al*.,2011).

RESULTS AND DISCUSSION

Isolation and efficiency of *Azotobacter* spp.:

Fifteen isolates of *Azotobacter* spp were isolated from Most Probable Number (MPN) tubes. The efficiency of N_2-fixation differed between the isolates (Table 1). The isolates reduced acetylene at a rate between 6.348 and 1381.023 nmoles C_2H_4/ml/day. The most active *Azotobacter* isolate (No. AZ 8) that isolated from the wheat field was selected for further experiments.

Table 1. Nitrogenase activity of *Azotobacter* spp. isolates

Isolate No.	Isolate origin	Nitrogenase activity (nmoles C_2H_4/ml/day)
AZ 1	Corn	1148.738
AZ 2		19.358
AZ 3		6.438
AZ 4		230.408
AZ 5	Rice	763.810
AZ 6		900.795
AZ 7		862.060
AZ 8	Wheat	1381.023
AZ 9		153.095
AZ 10		952.623
AZ 11		579.593
AZ 12		1064.623
AZ 13	Broad Bean	1137.398
AZ 14		433.748
AZ 15	Clover	1258.320

Isolation and efficiency of phosphate solubilizing bacteria:

Several dilutions of soil suspension were applied for plating technique using Pikovskaya's agar medium. Twenty-one bacterial colonies that showing clear zones around the colonies were isolated. Solubilization efficiency (SE) of each isolate was determined by spot inoculation technique. Five isolates (PSB 3, PSB 7, PSB 8, PSB 12 and PSB 14) were chosen for further studies due to their high solubilization efficiency % (SE%) 182.35, 173.33, 222.22, 170.00 and 194.11 %, respectively, (Table 2).

As for the mechanisms of phosphate bacterial solubilizing power, the five phosphate solubilizing bacteria were inoculated into Pikovskaya's liquid medium supplemented with tri-calcium phosphate or rock phosphate at the rate of 50 mg p per 100 ml culture media.

Tables (3 and 4) show the changes in pH values for Pikovskaya's medium supplemented with tri-calcium phosphate and\or rock phosphate as influenced by the inoculation with phosphate-solubilizing bacteria. Generally, there was a rapid decrease in pH within the first 3 days of incubation indicating the activity of isolates in degrading the organic carbon source, i.e., glucose, of the culture media releasing organic acids. In case of tri-calcium phosphate, the decrease in pH values is followed by a slight reduction and small fluctuations until the end of the experiment. In some instances, isolate PSB 3, PSB 7 and PSB 12 the pH tended to rise again. On the other side, on rock phosphate the decrease in pH is followed by a slight reduction up to the 7^{th} day of incubation then the pH tended to rise again within the last 7 days of the experiment and reaching a value most likely more than that of the initial. The reason for such rise in pH can be explained as due to a subsequent oxidation of organic acids produced in culture media and/or to the formation of natural substances (Hauka, 1986). Tables (3 and 4) also show the changes in water soluble phosphorus values for the same medium but supplemented with tri-calcium phosphate and/or rock phosphate and inoculated with the phosphate-solubilizing

bacteria. Both tri-calcium phosphate and rock phosphate were solubilized by phosphate-solubilizing bacteria but not in equal efficiency. The maximum release of soluble phosphorus from tri-calcium phosphate amounting 30.83, 25.35, 26.98, 29.64 and 36.52 mg p /100 ml culture media of the isolates of PSB 3, PSB 7, PSB 8, PSB 12 and PSB 14, respectively. On the other hand, the maximum release of soluble phosphorus from rock phosphate amounting 1.400, 2.082, 0.545, 0.371 and 1.095 mg p /100 ml culture media due to the isolates PSB 3, PSB 7, PSB 8, PSB 12 and PSB 14. The decrease in P content along with increasing the incubation period could be attributed to the utilization of P resulting in the fluctuating levels of P release. Moreover, availability of soluble phosphorus in the culture medium might also have an inhibitory effect on further phosphate solubilization. Excretory, toxic products may also responsible for such decline in P-solubilization (Hauka, 1986). The highest quantity of released phosphorus in the culture was for the isolate PSB 14 that isolated from the clover field was selected for further experiments. The current findings agree with those obtained by Hemalatha *et al*. (2013) and Walpola *et al*. (2014).

