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Assessment of plant growth promoting rhizobacteria activity under saline stress

Ahmed, Gh. Rahal[2]; Ehsan, A. Hanafy[1]; Rashed, A. Zaghloul[1]; Hamed, E. Abou-Aly[1]; Rasha, M. El-Meihy[1]

1- Fac. Agric., Moshtohor, Benha University
2- Soil, Water and Environment Research Inst. Agric. Research Center

Abstract

The present study was designed to isolate and characterize plant growth promoting rhizobacteria (PGPR) from different Egyptian salt-affected soils and to evaluate the in vitro bacterial mechanisms related to plant growth promotion and their tolerance for sea water and sodium chloride added in culture media. Two hundred and seven isolates of bacteria and actinobacteria were isolated from salt-affected soils of different Egyptian governorates namely, Kafr El-Shikh (Sakha), El-Qalubia (Meet Kenana), El-Behira (El-Nubaria) and Port Said (Sahl El-Teina). Obtained data showed that the two more potent isolates in nitrogenase activity were identified as *Azospirillum lipoferumD178* and *Azospirillum lipoferum D207*, while the isolates which gave high production of indole acetic acid were identified as *Paenibacillus alive D135* and *Bacillus pumilus D139*, while the isolates which appeared highly inorganic phosphate solubilization were identified as *Pseudomonas fluorescence D23* and Bacillus megaterium D159. The six identified strains were taken to examine their tolerance against different concentrations of sodium chloride (1-5%) and different dilutions of sea water (1:2 to 1:10 v/v). All strains under saline stress were able to produce indole acetic acid, gibberellines, catecolate-type siderophores and citric acid-type siderophoresas well as their ability to solubilize inorganic phosphates. All the PGPR activities in the supplemented medium with NaCl or sea water were indicative of their tolerance to the salt. A decrease in activities values has been reported with increasing salt concentration.

Key words: Auxins, gibberellines, NaCl, nitrogen fixation, PGPR, phosphate solubilization, sea water and siderophores.

Introduction

Plant growth promoting rhizobacteria (PGPR) are a group of the beneficial microorganisms to crops. PGPR are a heterogeneous group of soil bacteria which could be found in the rhizosphere. Most of PGPR are free living, associative or symbiotic soil bacteria (**Bent *et al,* 2001and Tilak *et al,* 2005**).

PGPR can produce direct or indirect effects on host plants. Indirect effects are those related to the production of metabolites such as antibiotics, siderophores or cyanogen (HCN) which increase plant growth by decreasing the activities of pathogens. While the direct effects of PGPR are by producing metabolites such as plant growth regulators (PGRs) that directly promote plant growth or by facilitating nutrient uptake by the plant (**Salamone *et al,* 2001**).The ability of phytohormons and siderophores productions, nitrogen fixation, phytopathogens antagonism, cyanogenesis, phosphate solubilization and ACC deaminase activities are the main plant growth promoting-related traits beneficial to plant. In addition to the above mentioned traits, PGPR must also be rhizosphere component, able to survive and flourish in soil following inoculation **(Young *et al,* 2006)**.

A more recent review quotes that one-third of the world's arable land resources are affected by salinity **(Qadir *et al,* 2000)**. Not surprisingly, the gradually increase in salt content in irrigated soils has been considered as one of the main threats against crop production **(Kotb *et al,* 2000)**. In general, salinity affects almost every aspect of the physiology and biochemistry of plants **(Cuartero *et al,* 2005)**.Under saline stress, PGPR have soon positive effects for plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, plant growth and yield **(Klopper *et al,* 2004 and Kokalis-Burelle *et al,* 2006).**

The aim of the present study is to isolate and identify plant growth promoting rhizobacteria and evaluate their PGPR-mechanisms in vitro in presence of either sodium chloride or sea water to be used as biofertilizers in salt-affected soils.

