# Evaluation of biological activities for salt-tolerant plant growth promoting rhizobacteria using different microbial carriers

**Hoda, R.A. El-Zehery[1]; Zaghloul, R. A[1]; Salem, A.A.[1], Abdel-Rahman, H.M.[1]andEnas, A. Hassan[2]**
**1-Agric. Microbiology Dept., Fac. Agric. Moshtohor, Benha Univ., Egypt**
**2-Agric. Microbiology Dept., Fac. Agric., Ain Shams Univ., Egypt**
**Corresponding Author:** ahmed.nayl@fagr.bu.edu.eg

**ABSTRACT**

Plant growth promoting rhizobacteria (PGPR) play an important role for improving plant growth and increasing productivity especially under stress condition. Fifty rhizobacterial isolates were isolated from the rhizosphere of the wheat plants. The rhizobacterial isolates were screened to select the most salt tolerant isolates. Then, the more tolerant isolates were evaluated for plant growth promoting activities such as ammonia, HCN, siderophores, nitrogenase, indole acetic acid (IAA) and gibberellins (GB) production as well as, root colonization ability, phosphate and potassium solubilization. The obtained data showed that, rhizobacterial isolate number (40) exhibited high records for most of PGPR activitiesand identified as*Paenibacillus polymyxa*MG309677.1using 16S rRNA gene sequencing techniques with 99% similarity. One of the problems which face production of microbial preparations for agriculture use is the maintenance of bacterial viability. In this respect, immobilization of *P. Polymyxa* MG309677.1 on three different carriers (peatmoss, compost and sawdust) was studied. Peatmoss and compost were used singly or combined with sawdust at two levels (50% and 75% sawdust).Bacterial populations, dehydrogenase activity, pH values and moisture contents were determined monthly up to six months of storage. Resultsrevealed that, using of peat moss either singly or combined with sawdust (50:50%) as a carrier gave the highest survival and population records for*P. polymyxa*MG309677.1even the end of storage period (180) days.

**Keywords:** ompost, peat moss, carriers, *Paenibacillus polymyxa*, survival and biological activities

## Introduction

One of the problems that face the production of microbial preparations for agriculture use is the maintenance of bacterial viability. High bacterial population in the rhizosphere improves the efficiency of these organisms**(Cheuk *et al*., 2003)**.High bacterial population can be maintained by the application of enriched compost as a carrier which supports their growth and activitiesduring the storage period and in soil**(Ahmad *et al*., 2008and Bonkowski, 2004)**. Indeed, after the introduction into the soil, the inoculant have to compete with native soil. Carrier materials that can offer nutrients to the inoculant providing a higher rate of inoculation success**.** In addition, the carrier is the major portion (by volume or weight) of the inoculant that helps to deliver a suitable amount of plant growth promoting rhizobacteria (PGPR) in good physiological condition.

The materials constituting the carrier can be included various origins; organic, inorganic, or synthesized from specific molecules. **(Trzciński et *al*., 2011).**Variety of materials used as carriers has been shown to improve the survival and biological effectiveness of inoculants by protecting bacteria from biotic and abiotic stresses. Suitable carrier should be cheap, easily used, mixable, packageable, and available. Also, the carrier must permit gas exchange, particularly oxygen, and has high organic matter content and high water holding capacity as well as must be non-toxic either to the bacterial inoculants or to the plant itself **(Ben Rebah *et al*., 2002)**.Furthermore, **Ferreira and Castro (2005)** stated that the carriers should have near neutral orreadily adjustable pH, be abundant locally at a reasonable cost and able to sterilize. These properties only indicate the potential for a good carrier, while final selection of carrier must be based on microbial multiplication and survival during storage, the general method of planting, equipment used for planting and acceptable cost.

Among carriers that can sustain high levels of microbial load, the peat is considered the most widely used carrier, but is not universally available. Alternatively, different materials such as industry by-products, compost, organic wastes, mineral soils, plant by-products, coal, perlite, and agro-industrial wastes have been tested as culture media for the microbial growth **(Stephens and Rask, 2000)**.

The present work was designed to study the effect of different carrier materials (peatmoss, compost and sawdust) for preparation of salt-tolerant PGPR formulation and evaluating the survival and biological efficiency of this strain till the end of the storage period.

## Materials and Methods

### Soil samples and carrier materials

Salt–affected soil samples were collected from different locations in Egypt (El-Behira, Kafer El-shikhand Alexandria Governorates) for isolating salt-tolerant PGPR.Mechanical and chemical analyses of soil **(Table 1)**. Compost, peat moss and sawdust were obtained from the farm of the Fac. of Agric. at Moshtohor, Benha Unive., Egypt. The physical and chemical characteristics of carrier materials were described in **Table (2).**

**Table 1.** Mechanical and chemical analyses of soil samples.

| Parameters | Unit | Soil (1) | Soil (2) | Soil (3) |
|---|---|---|---|---|
| | | **Mechanical analyses** | | |
| **Sand** | (%) | 50 | 53 | 25 |
| **Silt** | (%) | 27 | 15 | 55 |
| **Clay** | (%) | 23 | 32 | 20 |
| **Textural class** | | Clay loam | Light clay | Silty clay loam |
| | | **Chemical analyses** | | |
| **Organic matter** | (%) | 1.24 | 1.74 | 0.63 |
| **EC** | dS/m | 8.95 | 8.62 | 11.3 |
| **pH** | | 8.41 | 7.26 | 8.58 |
| **Soluble cations** | | | | |
| $Na^+$ | meq./L | 28.02 | 25.86 | 38.61 |
| $K^+$ | | 23.15 | 21.75 | 18.03 |
| $Ca^{2+}$ | | 18.63 | 21.8 | 24.74 |
| $Mg^{2+}$ | | 19.7 | 16.82 | 31.62 |
| **Soluble anions** | | | | |
| $CO_3^{=}$ | meq./L | Zero | Zero | Zero |
| $HCO_3^-$ | | 27.93 | 28.7 | 28.2 |
| $Cl^-$ | | 32.55 | 26.03 | 52.84 |
| $SO_4^{2-}$ | | 29.02 | 31.5 | 31.96 |

**Soil (1):** from El-Behira **Soil (2):** from Kafr El-Shikh,
**Soil (3):** from Alexandria

**Table 2.** Physiochemical properties of carriers used in this study.

| Properties | Peat moss | Compost | Sawdust |
|---|---|---|---|
| **PH** | 7.14 | 7.8 | 6.0 |
| **E.C ($dsm^{-1}$)** | 2.1 | 2.75 | 1.1 |
| **Total C(g/100g)** | 39.0 | 33.75 | 58.25 |
| **Total N (g/100g)** | 0.49 | 0.42 | 0.24 |
| **C/N ratio** | **78:1** | **80:1** | **243:1** |

**Isolation of salt-tolerant PGPR isolates**

The isolation process was carried out using pouring and streaking plates method on different specific microbiological media named Ashby's medium (**Abdel-Malek and Ishac, 1986)**, King's medium **(King *et al.*, 1954),** modified nutrient agar medium **(Atlas, 1995),** semi-solid malate medium **(Dobereiner, 1978)**, modified Bunt &Rovira agar medium **(Abdel-Hafez, 1966)**andstarch nitrate agar medium **(Waksman and Lechevalier, 1961)**.Isolates were sub-cultured several times on their specific media for purification and then maintained as a stock culture at 4-5˚C for the succeeding studies.