Table 2. Phosphate solubilization efficiency of phosphate-solubilizing bacteria (PSB) on Pikovskaya's agar medium

Isolate No.	Isolate origin	Solubilization Diameter (SD) mm	Growth Diameter (GD)mm	Solubilization Efficiency % (SE%)
PSB 1	Corn	14.5	9.50	152.63
PSB 2		13.0	10.0	130.00
PSB 3		15.5	8.50	182.35
PSB 4	Rice	7.00	5.50	127.27
PSB 5		6.50	5.00	130.00
PSB 6		7.00	6.00	116.66
PSB 7	Wheat	13.0	7.50	173.33
PSB 8		10.0	4.50	222.22
PSB 9		13.0	9.00	144.44
PSB 10	Broad Bean	13.0	11.5	113.04
PSB 11		10.5	7.00	150.00
PSB 12		8.50	5.00	170.00
PSB 13		8.00	6.50	123.07
PSB 14	Clover	16.5	8.50	194.11
PSB 15		12.5	7.50	166.67
PSB 16		13.5	9.00	150.00
PSB 17		12.5	9.00	138.88
PSB 18		13.0	8.00	162.50
PSB 19		15.0	9.00	166.67
PSB 20		22.5	17.0	128.57
PSB 21		12.5	8.00	156.25

Table 3. Changes in pH and water-soluble phosphorus (WSP) (mg/100 ml culture) as influenced by selected phosphate-solubilizing bacteria (PSB) on tri-calcium phosphate

Isolate No.	Incubation period (days)									
	3		7		14		21		28	
	pH	WSP	pH	WSP	pH	WSP	pH	WSP	pH	WSP
PSB 3	5.44	14.86	5.35	15.67	5.14	25.96	4.80	30.83	5.53	26.80
PSB 7	5.30	20.03	5.21	24.97	5.17	25.30	5.55	22.11	5.40	25.35
PSB 8	4.93	6.239	4.85	8.944	4.07	11.79	4.04	23.20	3.78	26.98
PSB 12	5.17	6.922	4.96	8.374	4.14	9.385	3.95	29.64	4.13	28.82
PSB 14	5.55	17.29	5.51	19.99	5.42	23.10	5.01	26.41	4.84	36.52

Initial pH value = 7.0. Initial soluble P = 3.20 mg/100 ml.

Table 4. Changes in pH and water-soluble phosphorus (WSP) (mg/100 ml culture) as influenced by selected phosphate-solubilizing bacteria (PSB) on rock phosphate

Isolate No.	Incubation period (days)									
	3		7		14		21		28	
	pH	WSP	pH	WSP	pH	WSP	pH	WSP	pH	WSP
PSB 3	4.59	0.839	4.06	1.400	5.96	0.062	6.16	nil	6.95	nil
PSB 7	4.87	0.340	4.46	2.082	6.10	nil	6.37	nil	7.63	nil
PSB 8	5.30	0.068	4.75	0.545	5.41	0.034	5.43	nil	5.61	nil
PSB 12	5.44	0.126	4.51	0.371	5.40	0.038	5.48	nil	5.50	nil
PSB 14	4.14	1.095	4.23	0.707	5.91	0.071	6.43	0.023	7.43	nil

Initial pH value = 7.0. Initial soluble P = nil.

Isolation and efficiency of Potassium-releasing bacteria:

Five isolates of potassium-releasing bacteria had translucent, protuberant, mucoid and like tear eye colonies were isolated on Alexandroov's medium. Table (5) indicate the evaluation of potassium amounts released from mica-muscovite due to the selected isolates of Potassium- releasing bacteria. Results showed that all the tested isolates can release potassium from mica but not in equal efficiency. The most efficient isolate namely KRB-2 that isolated from the rice field can release 7.05 ppm after 6 weeks incubation at 30°C, so it was used for the further experiments.