Materials and methods

Isolation and purification of PGPR

After collection of salt-affected soil samples from El-Nubaria, Port said, Kafr El-Shikh and Qalubia Governorates, the isolation process was carried out by using pouring and streaking plate methods on different specific bacteriological media named Ashby's modified medium **(Abd El-Malek and Ishac, 1986**), King's medium **(King *et al,* 1954)**, Modified nutrient agar medium **(Jacobs & Gerstein,**

1960), Semi–solid malate medium **(Dobereiner, 1978)**, Modified Bunt and Rovira agar medium modified by **(Abdel-Hafez, 1966)** and Starch nitrate agar medium **(Waksman and Lechevalier, 1961)**.
Two hundred and seven isolates belonging to bacteria and actinobacteria were isolated. The isolates were sub-cultured on their specific media for purification process and then maintained as a stock culture at 4-5˚C for subsequent studies**.**

Screening of PGPR isolates
Primary and secondary screening of PGPR isolates were depended on indole acetic acid (IAA) production, phosphate solubilization and siderophores production. From the obtained results of the primary screening, ten isolates were selected for the secondary screening. Those ten isolates gave high results in all tested parameters. The secondary screening was carried out under sodium chloride concentrations. Only six isolates were the most potent at all examined parameters.

Identification of the most potent isolates
The most potent isolates which showed high results in all examined parameters were identified according to **Bergy's Manual of Systematic Bacteriology (2005)**. The stock cultures of the six identified strains were maintained on King's medium **(King *et al,* 1954)** for *Pseudomonas fluorescence D23*, *Bacillus pumilus D135* and *Paenibacillus alvie D139* ; semi – solid malate medium **(Dobereiner, 1978)** for *Azospirillum lipoferum D178*, *Azospirillum lipoferum D207*and Modified Bunt and Rovira agar medium **(Abdel-Hafez , 1966)** for *Bacillus megaterium D159* at 5 °C for subsequent studies.

Tolerance of the identified PGPR for saline stress
This experiment was carried out to determine the activity of the identified PGPR strains in presence of different concentrations of sodium chloride and sea water. Sodium chloride was added to specific media to give final concentrations of 1, 2, 3, 4 and 5%. On the other hand, sea water, from Suez Canal, Ismailia governorate was diluted with fresh distilled water at five ratios namely1:2, 1:4, 1:6, 1:8 and 1:10 according to **Noufal *et al,* (2008)**.These ratios were chemically analyzed as described in **Table 1**.

Table 1. Chemical analysis of Sea water and the five dilutions.

Parameters		Unit	Sea water	1:2 ratio	1:4 ratio	1:6 ratio	1:8 ratio	1:10 ratio
Ph			7.32	7.48	7.20	7.06	6.89	6.82
EC		**dS/m**	53	17.9	10.6	7.57	5.88	4.82
Soluble cations	**Ca^{+2}**	**Meq/l**	26.0	8.67	5.20	3.70	2.89	2.36
	Mg^{+2}		111.0	37.0	22.2	16.0	12.3	10.1
	Na^{+}		390.5	130.2	78.41	55.98	43.38	34.93
	K^{+}		12.5	4.17	2.4	1.88	1.38	1.14
Soluble anions	**Cl^{-}**	**Meq/l**	445.0	148.3	89.0	64.0	49.4	39.4
	$CO_3^{=}$		Zero	Zero	Zero	Zero	Zero	Zero
	HCO_3^{-}		37.0	12.4	7.40	5.28	4.11	3.56
	$SO_4^{=}$		58.0	19.3	11.6	8.29	6.44	5.57

Specific media were prepared by adding the components of the media to sea water dilutions which previously mentioned.

Assessments of PGPR activities
Nitrogenase activity was measured as a guide for nitrogen fixing ability by using the acetylene reduction technique given by **Diloworth (1970).** Inorganic phosphate solubilization was detected on Pikovskaya's (PVK) medium **(Pikovskaya, 1948)** according to the method described by **(Naik *et al,* 2008)**. Indole acetic acid and gibberellines production were determined according to **Gilickmann and Dessaux (1995) and Graham and Henderson (1960),** respectively. Qualitative assessment of siderophores was achieved on Tryptic soy agar (TSA) (Difico) according to **Alexander and Zuberer(1991).** Catecholate-type siderophores were determined according to **Carson *et al* (1992)** and citric acid production was carried out according to the method described by **Marrier and Boulets (1958)**.

Results and discussion

Isolation of PGPR
Two hundred and seven isolates of bacteria and actinobacteria were isolated from different salt-affected soils of Egyptian governorates namely, Kafr El-Shikh (Sakha), El-Qalubia (Meet Kenana), El-Behira (El-Nubaria) and Port Said (Sahl El-Teina). PGPR isolations were performed using different bacteriological media namely Ashby's modified medium, King's medium, Modified nutrient agar medium, Semi–solid malate medium, Modified Bunt and Rovira agar medium and Starch nitrate agar medium.

