Original Research Article

Characterization And Isolation of Halotolerant Phosphate Solubilizing Bacteria from Mangrove Soil

ABSTRACT

On average less than 1% of the total phosphorous present in soils is available to plants, making phosphorous one of the most limiting macronutrients for crop productivity worldwide. The aim of this work was to isolate, select halotolerant phosphate solubilizing bacteria (PSB) and using them as biofertilizers in the presnces of salinity condition. Bacterial diversity from Mangroves ecosystems has been studied for their unique biochemical processes and their use in various applications. soil sample from Mangrove Forest in Ras Muhammad were collected for the isolation of bacteria. Thirteen bacterial isolates were screened for production of different PGPR traits. The most potent microbial candidates were identified by rRNA gene sequencing analysis and uploaded in GenBank as *Bacillus safensis* [OR341132] and *Cytobacillus firmus* [OR431197] which showed ability to tolerate salt concentration up to 10% , can fix nitrogen by growing on nitrogen free media with excellent growth and characterized qualitatively and quantitative solubilize p more than 100µg/ml of insoluble phosphate , hydrolyse phytate and produced IAA showing the highest value in the range of 94.54, 220,38 µg/mL respectively. The study has overall concluded that bacteria isolated from mangroves soil could use in various applications. In pot trials performed in a greenhouse the isolated strains *B.saf* and *c.fir* were able to significantly improve levels of assimilated phosphate, compared to non-inoculated plants co-inoculation revealed its superiority. this study focused on the behavior of our strains as halotolerant plant growth promoting rhizobacteria [HT-PGPR] and phosphate solublising bacteria [PSB] on the health of barley [genotype Giza123] plant under salinity condition during thirty days.

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Keywords: *Salinity,Bacillus safensis , Cytobacillus firmus,* ,IAA, HT-PGPR, phytate hydrolysing, Barley, proline.

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1. INTRODUCTION

Beneficial microorganisms that have evolved to high salt levels have been found naturally in mangrove soil. There have been reports of a distinctive, rich, and varied native microflora in mangrove soils [1]. Due to intensive biological salinization of

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mangroves and recurring tidal seawater immersion, mangrove soil has a high salinity [2].

Salinity in the soil is one of the primary stressors that restrict agricultural productivity worldwide. It affects the success of reproduction, plant growth and development, and seed germination [3] and [4].

The utilization of beneficial bacteria adapted to high salt conditions offers a potential option for increasing crop yield in areas afflicted by salinity, given that reclamation of the saline soil is a difficult process [5].

Numerous bacteria linked to salt stress adaptation have been found to be abundant in the rhizosphere of salt-tolerant plants, according to earlier research [6] [7]. Compared to the microbiota in bulk soils, the microbiota in rhizosphere soils of salt-tolerant plants exhibited a higher number of genes that support plant growth and mitigate salt stress [8].

Even in saline alkaline soil, halotolerant plant growth-promoting rhizobacteria (ht-PGPR) boost agricultural output in terms of plant health and productivity by reducing salinity damages in a variety of crops where salt stress is greatly decreased [9]. [10].

Environmental issues include detrimental effects on the food chain, imbalances in soil nutrients, and devastation of the soil microbial ecosystem can also result from the overuse of phosphate fertilizers [11].

In addition to being directly involved in nucleic acid synthesis, cell division, and the development of new tissues, phosphorus is a second essential macronutrient for plants after nitrogen. It is also required for a number of cellular functions, including photosynthesis, the metabolism of carbohydrates, energy production, with levels up to 0.2% of the dry weight of plants, phosphorus is also necessary for plant growth and development [11]. Additionally, it is a component of phospholipids, which make up cell membranes [12].

Between 60 and 70 percent of the p element present in the soil is found in both organic and inorganic forms. Only 0.1% of the soil phosphate that is accessible, nevertheless, is prepared for plants to directly absorb. In order to create inorganic forms of free solubilizing phosphate, the remaining phosphate needs the help of phosphate solubilizing microorganisms. Both types of phosphate must be mineralized and dissolved by plants in nature, which requires the assistance of the microbial community [13].

Phytic acid as phytate is one of the most common forms of organic p in soil, accounting for around 50%-60% of the total. Furthermore, phytic acid is a source of organic p that plants cannot absorb. The presence of phytase-producing bacteria in the mangrove environment is critical for the breakdown of organic p, which increases phosphorus availability and serves as a plant nutrient. As a result, it is essential to the soil's p cycle [14].

