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Characterization of Plant Growth Promoting Bacteria (PGPB) as a Tool for Alleviation Salt Stress of Medicinal Plants

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ABSTRACT

Salinity and drought are two of the most serious abiotic problems for agriculture productivity worldwide. Halophilic and halo-tolerant plant growth promoting rhizobacteria (HT-PGPR) are as an efficient eco-friendly tool to alleviate salt and drought stress. In this study, 40 isolates were isolated from rhizospher of 5 wild medicinal plants (*Zygophyllum album*, *Salsola tetrandra*, *Limoniastrum monopetalum*, *Halocnemum strobilaceum* and *Sarcocornia fruticosa*) grown at coastal ooletic sand dunes habitat at Mersa Matruh, Cleopatra, GPS reading is 31° 22.460 N, 27°11.009 E, Matrouh government. For initial screening, the results showed that 17 isolates gave positive reaction for IAA detection. While 30 isolates can produce exopolysaccharides, as well as, two different isolates are positive for phosphors (P) and potassium (K) solubiliztion. The forty isolates had different growth on nutrient agar medium supplemented with NaCl ranged from 3-15%. The efficient isolates (18) from the first screening were tested for their biological characteristics to mitigate salt stress; proline, total sugar, IAA production, antioxidant activity and P, k solubilization. The most efficient three isolates namely, Li1, Li8 and Sar13 were identified by 16s rRNA gene. These three strains are study their properties to alleviate the drought stress by grown them in different concentrations of 6000 Polyethylene glycol (PEG) and recorded the optical density (O.D) at 600 nm.

Keywords: PGPB, Salt stress, drought stress, medicinal plants, halophilic bacteria, proline

1. Introduction

Plants facing a lot of abiotic stresses through their life, the harmful abiotic stress are salinity and drought. The acceleration in climate change nowadays increases salinization of soils around the world. Soil salinity is one of the biggest abiotic stress globally expand affected plant productivity by ionic imbalance in plant lead to nutrient deficiency, osmotic stress, hormonal imbalance decrease rate of photosynthesis and regenerate of reactive oxygen species (ROS) (Kumar *et al.,* 2020).

Soil salinization refer to high electrical conductivity (EC), low in water potential that happened when there is a high concentration of salt ion such as sodium, calcium, magnesium, potassium, sulphate, chloride, carbonate and bicarbonate (Shahid *et al.,* 2020). Many plants, especially medicinal plants, are salt sensitive and do not have effective salt tolerance mechanisms. Therefore, PGPB in their rhizosphere and beneficial plant- growth bacteria, produce active metabolites in response to salt stress (Krishnamoorthy *et al.,* 2022).

Physical and chemical methods of treatment of saline soils, include leaching, flushing, scraping and addition of lime and gypsum, are not sustainable methods (Egamberdieva *et al.,* 2019). Halophilic and halotolerant plant growth promoting rhizobacteria (PGPR) are beneficial bacteria which can colonie in

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the plant root and enhancing the plant growth. The use of theses HT-PGPR increasing in agriculture and offers an alternative way to replace chemical fertilizers and pesticides (Wahab *et al.,* 2023).

So, recent researches, gave attention to Halophilic and halo-tolerant plant growth promoting rhizobacteria (HT-PGPR) as a green and sustainable solution for soil salinization (Mokrani *et al.,* 2022). Using of Halophilic and halo-tolerant plant growth promoting rhizobacteria elicited many mechanisms to cope with salt and drought stress to increases the tolerance of plants to those abiotic stresses (Sunita *et al.,* 2020). Many genera of salt tolerant and halophilic microorganisms have been isolated from extremely saline soils. (Niu *et al.,* 2018). These plant growth salt tolerant plant beneficial microbes have great importance in agriculture. They have potential improve to plant productivity in arid and semiarid regions through different physiological and molecular mechanism. Which include modification in root system, antioxidants, exopolysaccharides, phytohormones productions, uptake of minerals, synthesis of osmolytes (proline, sugars), increase organic matter, improve water retention and soil structure (Egamberdieva *et al.,* 2019). The two halotolerant bacteria *Halomonas* sp. G11, and *Piscibacillus salipiscarius* E5 were confirmed firstly *in vitro* for their PGP activities like indole acetic acid, phosphate solubilization, exopolysaccharides productions, siderophore, and osmolytes. These bacteria support plant growth under salt stress (up to 200mM NaCl) (Naili *et al.,* 2018).

Many studies have isolated rhizobacteria from the rhizosphere of halophytic plants and have shown their plant growth promoting properties, such as IAA (indole acetic acid), phosphate solubilization, siderophore production and enzymatic activity (Xia *et al.,* 2020). Kearl *et al.* (2019) isolated bacteria from the rhizosphere of the halophytes *Salicornia rubra, Sarcocornia utahensis and Allenrolfea occidentalis*.. Several studies have used halophilic and halotolerant plant growth promoting microorganisms from roots of halophytic plants to attenuate the effect of salt stress on economic importance plants, such as *Beta vulgaris L., Salicornia sp., Medicago sativa, Triticum aestivum* L. (Babar *et al.,* 2021), *Solanum lycopersicum, Arabidopsis thaliana, Zea mays L.,* and *Cucumis sativus* (Leontidou *et al.,* 2020). This study focuses for isolation and identification of salt stress tolerant bacteria from rhizospheric of some medicinal plants.

