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Evaluation of Plant Growth Promoting of Salt-tolerant Rhizobacteria Isolated from Egyptian Saline Soils

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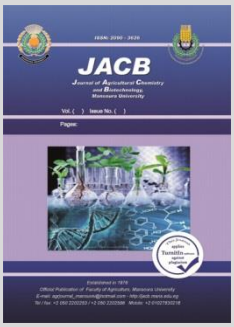

ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are alternative strategy for mitigating the negative impacts of salinity stress on plants. The aim of this study was to isolate native PGPR with high plant growth promoting traits from Egyptian soils which could be used as an environmentally eco-friendly technique for alleviating salinity stress. Twenty-five *Azotobacter* spp. were isolated from Most Probable tubes, then all isolates were tested to grow in different concentrations of NaCl for salt tolerance. The highest tolerant *Azotobacter* spp. were seven isolates which then were tested for their ability to fix nitrogen *in vitro* using acetylene reduction technique. The most active *Azotobacter* isolate was (No. AZ 1) which recorded 27.70 nmoles C_2H_4/ml/h. On the other side, twenty-five phosphate solubilizing bacteria were isolated from Pikovskaya's agar plates. All isolates were tested for salt tolerance and four isolates were selected **f**or further understanding the mechanism of phosphate solubilization and its relationship to pH in liquid Pikovskaya's medium supplemented with tri-calcium phosphate. The highest quantity of released phosphorus in the culture (16.31 mg p /100 ml) was for the isolate PSB 14 which selected for further studies. As well as isolates AZ 1 and PSB 14 showed high effectiveness in the production of indole acetic acid (67.87 and 8.99 µg/ml), respectively. Based on the 16S rRNA sequence analysis, AZ 1 and PSB 14 were identified as *Azotobacter salinstris* ON809700 and *Bacillus amyloliquefaciens* ON818999. Based on this study, these salt-tolerant isolates could add benefits to plant grown in saline conditions.

***Keywords*:** Salinity, PGPR, nitrogen, phosphate, IAA.

INTRODUCTION

Increasing of agricultural soil salinity is one of the most significant recent consequences of global climate change (Ilangumaran and Smith, 2017). Saline soil contains more soluble salts, such as Na ions, which can combine to form compounds such as NaCl, Na_2CO_3, and Na_2SO_4 in the soil (Choudhary and Kharche, 2018), which negatively impacts physical, chemical, and biological properties of the soil (Hamid *et al*., 2021). Furthermore, soil salinization inhibits germination (Gong *et al*., 2018), decrease plant nutrient uptake, particularly of the essential macronutrients like nitrogen (Gondek *et al*., 2020) and causing a physiological dehydration to plants (Ilangumaran and Smith, 2017). Hence, salinity reduces plant growth and productivity, making it difficult to satisfy food demands as the world population increase. Several beneficial associations between plants and microorganisms have been demonstrated by numerous studies such as classic legume–rhizobia symbiosis (Sheteiwy *et al*., 2021), and plant-arbuscular mycorrhiza symbiosis (El-Sawah *et al*., 2021). Plant roots excrete nutrient sources in the rhizosphere which support higher microbial population than the bulk soil (Gao *et al*., 2020). Free-living beneficial bacteria dwelling in the rhizosphere that exert beneficial activities are known as plant growth promoting rhizobacteria (PGPR). The application of plant growth promoting rhizobacteria as natural eco-friendly tools was found to be effective in mitigating the detrimental effects of salt stress conditions on plants (Kapadia *et al*., 2022). Several mechanisms for plant growth promoting rhizobacteria to mitigate salinity have been reported, including improved plant water relations, ion homeostasis, and photosynthetic efficiency in plants (Khumairah *et al*., 2022). The increased input of Na^+ and Cl^- ions can induce nutrient imbalances, however beneficial microbes can reduce these imbalances by increasing the mineral nutrient exchange of both macro and micronutrients by improving mineralization, rhizosphere pH alterations through organic acids production, and metal chelation through siderophore production (Ilangumaran and Smith, 2017). As well as PGPR were reported to enhance plant survival and its growth in the saline environment by means of producing biofilms, indole-3-acetic acids (IAA), exopolysaccharide (EPS), polyamines, aminocyclopropane-1-carboxylate (ACC) deaminase, and through phosphate solubilization (Ilangumaran and Smith, 2017). The aims of this study were isolation of rhizosphere bacteria possessing plant growth-promoting traits from plants grown in saline soils. The isolates were screened in vitro for salt tolerance and for their plant growth-promoting traits. The superior isolates were selected to identification using 16S rRNA sequence for further studies.