**Screening for prospective PGPR characteristics**

**Salt tolerance of PGPR isolates**

Primary screening of rhizobacterial isolates were conducted under saline stress in presence of different sodium chloride concentrations using nutrient broth to give final concentrations of 2, 4, 6, 8, 10, 12, 15, 18 and 20%. After inoculation, cultures were incubation at 37 ˚C for 7 days in rotary shaker (150 rpm).

**Biological activities of PGPR isolates**

Secondaryscreening was depended on the production of Indole acetic acid (IAA), gibberellins, sidrophorse, hydrogen cyanide (HCN), ammonia and nitrogenase enzyme. Moreover, colonization capability, phosphate and potassium solubilization were considered under salinity condition (4% NaCl).

The ability for IAA production was determined using Salkowski's reagent according to the method described by **Gilickmann and Dessaux, 1995.** Estimation of gibberellic acid (GA) production was colorimetrically achieved according to the method of **Holbrook *et al*. (1961)**.Production of siderophores by the PGPR isolates were detected according to **Alexander and Zuberer (1991).**Whereas, detection of catechol-type siderophoreswere measured using a method of **Carson *et al*. (1992)**.While,Production of HCN and ammonia were estimated qualitatively according to the method described by **Lorck (1948)**and**Cappuccino and Sherman (1992),** respectively**.**

Nitrogenase activity was estimated as a guide for nitrogen fixation using the acetylene reduction technique given by **Dilworth (1970).** The bacterial isolates which isolated on specific nitrogen free media were screened for nitrogen fixation ability and results expressed as Nmole $C_2H_4$/day/100 ml culture.

Phosphate-solubilization ability was qualitatively detected on Pikovskaya agar mediumaccording to the method described **by Nguyen *et al*.(1992).**

$$\textbf{Solubilization efficiency (SE)} = \frac{\textbf{Solubilization diameter (cm)}}{\textbf{Growth diameter (cm)}} \times 100$$

While, phosphate solubilization was quantitatively determined according to the method described by **Nautiyal (1999).**

Qualitative and quantitativeof potassium solubilization were estimated according to the method described by**Manib *et al*.(1986).**

**Root colonizationassay**

Roots colonization ability was estimated by method described by **Mae and Ohira (1981)**.

**Bacterial identification using 16S rRNA sequences**

The most potent isolate was completely identified using 16S rRNA sequence technique as the following: The isolate was grown in nutrient broth on a rotary shaker (120 rpm) at 28°C for 24 hours. Bacterial Gene Jet genomic DNA purification Kit (Thermo K0721) was used to extract DNA according to SIGMA company instructions.

**Phylogenetic analysis**

The obtained sequence for 16S rRNA gene was analyzed by VecScreen tool for vector contamination (http://www.ncbi.nlm.nih.gov/tools/vecscreen/). Also, NEbcuter V2.0 was used to create a restriction map and to identify the GC content of the obtained sequence **(Vincze *et al*. 2003**, http://nc2.neb.com/NEBcutter2/). ORF finder software was used to obtain possible ORFs of the obtained sequence. Also, Jalview software was used to show SNPs and consensus resulted from the alignment of our bacterial isolate obtained sequence and the nearest bacterial strain in NCBI database (http://www.jalview.org/).

The sequence was registered in NCBI database under accession number MG309677.1(http://www.ncbi.nlm.nih.gov/nuccore/MG309677.1).Construction of the phylogenetic tree was done by using Clustal Omega and MEGA6 software.

**Effect of carriers on survival and viability of the identified bacteria.**

An application experiment was carried out to investigate the effect of carrier on the population and the activity of the rhizobacterial strain.Three different carriers were used namely, compost, peat moss and sawdust. Compost and peat moss were used singly or combined with sawdust at two rates(50% and 75% sawdust). Bacterial populations, dehydrogenase activity, the pH values and the moisture contents were monthly evaluated up to six months of storage.

**Preparation of inoculum**

Nutrient broth was inoculated with rhizobacteria strain in 250 ml Erlenmeyer flasks, incubated at 30°C for 48 hours in a shaker incubator at 100 rpm. The culture containing a bacterial population of about$\times 10^7$cellsml$^{-1}$was used for mixing with the carriers.

**Preparation of carrier materials**

The carrier materials**(Table 3)** were dried and neutralized using lime if acidic or HCl if alkaline and packed in opaque low density polypropylene bags with thickness of about 75 µm and then sterilized according to the procedure reported by**(Somasegaran*et al*., 1994).**

**Table 3.** Carrier preparations that used for this study

| Treatments | Description |
|---|---|
| **T1** | Compost (100%) |
| **T2** | Compost (50%) + Sawdust (50%) |
| **T3** | Compost (25%) +Sawdust (75%) |
| **T4** | Peat moss (100%) |
| **T5** | Peat moss (50%) + Sawdust (50%) |
| **T6** | Peat moss (25%) + Sawdust (75%) |
| **T7** | Sawdust (100%) |

Bacterial culture in late log phase was inoculated in carrier preparations aseptically to obtain 35% moisture content. The treatments which do not receive inoculantwhere moistened with sterilized broth of required quantity.

**Determination of viability ofthe carrier based inoculum**

The inoculated pages were stored at room temperature and screenedforcell population, viable cell respiration (dehydrogenase activity), moisture content and pH monthly up to six months of storage.

The standard plates count method was used as described by **Chevallierdand Lacroute, (1980).**While, dehydrogenase activity was assayed in carriers samples according to **Glathe and Thalmann (1970)**. Moisture contents of the carrier based inoculum samples were determined according to **pepper *et al*.,(1995)**.PH values were periodically determined using digital pH meter apparatus (JENWAY, 3510 Bench pH/mV Meter, UK).