2. Material and Methods

2.1. Collection of Plant Sample and PGPB isolation

Five rhizosphere samples of wild medicinal plants (*Zygophyllum album*, *Salsola tetrandra*, *Limoniastrum monopetalum*, *Halocnemum strobilaceum* and *Sarcocornia fruticosa*), were used to isolate salt-tolerant growth promoting bacteria (PGPB). These samples were collected from coastal ooletic sand dunes habitat at Mersa Matruh, Cleopatra, GPS reading is 31° 22.460 N, 27°11.009 E, Matrouh government. The electrical conductivity (EC) of cultivated soil by these plants was measured using the saturated paste extract method.

2.2. Isolation from saline soils and screening of bacterial cultures for salt tolerance

The bacterial isolates were isolated by employing Serial Dilution Agar Plating Method (Ben-David and Davidson, 2014). Approximately 20 ml of molten medium (45°C) of nutrient agar amended with 3% NaCl was poured in Petri dishes containing 1 ml of dilution then were mixed thoroughly by rotating the plates several times. The plates were allowed to set then inverted and incubated at 30°C for 24-48 hours. The colonies which obtained were further sub cultured to get colonies in pure. The evaluation of collected isolates to salinity stress was carried out by growing the collected isolates at different concentration of NaCl (3%, 6%, 9%, 12% and 15%) by streaking method (using 3 replicates) at 28 - 30 °C on nutrient agar for 2 days. This method was carried out according to Quadri *et al.* (2016). The growth was observed and recorded as low (+), good (++), very good (+++).

2.3. Qualitative and Quantitative assay for IAA production

The ability of collected bacterial isolates to produce indole acetic acid was qualitatively assayed by growing the tested isolates on nutrient agar medium supplemented by 1mM tryptophan for 3 days/28°-30°C. After the incubation period salkowski reagent was added to the plates. Colonies with pink color was positive IAA production. While the quantitative assay was performed in culture supernatant of selected isolates by the colorimetric method using Salkowski reagent as described by Bric *et al.* (1991).

2.4. Phosphorus solubilizing Ability

For soluble phosphate detection, a loop of active culture of each isolate was spotted on Pikovskaya's agar medium (Subba Rao, 1982). After 4 days of incubation period at 30˚C, a clear zone around the growing colony was considered a positive result. The screening was evaluated based on index of phosphate solubilization [The ratio of the total diameter (colony + halo-zone) and the colony diameter] which according to Edi-premono et al (1996). The amount of soluble phosphate for the most efficient isolates was determined in culture supernatant by the colorimetric technique as describe by Jackson, (1958). One hundred milliliters of Pikovskaya's broth medium, amended with 0.5% tricalcium phosphate, were inoculated with standard inoculum of each of the most potent bacteria then incubated at 28-30 ˚C under shake condition (150 rpm) for 14 days. The amount of soluble phosphate was determined in culture filtrate by colorimetric method at 530 nm according to Jackson (1958).

2.5. Potassium solubilizing ability

One gram Feldspar powder was added to one liter of modified Aleksandrov agar medium (Zahra, 1969), as the sole source of K to test the K-solubilization ability of tested isolates. After poured the medium to plates and solidified, the tested culture was looped in medium, the clear zone around the colonies considered as a positive result. As well as, the quantitative amount of soluble potassium was determined by one hundred ml flasks containing 50 ml of Modified Aleksandrov`s broth medium (Zahra, 1969) that was inoculated by one ml. standard inoculum (10^7 cfu /ml) of the tested isolates individually, then incubated at 28-30˚C under shake conditions (150 rpm) for 15 days. Cell free culture was used to determine soluble K using flame photometer as described by Sugumaran and Janarthanam (2007).

2.6. Exopolysaccharides production

The tested isolates were assessed for exopolysaccharides production by growing the isolates on ATCC no.14 medium for seven days at 28-30 °C. Positive results showed as bacterial colonies form slime or mucus thick with the category of low level (+) to very good (+++) which form a thick slime (mucoid). The ATCC no. 14 Medium containing: 0.2 g KH_2PO_4, 0.8 g K_2HPO_4, 0.2 g $MgSO_4.7H_2o$, 0.1 g $CaSO_4.2H_2O$, 2.0 mg $FeCl_3$, Trace of $Na_2MoO_4.2H_2O$, 0.5 g yeast extract, 20 g Sucrose and 20 g Agar, pH 7.2. For determination of exopolysaccaride, pure culture of tested bacterium, that form thick slime (mucoid) on ATCC no. 14 medium, was grown in 50 ml liquid medium ATCC no. 14 and incubated at 28-30 °C for three days under shaking condition (200 rpm). At the end of incubation period, the cells were harvested by adding 500 µl EDTA (1 mM), and then shaken until homogeneous and centrifuged at 1000 rpm for 10 min. The supernatant was separated from the bacterial cell deposition was taken, coupled with cold acetone solution then deposition of biomass in the form of exopolysaccharide then washed with distilled water and dried at 60°C for 24 hours or until dry weights obtained were fixed (Pawar *et al.,* 2013).