MATERIALS AND METHODS

Soil samples collection

Five soil samples were collected from different fields of clover, rice, lettuce, cabbage, and sugar beet from

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Gamassa region, Dakhalia Goverenment, Egypt. Electrical conductivity (EC) and pH of these soil samples were determined by the method described by Jackson (1973). Then, soil samples were separated from plant root residues and dirt. Approximately 300 g of soil sample was put in the sample bag and transported to the laboratory on the same day for isolation and characterization work. The samples were kept at 4°C until bacterial isolation.

Isolation of *Azotobacter*

Tubes containing 5 ml of Ashby's medium (Abd-El-Malek and Ishac, 1968) were inoculated with 1 ml aliquots of prepared serial dilutions of rhizospheric soil and incubated at 30 °C for 15-21 days. Positive tubes were recognized by the formation of surface fine brown pellicles. For purification a loopful of the culture was transferred to 20 ml sterile tap water in a bottle containing glass beads, mechanically shaken for 30 min in order to fragment the mucous around the cells which usually carries contaminants, then used for streaking on plates. Developing colonies were picked and re-purified at least five times, till proved no contamination. Cultures were considered pure when microscopical examination revealed only typical *Azotobacter* and no contaminating microorganisms. Single colonies were kept in Ashby's agar slants and maintained at 4°C.

Isolation of phosphate-solubilizing bacteria

The phosphate-solubilizing bacteria were isolated by pouring plate technique on Pikovskaya's medium (Pikovskaya, 1948). In brief, ten g of each soil sample were added to flasks containing 90 ml distilled water. Tubes were shacked for 20 min at 120 rpm. One ml of 10^{-3} to 10^{-5} dilutions of each soil samples solution was added to Pikovskaya's agar plates. Plates were incubated at 30 °C for 7 days. Bacterial phosphate solubilizers were detected by clear zones around the colonies. Streaking plates and single colony isolation were used to purify representative isolates. Pure colonies were kept on Pikovskaya's agar slants and maintained at 4°C.

Effect of salt on growth of isolated bacteria

NaCl with different concentrations (0%, 2%, 4%, 6%, 8% and 10%) were added to Ashby's medium (Abd-El-Malek and Ishac, 1968) and nutrient broth medium (Skerman, 1967). Five ml of each medium was added into each test tube. These media were sterilized in autoclave at 121°C for 15 min. Three replicates test tubes were inoculated by the tested isolates. All cultures were incubated for 3 days at 30°C and the cells were well mixed by vortex. The optical density (OD) was measured by spectrophotometer 600 nm.

Estimation of nitrogenase activity

The Capacity of *Azotobacter* isolates to fix nitrogen was assayed by acetylene reduction technique on Ashby's medium (Abd-El-Malek and Ishac, 1968) according to Hardy *et al*. (1973).

Estimation of phosphate solubilization

Solubilization of phosphate was estimated on Pikovskaya's agar plates supplemented with tri-calcium phosphate (TCP) using spot inoculation as described by Hauka *et al*. (2017) using the following equation:

$$\text{Solubilization Efficiency (SE\%)} = \frac{\text{Solubilization Diameter (SD)}}{\text{Growth Diameter (GD)}} \times 100 \quad (1)$$

Then the selected isolates were grown in 100 ml sterile Pikovskaya's liquid medium supplemented with tri-calcium phosphate at the rate of 50 mg P/100 ml as a source of insoluble phosphorus. Incubation was performed at 30 ^{0}C at 150 rpm. The determination of water-soluble phosphorus and pH values were carried out initially and after 3, 7, 14, 21 and 28 days of incubation by the method described by Hauka *et al*. (2017)

IAA- detection

Bacterial isolates were grown in Ashby's medium (Abd-El-Malek and Ishac, 1968) and nutrient broth medium (Skerman, 1967) supplemented with 0.1% tryptophan according to Ahmad *et al*. (2005). IAA was determined 530 nm as described by Pilet and Chollet (1970).

Identification of the potent bacterial isolates

Bacterial isolates were identified by Sigma Scientific Services Co., using 16s rRNA sequence. The sequences obtained were then compared with the existing sequences at the NCBI database. The MEGA 11.0 software was used for multiple sequence alignment of 16S rRNA sequences. The accession numbers were obtained from the NCBI GenBank database for 16S rRNA sequences of bacteria.

Statistical analysis

The data presented are the means of four replicates ± standard deviations (SD). The data were analyzed using SPSS v25.0 (SPSS, Inc., Chicago, IL, USA), and means were separated using Duncan's multiple-range tests at $P<0.05$.