Screening of PGPR *The primary screening* was performed depending on IAA production, phosphate solubilization, siderophores production and growth on N_2-free medium. Data in Table 2indicated that 3% of the isolates were negative for IAA production. Moreover, 11% of total isolates produce highly amounts of indole acetic acid. Therefore, these isolates were subjected again to secondary screening under saline stress. These results are in agreement with those obtained by **Tsavkelova *et al* (2006)**who reported that the ability to synthesis IAA has been detected in many rhizobacteria, symbiotic and free living bacterial species as well as in pathogenic bacteria. Also, **Glick (1995)** and **Probanza *et al* (1996)**reported that different bacterial species and strains belonging to the genera *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Herbaspirillum*, *Burkhlderia* and *Bacillus* can be able to produce indole acetic acid and different types of plant growth regulators (PGRs).Regarding the inorganic phosphate solubilization by all investigated isolates, data in **Table 2** indicated that 67.6 % of isolates were able to solubilize rock phosphate on PVK plates. Whereas, 32.4% were not able to solubilize phosphate on the same medium. Also results in **Table 2** showed that 9.2% of isolates gave the highest producing for available phosphorus in culture medium. This result may be due to the production of organic acids and decreasing the pH of medium and enhancing phosphorus availability in microbial culture medium **(Hariprasad and Niranjana, 2009)**. Whereas, about 34.3% of isolates were not able to solubilize rock phosphate in broth medium.

Table 2. Qualitative and quantitative screening of PGPR isolates.

Qualitative assessments					
% of isolates	Growth on N_2-free medium	% of isolates	Growth on TSA medium	% of isolates	Growth on PVK agar medium
15.5	+	35.7	+	67.6	+
84.5	-	64.3	-	32.4	-

Quantitative assessments							
IAA production		Available phosphorus		Types of siderophores			
% of isolates	µg/ml	% of isolates	µg/ml	% of isolates	Cat.	% of isolates	Cit.
3	ND	34.3	ND	54.6	ND	46.8	ND
61	4-40	31.8	0-10	33.3	+	41.0	+
25	41-60	21.3	11-30	12.6	++	11.6	++
11	60-100	9.2	31-140	7.2	+++	0.5	+++

Cat. : catecholate-types Cit. : citric acid-types ND: not detected
+: low ++: moderate +++: high

Similar results were observed by **Nautiyal (1999)** who stated that organic acid production is an important mechanism in 'P' solubilization but not the sole mechanism. Also, **Gupta et al (1994) and Illmer *et al* (1995)** reported that phosphate solubilization is due to organic acid production in liquid media.

Concerning siderophores production by PGPR isolates, data in **Table 2** indicated that 35.7% of isolates were able to grow on TSA medium and produce siderophores. Whereas, the other isolates showed negative results. These results are in accordance with the findings of **Alvarez *et al* (1995)** who reported that most of PGPR were able to produce siderophores on TSA medium. Also, **Rachid and Ahmed (2005)** reported that most of PGPR strains are able to produce siderophores in media amended with 8-hydroxyquinoline which reduce the concentration of iron. In case of verifying the nature of the produced siderophores, data in **Table 2** showed that the isolates were able to produce two types of siderophores such as catecholate and citric acid.28.5% of the isolates were able to produce two types of siderophoreswhile22.2% aren't able to produce any siderophores types. These results are in harmony with **Lacava *et al* (2008)** who indicated that some bacterial strains produce hydroxymate-type siderophores, while the others produce catecholate-types. In a stable of iron limitation, the siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins.

The secondary screening was carried out to investigate the ability of isolates which gave high results in the primary screening to determine some other characters under saline stress.

Data illustrated by **Fig 1** indicated that the selected isolates were able to produce IAA under different NaCl concentrations. Generally, the amount of IAA were decreased at high sodium chloride concentrations (4 and 5%), but these amounts were high at 1% NaCl. Furthermore, the isolates number 30, 129 and 132 were the highest producing for IAA at 1% NaCl. Whereas, their ability to produce IAA was decreased under all other NaCl concentrations.