2.7. Proline determination

Proline production was estimated for selected cultures as described previously by Abou-Aly *et al.* (2019) as the following; 2.0 ml of bacterial supernatant and 2.0 ml of glacial acetic acid were added to 2.0 ml of acid ninhydrin (2.5 g of ninhydrin was dissolved in 60ml of glacial acetic acid and 40ml of 6M of phosphoric acid) in a glass tube, and put in a boiling bath for 1hr, and transferred to an ice bath. After that, 4ml of toluene was mixed for 15–20sec. The sample was red in spectrophotometer at 520nm and the toluene was used as a blank sample.

2.8. Sugars determination

The total sugar was quantified by 3, 5-dinitrosalicylic acid (DNS) method as described by Tasun *et al.* (1970) in cell free culture of selected bacterial cultures.

2.9. Antioxidant assay

The free radical scavenging DPPH assay was used to evaluate the antioxidant potential of bacterial supernatant. The percentage of inhibition of free radical formation (I%) was estimated in 1ml of cell free bacterial culture using standard method as described by Burits and Bucar (2000) and that was calculated by the following formula**:**

I% = (A blank – A sample / A blank) × 100

When *A blank* is the absorbance of the control re-action (which containing of all reagents except the test sample) and *A sample* is the absorbance of the test sample.

2.10. Drought tolerance

The most efficient isolates were examined their ability to modulate the drought tolerance by growing the isolate on nutrient broth medium supplemented with different concentration of Polyethylen glycol 6000 (PEG) (0% - 2% - 4% - 6%). One hundered milliliters of nutrient broth was inoculated with 1% stander inoculum (10^7 cfu/ml) then incubated at 28°-30° C for 7 days at 150 rpm shaking conditions, the growth was measured at 600 nm (JASCO V-630 spectrophotometer), while using a sterile medium as a blank, three replicate used for each isolate according to Sandhya *et al.* (2009) and Vardharajula and Shaik (2014). The optical density (OD) values of drought-tolerant isolates which were divided into 4 categories as highly sensitive OD < 0.3; sensitive OD 0.3 to 0.39; tolerant OD 0.4 to 0.5, and completely tolerant OD > 0.5 (Susilowati *et al.,* 2018).

2.11. Identification of selected cultures

The molecular identification was done by extracting genomic DNA using the modified protocol of Ausubell *et al.* (1988) and amplification of 16S rDNA according to Lane (1991) by Sigma Company. The sequences were identified using the nucleotide BLAST tool from the National Center for Biotechnology Information (NCBI), and a phylogenetic tree was constructed using the neighbor-joining method with MEGA v.10 software. The identified sequences for the selected isolates were deposited in the NCBI gene bank with an accession number.

2.12. Statistical Analysis

All data were analyzed statistically according to Snedecor and Cochran (1980) using program SPSS version No.23 by using one way of analysis of variance (ANOVA).

3. Results

3.1. Isolation of salt-tolerant bacterial isolates

Five rhizosphere of wild medicinal plants (*Zygophyllum album*, *Salsola tetrandra*, *Limoniastrum monopetalum*, *Halocnemum strobilaceum* and *Sarcocornia fruticosa*), growing in extermily saline soil (EC ranged from 20.80 to 32.12 dS/m) shown in Table (1) were used as sources for isolation salt-tolerant bacteria. Forty isolates were obtained from the rhizosphere of these samples. By studying their morphological properties, it was found twenty five (25) isolates short rods and fifteen (15) isolates bacilli. The percentages of isolates from total isolates were 62.50%, and 37.50 % respectively.

Table 1: The electrical conductivity (EC) of cultivated soil by collected plant samples

Collected Plant	EC (dS/m)
Zygophyllum album	30.16
Salsola tetrandra	32.12
Limoniastrum monopetalum	20.80
Halocnemum strobilaceum	25.76
Sarcocornia fruticosa	26.80

3.2. Primary screening for salt tolerance and PGP activities

The isolated bacterial cultures (40) were screened for their salt tolerance ability at different salt concentrations and their plant growth-promoting traits such as indole acetic acid (IAA), exoploysaccharides production, potassium (K) and phosphorus (P) solubilization. Fig (1) presents the number of isolates that grew at each salt concentration. The highest number of isolates (40) was

observed to grow at a 3% NaCl concentration. It was observed that the numbers of positive isolates were decreased as the NaCl concentration increases. Where (29) isolate out of (40) grew at 15% NaCl. Although it was recorded a decreasing trend, a significant number of isolates can grow at higher salt concentrations (9%, 12% and 15%), suggesting that these isolates have a certain level of salt tolerance. Fig (2) the ability of isolates to grow at high salt concentrations indicates to their potential use in promoting the growth of plants under saline conditions because of their abilities to alleviate salt stress in plants by several mechanisms. The recorded results show that the isolates exhibit variability in their growth response under each salt concentrations ranged from low growth (+) to high growth (+++). Notably, 23 isolate tolerated different salt concentrations up to 15% with amount of growth compared to other isolates (Zy1, Zy3, Zy6, Zy7, Sa8, Li2, Li3, Li5, Li9, Li11, Ha3, Ha5, Ha6, Sar1, Sar2, Sar7, Sar8, Sar9, Sar11, Sar12, Sar13, Sar14 and Sar15). They grew with the same efficiency up to 15% salt.