RESULTS AND DISCUSSION

Results:

Chemical properties of collected soil samples

Data regarding chemical properties of collected soil samples (EC and pH) is presented in Table 1. Electrical conductivity (dSm^{-1}) which was determined in examined soil samples S1, S2, S3, S4, and S5 being 2.3, 5.1, 4.9, 2.4, and 3.9 respectively. However, pH values were ranged between 7.83 and 8.45.

Table 1. Chemical properties of collected soil samples

Soil sample No.	Cultivated plant	EC values (dSm^{-1})	pH values
S1	Clover	2.3	8.08
S2	Rice	5.1	8.45
S3	Lettuce	4.9	8.08
S4	Cabbage	2.4	8.04
S5	Sugar beet	3.9	7.83

Isolation of salt-tolerant nitrogen fixing bacteria

Twenty-five of *Azotobacter* spp. were isolated from Most Probable tubes and were tested to grow in different concentrations of NaCl (0%, 2%, 4%, 6%, 8%, and 10%) are presented in Table 2. To varying NaCl concentrations, all isolates demonstrated the ability to grow at all salinity concentrations tested. Among isolates, seven isolates (AZ 1, AZ 2, AZ 3, AZ 8, AZ 9, AZ 16, and AZ 24) were selected to their high ability to grew under 10% NaCl. As well as these seven isolates were tested for their ability to fix nitrogen invitro using acetylene reduction technique (Table 3). The efficiency of N_2-fixation differed between the isolates. The isolates reduced acetylene at a rate between 0.11and 27.70 nmoles C_2H_4/ml/h. The most active *Azotobacter* isolate (No. AZ 1) that was selected for further experiments.

Table 2. Effect of NaCl concentrations on growth of Azotobacter spp.

Isolate No.	Growth of *Azotobacter* spp. expressed as OD (600 nm) at various salinity concentrations					
	0% NaCl	2% NaCl	4% NaCl	6% NaCl	8% NaCl	10% NaCl
AZ 1	2.153±0.039a	1.890±0.018a	1.571±0.050a	1.199±0.010a	0.858±0.040a	0.404±0.008a
AZ 2	1.892±0.043c	1.627±0.010c	1.290±0.019bc	1.045±0.035b	0.750±0.034c	0.348±0.043ab
AZ 3	1.584±0.075h	1.283±0.040j	1.100±0.002c-e	0.954±0.036c	0.641±0.023d	0.238±0.019b-d
AZ 4	1.708±0.006fg	1.430±0.010f	1.036±0.040ef	0.808±0.028d	0.447±0.016g	0.158±0.035c-e
AZ 5	1.574±0.015h	1.204±0.007k	0.895±0.024f-h	0.599±0.018e-g	0.293±0.021kl	0.088±0.013e-g
AZ 6	1.685±0.022fg	1.269±0.058j	0.896±0.080f-h	0.499±0.078hi	0.252±0.047l	0.092±0.023e-g
AZ 7	1.810±0.013d	1.502±0.010e	1.141±0.030c-e	0.790±0.073d	0.503±0.009f	0.200±0.009c-e
AZ 8	2.065±0.047b	1.782±0.019b	1.409±0.016ab	1.023±0.032bc	0.802±0.006b	0.389±0.018a
AZ 9	1.910±0.023c	1.593±0.020cd	1.281±0.041b-d	0.995±0.025bc	0.732±0.043c	0.350±0.032ab
AZ 10	1.471±0.050ij	1.171±0.050k	0.838±0.121gh	0.635±0.024e	0.355±0.001ij	0.142±0.023d-f
AZ 11	1.676±0.057fg	1.347±0.036g-i	1.105±0.008c-e	0.787±0.037d	0.386±0.021hi	0.159±0.053c-e
AZ 12	1.564±0.014h	1.204±0.016k	0.876±0.053f-h	0.623±0.011ef	0.322±0.010jk	0.092±0.020e-g
AZ 13	1.391±0.021k	1.187±0.016k	0.900±0.077f-h	0.673±0.061e	0.395±0.017hi	0.146±0.040d-f
AZ 14	1.426±0.025jk	1.207±0.003k	1.006±0.014e-g	0.611±0.167e-g	0.409±0.008gh	0.201±0.010c-e
AZ 15	1.660±0.034g	1.296±0.006ij	1.093±0.027de	0.796±0.017d	0.511±0.043f	0.187±0.019c-e
AZ 16	1.228±0.014mn	1.075±0.021l	0.799±0.029h	0.525±0.041g-i	0.272±0.040kl	0.239±0.303b-d
AZ 17	1.177±0.008n	0.949±0.034m	0.854±0.033f-h	0.545±0.031f-h	0.281±0.018kl	0.014±0.007g
AZ 18	1.305±0.013i	1.152±0.046k	0.742±0.026h	0.454±0.022i	0.202±0.004m	0.021±0.010fg
AZ 19	1.238±0.022m	1.038±0.053l	0.866±0.047f-h	0.627±0.023ef	0.311±0.009jk	0.089±0.015e-g
AZ 20	1.501±0.012i	1.199±0.010k	0.802±0.012h	0.542±0.029f-h	0.321±0.026jk	0.156±0.011c-e
AZ 21	1.774±0.057de	1.396±0.022fg	1.146±0.029c-e	0.799±0.023d	0.380±0.040hi	0.161±0.042c-e
AZ 22	1.664±0.027g	1.316±0.016 h-j	1.131±0.039c-e	0.845±0.032d	0.428±0.003gh	0.133±0.036d-g
AZ 23	1.696±0.004fg	1.368±0.044gh	1.468±0.459a	0.951±0.029c	0.588±0.029e	0.203±0.006c-e
AZ 24	1.794±0.015d	1.563±0.048d	1.268±0.041b-d	0.940±0.028c	0.534±0.058f	0.284±0.023a-c
AZ 25	1.731±0.038ef	1.339±0.043f	1.047±0.026ef	0.796±0.020d	0.513±0.014f	0.176±0.039c-e