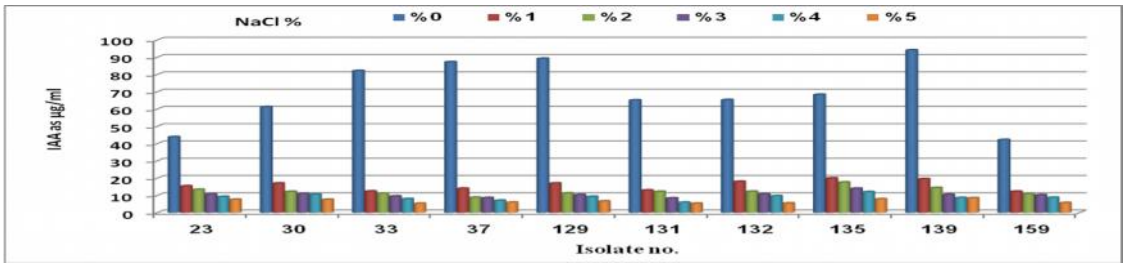

Fig 1.IAA production by selected PGPR isolates under saline stress

Data also indicated that the isolates number 135 and 139 produced the highest amounts of IAA at different sodium chloride concentrations. Therefore, the isolates number 135 and 139 were chosen and identified to be used in the further studies.

Similar results were observed by **Bochow *et al* (2001) and Woitke *et al* (2004)**who reported that PGPR have positively influenced plant vitality and the ability of the plant to cope with a biotic stressing conditions such as drought and salinity. In this respect, **Idriss *et al* (2002)** stated that *B. subtilis* has many mechanisms such as IAA production in its interacts with plants, especially as an a biotic stress mediator.

The ten selected isolates from the primary screening were examined also for their ability to solubilize inorganic phosphate and enhance phosphorus availability in culture media under saline stress. In this experiment, PVK broth medium amended with NaCl was used and inoculated with each isolate and incubated at 30ºC for 4-5 days, then available phosphorus was determined spectrophotometerically at 600 nm.

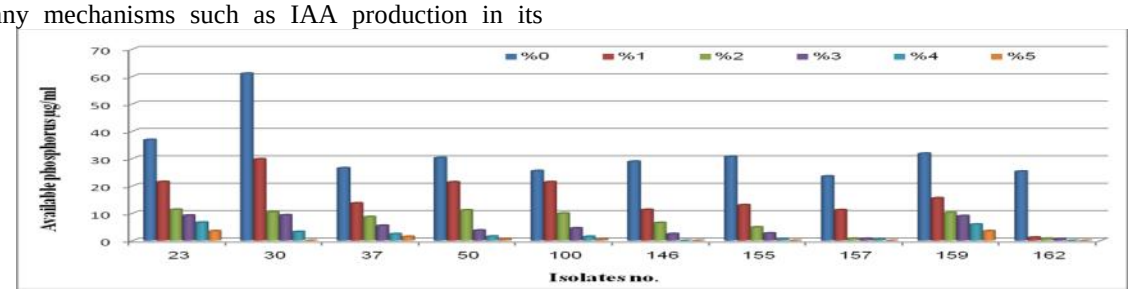

Fig 2. Phosphorus availability by selected PGPR isolates under saline stress

Data illustrated by **Fig 2** revealed that the isolate number 131producedhigh amounts of available phosphorus at 1% sodium chloride, whereas, at high sodium chloride concentrations the solubility of phosphorus was not observed. On the other hand, the isolates number 23 and 159 solubilize inorganic phosphate at all tested sodium chloride concentrations. Therefore, those two isolates were chosen for identification.

These results are in harmony with **Naik *et al* (2008)** who stated that PGPR strains which have been reported as phosphate solubilizers are capable for improving plant nutrients uptake, tolerance to stress such as salinity, metal toxicity and pesticide. The presence of PGPR in the rhizosphere enabled plants to achieve high levels of biomass in soil despite extreme stress conditions **(Gehardt *et al*, 2006).**

Nitrogenase activity of each isolate grown on N_2-free media was examined under different sodium chloride concentrations. To achieve this test, each of the selected ten isolate was cultured on specific N_2-free medium amended with sodium chloride at different concentrations, then incubated at 30ºC for 4-5 days, nitrogenase activity was measured by Gas chromatography (GC).