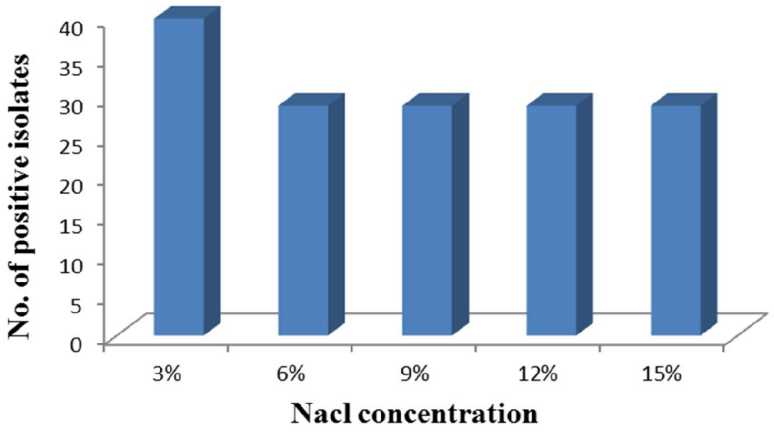


Fig 1: The number of isolated bacterial cultures grew under different concentrations of salt stress (NaCl %)

3.2.1. IAA Production

IAA is a phytohormone, at auxins category play a role in plant growth and development, like seed germination, root development and plant tolerance against stresses (Saberi Riseh *et al.,* 2021). The detection of IAA for collected isolates refers that seventeen (17) isolates out of 40 isolates can produce IAA ranged from less value (+) to high value (+++). The quantity assessment of indole acetic acid for positive isolates ranged from 0.10 to 26.17 ppm (Table 2), registered the maximum production by isolates Li6, Li13 and Li8 being 26.17, 24.57 and 22.81, respectively.

3.2.2. Phosphorus and Potassium solubilization

Phosphorus and potassium are plant macronutrients, which play a vital role in plant developments. The results of detection method of phosphorus solubilization revealed that two isolates only (Li8 and Sar13) out of 40 isolates can solubilize phosphorus on Pikovskaya's agar medium, where their phosphorus solubilizing index were 1.66 and 1.57, respectively. While the quantity assessments of soluble P of these isolates in liquid culture were 69.64 and 56.51 ppm as respective order.

For potassium solubilization**;** it was found that two isolates only (Sar13, Li4) out of 40 isolates can solubilize potassium giving 1.10 and 0.49 as potassium solubilizing index, respectively on Aleksandrov agar medium. While the soluble potassium quantity for these isolates were 1.14 and 0.57 ppm, respectively.

3.2.3. Exopolysaccharides production

Exopolysaccharides play role in mitigate salt and drought stress. Thirty isolates out of 40 isolates (75%) can qualitatively produce exopolysaccharide polymers on ATCC no.14 agar medium by different response ranged from less level (+) to very good (+++). Fifteen isolates gave high slime or mucus thick exopolysaccharide (++) and two isolates only recorded the highest thick slime (mucoid) (+++) (Li8 &

Li7). Whereas the other isolates gave low growth (+). Table (2) summaries the PGP activities of the most efficient cultures (18) considering the IAA production and salt- tolerance ability as the main goals in addition to positive results in two or more of PGP parameters. In the further study, these isolates were examined their ability to mitigate the salt stress by different mechanisms (proline, total sugar and antioxidants production).

Table 2: The plant growth promoting activities of the most efficient bacterial isolates to alleviate salt stress

Plant source	Isolates code	IAA Production (PPM)	Proline production (PPM)	% Antioxidants	Exopolysaccharide production	NaCl % concentration			
						6%	9%	12%	15%
Zygophyllum album	Zy3	0.58^{ab}	0.0^{a}	33.67^{c}	++	++	++	++	++
	Zy4	16.46^{i}	0.046^{a}	41.20^{h}	+	++	++	++	+
	Zy6	0.51^{ab}	0.0^{a}	34.05^{cd}	+	++	++	++	++
Salsola tetrandra	Sa2	10.31^{gh}	0.180^{b}	45.26^{i}	+	++	++	++	+
	Sa7	0.10^{ab}	0.294^{c}	57.71^{m}	+	++	++	++	+
Limoniastrum monopetalum	Li1	10.79^{h}	0.275^{c}	36.37^{f}	++	++	++	++	+
	Li4	0.0^{a}	0.266^{c}	56.66^{L}	+	++	++	++	+
	Li6	26.17^{L}	0.171^{b}	35.33^{e}	++	++	++	++	+
	Li8	22.81^{J}	0.275^{c}	34.71^{de}	+++	++	++	++	+
	Li10	1.24^{bcd}	0.047^{a}	47.02^{j}	+	++	++	++	+
	Li13	24.57^{K}	0.0^{a}	47.92^{k}	+	++	++	++	+
Halocnemum strobilaceum	Ha6	2.65^{e}	0.0^{a}	29.91^{a}	++	++	++	++	++
Sarcocornia fruticosa	Sar7	0.94^{bc}	0.0^{a}	38.09^{g}	++	++	++	++	++
	Sar9	1.00^{bc}	0.0^{a}	35.57^{ef}	++	++	++	++	++
	Sar11	7.70^{f}	0.0^{a}	33.26^{c}	++	++	++	++	++
	Sar12	1.72^{cd}	0.0^{a}	31.97^{b}	++	++	++	++	++
	Sar13	9.60^{g}	0.0^{a}	32.16^{b}	++	++	++	++	++
	Sar14	2.01^{de}	0.0^{a}	33.61^{c}	+	++	++	++	++
P- Value		0.008	0.002	0.001	-	-	-	-	-
LSD		1.43	0.09	0.97	-	-	-	-	-