Data included are means ± SD; different letters within the same column indicate significant differences between means after Duncan's multiple-range test at $P \leq 0.05$

Table 3. Nitrogenase activity of *Azotobacter* spp. isolates.

Isolate No.	Nitrogenase activity (nmole C_2H_4/ml/h)
AZ 1	27.70
AZ 2	0.92
AZ 3	1.36
AZ 8	0.62
AZ 9	4.19
AZ 16	9.88
AZ 24	0.11

Isolation of salt-tolerant phosphate soulibilizing bacteria

Twenty-five bacterial isolates were isolated from Pikovskaya's agar plates, these bacteria was showing clear zones around the colonoies indicating the soulibilize of tri-calcium phosphate (Table 4). These bacteria were tested to grow in different concentrations of NaCl (0%, 2%, 4%, 6%, 8%, and 10%) are presented in Table 5. Among bacterial isolates, four isolates (PSB 1, PSB 6, PSB 7, and PSB 14) were selected to their high ability to grew under 10% NaCl for futhur studies. For further understanding of the mechanism of phosphate soulibilization and its relationship to pH, the four isolates were inoculated into Pikovskaya's liquid medium supplemented with tri-calcium phosphate.

Table 4. Phosphate solubilization efficiency using Pikovskaya's agar medium.

Isolate No.	Growth Diameter (GD) mm	Solubilization Diameter (SD)mm	Solubilization Efficiency % (SE%)
PSB 1	5.00±0.81a	10.25±0.95a	207.08±19.16hi
PSB 2	3.12±0.25bc	8.37±0.47b	268.45±10.88d-i
PSB 3	2.75±0.28cd	7.25±0.28c	265.83±29.86e-i
PSB 4	2.75±0.28cd	8.75±0.28b	321.66±44.26a-e
PSB 5	3.25±0.28b	8.87±0.25b	274.40±21.06d-h
PSB 6	2.00±0.00e-g	7.25±0.28c	362.50±14.43ab
PSB 7	2.00±0.00e-g	7.00±0.00c	350.00±00.00a-c
PSB 8	2.12±0.25e-g	6.25±0.28de	296.25±26.88b-f
PSB 9	2.00±0.00e-g	6.75±0.28cd	337.50±14.43a-d
PSB 10	2.25±0.28ef	6.00±0.00ef	270.00±34.64d-i
PSB 11	2.12±0.25e-g	5.25±0.28f-h	250.00±35.35e-i
PSB 12	2.25±0.28ef	5.50±0.57f-h	247.50±41.12f-i
PSB 13	2.25±0.28ef	5.62±0.25e-g	252.50±27.23e-i
PSB 14	1.37±0.25i	5.00±0.00gh	375.00±83.33a
PSB 15	2.37±0.25de	5.25±0.28f-h	223.75±35.44g-i
PSB 16	2.25±0.28ef	5.50±0.57f-h	245.00±5.77f-i
PSB 17	1.50±0.00ih	5.50±0.00f-h	366.66±0.00a
PSB 18	2.00±0.00e-g	4.00±1.08i	200.00±54.00i
PSB 19	1.75±0.28g-i	5.37±0.25f-h	314.58±61.00a-f
PSB 20	1.75±0.28g-i	3.75±0.86i	222.91±77.39g-i
PSB 21	1.87±0.25f-h	5.12±0.25gh	277.08±39.30d-h
PSB 22	1.87±0.25f-h	4.75±0.64h	258.33±58.92e-i
PSB 23	1.75±0.28g-i	5.37±0.47f-h	314.58±66.79a-f
PSB 24	1.87±0.25f-h	5.12±0.25gh	279.16±58.33d-g
PSB 25	1.87±0.25f-h	5.25±0.28f-h	283.33±35.35c-g