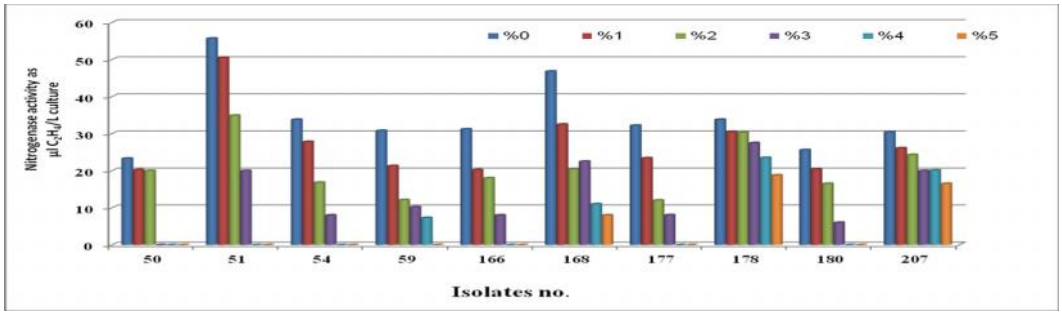

Fig 3. Nitrogenase activity by selected PGPR isolates under saline stress

Obtained results graphically illustrated by **Fig 3** showed that the isolates number 59, 168, 178 and 207 were the most potent isolates for nitrogenase activity. When sodium chloride was applied at concentrations (4 and 5%), N_2-ase activity of the isolates number 59 and 168 was decreased sharply with increasing salinity. On contrast, the nitrogenase activity of isolates number 178 and 207 was affected gradually under all tested sodium chloride concentrations.

Also, data illustrated by **Fig 3** showed that seven isolates lost their ability to fix atmospheric nitrogen at 5% sodium chloride concentration. Therefore, the isolates number 178 and 207 were chosen and identified as nitrogen fixers under saline stress.

These results are in agreement with those mentioned by **Saleen *et al* (2002)** who stated that the free living nitrogen fixing bacteria (*Azotobacter and Azospirillum*) were able to fix nitrogen without any decrease up to 100m*M*NaCl but in the presence of 250m*M* the activity reduced by 35-44%.

Identification of the most potent isolates.

Isolates which were capable to produce IAA (D135 and D139), available phosphorus (D23 and D159) and high nitrogenase activity (D178 and D207) under saline stress were purified and subjected to detail morphological and physiological studies according to **Baldani *et al.* (2005)** for *Azospirillum* sp.; **Palleroni (2005)** for *Pseudomonas* sp. and **Reva *et al.* (2001)** for *Bacillus* spp. and *Paenibacillu*s sp.

From the morphological characteristics, staining properties, spore formation and physiological properties, it was clear that the isolates could be identified as *Paenibacillus alive D135*, *Bacillus pumilus D139*, *Bacillus megaterium D159*, *Pseudomonas fluorescence D23*, *Azospirillum lipoferumD178* and *Azospirillum lipoferum D207*.

Tolerance of identified PGPR strains for saline stress

Nitrogenase activity

From data recorded in **Table 3** it was obvious that two out of six identified PGPR strains (*A. lipoferum D178* and *A. lipoferum D207*) were able to fix atmospheric nitrogen under saline stress. Obtained data clearly indicated that nitrogenase activity was higher under sodium chloride concentrations than those of different sea water dilutions. The highest N_2-ase activity was observed by *A. lipoferum D207* at 1% sodium chloride. On the other hand, the lowest nitrogenase activity was observed when *A. lipoferumD178*was tested at (1:2) ratio of sea water. These results are in harmony with **Tripathi *et al* (2002)** who reported that growth of *A. brasilense* in media is not sensitive to high NaCl concentrations (200-400m*M*). Also, **Bashan *et al* (2004)** and **Barassi *et al* (2007)**reported that *Azospirillum* spp. were not only able to fix atmospheric N, but also help plants to minimize the negative effects of abiotic stresses such as salinity.

Table 3. Nitrogenase activity for PGPR under saline stress

Strains		*B. megateriu m D159*	*Ps. fluorescen ce* *P. alvie D139*	*B. pumilus D135*	*A. lipoferum D178*	*A. lipoferum D207*
Nitrogenase activity as µg C_2H_4. $hr.l^{-1}$ culture.						
Control*		ND	ND	ND	ND	33.0	30.4
NaCl (%)	1	ND	ND	ND	ND	30.5	26.1
	2	ND	ND	ND	ND	30.5	24.3
	3	ND	ND	ND	ND	27.5	20.0
	4	ND	ND	ND	ND	23.0	20.1
	5	ND	ND	ND	ND	18.8	16.5
Sea water : Distilled water	1:2	ND	ND	ND	ND	10.0	12.2
	1:4	ND	ND	ND	ND	17.2	15.0
	1:6	ND	ND	ND	ND	21.1	19.6
	1:8	ND	ND	ND	ND	21.3	22.4
	1:10	ND	ND	ND	ND	26.0	24.0

*N_2-ase activity by all strains without NaCl or sea water.