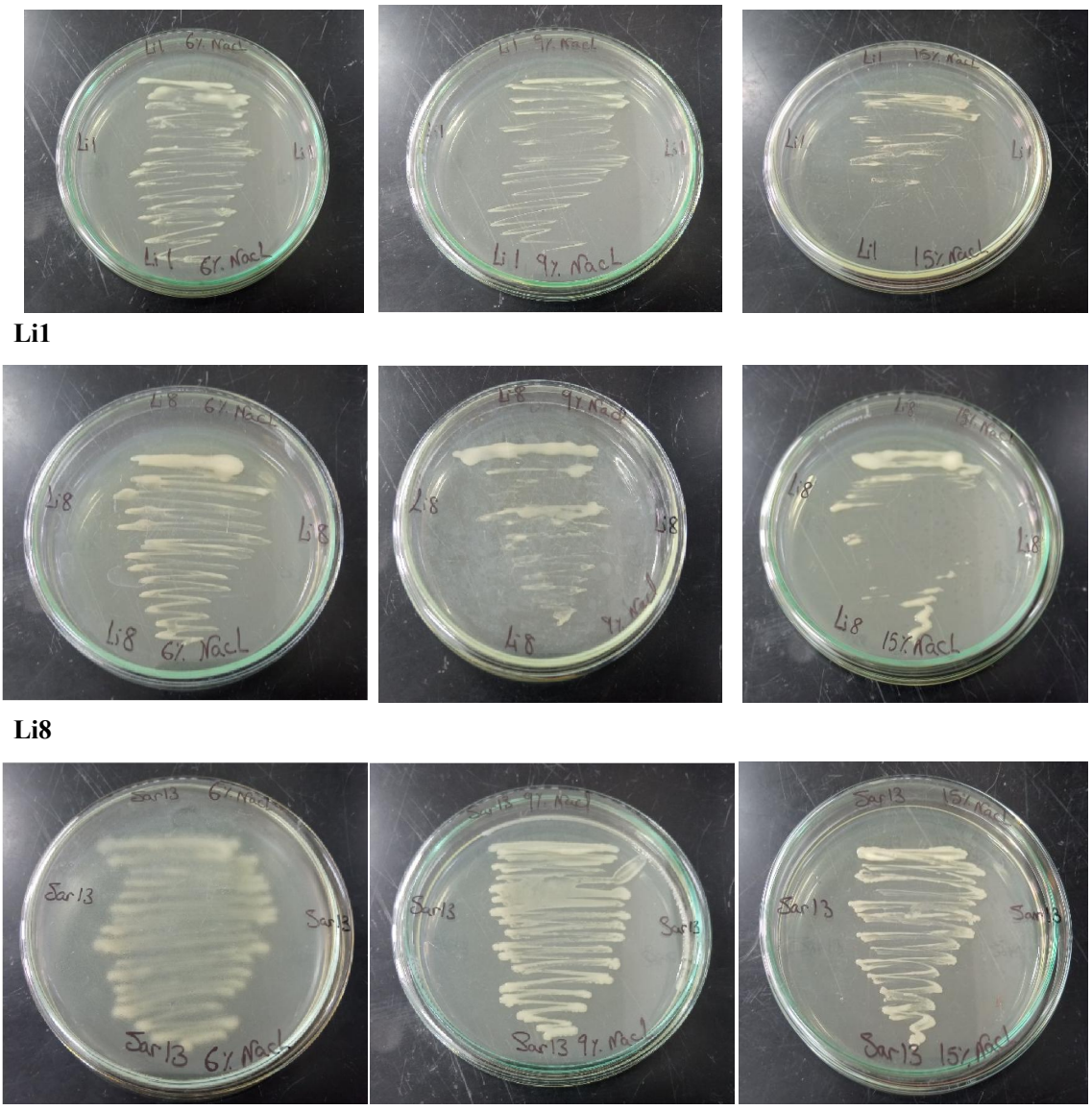


Fig. 2: The ability of some isolates to grow at high salt concentrations (15% NaCl)

3.3. Secondary screening for proline and sugar production

Osmolytes (proline and sugars) are neutral substances that protect proteins and other cell membranes from different stress. Salt stress-induced proline buildup was observed to be considerably higher in salt challenged stress. Table (2) shows eight isolates produce proline ranged from 0.046 to 0.294 ppm. The higher producer isolates were Sa7, Li1, Li4 and Li8 recording 0.294, 0.275, 0.266, 0.275 ppm respectively. For sugar production, isolates Zy6 (0.01ppm) and Sar11 (0.41 ppm) were the only two out of eighteen (18) isolates that can produce sugar measured as total sugar.