The obtained results agree with those obtained by Afify and Bayoumy (2001) who tested some bacterial strains isolated from rhizosphere have ability to release K, also Zhang and Kong (2014) who tested 27 KRB strains isolated from tobacco rhizosphere for their ability to release K, and reported that all the examined bacteria could release K but not in equal efficiency. The ability of the KRB in releasing k was ranged from 0.59 ppm to 4.4 ppm.

Table 5. Changes in water soluble potassium (WSK) (ppm) values as influenced by selected potassium-releasing bacteria (KRB) on mica-muscovite

Isolate No.	Isolate origin	Incubation period (weeks)				
		2	4	6	8	10
KRB 1	Corn	2.89	3.40	4.04	4.42	3.91
KRB 2	Rice	3.59	4.87	7.05	4.94	3.53
KRB 3	Wheat	2.44	2.63	5.00	4.36	2.63
KRB 4	Clover	2.69	3.40	4.74	3.27	3.01
KRB 5		2.82	3.33	4.36	2.89	2.50

Initial soluble K = 2.05 ppm.

Identification of the most efficient isolates:

Data in Table (6) show the morphological and biochemical characteristics of isolates AZ 8, PSB 14 and KRB 2. Certain tests were performed for each isolate and the obtained data showed that isolate AZ 8

cells were rods to ellipsoid shaped with cell dimensions (2-2.5 µm) × 3 µm, Gram-negative, capsulated, motile, non-spore forming, formed dark brown pigments in old cultures, catalase positive. Hydrolyzing starch, not hydrolyzing casein, can assimilate mannitol, glucose, fructose, maltose, sucrose, sorbitol and starch as a sole carbon sources. Isolate PSB 14 cells were rod shaped with cell dimensions (0.5-0.8 µm) × (2-3 µm), Gram-negative, non-motile, non-spore forming, catalase positive, not hydrolyzing starch, casein and lipids, positive for Indole and V. P. tests, can assimilate arabinose, fructose, galactose, glucose, maltose, mannitol, xylose, sucrose and sorbitol as a sole carbon sources, cannot assimilate lactose as a sole carbon source. Isolate KRB 2 cells were rod shaped with cell dimensions (0.5 µm) × (1-1.5 µm), Gram-negative, non-motile, non-spore forming, catalase positive, not hydrolyzing starch, casein and lipids, can assimilate glucose, galactose, fructose, mannose, arabinose, maltose. Mannitol, sorbitol and xylose as a sole carbon sources. According to the morphological, biochemical characteristics and 16S rRNA sequence analysis (Macrogene Comp., Korea), the isolates showed close proximity with *Azotobacter chroococcum* MF135558, *Klebsiella oxytoca* MF135559 and *Rhizobium pusense* MF135560 (Table 7) according to the GeneBank database were achieved in BLASTN searches at the National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov).

Table 6. Morphological and biochemical properties of isolates AZ 8, PSB 14 and KRB 2

Isolates	Isolate AZ 8	Isolate PSB 14	Isolate KRB 2
Morphological characteristics			
Shape	Rods to ellipsoid	Rods	Rods
Cell dimensions (µm)	(2-2.5) × 3	(0.5-0.8) × (2-3)	(0.5) × (1-1.5)
Spore forming	–	–	–
Capsule formation	+	nd	nd
Motility	+	–	+
Pigment	Brown-black	nd	nd
Gram stain	–	–	–
Biochemical characteristics			
Catalase production	+	+	+
Starch hydrolysis	+	–	–
Casein hydrolysis	–	–	–
Lipase production	nd	–	–
Indole produced	nd	+	nd
Voges-Proskauer test	nd	+	nd
Growth at			
1 % NaCL	nd	nd	+
2 % NaCL	nd	nd	+
Assimilation of sugars			
Glucose	+	+	+
Galactose	nd	+	+
Fructose	+	+	+
Mannose	nd	nd	+
Arabinose	nd	+	+
Maltose	+	+	+
Lactose	nd	-	nd
Mannitol	+	+	+
Sorbitol	+	+	+
Xylose	nd	+	+
Sucrose	+	+	nd
Starch	+	nd	nd

+ = positive - = negative nd = not determined.