Phosphorus availability

Results in **Table 4** indicated that the six strains could solubilized phosphorus in culture medium at all sodium chloride concentrations and sea water dilutions. Data also showed that the produced amounts of available phosphorus were high when sodium chloride was added to the culture medium at concentration of 1% and when sea water was diluted at ratio of (1:10). The same trend of results was observed with all the investigated strains.

Phosphorus availability by PGPR strains may be due to their ability to exert organic acids and reduce pH of culture media. Similar data were observed by **Hariprasad and Niranjana (2009)** who reported that the ability of phosphate solubilizing microorganisms to solubilize phosphate complexes could be attributed to their ability to reduce pH of the surroundings, either by releasing organic acids or protons. Respecting the effect of saline stress on phosphorus availability, data in **Table 4** revealed that *B. megaterium D159* and *Ps. fluorescence D23* were the higher in solubilizing phosphorus. While, *A. lipoferum D207* lost its ability to solubilize phosphate when sodium chloride was added to culture medium at concentration of 5%.

Table 4.Phosphorus availability by the PGPR strains under saline stress

Strains		*B. megaterium D159*	*Ps. fluorescence D23*	*P. alvie D139*	*B. pumilus D135*	*A. lipoferum D178*	*A. lipoferum D207*
Available phosphorus as µg/ml							
Control*		11.80	16.83	0.48	0.24	ND	3.09
NaCl (%)	1	1.53	1.48	0.75	0.73	0.27	0.37
	2	1.18	1.34	0.52	0.70	0.15	0.32
	3	1.00	1.19	0.31	0.46	0.15	0.14
	4	0. 92	0.64	0.29	0.22	0.08	0.09
	5	0.48	0.47	0.14	0.17	0.08	0.00
Sea water : Distilled water	1:2	1.8	2.0	0.74	0.52	0.61	0.55
	1:4	1.9	2.1	0.74	0.82	0.80	0.76
	1:6	2.4	2.2	0.84	0.96	0.81	0.90
	1:8	2.3	2.3	0.86	1.13	1.11	1.00
	1:10	2.4	2.2	0.92	1.42	1.24	1.15

*Available phosphorus production by all strains on PVK medium without NaCl or sea water.

In general, the available phosphorus amounts were higher under all applied sea water dilutions than sodium chloride concentrations. In this respect, **Hamid (1999) and Bacilio *et al* (2004)** reported that the use of PGPR and mycorrhizal fungi which have been reported as phosphate solubilizers to promote plant growth in saline soils is a developing technology.

Indole acetic acid production

Results in **Table 5** clearly indicated that all strains produced IAA under all NaCl and sea water concentrations. Generally, IAA amounts decreased at 5% sodium chloride and (1:2) sea water dilution. Data also emphasized that the highest produced amounts of IAA were observed by *P. alive D135* and *B. pumilus D139* when sea water was diluted at ratio of (1:10). On the other hand, the lowest amounts of IAA were produced by *B. megaterium D159* and *A. lipoferumD178* at 5% sodium chloride.

Table 5. Indole acetic acid production by PGPR strains under saline stress

Strains		*B. megaterium D159*	*Ps. fluorescence D23*	*P.alvie D135*	*B.pumilus D139*	*A.lipoferum D178*	*A.lipoferum D207*
IAA amounts as µg/ml							
Control*		22.2	43.8	68.4	94.1	17.5	33.6
NaCl (%)	1	2.58	13.49	19.98	19.49	3.76	2.85
	2	2.12	9.24	17.59	14.39	3.44	2.06
	3	1.96	7.15	13.99	10.44	2.87	1.55
	4	1.60	5.65	11.99	8.64	1.90	1.21
	5	1.00	3.42	5.09	6.39	1.00	1.18
Sea water : Distilled water	1:2	3.48	1.75	14.9	13.7	4.35	3.23
	1:4	5.39	4.11	18.4	19.5	4.91	5.05
	1:6	7.19	2.72	27.7	21.8	5.51	3.83
	1:8	4.91	8.53	35.1	34.3	7.41	7.35
	1:10	9.99	10.6.	35.0	38.8	8.54	9.94

*Indole acetic acid production by all strains without NaCl or sea water.