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Excessive usage of chemical fertilizers over time can lead to nutritional imbalances in soil. The goal of applying biofertilizer to rhizosphere soil is not to add soil nutrients, but to increase the availability of nutrients for plants through the use of microorganisms. Biofertilizer is a biological fertilizer that contains bacteria that help plants thrive. [15]

The discovery of phosphate solubilizing bacteria (psb) provides a new solution to the problem of effective phosphorus deficiency in soils; psb play an important role in the soil phosphorus cycle by mineralizing organic phosphorus through acid secretion and hydrolyzing inorganic phosphorus minerals through enzyme activity, thus solubilizing insoluble phosphorus and increasing the amount of available phosphorus in soils [11].

(PGPR) are the type of bacteria that promote plant growth. Rhizosphere microorganisms have emerged as a crucial instrument for protecting plant health in an environmentally responsible way. The growth of plants may be impacted by these microbes. Through nitrogen fixation, mineral nutrient solubilization, mineralization of organic substances, and phytohormone synthesis, PGPR possessed direct mechanisms that either improve nutrient availability or facilitate nutrient uptake. The development and yield of numerous plants, including bacillus spp. Isolated from mangrove soil, have been found to be influenced by PGPR [16].because p is bound by metal cation complexes like al, Ca, and Fe, which render it immobile in the soil, 75–90% of p is unavailable for plant use. PSB plays a significant role in helping plants overcome this limited availability of p. Metal cations that were initially bonded to P elements will be bound by the organic acid compounds that psb can create. In order to make p elements available to plants, they can be extracted from their bindings with cations. [15]

Meanwhile, the solubilization of organic phosphate through mineralization is carried out by nonspecific acid phosphatases, alkaline phosphatases, and phytases that are naturally produced by bacteria. By generating phosphate-soluble enzymes and solubilizing the inorganic p molecule, the microbial population can utilize the p content in soil [13].

As a member of the phosphatase enzyme class, phytase (myo-inositol hexakisphosphate phosphohydrolases) can hydrolyze phosphates that contain organic compounds like phytate, releasing inorganic phosphorus in the process. One of the most significant sources of phytase synthesis is bacteria. Numerous phytase-producing bacterial species, including bacillus, enterobacter, pseudomonas, klebsiella, proteus, lysinibacillus, and staphylococcus, have been isolated from mangrove environments [14].

Barley (hordeum vulgare, l.) Is the world's fourth most extensively grown cereal, following wheat, rice, and corn. Around 70% of barley produced worldwide is utilized directly or indirectly for animal feed, with the remaining portion being used for malting and beer manufacture [17]. Compared to other cereal crops, barley is distinguished by its capacity to thrive in challenging environmental circumstances such salt and drought [18].

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2. MATERIAL AND METHODS

2.1. Site description and Sample collection:

Mangroves soil sample was collected from Mangrove forest in Ras Muhammad Nature Reserve located 12 km from the city of Sharm El Sheikh at the southern extreme of the Sinai Peninsula, overlooking the Gulf of Suez on the west and the Gulf of Aqaba to the east (Coordinates: 27°46′10″N 34°12′35″E). Soil was collected from 4cm deep area using a sterile spatula. The soil sample was collected from the topsoil from 4 - 5 cm depth under the root surface of the mangrove plant. The soil sample was collected in sterile plastic bags, kept in an icebox, transported to the Laboratory and stored at 4ºC until use. The characteristics of the collected soil were done according to the methods described by [19] [20]. Some physical and chemical soil properties are recorded in Table (1).

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Table 1. Some physical and chemical properties of the experimental mangrove`s soil

Particle size distribution %				Textural Class	Physical and chemical properties										
Clay	Silt	Sand	Silt clay	Sandy Loamy	Sp	EC	pH	Soluble cations				Soluble anions			
13.52	12	74.4	25.52					K^+	Ca^{++}	Mg^{++}	Na^+	SO_4^{--}	CO_3^{--}	HCO_3^-	Cl^-
					22	43.9	9.41	14	57	103	484.5	174	9	0.5	475

EC [dSm^{-1} (1:5)], pH [1:2.5], soluble cations and anions [meq. L^{-1}]

2.2. Isolation of bacteria:

Using sterile normal saline (0.75% NaCl), soil suspensions were made. Plates of Sterile Nutrient Agar were used to isolate bacteria. The plates were incubated for twenty-four hours at 37ºC. Bacterial colonies that were well isolated after incubation were chosen and subcultured on sterile nutrient agar plates for purification. Characteristic analysis was done on the bacterial isolates [21] .