3.3.1. Antioxidants activity

Increasing salinity causes reactive oxygen species (ROS) generation. The primary cause of ROS generation is the over-reduction of photosynthetic activity (Keswani *et al.,* 2019). The HT-PGPR are known for alleviate antioxidant defense mechanisms in plants to react against oxidative stress caused by salt stress. All the eighteen (18) selected isolates have antioxidant activity ranged from 29.91% to

57.71% (Table 2). The minimum antioxidants activity was achieved by isolate Ha6 (29.91%), while the superior antioxidants activity with Sa7 (57.71%).

3.4. Molecular Identification of selected cultures

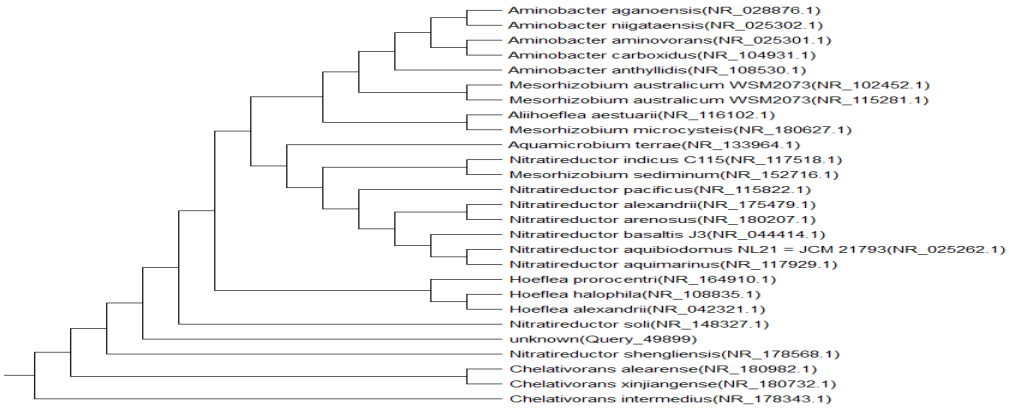
From the previous results, the isolates Li1, Li8 and Sar13 are Gram-negative, short-rod-shaped, showed significant salt-tolerant and PGPR activities. So they were selected to identify. These isolates were sequenced for amplified 16SrRNA gene. The obtained resulting genomes were employed for phylogenomic and comparative genomic analysis with the reference to the Gene Bank data base of the National Centre for Biotechnology Information (NCBI) using BLAST function subsequently a phylogenetic analysis. The phylogenetic trees of selected bacterial isolates were drawn (Fig. 3). It was observed that they are belonging to three genuses. Isolates Li1 has a similarity 98.21% with *Nitratireductor shengliensis*, isolates Li8 has a similarity 97.67% with *Roseibium album*, isolate Sar13 has a similarity of 99.88% with *Halomonas elongate.* The results of BLAST analysis which have sequences with ≥97% show the same genus. Sequences with ≥99% similarity achieved the same species (Drancourt *et al.,* 2000). They were accessed in the gene bank with accession number as mentioned in Table (3).

Table 3: Species identical to selected bacterial cultures isolated from rhizosphere of medicinal plants by gene encoding of 16S rRNA

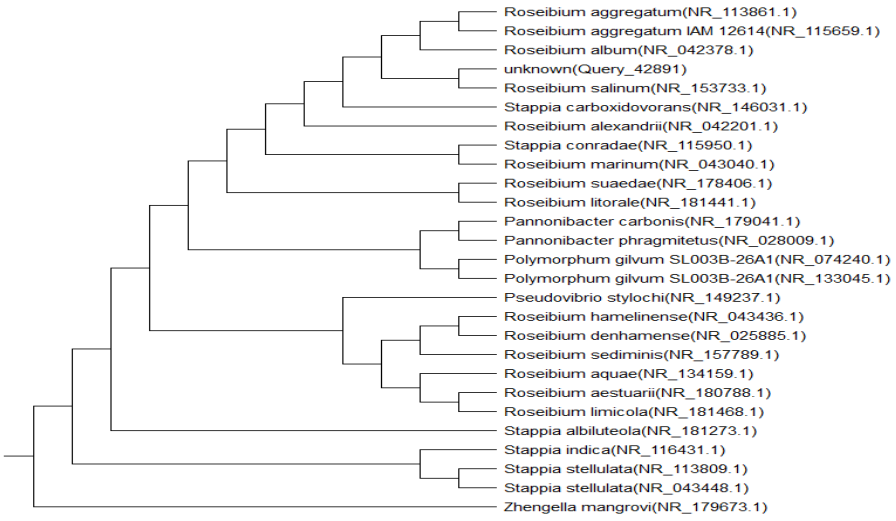
Isolates code	Closest relatives	Similarity (%)	Accession No.
Li1	*Nitratireductor shengliensis*	98.21%	PQ058684
Li8	*Roseibium album*	97.67%	PQ059401
Sar13	*Halomonas elongate*	99.88%	PQ059853