Data included are means ± SD; different letters within the same column indicate significant differences between means after Duncan's multiple-range test at $P \leq 0.05$

Table 5. Effect of NaCl concentrations on the growth of phosphate solubilizing bacteria

Isolate No.	Growth of Phosphate solubilizing bacteria expressed as OD (600 nm) at various salinity concentrations					
	0% NaCl	2% NaCl	4% NaCl	6% NaCl	8% NaCl	10% NaCl
PSB 1	2.893±0.002a	2.745±0.038a	2.402±0.012ab	2.087±0.010b	1.728±0.020a	1.199±0.001a-c
PSB 2	2.813±0.012b	2.546±0.042b-d	2.247±0.034b-e	1.847±0.024d	1.559±0.003c	1.101±0.096c
PSB 3	2.362±0.023m	2.192±0.007hi	1.924±0.009g-k	1.643±0.029i	1.308±0.019hi	0.929±0.015d-f
PSB 4	2.567±0.032i	2.423±0.009ef	2.187±0.013b-f	1.737±0.022fg	1.429±0.011fg	0.898±0.104d-g
PSB 5	2.559±0.007ij	2.322±0.005fg	2.001±0.007f-j	1.748±0.027f	1.349±0.020h	0.963±0.032de
PSB 6	2.752±0.009e	2.615±0.033bc	2.302±0.045b-d	1.946±0.018c	1.653±0.036b	1.130±0.029c
PSB 7	2.768±0.009de	2.513±0.014c-e	2.204±0.023b-f	1.805±0.012e	1.452±0.027e-g	1.160±0.024bc
PSB 8	2.540±0.014j	2.322±0.015fg	2.124±0.008c-h	1.789±0.001e	1.405±0.011g	0.987±0.045d
PSB 9	2.651±0.017gh	2.442±0.026de	2.147±0.004c-g	1.863±0.030d	1.302±0.013i	0.798±0.109gh
PSB 10	1.544±0.022q	1.436±0.013l	1.261±0.005m	1.042±0.029n	0.889±0.019m	0.802±0.012gh
PSB 11	2.547±0.008ij	2.505±0.015c-e	2.325±0.013a-c	1.936±0.020c	1.468±0.051d-f	0.765±0.045h
PSB 12	2.783±0.010cd	2.297±0.068gh	1.867±0.054i-k	1.487±0.018k	1.207±0.002j	0.840±0.045f-h
PSB 13	2.799±0.006bc	2.739±0.024a	2.296±0.005b-d	1.849±0.032d	1.506±0.017d	0.804±0.085gh
PSB 14	2.682±0.006f	2.637±0.019b	2.514±0.030a	2.138±0.006a	1.689±0.020ab	1.297±0.076a
PSB 15	2.240±0.008o	2.138±0.005i	1.915±0.025h-k	1.661±0.046hi	1.170±0.052j	0.754±0.131h
PSB 16	2.675±0.009fg	1.894±0.039j	1.721±0.060k	1.362±0.025m	0.956±0.034l	0.531±0.020j
PSB 17	1.729±0.014p	1.587±0.061k	1.244±0.003m	1.066±0.051n	0.695±0.017n	0.385±0.038k
PSB 18	2.362±0.020m	2.309±0.007g	2.139±0.033c-h	1.801±0.004e	1.203±0.022j	0.649±0.034i
PSB 19	2.402±0.010l	2.432±0.275d-f	1.938±0.017g-k	1.585±0.024j	1.292±0.021i	0.900±0.012d-g
PSB 20	2.388±0.007l	2.307±0.002g	2.066±0.005e-i	1.702±0.013gh	1.497±0.019de	1.135±0.022c
PSB 21	2.757±0.005e	2.443±0.025de	2.233±0.049b-e	1.800±0.024e	1.585±0.027c	1.253±0.026ab
PSB 22	2.332±0.012n	2.220±0.008g-i	2.086±0.055d-i	1.433±0.011l	1.447±0.039fg	0.867±0.023e-h
PSB 23	2.458±0.020k	2.162±0.013i	1.829±0.018jk	1.399±0.009m	1.102±0.010k	0.814±0.081gh
PSB 24	2.629±0.014h	2.190±0.028hi	1.824±0.016jk	1.714±0.003fg	0.901±0.010m	0.412±0.092k
PSB 25	2.661±0.011fg	2.459±0.039de	1.460±0.575l	1.696±0.025gh	1.269±0.052i	0.999±0.026d