Table 7. Isolates accession numbers in NCBI gene bank

Isolate No.	Bacterial Species	Sequence length (bp)	Similarity (%)	Accession number
AZ 8	*Azotobacter chroococcum*	800	99	MF135558
PSB 14	*Klebsiella oxytoca*	800	99	MF135559
KRB 2	*Rhizobium pusense*	800	99	MF135560

REFERENCES

Abd-El-Malek, Y. and Y. Z. Ishac (1968). Evaluation of methods used in counting *Azotobacter*. J. Appl. Bact., 31: 267-275.

Afify Aida, H. (1982). Studies on silicate bacteria. M. Sc. Thesis, Fac. Agric., Mansoura Univ., Egypt.

Afify Aida, H. and Samia M. M. Bayoumy (2001). Effect of certain silicate bacteria on primary silicate minerals. J. Agric. Sci. Mansoura Univ., 26: 3111-3125.

Altschul, S. F., T. L. Madden, A. A. Schaffer, J. H. Zhang, Z. Zhang, W. Miller and D. J. Lipman (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res., 25:3389-3402.

Bashan, Y. and L. E. de-Bashan (2005). "Plant Growth-Promoting. Encyclopedia". Soils Environ., 1: 103-115.

Bhattacharjee, R. B. and U. Dey (2014). Biofertilizers, a way towards organic agriculture. Afr. J. Microbiol. Res., 8: 2332-2342.

Bhattacharjee, R. B., A. Singh and S. Mukhopadhyay (2008). Use of nitrogen-fixing bacteria as biofertilizer for non-legumes: "prospects and challenges". Appl. Microbiol. Biotechnol., 80: 199-209.

BLASTN, "the National Center for Biotechnology Information (NCBI) Web site"(http://www. ncbi. nlm.nih.gov)."

Boltz, F. D. and G. M. Mellon (1948). Spectrophotometric determination of phosphorus as molybdophosphoric acid. Anal. Chem., 20: 749-751.

Han, H. and K. Lee (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant soil Environ., 52: 130-136.

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

Hauka, F.I.A. (1986). Studies on the phosphate-dissolving bacteria. Ph. D. Thesis, Fac. Agric., Mansoura Univ., Egypt.

Hemalatha, N., N. Raja, A. Jayachitra, A. Rajalakshmi and N. Valarmathi (2013). Isolation and characterization of phosphate solubilizing bacteria and analyzing their effect on *Capsicum annum* L. Inter. J. Biol. Pharm. Res.,4: 159-167.

Jackson, M. L. (1967). "Soil Chemical Analysis". Prentice-Hall of India, Private & Ltd. New Delhi, India.

Liang, X., J. W. Hall, J. Yan, M. Yan, K. Doll, R. Bey and Y. Ji (2011). Identification of single nucleotide polymorphisms associated. *PLoS ONE*. 6: 1-10. (www.plosone.org).

Liu, D., B. Lian and H. Dong (2012). Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. Geomicrobiol. J., 29: 413 - 421.

Meena V. S., B. R. Maurya, J. P. Verma, A. Aeron, A. Kumar, K. Kim and V. K. Bajpai (2015). Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. Ecol. Engine., 81: 340-347.

Panday. D, P. Schumann and S. K. Das (2011) *Rhizobium pusense* sp. nov., isolated from the rhizosphere of chickpea (*Cicer arietinum* L.). Inter. J. Syst. Evol. Microbiol., 61: 2632-2639.