**Results and Discussion**

**Isolation and screening processes of salt-tolerant PGPR**

Fifty rhizobacterial isolates were isolated from the rhizosphere of the wheat plants.The primary screening of the rhizobacterial isolates was achieved under saline stress to investigate the most salt tolerant isolates.From the obtained data (Fig1), all tested rhizobacteria isolates showed salt tolerance up to 6% sodium chloride. While, 12%,10% and 12% of the tested isolates sowed salt tolerance at sodium chloride concentrations of 10%, 12% and 15%, respectively. Only 28% of the examined isolates showed salt tolerance at concentration of 20% sodium chloride. Salt tolerance of most of the rhizobacterial isolates could be attributed to the adaptability of these rhizobacterial isolates in their original habitat (Salt–affected soil samples that were collected from different places in Egypt).

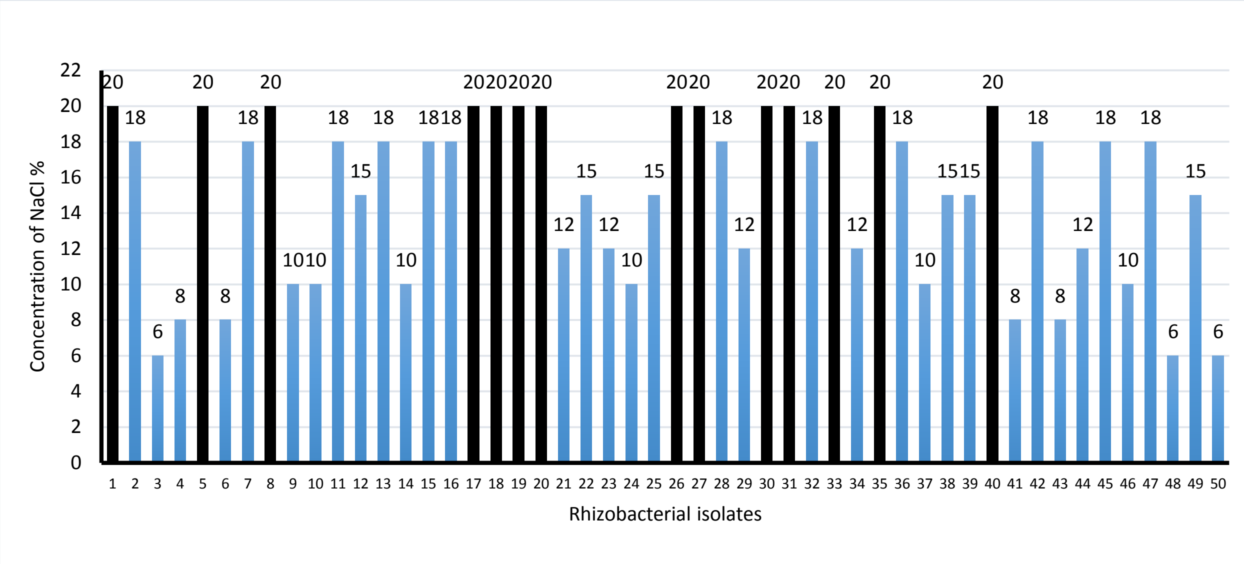


**Fig (1):** Salt tolerance of the rhizobacterial isolates

More tolerant isolates (14 isolates) that grew up to 20% NaCl were screenedfor their putative PGPR activities.Screening was depended on the production of Indole acetic acid (IAA), gibberellins, sidrophorse, hydrogen cyanide (HCN), ammonia and nitrogenase enzyme. Moreover, colonization capability, phosphate and potassium solubilization were considered (Table 4).

From the obtained results, rhizobacterial isolate number (40) exhibited high records for most of PGPR activities. The capability of IAA and gibberellin production by the rhizobacterial isolate number (40) was evaluated which being16.2 µg/ml and 10.25 µg/ml, respectively. Concerning HCN, siderophores and ammonia production, rhizobacterial isolate number (40) showed the highest qualitative production of above mentioned. In the same manner, 200%as phosphate solubilization efficiency was observed. Moreover, rhizobacterial isolate number (40) exhibited good efficiency for potassium solubilization being 57ppm as soluble potassium. Superiority for nitrogen fixation was found based on nitrogenase activity being 24N moles $C_2H_4$/day/100 ml.

Finally, rhizobacterial isolate number (40) recorded good ability to colonize the roots of wheat that directly enhanced the shoot length and root lengthof wheat plants comparing with un-inoculated plants.

The obtained results are in agreement with **Parihar *et al.* (2015)** reported that plant growth promoting rhizobacteria such as *Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Paenibacillus polymyxa,Pseudomonas* sp, *B. pantothenticus, B. circulans* has been reported to release potassium in accessible form from potassium bearing minerals in soils and support ecofriendly crop production. Thus, application of plant growth promoting rhizobacteria as biofertilizer for improvement of plant growth and production.

**Bacterial identification using 16S rRNA sequences**

The most potent isolate (isolate No.40) was chosen and identified by 16S rRNA gene sequence analysis to ascertain their taxonomic positions **(Figs. 2, 3 and 4)**.

Squencing result was registered in NCBI database under accession number, MG309677.1. Analysis of the obtained sequence via Vecscreen database showed no contamination with vector sequence.

The FASTA homology showed that the16S rRNA gene sequence of the current isolate (ACC. no. MG309677.1) had 99% nucleotide similarity with that of *P. polymyxa* strain recorded in NCBI database (ACC. no. GQ375783.1).This result was confirmed by the phylogenetic position of the obtained isolate, forming polyphyletic clade with *P. polymyxa*, but with an obvious phylogenetic distance **(Fig. 2)**. Also, the restriction Map of the obtained 16S rRNA partial sequence was done **(Fig. 3)**. Calculating the pairwise alignment analysis, exhibited 4 SNPs between the sequence of the obtained isolate and the nearest registered bacterial strain in NCBI database, *P.polymyxa* (GQ375783.1), for 16S rRNA gene **(Fig.4).**