Produced amounts of IAA in the medium supplemented with NaCl or sea water is indicator to their degree of salt tolerance. A decrease in IAA amounts has been reported with increasing concentrations of salt **(Bano, 2009)**. Also, **Naz *et al* (2009)** isolated PGPR producing plant growth regulators from weeds growing in dry salty environment and the strains exhibited their tolerance when tested on saline media.

Gibberellins production

Data in **Table 6** showed that all investigated strains possess the ability to produce gibberellines under saline stress. Generally, gibberellines amounts reduced at high NaCl concentrations and sea water dilutions.

Table 6. Gibberellins production by PGPR strains under saline stress

Strains		*B. megaterium D159*	*Ps. fluorescence D23*	*P. alvie D135*	*B. pumilus D139*	*A. lipoferum D178*	*A. lipoferum D207*
		Gibberellines amounts as µg/ml					
Control*		865.2	200.1	809.4	288.7	147.6	120.0
NaCl (%)	1	810.2	175.3	712.7	250.2	135.8	102.4
	2	600.4	148.3	599.2	189.9	99.7	93.2
	3	386.2	135.8	522.9	178.9	83.5	88.1
	4	200.4	122.0	500.0	145.2	68.9	82.9
	5	165.0	110.7	487.1	99.9	35.8	53.7
Sea water : Distilled water	1:2	154.8	66.8	382.5	112.7	178.9	30.8
	1:4	173.0	80.9	506.4	299.0	180.0	78.5
	1:6	195.2	134.2	671.7	385.5	189.6	90.7
	1:8	200.7	197.4	711.2	400.6	199.3	111.3
	1:10	255.5	233.7	820.4	418.4	238.9	132.7

*Gibberellines production by all strains without NaCl or sea water.

P. alvieD135 was the highest producer of gibberellines under all sodium chloride and sea water concentrations followed by *B. megaterium D159*. Whereas, *A. lipoferum D207* was the lowest producer of gibberellines. Also, data in **Table 6** mentioned that the lowest amounts of gibberellines were produced when sea water was used at ratio of(1:2) with all investigated strains. Data also provided that when *B. pumilus D139* examined for gibberellines production at 5% sodium chloride the produced amounts showed sharp drop. This trend of results was observed with *A. lipoferum D178* and *A. lipoferum D207*.

These results are in accordance with **Egamberdieva (2009)** who recommended that it is possible to use GAs producing PGPR strains to alleviate salt stress of wheat grown in soil under salinity conditions.

Siderophores production

Data recorded in **Table 7** indicated that all investigated PGPR strains were able to produce siderophores under sea water and sodium chloride concentrations. Data in **Table 7** clearly indicated that one out of the six investigated strains*(B. megaterium D159*) was not able to produce siderophores on TSA plates with increasing salinity. Whereas, data showed that the same strain was the highest producer for catecholate-types at all applied sodium chloride concentrations followed by *P. alive D135* then *A.lipoferumD178*.

Except of *A. lipoferum D178*, it was clearly noticed that all investigated strains produced catecholate-types under sea water dilutions. Although *B. pumilus D139* was able to produce catecholate-types under all sea water dilutions, in contrast under high sodium chloride concentrations (3, 4 and 5%), this strain lost its activity. The same trend of results was observed with *Ps. fluorescens D23.*

Data in **Table 7** showed that *Ps. fluorescens D23* was the only investigated PGPR strain which was able to produce citric acid-types under all tested sodium chloride concentrations. Also, data revealed that *B. pumilus D139* produced two types of siderophores at all applied sea water dilutions.

These results are in harmony with **Rachid and Ahmed (2005)** who reported that strains of *Pseudomonas fluorescens* were able to produce siderpphores in media amended with 8-hydroxyquinoline which reduce the concentration of iron. Also, **Lacava *et al* (2008**) mentioned that some bacterial strains produce hydroxymate-type siderophores, while the others produce catecholelate-types. In a stable of iron limitation, the siderophore-producing microorganisms are also able to bind and transport the iron-siderophore complex by the expression of specific proteins.