Screening Salt Tolerance of Bacterial Isolates

In test tubes, 10 milliliters of nutritional broth were made with different concentrations of NaCl (0, 2, 4, 8, 10, and 20%). After that, 0.1 ml of cultures extracted from mangrove soil were added to the broth, and it was incubated for 48 hours at 28 °C. A spectrophotometer was used to measure optical density at 600 nm in order to track development during incubation [22].

Characterization of the isolated bacteria for Plant Growth Promoting (PGPR)Traits

2.2.1. Production of auxin (IAA)

To produce auxin (IAA), a medium containing 1% peptone water and 1% tryptophan was employed. The 24 old cultures were injected into the medium and incubated for 24 hours at 28±2ºC. Following incubation, the medium was centrifuged at 5000 rpm for ten minutes. For IAA determination, 1 mL of supernatant was combined with 4 mL of Salkowski's reagent. The formation of pink color suggested the generation of IAA. The optical density was measured at 540 nm. The concentration of IAA was calculated using the standard IAA [23].

2.4.2. Nitrogen fixation activity

The bacterial isolates were tested for nitrogen-fixing ability on Jensen's agar following the method described by [24]. Pure bacterial isolates were inoculated onto nitrogen-free Jensen's medium and kept for incubation at 28 ◦C for 72 h [24]. Bacterial isolates showing growth on Jensen's plates were considered to be able to fix atmospheric nitrogen

2.4.3. Assessment of phosphate solubilization

2.4.3.1. Qualitative estimation of solubilized Phosphate:

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The ability of the isolated bacteria to solubilize TCP (insoluble $Ca_3(PO4)_2$) was studied. So, sterilized National Botanical Research Institute phosphate agar medium (NBRIP) was placed into sterilized Petri plates containing TCP. The bacterial strains were placed on plates with (NBRIP) agar.

(NBRIP) agar medium comprising /L: glucose 10 g; $Ca_3(-PO4)_2$ 5 g; $MgCl_2$_6H2O 5 g; $MgSO_4$_$7H_2O$ 0.25 g; KCl 0.2 g; $(NH4)_2SO4$ 0.1 g; pH-7.0. PSB, or phosphate-solubilizing bacteria, evolved after colonies that formed clear surrounding halos. The isolated PSB was also screened in triplicate for their potential to render the blue hue of the NBRIP - bromophenol blue broth medium (pH-7.0) (NBRIP-BPB), due to the generation of organic acid and a drop in pH [25].

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2.4.3.2. Quantitative Estimation of Phosphate Solubilization

In triplicate, bacterial isolates were inoculated into Erlenmeyer flasks with 50 ml of NBRIP broth devoid of bromophenol. The control was a non-inoculated media. The flasks were shaken at 100 rpm for seven days at 37 °C in an incubator shaker. After ten days, flasks were gathered and centrifuged for ten minutes at 10,000 rpm. After the supernatant was filtered using Whatman No.1 filter paper and a 0.22 lm Millipore membrane, it was utilized to determine the amount of phosphate released spectrophotometrically (880 nm) in triplicate using Murphy and Riley's standard procedure. [25].

2.4.4. Screening of phytate hydrolysing:

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The obtained bacteria were streaked on Phytase Screening Media (PSM) and incubated at 37 °C [26]. PSM was made by dissolving 3g glucose, 1g tryptone, 1g sodium phytate, 0.3g CaCl2, 0.5g MgSO4, 0.04g MnCl2, 0.0025g FeSO4, and 15g agar in 1 liter of pure water. A clear zone surrounds the colony of phytase-producing bacteria. The chosen isolates were then purified and kept on LB agar medium.

2.5. Molecular identification of bacterial isolates:

The bacterial isolates were cultivated in a test tube containing 10 ml autoclaved nutrient broth medium [27] and incubated at 28ºC for 48 hours. Cultures were sent to the molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. The extracted DNA samples were shipped to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and 16S gene sequencing. PCR was performed using two universal primers namely 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTT ACGACTT-3'). The PCR product was reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. The purified PCR product was sequenced in the sense and antisense directions using 27F and 1492R primers with the incorporation of dideoxy nucleotides (dd NTPs) in the reaction mixture [28]. The obtained sequences were analysed using Basic Local Alignment Search Tool (BLAST) from the National Center of

Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done using MegAlign (DNA Star) software version 5.05.