**Table 4.** Biological activities of the most salt tolerant rhizobacteria isolates.

| No.of isolate | Features of PGPR | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Phosphate solubilization | | Silicate solubilization | | Root colonization | | |
| | IAA (µg/mL) | Gibberellin | Sidrophorse | Catechol | HCN | Ammonia | Solubilization efficiency (%) | solubilized Phosphate (ppm) | Qualitative (%) | Quantitative (ppm) | Shoot (cm) | Root (cm) | N2-ase (N moles $C_2H_4$/ day/ 100ml culture) |
| 1 | 7.32 | 22.62 | + | + | +++ | + | 80 | 3.96 | ++ | 33.6 | 1.8 | 1.1 | 0.72 |
| 5 | 18.52 | 4.2 | + | + | ++ | + | 80 | 3.99 | ++ | 31.4 | 2.5 | 2.3 | 2.232 |
| 8 | 2.675 | 4.75 | - | - | ++ | - | 320 | 45.26 | ++ | 33.1 | - | - | ND |
| 17 | 3.4 | 9.43 | - | + | ++ | - | 220 | 13.05 | - | 11.8 | - | - | ND |
| 18 | 6.975 | 0.625 | - | - | + | - | 180 | 12.34 | - | 7.6 | 1.4 | 1.5 | ND |
| 19 | 5.025 | 22.75 | - | - | ++ | - | ND | 9.56 | - | 8.2 | - | - | ND |
| 20 | 2.975 | 18.13 | - | + | - | - | ND | 8.74 | - | 8.6 | - | - | 0.72 |
| 26 | 2.15 | 0.865 | + | - | ++ | - | ND | 11.19 | - | 5.2 | 1.8 | 1.5 | ND |
| 27 | 2.15 | 2.15 | - | - | ++ | - | ND | 6.28 | - | 8.5 | 2.1 | 1.9 | ND |
| 30 | 1.475 | 1.475 | - | - | + | - | ND | 6.83 | + | 75.5 | - | - | ND |
| 31 | 1.875 | 1.875 | - | - | + | - | 120 | 2.95 | - | 11.3 | 1.5 | 1.3 | ND |
| 33 | 2.05 | 2.05 | - | + | ++ | - | 300 | 25.72 | - | 9.2 | - | - | ND |
| 35 | 10.25 | 9.27 | ++ | + | +++ | + | 200 | 7.32 | + | 36 | 1.2 | 0.7 | 8.4 |
| 40 | 16.2 | 10.25 | ++ | + | +++ | + | 200 | 14.63 | ++ | 57 | 2.2 | 1.5 | 24 |

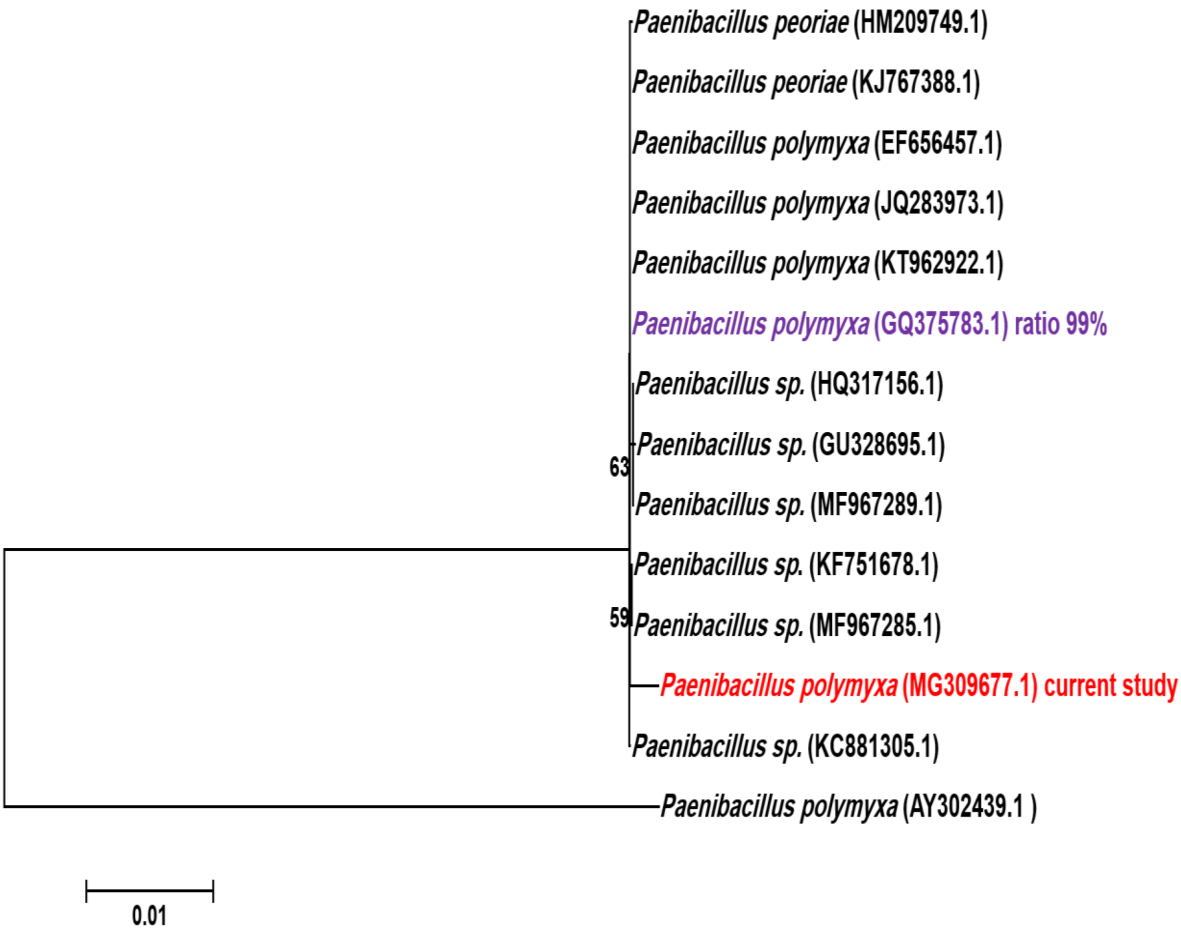


**Fig.2. Phylogenetic tree recovered from maximum likelihood and neighbor joining analyses of the 16S rRNA gene Partial sequences.**