Table 7. Siderophores production by PGPR under saline stress

Strains		*B. megaterium D159*		*Ps. fluorescence D23*		*P. alvie D135*		*B. pumilus D139*		*A. lipoferum D178*		*A. lipoferum D207*	
		Types of siderophores											
		Cat.	Cit.	Cat.	Cit.	Cat.	Cit.	Cat.	Cit.	Cat.	Cit.	Cat.	Cit.
Control*		+++	++	++	++	+++	++	+	ND	+	ND	+	+
NaCl (%)	1	+++	+	++	++	+++	++	+	+	+	+	+	+
	2	+++	+	+	+++	+	ND	+	+	++	++	+	+
	3	+++	ND	+	+	+	ND	ND	+	+	+	+	+
	4	+	ND	ND	+	+	+	ND	ND	+	+	+	ND
	5	+	+	ND	+	ND	+	ND	ND	+	ND	ND	ND
Sea water : Distilled water	1:2	+	ND	+	+	ND	ND	+	+	ND	+	+	ND
	1:4	++	+	+	+	+	+	+	+	++	+	+	+
	1:6	++	+	++	ND	+	++	+	+	++	+	+++	++
	1:8	+++	+	++	ND	+	+	+	+	++	+	++	+
	1:10	+++	++	++	+	+	+	+	+	+	+	+	++

Cat. : catecholate-types Cit. : citric acid-types ND: not detected
+: low ++: moderate +++: high

Conclusion

This study indicated that about six strains of the obtained PGPR isolates possessed the ability to grow under saline stress and produced high indole acetic acid and gibberellines, solubilize inorganic phosphate, produce two types of siderophores and fix atmospheric nitrogen. These strains have the potential to be used as plant biofertilizers and biostimulant for plant growth performance improvement under saline stress.

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الملخص العربى

تقييم نشاط الريزوبكتريا المشجعة لنمو النبات تحت الإجهاد الملحى

أحمد غنيم رحال[٢]– إحسان احمد حنفى[١]- راشد عبدالفتاح زغلول[١] – حامد السيد أبوعلى[١] – رشا محمد الميهى[١]

١- كلية الزراعة - جامعة بنها

٢- معهد بحوث الأراضى والمياه والبيئة-مركز البحوث الزراعية

تم إجراء هذا البحث بهدف عزل وتعريف بعض الريزوبكتريا المشجعة لنمو النباتات من الأراضى المصرية المتأثرة بالأملاح، حيث تم تقييم بعض الأنشطة الحيوية لهذه الميكروبات والتى تؤثر على خصوبة التربة وذلك تحت ظروف الإجهاد الملحى. وفى هذه الدراسة تم عزل ٢٠٧ عزلة من الكائنات الحية الدقيقة من مناطق سخا (محافظة كفر الشيخ) والنوبارية (محافظة البحيرة) وسهل الطينة (محافظة بورسعيد) وميت كنانة (محافظة القليوبية). وقد أوضحت النتائج انه يوجد عزلتين أبدت نشاط عالى فى انزيم النيتروجينيز وتم تعريفهم على انهم *Azospirillum lipoferum D178* and *Azospirillum lipoferum D207* . أيضا أوضحت النتائج وجود عزلتين تم تعريفهم على أنهم *Paenibacillus alive D135* and *Bacillus pumilus D139* لهم نشاط عالى فى إنتاج إندول حامض الخليك. بينما وجد أن هناك عزلتين لهم نشاط عالى فى إذابة الصخر الفوسفاتى تم تعريفهم على أنهم *Pseudomonas fluorescence D23* and *Bacillus megaterium D159*.

تم دراسة كفاءة السلالات السابق تعريفها من حيث بعض الأنشطة الحيوية والتى لها علاقة بخصوبة التربة تحت ظروف الإجهاد الملحى، وأوضحت النتائج بصورة عامة أن كل السلالات التى اختبرت لها القدرة على إنتاج اندول حامض الخليك و الجبريللينات والسيدروفورس بكلا نوعيها (الكاتيكولات وحامض الستريك) بالإضافة لقدرتها على إذابة صخر الفوسفات وذلك فى وجود تركيزات مختلفة من كلوريد الصوديوم (١- ٥% **وماء البحر** (١:٢- ١:١٠).

وفى ضوء النتائج المتحصل عليها من هذه الدراسة يمكن أن يوصى بإستخدام مثل هذه الكائنات الحية الدقيقة كمخصبات حيوية ومنشطات لنمو النبات بالإضافة لقدرتها على تقليل الحدة المرضية للأمراض التى تسببها الميكروبات المحمولة عن طريق التربة من خلال قدرتها على إنتاج مركبات السيدروفورس وذلك تحت ظروف الأراضى المتأثرة بالأملاح.