Pikovskaya, R. I., (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya. 17: 362-370.

Rodríguez, H. and R. Fraga (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv., 17: 319-39.

Sheng, X. F. (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol. Biochem., 37: 1918-1922.

Srinivas. N. P. and C. Hemalata (2014). Study of phosphate solubilizing microorganisms & their effect on plant growth. Bionano Frontier. 7:50 - 52.

Thompson, J. P. and V. B. D. Skerman (1979). *Azotobacteraceae*: The Taxonomy and Ecology of the Aerobic Nitrogen-Fixing Bacteria, Academic Press, London.

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

Walpola, C. B., K. Arunakumara, H. M. Yoon (2014). Isolation and characterization of phosphate solubilizing bacteria *Klebsiella oxytoca* with enhanced tolerant to environmental stress. Afr. J. Microbiol. Res., 8: 2970-2978.

Woodward, B. W., M. Carter and R. J. Seidler (1979). Most nonclinical *Klebsiella* strains are not *K. pneumoniae sensustricto*. Curr. Microbiol., 2: 181-185.

Yao, A., H. Bochow, S. Karimov, U. Boturov, S. Sanginboy and A. Sharipov (2006). Effect of FZB 24 *Bacillus subtilis* as a biofertilizer on cotton yields in field tests. Arch. Phytopathol. Plant Protec., 39: 323-8.

Zahra, M. K. (1969). Studies on silicate bacteria. M. Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Zhang, C. and F. Kong. (2014). Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Appl. Soil Ecol., 82 :18-25.

تقييم كفاءة بعض بكتيريا المجال الجذرى المعزولة من الأراضى المصرية معملياً كمسمدات حيوية.

فتحى اسماعيل حوقه ، عايدة حافظ عفيفى و أحمد محمود السواح.

قسم الميكروبيولوجيا – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

يعتبر النيتروجين والفوسفور والبوتاسيوم أهم العناصر الكبرى التى يحتاجها النبات ، وتعتبر قدرة ميكروبات التربة على امداد النبات بهذه العناصر فى صورة ميسرة من أهم خصائص الميكروبات المستخدمة كمسمدات حيوية. فى هذه الدراسة تم عزل ١٥ عزلة من بكتيريا الأزوتوباكتر واختبار قدرتها على تثبيت النيتروجين الجوى عن طريق اختبار اختزال الأسيتيلين حيث تراوحت كفاءتها من ٦.٣٤٨-١٣٨١.٠٢٣ نانومول / مل/ يوم ، وكانت أكثرها كفاءة العزلة رقم ٨ ، وتم تعريفها على أنها أزوتوباكتر كروكوكم. كما تم عزل ٢١ عزلة بكتيرية مذيبة للفوسفات المعدنية غير الذائبة وتم اختبار كفاءتها مبدئياً عن طريق تقدير كفاءة الإذابة كنسبة مئوية على أطباق أجار صلبة ، حيث تم إختيار ٥ عزلات –هى الأكثر كفاءة- واختبرت قدرتها على إذابة الفوسفور فى بيئات سائلة تحتوى فوسفات الكالسيوم الثلاثية وصخر الفوسفات ، وكانت أكثر العزلات كفاءة هى رقم ١٤ حيث حررت ٣٦.٥٢ ملجم فوسفور/ مل و ١.٠٩٥ ملجم فوسفور / مل من فوسفات الكالسيوم الثلاثية وصخر الفوسفات على الترتيب ، وتم تعريفعها على أنها كليبسيلا أوكسيتوكا. كما تم عزل خمس عزلات بكتيرية محررة للبوتاسيوم على بيئة ألكسندروف المعدلة ، وتم اختبار كفاءتها لتحرير البوتاسيوم من الميكا مسكوفيت ، وجميع العزلات استطاعت تحرير البوتاسيوم وكانت أكثرها كفاءة العزلة رقم ٢ وتم تعريفها على أنها رايزوبيم بيوزينس.