2.6. Greenhouse Pot Experiments

2.5.1. Experimental design and treatments

Barley plant (*Hordeum vulgare* Giza 123) was cultivated in the two types of saline soils (Sandy and clay loamy) in a pot experiment in the Agriculture Research Center greenhouse in Giza, Egypt. The sandy soil obtained from the experimental farm of El Kassasien Research Station, Ismailia Governorate, Egypt, also the other soil obtained from Khaled ibn El-Waled village (32° 00' to 32° 15' N and 30° 50' to 31° 15' E), Sahl El-Husseiniya,

To investigate the potential of the effectiveness isolated halotolerant phosphate-solubilizing bacteria (PSB) on some soil chemical properties and plant parameters. The characteristics of the investigated soils before cultivation were done according to the methods described by [19] [20]. The experimental treatments on the both types of saline soils (Sandy and clay loamy) included 4 treatments for each type [control, *bacillus safensis (B. safensis), Cytobacillus firmus (C. firmus)* and Mix treatment (*B. safensis* + *C. firmus*)]. Some physical and chemical soil properties before planting are recorded in Table (2). According to the recommendations of the Egyptian Ministry of Agriculture, a mineral fertilizer (N, P, K) was applied.

Table 2. physical and chemical properties of the both experimental saline soils

Particle size distribution %				Textural class	Chemical properties					
Clay	Silt	Sand	Coarse Sand		O.M.	EC	pH	Available Macronutrients		
								N	P	K
2.81	3.54	3.65	70	Sandy	.34	4.31	7.75	39	8.1	50
.66	36.45	22.69	4	Clay loam	.52	10.25	8.45	67.25	9.5	100

O.M. [%], EC [dSm^{-1} (1:5)], pH [1:2.5], Available macronutrients [mg kg^{-1}]

2.5.2. Soil analysis

Soil samples were gathered during a 30-day period. These samples were then air-dried, crushed, properly mixed, and sieved to ensure they fit through a 2mm sieve. The generated samples were then stored until they could be assessed for various chemical properties in accordance with [20]. Total bacterial counts were evaluated 30 days after planting, in accordance with [29].

Plant samples

The plant specimens were weighed, then oven dried at 70°C until they reached a constant dry weight. The dried materials were then digested using a mixture of H2SO4 and H2O2, as reported in [19]. The digested samples were next analyzed for nutritional content, specifically nitrogen (N), phosphorus (P), and potassium (K), using the designated methods given in [20]. Furthermore, the total nutritional content of the plant was calculated as mg/kg plant and percentage (%). The measurement of photosynthetic pigments, including chlorophyll and carotenoids, was performed in the foliage of numerous plant species during a 30-day period

using the method of [30].The estimates were based on formulae derived by [31], and pigment concentrations were represented in $mgFw^{-1}$.

The proline content of plant tissues was determined as described by [32]. The proline content was estimated as mg/g fresh weight using a predefined calibration curve for proline.

2.6. Statistical analysis

The acquired data underwent statistical analysis of variance using a computer software CoStat Software (2004)

3. RESULTS AND DISCUSSION

3.1. Isolation of bacteria

From mangrove soil 13 bacterial strains were isolated. Screening Salt Tolerance and characterization of the isolated bacteria for Plant Growth Promoting (PGPR) Traits were carried out.

3.2. Screening Salt Tolerance of Bacterial Isolates

Bacterial isolates from mangrove soil were screened for their salt tolerance potential, where thirteen bacterial isolates showed excellent growth in control (without NaCl) and in the 2,4% NaCl supplemented medium. 13 isolates showed good and moderate growth, at 8%; at 10% M5 and M12 showed excellent while 11 isolates showed moderate or no growth colonies, on the other hand M5, M12 didn't grow, at 20% salt concentration. This implies that the isolates were moderately halophilic to extremely halophilic M5 and M12 isolates were able to tolerate up to 10% NaCl.

Table 3. Growth pattern of 13 isolates at different NaCl concentrations.

Isolates	0%	2%	4%	8%	10%	20%
M1	+ + +	+ + +	+ + +	+ +	-	-
M2	+ + +	+ + +	+ + +	+ +	-	-
M3	+ + +	+ + +	+ + +	+ +	-	-
M4	+ + +	+ + +	+ + +	+ + +	+	-
M5	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	-
M6	+ + +	+ + +	+ + +	+ + +	+	-
M7	+ + +	+ + +	+ + +	+ + +	+ +	-
M8	+ + +	+ + +	+ + +	+ + +	+ +	-
M9	+ + +	+ + +	+ + +	+ + +	+ +	-
M10	+ + +	+ + +	+ + +	+ + +	+ +	-
M11	+ + +	+ + +	+ + +	+ + +	+ +	-
M12	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	-
M13	+ + +	+ + +	+ + +	+ +	-	-

('++++' excellent growth,'+++' good growth,'++' moderate growth,'+' weak growth,'- 'no growth)

Many marine species are mild halophiles (3% w/v NaCl in sea water). Moderate halophiles thrive best at 3-15% w/v NaCl, extreme halophiles at 25% w/v NaCl (halobacteria and halococci), and borderline extreme halophiles at at least 12% w/v salt [33].