Data included are means ± SD; different letters within the same column indicate significant differences between means after Duncan's multiple-range test at $P \leq 0.05$

Data in Fig. 1 show the changes in pH values for Pikovskaya's medium supplemented with tri-calcium. 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. The decrease in pH values is followed by a slight reduction until 21 days of incubation, then the pH tended to rise again. Fig. 2 show the changes in water soluble phosphorus values supplemented with tri-calcium phosphate and inoculated with phosphate-solubilizing bacteria. The maximum release of soluble phosphorus from tri-calcium phosphate amounting 14.14, 9.53, 15.58 and 16.31 mg p /100 ml culture media of the isolates of PSB 1, PSB 6, PSB 7, and PSB 14, respectively. The highest quantity of released phosphorus in the culture was for the isolate PSB 14 that selected for further experiments.

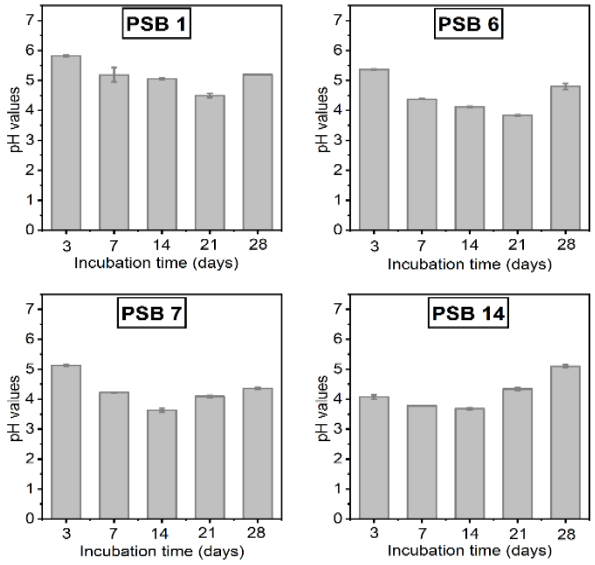

Fig 1. Changes in pH values as influenced by the activity of phosphate-solubilizing bacteria on tri-calcium phosphate. Data included are means ± SD

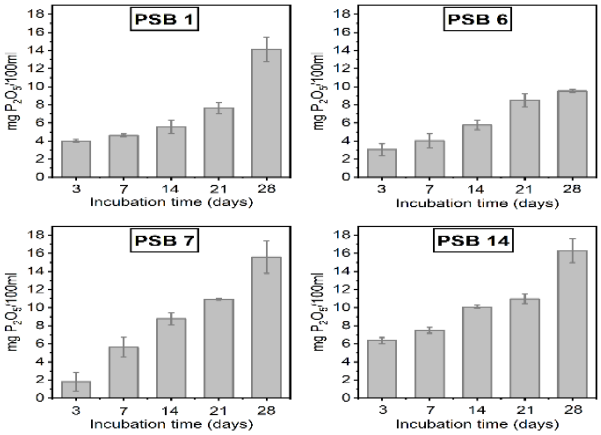

Fig 2. Changes in water soluble phosphorus (WSP) (mg/100 ml culture) as influenced by the activity of phosphate-solubilizing bacteria on tri-calcium phosphate. Data included are means ± SD

Indole acetic acid production by the potent isolates

Data in Fig. 3 showed the production of indole acetic acid (IAA) by AZ 1 and PSB 14 up to 8 days of incubations.

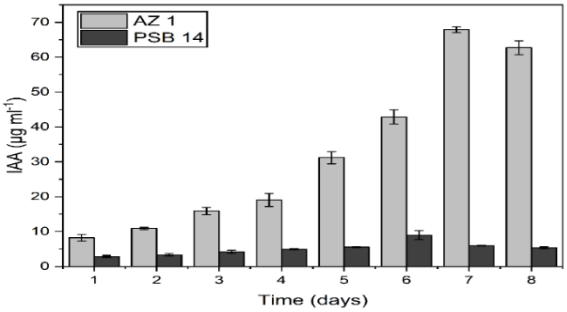

Fig 3. IAA production by bacterial isolates. Data included are means ± SD

As shown from the results, the two isolates could produce IAA but not in equal efficacy. For AZ 1, was detected from the first day and gradually increased up to the the 7th day (67.87 µg/ml) then the production decreased. For PSB 14, was detected from the first day and gradually

increased up to the the 6th day (8.99 µg/ml) then the production decreased.