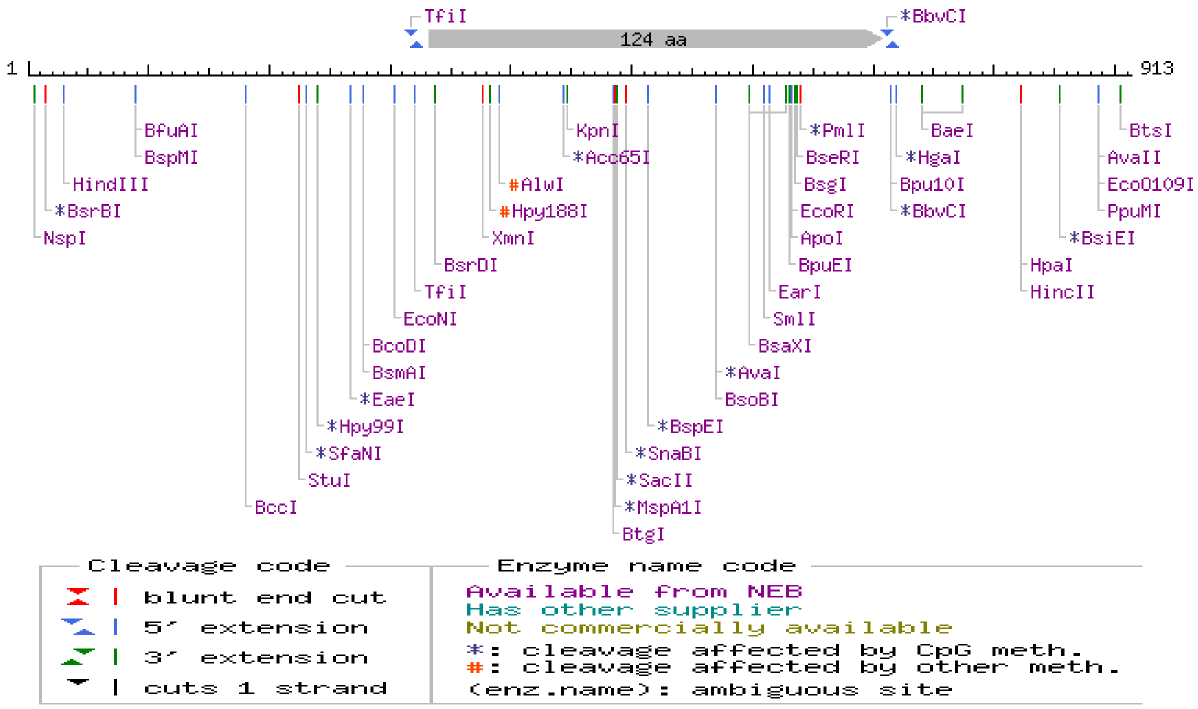


**Fig. 3. Restriction Map of the obtained 16S rRNA partial sequence**

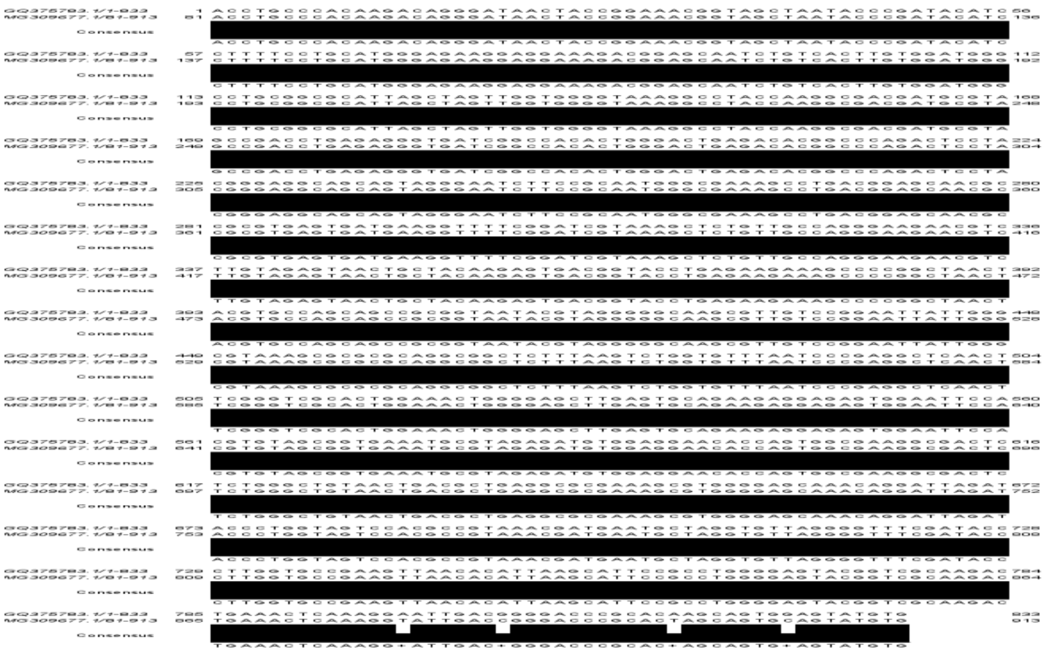

**Fig.4.** Single nucleotide polymorphism (SNPs) showed 4 SNPs between the obtained isolate, KM491552.1, and the nearest one on NCBI data base, AJ880396.1 based on pairwise alignment analysis method.

**Effect of carriers on Survival ofP. Polymyxa MG309677.1 in different carriers**

Data presented in **Table 5** revealed that, using of peat moss either singly or combined with sawdust (50:50%) as a carrier gave the higher survival and population records for *P. polymyxa*MG309677.1. The highest population of *P. polymyxa*MG309677.1 showed with peat moss (100%) as a carrier. The population rates increased with increasing the incubation period up to 90 days and thereafter decreased. This result was observed in various treatments.These results can be explained as carrier materials can offer nutrients to the inoculant providing a higher rate of inoculation success**.**Various types of materials are used as carrier for seed or soil inoculation to improve the survival and biological effectiveness of inoculants by protecting bacteria from biotic and abiotic stresses (**Malusa *et al*. 2012)**. Carrier is a delivery vehicle which is used to transfer live microorganism in a good physiological condition from an agar slant of laboratory to a seed/rhizosphere (**Smith 1992)**. Since a suitable carrier plays a major role in formulating microbial inoculants, the use of any ideal carrier material is important in the production of good quality microbial inoculants. Of these materials, the neutralized peat has been found as the better carrier material for inoculant production **Bashan, 1998**.

The obtained data showed that the survival rate of*P. polymyxa*MG309677.1 in all peat moss treatments was better than that in compost. As expected, using of sawdust solely (100%) showed the lowest population rates and survival for all examined rhizobacterial strain. The obtained results are in harmony with those of **Phiromtan*et al*. (2013)** who evaluated the effect of various carriers and storage temperatures on survival of *Azotobacter vinelandii* NDD-CK-1. Obtained results showed that peat moss either singly or combined with compost as a carrier gave the higher survival and population records.