As can we see in table 3 with the increase in the salt concentration there was a decline in the numbers of bacteria able to grow with only 2 isolates growing at 10% salt concentration.

Two species isolate (M5 and M12) able to tolerate high saline conditions and high alkalinity environments. under soil salinity stress the activity and metabolism of the microorganisms decreased.

Life under salinity stress requires the microflora to maintain the osmotic balance between the cytoplasm of the microbes and the surrounding environment. These findings are consistent with [34], who stated that when the concentration of salt in the surrounding medium of the cells increases and becomes high, the solute concentration inside the cells loses water, posing the risk of the cell drying out. Microbes needed to be able to survive at high salt or solute concentrations in the medium in order to maintain an equal high concentration in the cell cytoplasm. The creation and accumulation of tiny organic molecules can increase the solute content in the cell's cytoplasm. The formation and accumulation of tiny organic molecules, known as compatible solutes, can increase the solute concentration in the cell cytoplasm because they do not interfere with cellular activities [5].

Characterization of the isolated bacteria for Plant Growth Promoting (PGPR) Traits

3.2.1. Production of auxin (IAA)

Thirteen bacterial isolates tested were able to produce moderate quantity of IAA. 6 isolates out of 13 produced IAA levels more 15 µg/ml. The isolates that produced the most IAA were M5 and M12 in table (4).

Table 4. Characterization for Plant Growth Promoting (PGPR) Traits of 13 isolates

Isolates	IAA (µg/ml)				P [µg/ml]				N fixation	P solubilization	Phytase
	Average	±	SE	Rank	Average	±	SE	Rank			
M1	15.02	±	0.10	de	17.57	±	0.35	i	-	-	+
M2	12.77	±	0.15	e	25.96	±	0.54	h	-	-	+
M3	13.04	±	0.09	e	53.84	±	1.02	f	-	-	+
M4	17.39	±	0.31	cd	63.76	±	1.92	e	-	-	-
M5	94.54	±	0.29	b	102.39	±	1.45	a	+ + +	+ + +	+ + +
M6	13.49	±	0.29	e	65.95	±	3.03	e	-	+	-
M7	14.01	±	0.12	e	48.20	±	1.33	f	-	+	-
M8	13.77	±	0.29	e	73.81	±	2.32	d	+	+	+
M9	13.62	±	0.31	e	92.85	±	1.41	b	-	+	-
M10	14.97	±	0.32	de	34.81	±	2.43	g	-	+	-
M11	19.10	±	0.20	c	47.79	±	1.47	f	-	+	-
M12	220.38	±	2.56	a	107.63	±	4.14	a	+ + +	+ + +	+ + +
M13	15.89	±	0.48	de	80.47		0.90	c	-	+	-

IAA [LSD0.05=2.1982], P [LSD0.05=505572], P-solubilization ('+++' excellent growth and clear halo zone , ''+' weak growth and small clear halo zone, and '-' no growth), N-fixation (''+' weak growth and '+++' excellent growth'-' no growth),

Thirteen halotolerant isolates were positive for IAA production, with M5 and M12 showing the highest production in the range of 94.54, 220,38 µg/mL respectively . This is contrary to [35], which said that Endophytic bacteria Bacillus safensis were examined for their ability to increase the formation of indole-acetic acid (IAA), and that IAA produced by halotolerant PGPR plays an important role in conferring salt tolerance. In this investigation, the majority of the isolates produced IAA, which solubilized phosphate. This was seen to be

consistent with the findings of previous investigations published by [36]. IAA is a natural AUX created by plants and HT-PGPR, which was necessary for plant growth and development and helps plants resist environmental stress. [37]

Nitrogen fixation activity

To screen for plant growth promoting traits of the halotolerant bacterial isolates, 13 bacterial isolates were examined for nitrogen fixation. All Isolates showed no growth on N -free media except M8 which showed weak growth and also, M5 and M12 showed excellent growth. Nitrogen is an essential component of chlorophyll and is involved in photosynthesis. Nitrogen-fixing PGPB converted gaseous nitrogen (N2) into ammonia (NH3), making it available to host plants [38]. Nitrogen is equally crucial to plants, as a vital component of chlorophyll, amino, and nucleic acids, and it has been previously shown that the concentration of nitrogen in non-leguminous plants can be raised by the connection with Bacillus species [39].