Identification of the most potent isolates

According to the 16S rRNA sequence analysis, the isolates showed close proximity with *Azotobacter salinstris* and *Bacillus amyloliquefaciens* (Table 6) according to the Gene Bank data base were achieved in BLASTN searches at the National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov). The sequences of these bacteria are now available at the NCBI data bank under the accession numbers ON809700 and ON818999, respectively (Fig. 4).

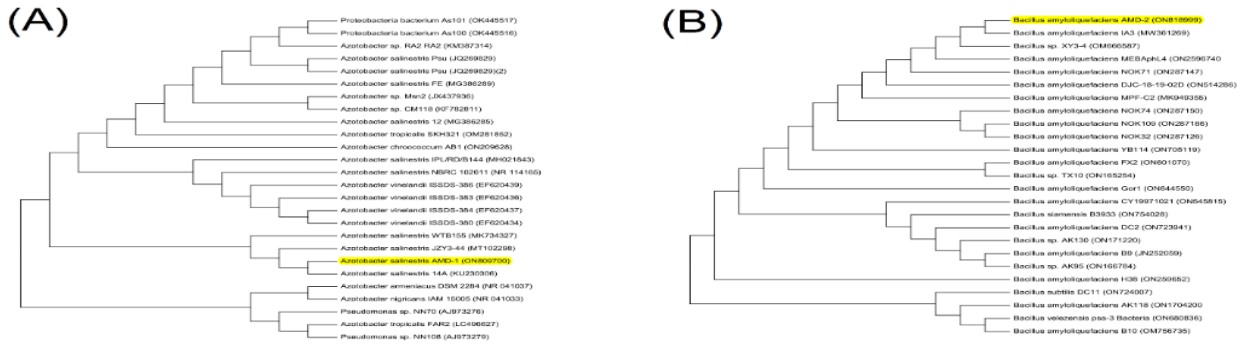

Fig 4. Phylogenetic trees of (A) *Azotobacter salinestris* ON809700 (B) *Bacillus amyloliquefaciens* ON818999

Table 6. Isolates accession numbers in NCBI Gene Bank:

Isolate No.	Bacterial Species	Sequence length (bp)	Accession number
AZ 1	*Azotobacter salinstris*	800	ON809700
PSB 14	*Bacillus amyloliquefaciens*	800	ON818999

Discussion:

Salinity has a negative impact on most plants and reduce their growth and productivity, as a result, using appropriate methods to reduce or eliminate these effects is critical. (Ilangumaran and Smith, 2017). As a method, using halotolerant plant growth promoting rhizobacteria could add a benefit in improving growth and productivity of plants under salinity stress conditions (Kapadia *et al*., 2022). Hence, isolating native PGPR with high plant growth promoting traits from Egyptian soils could provide a reliable foundation for selecting the most effective ones. For this purpose, we isolated twenty-five *Azotobacter* isolates from saline Egyptian soils (Table 1) and were tested to grow in different concentrations of NaCl (Table 2). Results showed that all isolates have the ability to grow at all salinity concentrations at varying degrees with seven potent isolates which are selected due to high ability to grew under 10% NaCl. The seven isolates also have the ability to fix nitrogen effectively and the most active one is AZ 1 which could be used for future in vivo studies (Table 3).

These results were in the line with El-Fadaly *et al*. (2019) who isolated 90 isolates of *Azotobacter* spp. from paddy saline soils of Damietta city, Damietta governorate, Egypt. Among isolates, twelve have high ability to grow under high NaCl concentrations. Another study by Khumairah et al. (2022) isolated three potential halotolerant plant growth-promoting rhizobacteria from different rhizo-microbiome of rice plant. These three potent isolates were found to produce indole-3-acetic acid and nitrogenase and were identified as *Pseudomonas stutzeri* and *Klebsiella pneumonia* based on molecular characteristics. In saline soil, due to the P fixation with calcium, aluminum, and iron, the amount of plant-available P is low (Sashidhar and Podile, 2010). Hence, one of the abilities of PGPR is to solubilize insoluble compounds of P through their ability to produce organic acids. Twenty-five phosphate solubilizing bacteria were isolated, then they were tested to grow in different concentrations of NaCl (Table 5).