**Table 5.** survival of *P. polymyxa* MG309677.1 in different carriers.

| Treatments | Plate count( X $10^6$ ) After (days) | | | | |
|---|---|---|---|---|---|
| | **30** | **60** | **90** | **120** | **180** |
| **T1** | 54 | 58 | 56 | 39 | 24 |
| **T2** | 64 | 68 | 76 | 58 | 32 |
| **T3** | 56 | 60 | 63 | 55 | 29 |
| **T4** | 68 | 74 | 83 | 48 | 39 |
| **T5** | 66 | 70 | 78 | 39 | 30 |
| **T6** | 59 | 63 | 66 | 58 | 28 |
| **T7** | 39 | 44 | 50 | 28 | 19 |

**For more details about treatments: T1 to T7 see Table 3**

The results revealed that types of carrier, storage temperatures and interaction between them showed significant effect on survival of *Azotobacter* during 7 to 90 days. The survival rate was the highest in PtLC, followed by PtCC, PtMC, and Pt which gave higher values in peat moss mixed different type of compost compared to peat moss only.

**Determination of respiration ratein different carriers' treatment.**

Dehydrogenase activity was determined refereeing to the respiration rate of the rhizobacterial strain*P. polymyxa*MG309677.1 in different carriers. The obtained data were tabulated in **Table 6.** Because of the strong relationship between the growth rate and the respiration rate, the superiority of peat moss solely or combined with sawdust (50:50%) as a carrier could be explained. According to dehydrogenase activity, all peat moss treatments exhibited a good capability as a carrier for the survival of *P. Polymyxa* followed by compost treatments.

This high bacterial population can be maintained by the application of enriched compost as carrier which supports their growth and activities**(Ahmad *et al*., 2008).**

**Table 6.** Dehydrogenase activity of rhizobacterial strain in different carriers treatment.

| Treatments | Dehydrogenase activity (µg TPF $g^{-1}$ dw $h^{-1}$) After (days) | | | | |
|---|---|---|---|---|---|
| | **30** | **60** | **90** | **120** | **180** |
| **T1** | 52.63 | 59.11 | 59.48 | 58.55 | 56.70 |
| **T2** | 61.06 | 62.63 | 63.28 | 63.02 | 62.26 |
| **T3** | 41.41 | 44.29 | 52.26 | 48.64 | 74.34 |
| **T4** | 249.97 | 267.11 | 314.82 | 301.20 | 196.79 |
| **T5** | 224.95 | 246.26 | 296.57 | 264.98 | 223.29 |
| **T6** | 133.97 | 169.18 | 205.76 | 195.68 | 186.50 |
| **T7** | 31.22 | 30.95 | 28.81 | 28.07 | 261.27 |

**For more details about treatments: T1 to T7 see Table 3**

**Moisture contents and pH values in different carriers.**

Moisture content and pH values of the examined different carriers were evaluated. The obtained data in **Fig 5 and 6**. Observed that moisture content, gradual decrease with the increasing of storage period in all carriers' treatments and could be attributed to the storage at room temperature and the gradual development and growth of the used rhizobacterial strain. The highest moisture content after 180 days observed with peat moss-based inoculum. While, the lowest moisture content after 180 days observed with compost (25%) + sawdust-based inoculum (75%).

A slight decline in pH was recorded in all carriers treatments up to 90 days of storage. While, an increase of the pH values was seen for all treatments after 120 days of storage.

These data are in accordance with the findings of **Ghazi (2017)**who evaluatedtwo different rice biochar preparations (biochar alone and biochar- vermiculite 50:50 ) in comparison with peat moss based carrier (peat moss: vermiculite 50:50) for their suitability for commercial production of *Rhizobia*. Carriers were evaluated over a period of 180 days for its moisture content, pH, survival of the microbial inoculant and respiration rate At the end of storing period (180 days), biochar based carrier recorded a maximum population of log 9.98 CFUg/1 of carrier with a maximum moisture content of 20%. Moreover, slight decreasein pH was recorded at the end ofstoring.

.