3.2.2. Assessment of phosphate solubilization:

P is essential for photosynthesis, respiration, carbohydrate metabolism, and energy storage and transfer in the form of adenosine diphosphate (ADP and ATP) and di- and tri-phosphopyridine nucleotides (DPN and TPN) [40];[41]. Apart from DNA and RNA structures, P played a role in root development, flower initiation, and seed and fruit development [41]. In addition, [42] successfully recovered 19 isolates of PSB from Mangrove soils.

Qualitative phosphate solubilization test:

All the 13 isolates were screened for Phosphate solubilization on NBRIP medium containing insoluble Ca3(PO4) (TCP) and showed positive result in primary screening. The screened bacteria were able to solubilize TCP on solid culture state by forming clear halo zone, with different degree, depending on the type of organism involved. Isolates M1, M2, M3 and M4 showed no growth while the excellent growth and halo zone take place with M5 and M12 isolates. Finally, the other isolates had weak growth and small halo zone. Also, phosphate solubilization ability of isolates was confirmed by using NBRIP-BPB broth medium. Solubilization of mineral phosphate in NBRIP-BPB media resulted in decolorization of blue color of the media after incubation. Finally, M5 and M12 were the most phosphate solubilizing bacteria (PSB).

The zone of solubilization of isolates after 8 and 15 days was in the same range as previously reported [10].

[43] indicated that the results were based on the kind, rate of release, and degree of dissemination of the metabolic chemicals responsible for phosphate solubilization, as well as the presence of isolates. This demonstrated that PSB is prevalent in the mangrove rhizosphere and helped mangrove plants receive P elements.

Quantitative phosphate solubilization test:

The in vitro phosphate solubility capability of each strain was tested in NBRIP broth without bromophenol [44].

As shown in table (4), 13 strains were characterized, and all the strains seem to be well adapted in the environment. On quantitative basis strains M5 and M12 solubilized more than 100µg/ml insoluble phosphate and considered as the most efficient P solubilizer. M3, M4, M6, M8, M9, M13 solubilized P in the range between 50µg/ml to 100µg/ml and considered

as high p solubilizers. M1, M2, M10, M7, M11 solubilized P below 50µg/ml, hence considered as low P solubilizer. These findings correspond with those obtained by [45], who isolated and described 20 strains, and all of the strains appear to be well suited to their environment.

[45] the formulation to quickly screen the phosphate-solubilizing bacteria qualitatively utilizing an effective media NBRIP containing Bromophenol Blue as a dye. As a result, the isolated bacterial strains were chosen for low and high phosphate solubilization, as well as qualitative decolorization of the dye.

3.2.3. Screening of phytate hydrolysing

In addition to free P, phytate is the most abundant organic P in soil and plant tissues. Phytases from various sources, including plants, animals, and microbes, catalyze the hydrolysis of phytate and release accessible forms of inorganic P. [36].

As documented in [46], PSB can convert insoluble phosphorus forms in soils into accessible phosphorus, which can be directly absorbed and utilized by plants via various mechanisms such as enzyme secretions (phytase and phosphatase) to produce dissolved phosphate. However, phytase is generated from microbial sources due to its increased phytase synthesis capacity. In the present study, phytase producing bacteria were isolated from mangrove soil .in table (4) Higher phytase activity was observed in 2 out of 13 bacterial strains M5, M12 were gave excellent growth and clear zone figure 1C. The majority of other isolated strains had shown no significant difference in the extracellular phytate-degrading activity was observed. except for M1, M2, M3, M8 which manifested low level of activity. The results obtained are consistent with those reported by [14], who found that 48 of the 68 bacteria isolated from mangrove sediments were phytase producers. This is determined by the existence of a clear zone around the colonies in solid PSM. The isolates M5 and M12 produced phytase with much higher activity.

Finally, the screening results revealed that the most potent isolates selected for identification and further studies were M5 and M12, which demonstrated the ability to tolerate salt concentrations of up to 10%, fix nitrogen by growing on nitrogen-free media with excellent growth, and were qualitatively characterized for their ability to solubilize phosphate and produce phytase enzyme, as shown in Figure 1 (A, B, C, and D).