Four isolates were selected to their high ability to grow under 10% NaCl and were inoculated in Pikovskaya's liquid medium supplemented with tri-calcium phosphate to study the correlation between pH and P solubilization. Generally, there was a rapid decrease in pH values within the first three days of the experiment, reflecting the high activity of organic carbon degradation bacteria leading to organic acids releasing which mainly contribute to the decreasing pH value. The increase in pH might be due to a subsequent oxidation of organic acids produced in the culture media or might be due to the formation of other natural substances (Gao *et al*., 2020). The most active isolate is PSB 14 which could be used for future in vivo studies. These results agree with the studies of Hauka *et al*. (2017) and Gao *et al*. (2020). Indole-3-acetic acid (IAA) is the most common plant hormone of the auxin class and it regulates various aspects of plant growth and development processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens (Fu *et al*., 2015). The ability of the potent isolates to produce IAA was tested in this study. The two isolates could produce IAA effectively. These results agree with the studies of El-Sawah *et al*. (2018) and Gao *et al*. (2020). As well as, these bacteria were identified according to 16S rRNA sequence sequences of these bacteria and are now available at the NCBI data bank *Azotobacter salinstris* ON809700 and Bacillus amyloliquefaciens ON818999, respectively. The current study suggested using these bacteria to alleviate salinity stress in plants grown in saline conditions.

CONCLUSIONS

In conclusion, the current study focused on identifying and characterizing the native PGPR from Egyptian soils to use the more superior isolates to improve growth and yield of plants grown under saline stress. It was found that some of the isolated bacteria showed marked PGP traits including high salt tolerance, nitrogen fixation, phosphate solubilization, and indole acetic acid production. Based on the 16S rRNA sequence analysis, The most potent isolates were identified as *Azotobacter salinstris* ON809700 and *Bacillus amyloliquefaciens* ON818999. These salt-tolerant isolates could be a simple, cost-effective, and efficient method that could add benefits to plant grown under salinity stress.

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تقييم النشاط المحفز لبكتيريا المحيط الجذري المتحملة للملوحة المعزولة من التربة المصرية ذات الملوحة العالية

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الملخص

تعد بكتيريا المحيط الجذري التي تعزز نمو النبات استراتيجية بديلة للتخفيف من الآثار السلبية لإجهاد الملوحة على النباتات ، وكان الهدف من هذه الدراسة عزل بكتيريا المحيط الجذري متحملة للملوحة وتقييم كفاءتها فى إنتاج المواد المحفزة لنمو النبات لاستخدامها كأسلوب صديق للبيئة للتخفيف من إجهاد الملوحة على النباتات المتأثرة به ، تم عزل خمسة وعشرون عزلة من بكتريا الأزوتوباكتر ، ثم تم اختبار جميع العزلات للنمو فى وجود تركيزات مختلفة من كلوريد الصوديوم. وقد أظهرت سبعة عزلات قدرة أكثر على تحمل تركيزات ملوحة أعلى ، ثم تم اختبار العزلات السبعة السابقة لقدرتها على تثبيت النيتروجين باستخدام تقنية اختزال الأسيتيلين ، حيث كانت العزلة رقم 1 أكثرها كفاءة والتى سجلت 27.70 نانومول إيثيلين / مل / ساعة على الجانب الآخر ، تم عزل خمسة وعشرون عزلة بكتيرية مذيبة للفوسفات على بيئة أجار بيكوفسكايا ، و تم اختبار جميع العزلات السابقة لتحمل الملوحة ثم تم اختيار أربعة عزلات لمزيد من الدراسة لآلية إذابة الفوسفات وعلاقتها بحموضة البيئة ، تم تلقيح بيئات سائلة تحتوى على ثلاثي فوسفات الكالسيوم غير الذائب بالعزلات الأربعة السابقة ، كانت أعلى كمية من الفوسفور المحرر في المزرعة (14.14 مجم / 100 مل) بواسطة العزلة رقم 14 والتى تم اختيارها لمزيد من الدراسات. وكذلك أظهرت العزلات AZ 1 و PSB 14 كفاءة عالية في إنتاج إندول حامض الخليك (67.87و 8.99 ميكروجرام / مل)على التوالي. وقد تم تعريف هذه العزلات بناءا على أنهما أزوتوباكتر ساليتريس و باسيلس أميلوليكويفاشينس ، ونستنتج من هذه الدراسة أنه يمكن لهذه السلالات البكتيرية المقاومة للملوحة أن تضيف فوائد للنباتات المزروعة تحت ظروف الإجهاد الملحى.