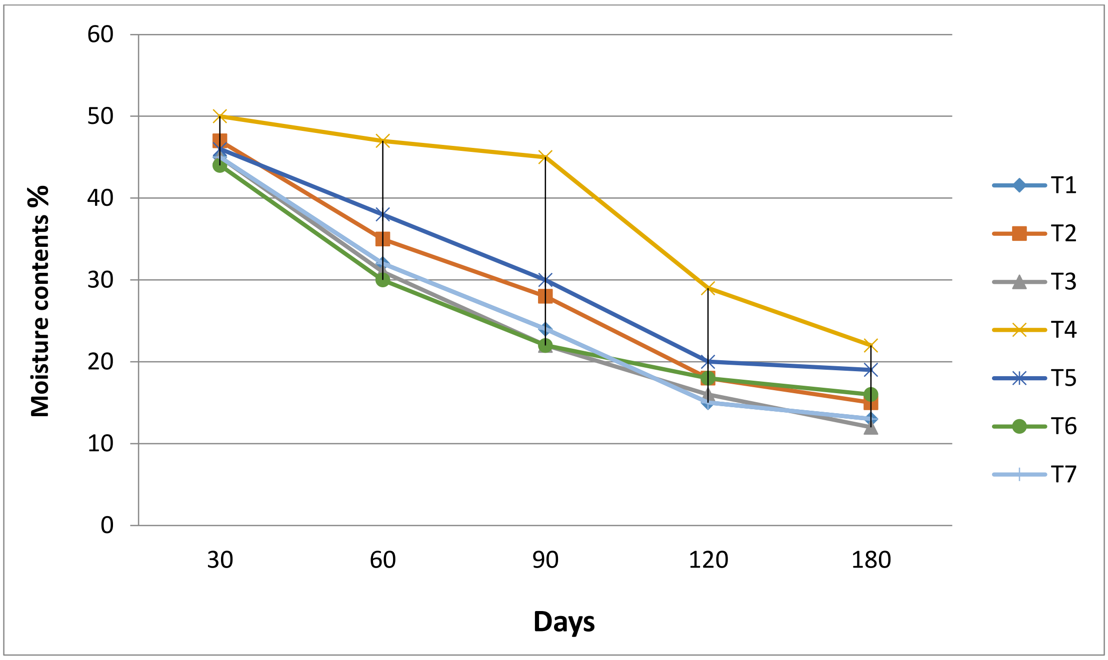


**Fig (5).** Moisture contents in different carriers' treatments.

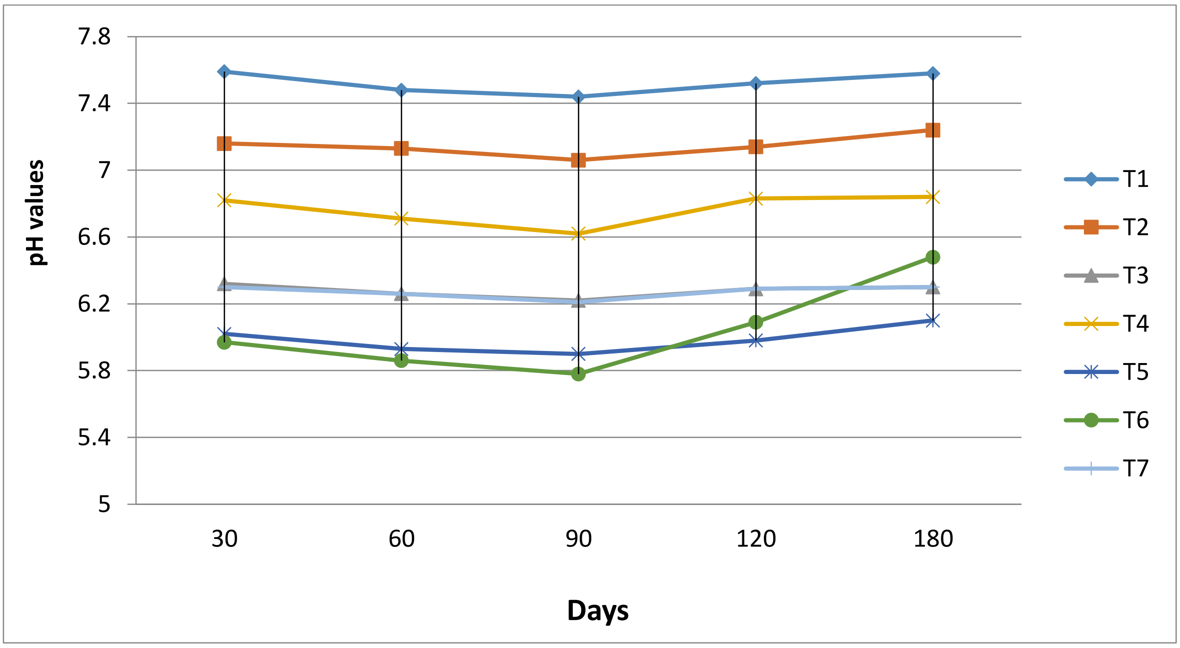


**Fig (6). PH values in different carriers treatment.**

## Conclusion

From the previous work it can be concluded that, carrier materials can offer nutrients to the inoculant providing a higher rate of inoculation success and were able to maintain the survival and biological efficiency of the plant growth promoting rhizobacteria *P. Polymyxa* MG309677.1even the end of storing period (180) days.

## Acknowledgment

The authors would like to express sincere appreciation to all staff members of Agric. Microbiology Dept., Faculty of Agriculture at Moshtohor, Benha University, for their kind cooperation.

## References

**Abdel-Hafez, A.M. (1966).** Some studies on acid producing micro-organisms in soil and rhizosphere with special reference to phosphate dissolvers. Ph.D. Thesis, Agric. Botany Dep. Fac. Agric., Ain Shams Univ., Egypt, p: 31.

**Abdel–Malek, Y. and Y. Z. Ishac (1986):** Evaluation of methods used in counting *Azotobacter*. J. Appl. Bacteriol., 31: 267-269.

**Ahmad, R.; A. Khalid; M. Arshad; Z.A. Zahir and T. Mahmood (2008):** Effect of composted organic waste enriched with N and L-tryptophan on soil and maize. Agronomy for Sustainable Development, 28: 299-305.

**Alexander, D.B. and D. Zuberer (1991).** Use of chrome azurol S reagents to evaluate siderophores production by rhizosphere bacteria. Boil. Fertile. Soils, 12:39-45.

**Atlas, R.M. (1995):** Handbook of Media for Environmental Microbiology, 2nd Ed. CRC Press, Boca Raton. 411-425.

**Bashan,Y. (1998)** Inoculants of plant growth promoting bacteria for use in agriculture.Biotechnol. Adv., 16:729–770.

**Ben Rebah, F.B.; R.D. Tyagi, and D. Prevost (2002):** Wastewater sludge as a substrate for growth and carrier for rhizobia: the effect of storageconditions on survival of *Sinorhizobium meliloti*.Bioresource Technology, 83: 145-151.

**Bloemberg, G. V. and B. J. Lugtenberg (2001):** Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biol., 4: 343-350.

**Bonkowski, M. (2004):** Protozoa and plant growth: The microbial loop in soil revisited. New Phytologist, 162: 617-631.

**Cappuccino, J.C. and N. Sherman (1992):** Microbiology: A Laboratory Manual (third), Benjamin/cummings Pub. Co., New York. 125-179.

**Carson, K.C.; M.J. Dilworth and A.R. Glenn (1992).** Siderophore production and iron transport in *Rhizobium leguminosarum* bv. viciae MNF710. J. Plant Nutr. 15(10):2203-2220.

**Cheuk W.; K.V. Lo; R.M.R. Branion and B. Fraser (2003):** Benefits of sustainable waste management in the vegetable greenhouse industry. J. Environ. Sci.Health Part B-Pesticides Food Contaminants, 38:855-863.

**Chevallierd,M. R. and F. Lacroute (1980)**: Expression of the cloned uracil permease gene of *Saccharomyces cerevisiae* in heterologous membrane .Embo. J., 1 (3): 375-377.

**Diloworth, M. J. (1970).** The acetylene reduction method for measuring biological nitrogen fixation. *Rhizobium* News Letters, 15(7): 155-161.

**Dobereiner, J. (1978):** Influence of environmental factors on the occurrence of *S. lipoferum* in soil and roots. Ecol. Bull. (Stockholm), 26: 343-352.

**Ferreira, E.M. and I.V. Castro (2005):** Residues of the cork industry as carriers for the production of legume inoculants. Silva Lusitana. 13, 159-167.

**Ghazi, A. A. (2017):** Potential for biochar as an alternate carrier to peat moss for the preparation of rhizobia bioinoculum. Microbiology. Res. J. Internatil., 18 (4): 1-9.

**Gilickmann, E. and Y. Dessaux (1995):** A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ. Micobiol., 61(2): 793-796.