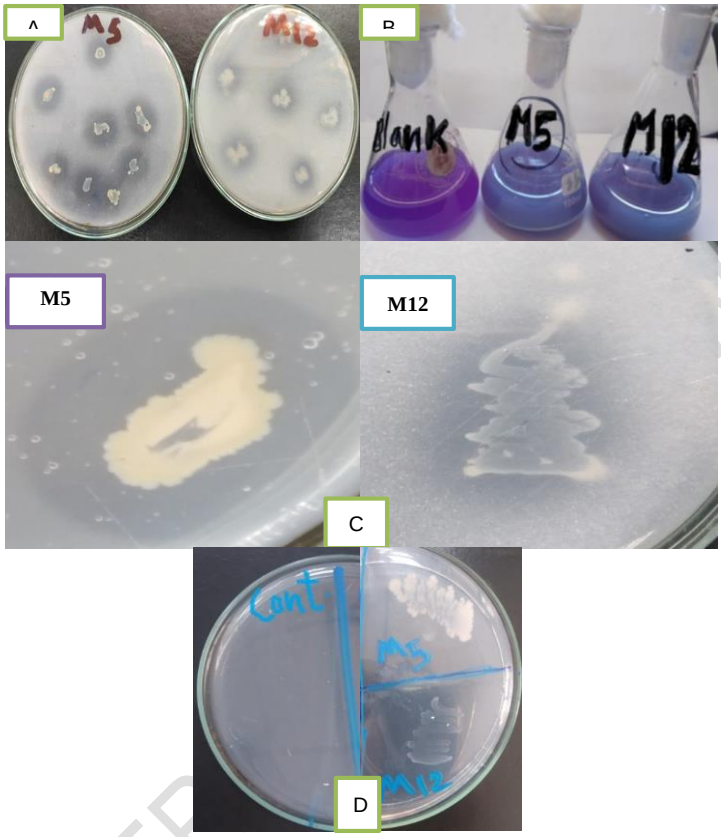

Fig. 1 Characterization of the most potent isolates (M5 and M12) for Plant Growth Promoting (PGPR) Traits

- **(A) Qualitative phosphate solubilization test of M5 and M12 on solid medium**
- **(B) Qualitative phosphate solubilization test of M5 and M12 isolates in liquid medium**
- **(C) Clear zone by phytase-producing isolates (M5 and M12) on PSM plate after 96 hours**
- **(D) Growth on N- free media**

3.3. Molecular identification of selected bacterial isolates by rRNA gene sequencing analysis

Isolates M5 and M12, phylogenetic trees based on 16S rDNA sequences of the bacterial isolates as shown in figures 2 and 3 (M5and M12) were identified in the present study as with Gen *Bacillus safensis* Bank accession OR341132 and Cytobacillus firmus with GenBank accession OR431197 (red arrow).

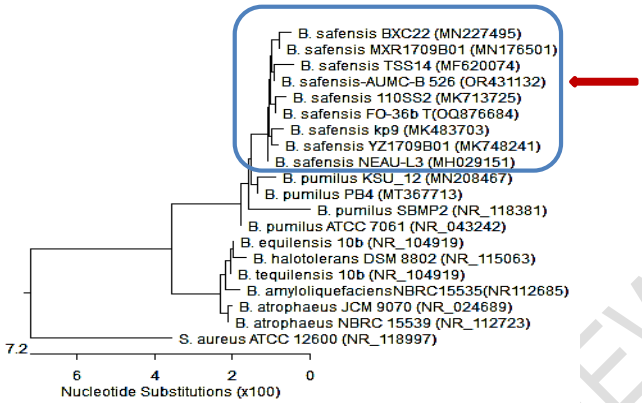

Fig. 2. Phylogenetic tree based on 16S rDNA sequencing of *Bacillus safensis* isolate M5, Strain AUMC-B526, GenBank accession no. OR341132 (arrowed) aligned with closely related sequences of bacterial strains accessed from the GenBank. This bacterial strain showed 99.30% - 99.79% identity and 99% - 100% coverage with several related strains including the type materials *B. safensis* strain FO-63b with GenBank accession no. (OQ876684). *Staphylococcus aureus* is included in the tree as an outgroup strain, *B. = Bacillus, S. Staphylococcus.*