3.5. Drought tolerance and exopolysaccharide production

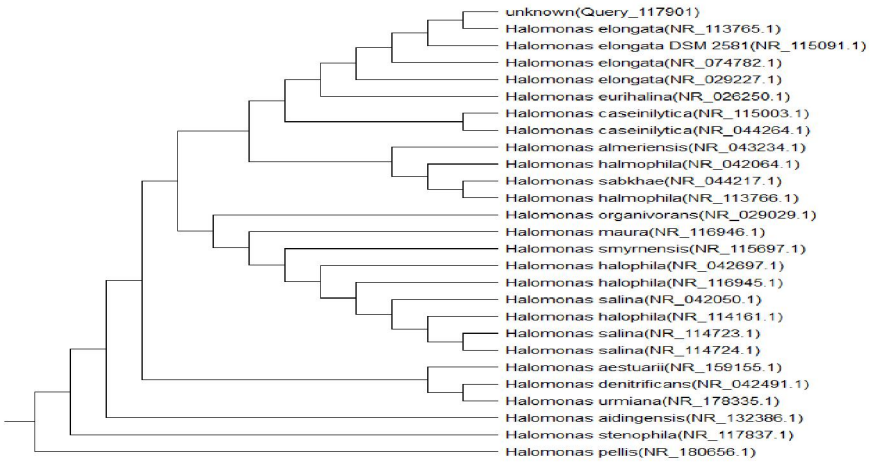
The identified bacteria were examined their drought tolerance and the amount of Exopolysaccharide production in broth culture. Fig. (4) shows that the most efficient strains can mitigate drought stress by determined the optical density (O.D 600 nm) of their growth on nutrient broth medium supplemented by different concentration of PEG 6000 (0% - 2% - 4% - 6%).The bacterial strains show varied responses of at different concentrations and the negative effects of Polyethylen glycol 6000 induced osmotic stress on growth that increased by increasing the concentrations. *Nitratireductor shengliensis* Li1 showed the best performance and exhibited the smallest decline in growth at different PEG-concentrations. It recorded 0 %, 13.7 %, 8.3% decline at 2%, 4%, 6% PEG concentration in comparison to without osmotic agent treatment (0%) respectively. *Roseibium album* Li8 showed better osmotic tolerance and exhibited 9.5 % (2%), 12.3% (4%) and 26.4% (6%) decline comparing to control (0%). *Halomonas elongate* Sar13 strain achieved the highest decrease in growth at 4% & 6% PEG concentrations. The determination of dry weight of EPS produced revealed that the identified bacteria produced dry weight of EPS ranged from 0.59 to 0.62 gram/ 50ml medium (Fig. 4).



Li 1



Li8



Sar13

Fig. 3: Phylogentatic tree for the three most efficient isolates (Li1, Li8 and Sar13)

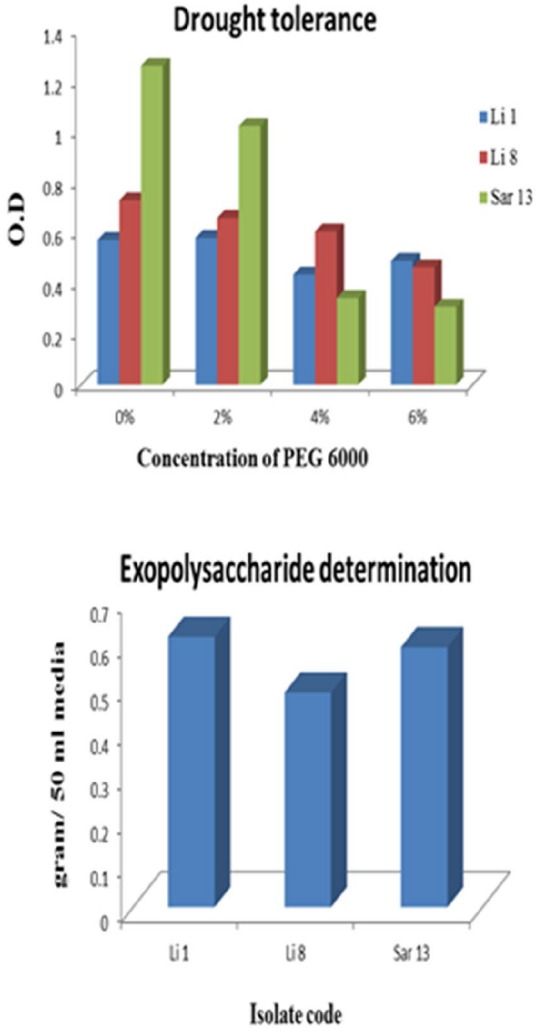


Fig. 4: Drought tolerance and dry matter of exopolysaccharide production of the most efficient strains

4. Discussion

Soil Salinity in Changing Plant Metabolites and Proteins Plant development can be hampered by salinity in the rhizosphere because roots cannot draw water from salt-accumulated soil. To grow in saline soils, plants change various biochemical and physiological strategies like ion exchange, hormonal stimulation and activation of antioxidant enzymes. In addition to mechanisms of plant adaption, plant growth-promoting rhizobacteria (PGPR) can improve salt tolerance in plants via production of antioxidants, ion homeostasis, phytohormones, volatile organic compounds ACC deaminase, extracellular polymeric substance (EPS), accumulation of osmolytes, activation of plant antioxidative enzymes, and improvement of nutrients uptake (Saberi Riseh *et al.,* 2021). In the present study, 40 bacterial isolates were obtained on nutrient agar medium supplemented with 3% NaCl. They were characterized as short rod and bacilli. It was found various responses of their growth at different concentrations of NaCl. twenty three bacterial isolates can grow with the same response up to 15% compared to other isolates. It would be beneficial to select isolates that demonstrate robust growth across a range of salt concentrations, particularly those that maintain higher growth rates at elevated salt levels. Egamberdieva *et al.* (2019) reported that salt-tolerant microbes can survive in osmotic and ionic stress. Various genera of bacteria that belong to salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) were isolated from sodic soils, saline, and extreme alkaline. Also many of them are known