**Glathe, H. and A. Thalmann (1970):** Uber die microbiello activitat and iher Beziehungen zu Fruchtbrkeitsmerkmalen einiger Acherboden unter besonderer Berucksichtigung der dehydrogenase akativitat (TCC. Redukation). Zbl. Bakt. Abt. II, 124: 1-23.

**Holbrook, A.; W.Edge; F.Bailey (1961):** Spectrophotometric method for determination of gibberellic acid. Adv Chem Ser., 28, 159-67.

**King, E. O.; M. K. Ward and D. E. Raney (1954).** Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clim. Med., 44: 301-307.

**Le Chevallier, M. W.; R. J. Seidler and T. M. Evans (1980**): Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Appl Environ Microbiol., 40 (5): 922–930.

**Lorck, H. (1948):** Production of hydrocyanic acid by bacteria. Physiol. Plant., 1: 142-146.

**Mae, T. and K. Ohira (1981).** The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). Plant Cell Physiol., 22:1067–1074.

**Malusa, E.; L.Sas-Paszt; J.Ciesielska (2012)** Technologies for beneficial microorganisms inocula used as biofertilizers. Sci World J., pp12.

**Manib, M.; M. K.Zahra; S. H. I. Abdel-Al and A.Heggo (1986).** Role of silicate bacteria in releasing K and silicone from biotite and orthoclase. In: Soil biology and conservation of the biosphere.

Szegi, J. Ed. Akademiai Kiado, Budapest. pp. 733-743.
**Nadège, A.; A. Pacôme and A. Adolphe (2015):** Response of maize (*Zea mays* l.) crop to biofertilization with plant growth promoting rhizobacteria and chitosan under field conditions. J.Experim. Biolo and Agric. Sci., 3 (6):84-55.
**Naik, P. R.; G. Raman; K.B. Narayanan and N. Sokthivel (2008):** Assessment of genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads isolated from rhizospheric soil. BMC Microbiol., 8 (230):1-14.
**Nautiyal, C.S. (1999).** An efficient microbiological growth medium for screening phosphate solubilising microorganisms. FEMS Microbiol. Lett., 170: 265–270.
**Nguyen, C.; W.Yan and T. F. Le (1992).** Genetic variability phosphate solubilizing activity of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) P.D. Orton. Plant Soil., 143:193–199.
**Parihar P.; S. Singh; V. P. Singh and S. M. Prasad (2015):** Effect of salinity stress on plants and its tolerance strategies: a review. Environ. Sci. Pollut. Res., 22, 4056–4075.
**Patten, C.L. and B.R. Glick (2002)**: Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and stationary-phase sigma factor Rpos. Can. J. Microbiol., 48: 635-642.
**Pepper, I.; Gerba, C.andJ.Brendecke(1995):** Environmental Microbiology2nd Edition: A Laboratory Manual. pp232
**Phiromtan, M.; T. Mala and P. Srinives (2013):** Effect of various carrier and storage temperature on survival of *Azotobacter vinelandii* NDD-CK-1 in powder Inoculant. Modern Appl. Sci., 7(6): 152-159.
**Priyanka P. and S. S. Sindhu (2013):** Influence of nutritional and environmental conditions. J.Microbio. Res., 3 (1): 25-31.
**Smith, R.S. (1992)** Legume inoculant formulation and application. Can J Microbciol., 38:485–492
**Somasegaran P and H. S. Hoben (1994):** Handbook for Rhizobia. In: Methods in legume - *Rhizobium* technology. Springer- Verlag, New York. 45-85.
**Stephens, J. H. and H. M. Rask (2000):** Inoculant production and formulation. Field Crops Res., 65: 249-258.
**Trzciński, P; E. Malusá and L. S. Paszt (2011):** Survival of PGPR in beads of biodegrdable polimer. In: Proceedings of the Ogólnopolską Naukową Konferencję Ekologiczną ,Osiągnięcia i Możliwości Rozwoju Badań i Wdrożeń w Ekologicznej Produkcji Ogrodniczej; Skierniewice, Poland. 181-182.
**Vincze, T.;J. Posfai and R. J. Roberts (2003):** NEBcutter: a program to cleave DNA with restriction enzymes Nucleic Acids Res., 31: 3688-3691
**Waksman, S.A. and H.A. Lechevalier (1961).** The actinomycetes Vol. II classification, identification and description of genera and spices. Williams and Wilkins Co., Baltimore. USA.

## تقييم الأنشطة الحيوية للريزوبكتريا (المتحملة للملوحة) والمشجعة لنمو النبات بإستخدام حوامل ميكروبية مختلفة.

هدي رشوان احمد الزهيري[1]و راشد عبد الفتاح زغلول [1] و أحمد عبد الخالق سالم [1]و هاني محمد احمد عبدالرحمن[1] و إيناس عبد التواب حسن[2]
١- قسم الميكروبيولوجيا الزراعية – كلية الزراعة بمشتهر – جامعة بنها – مصر.
٢- قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة عين شمس – مصر.

من المعروف أن البكتريا المشجعة لنمو النبات تقوم بدور هام في تحسين وزيادة إنتاجية المحاصيل تحت ظروف الأراضي الملحية . في هذا البحث تم عزل ٥٠ عزلة من منطقة الريزوسفير لمحاصيل مختلفة نامية في أراضي ملحية.تم تقييم أنشطة هذه العزلات والتي تضمنت إنتاج الأمونيا ،سيانيد الهيدروجين ،السيدروفوز، أنزيم النيتروجينيز، حمض الخليك والجبرللين، إستعمار البكتريا للجذور وإذابة كلا من مركبات الفوسفات والبوتاسيوم.ولقد أوضحت نتائج هذه الدراسة أن ٤٠ عزلة قد أظهرت نشاط عالي لمعظم الأنشطة الحيوية سالفة الذكر وذلك من خلال عملية التقييم الأولي لهذه العزلات.وكذلك تم تعريف أفضل العزلات من حيث الأنشطة الحيوية بإستخدام تقنية 16SrRNA. حيث أوضحت نتائج التعريف هي *Paenibacillus Polymyxa* .وبخصوص تأثي إستخدام الحوامل المختلفة علي أنشطة هذه السلالة وفترة بقائها أثناء التخزين فقد أوضحت النتائج أن إستخدام البيت موس مختلطا مع نشارة الخشب بنسبة ١:١ أدي الي أعلي نشاط من حيث الأنشطة الحيوية التي درست أثناء فترة التخزين والتي استمرت ١٨٠ يوم.