to mitigate various abiotic and biotic-stresses in plants. Potential PGPR enhancing the productivity of plants facing salt-stress by ST-PGPR. This can be exploited for the reclamation of saline agro-ecosystems. The obtained bacterial isolates were evaluated for their ability to produce IAA and exoploysaccharides, potassium (K) and phosphorus (P) solubilization. Seventeen isolates can produce IAA, and the maximum production was registered by isolates Li6, Li8 and Li13. Our results similar to Xia *et al.* (2020) who isolated bacteria from the rhizosphere of halophytic plants and have shown their plant growth promoting characteristics, such as IAA (indole acetic acid) and siderophore production, phosphate solubilization and enzymatic activity) The uptake of macronutrients such as nitrogen, phosphorous, and potassium reduced under salinity stress. The HT- PGPB improves the solubility of (P) and (K) and makes the nutrients in available form by metal chelating mechanism (siderophores production), ion exchange (pH change), acidification (organic acid) under salt stress (Rashid *et al.,* 2016) and produces exopolysaccharide alleviates salt stress through many mechanisms (reducing the Na^+ content for plant uptake - soil aggregation – holding the free phosphorous and circulation of nutrient – protect plant from infection play a defense role – increasing water holding capacity decrease osmotics stress, formation of water rich layer around the root surface) (Wahab *et al.,* 2023). HT- microorganisms improved salt tolerance ability in plant by osmolytes such as proline production through increasing antioxidants activitis, photosynenthatic activity and plant growth (Saberi Riseh *et al.,* 2021). Osmolytes are neutral substances that help to protect proteins and other cell membranes from different stress events that affect metabolite of cells. Salt stress-induced proline build-up was noticed to be frequently higher in salt challenged (300 mM). Inoculation of salt-stressed soybean plants with *Bacillus firmus* SW5 increased proline levels by up to 23.1%. The synthesis of proline gene (P5CS1) expression was up-regulated dramatically in *Enterobacter* sp. EJ01-infected plants under stressful conditions (Hmaeid *et al.,* 2019 and Acosta *et al.,* 2020). So in this study, it was determined the proline and total sugar content of selected isolates giving eight isolates out of 18 isolates produce proline and the highest amount was recorded by Sa7, Li1, Li4 and Li8. Two isolates only can produce soluble sugar. All tested isolates (18) have antioxidant activity and achieved the minimum activity by isolate Ha6 (29.91%) and the maximum activity with Sa7 (57.71%). The most efficient isolates which were genetically identified as *Nitratireductor shengliensis*, *Roseibium album* and *Halomonas elongate* with similarity ranged from 97% to 99%.

The biotic and abiotic stress for plant lead to increase the reactive oxygen species (ROS), which have a strong oxidizing reaction on compound of cell and the cell itself. So, the HT-PGPB plays an essential role by producing antioxidants compound to detoxifying the ROS effect on plants under salinity stress (Saberi Riseh *et al.,* 2021).

And the HT-PGPB decreases the drought stress, by producing many extracellular polymeric substances (EPS) proline – sugars - exopolysaccharides – formation of biofilm). EPS in the biofilm complex increase the water holding capacity of soil and improves the water activity in plants under stress conditions of salinity and drought (Costa *et al.,* 2018). *Nitratireductor shengliensis* Li1 can survive at different concentrations of PEG 6000 with the similar ability by increasing the concentrations and produces 0.62 gm dry weight EPS/ 50 ml broth medium. *Halomonas elongate* Sar13 produces 0.59 gm dry weight EPS/ 50 ml broth medium and its growth reduced by 69% at 6% PEG 6000. The growth of *Roseibium album* Li8 reduced by 29.6% comparing to 2% PEG 6000. *Halomonas elongata* strain K4 is an endophytic bacterial strain that was isolated from roots of *Cyperus conglomeratus* collected at the Red Sea coast in Thuwal, Saudi Arabia under salt stress (Lafi *et al.,* 2016). HT-PGPR have significant role for increasing productivity of rice under salinity stress (200 mM NaCl) (Sultana *et al.,* 2020). Also, inoculation with salt-tolerant *Halomonas variabilis* HT1 increased the growth of chickpea and soil aggregation with roots under high salt concentrations (up to 200 mM NaCl) (Sandhya and Ali, 2015).

5. Conclusion

There is a rapid increasing in salination and drought soil around the world. These problems accelerate due to the climate change. So, we must to look for safety solution. Using microbial agent's formula is an ecofriendly solution for salt and drought. HT- PGPB has a several solutions to alleviate salt and drought in soil. In this study, these three HT-rhizobacteria (*Nitratireductor shengliensis* Li1 strain PQ058684, *Roseibium album* Li8 strain PQ059401 and *Halomonas elongata* Sar13 strain PQ059853), can be used as a potenitial strategy to improve the medicinal plants growth under salt and drought stress.

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