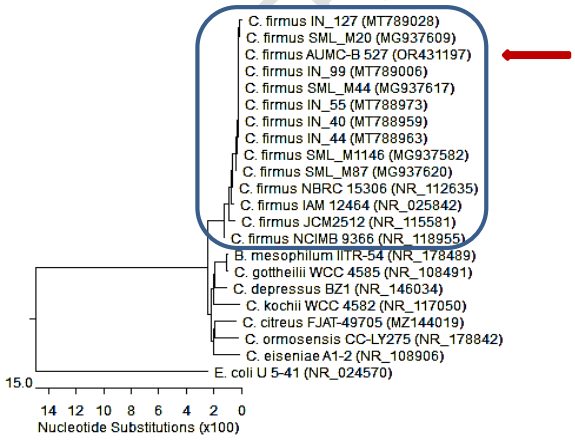

Fig. 3. Phylogenetic tree based on 16S rDNA sequencing of *Cytobacillus firmus* isolate M12, Strain AUMC-B526, GenBank accession no. OR431197 (arrowed) aligned with closely related sequences of bacterial strains accessed from the GenBank. This bacterial strain showed 99.30% - 99.79% identity and 99% - 100% coverage with several related strains including the type materials *C. firmus* strain NBRC15306 with GenBank accession no. (NR_112635). *Escherichia coli* is included in the tree as an outgroup strain, *C. = Cytobacillus, E.= Escherichia.*

Bacillus bacteria are the largest extracellular phytase-producing bacteria and are efficient at mineralizing phytate. [14]

In addition, C. firmus, a bacilli class member, was discovered to produce high-quality IAA [5]. This is consistent with the findings (Bacillus sp.) obtained from the mangrove waters of the Pasuruan Kraton Coast. Bacillus spp. occupy 50% of the mangrove environment as well. [47].

Greenhouse Pot Experiments:

Salt stress has a deleterious impact on plant morphology, physiology, and biochemistry. High salt concentrations can reduce soil porosity and water conductivity [48]. Furthermore, it encourages us to conduct additional research on our isolates Bacillus safensis (B. safensis) OR341132 and Cytobacillus firmus OR431197 (C. firmus) as Halotolerant Plant growth-promoting rhizobacteria (HT-PGPR) and Phosphate Solubilizing Bacteria (PSB) on parley (genotypes Giza 123), which is widely cultivated in Egypt as feed. [47] uses some of the indigenous bacteria that are PGPB from saline habitats as Bacillus to fix nitrogen, dissolve phosphate, and create IAA. Their application as biofertilizers and biological control agents enhances mineral content, plant tolerance to environmental challenges, protects plants from infections, N elements, P elements, and the formation of indole-3-acetic or IAA.

3.3.1. Available nutrients in soil after 30- days of planting in saline soils:

Table (5) showede the availability of soil N, P, and K after 30 days. All treatments considerably increased available N, P, and K in soil when compared to the control treatment. The combination inoculation treatment (B. safensis + C. firmus) produced the highest soil N, P, and K values. Meanwhile, in the *B. safensis* and *C. firmus* treatments, N, P, and K values in the root rhizosphere rose as compared to the control. Sandy soil had the maximum nutrient content (N, P, and K) at 150.5, 31.74, and 164.5 mg kg-1, while clay loamy soil had 172.53, 38.15, and 184.57 mg kg-1.

Plants require macronutrients (N, P, K) and micronutrients (Fe, Zn, B, Cl, Mo, Mn, Cu, and Ni) for optimal growth [49]. As biofertilizers, inoculants of HT-PGPR supply plants with additional necessary and trace elements for improved plant growth and development, which if deficient, this has an unfavorable effect on plants, resulting in lower yield and tolerance in agricultural plants [50].

In table (5), data showed a considerable increase in N, P, and K elements in the inoculation treatment compared to the control. Dual inoculation (B. safensis and C. firmus) resulted in a more significant rise. The data provided above confirm that our isolates can manufacture phytase enzymes. In our pot experiment, results in table (5) showed that adding phosphate in the soil increased zinc analysis marginally. Sand soil and clay loamy soil had the highest zinc levels (3.23 and 3.7 mg kg-1, respectively).

These findings supported the importance of *B. safensis* and *C. firmus* strains as the most potent phosphate solubilizes. Phosphorus is a key ingredient that inhibits zinc uptake in soil. When the ratios of P and Zn are within legal limits, they may work synergistically in plant processes such as root growth, photosynthesis, and respiration. [51].

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Table 5. Available macronutrients (N, P, K) and Zinc (micronutrient) in soil after 30 days of planting barley plant in saline soils (sandy and clay loamy).

Soil type	Treatment	Nutrients (mg/Kg)							
		N		P		K		Zn	
		Mean	rank	Mean	rank	Mean	rank	Mean	rank
sandy	Control	54.10	d	8.58	g	66.83	h	0.85	f
	B safensis	101.83	f	12.06	e	100.61	g	1.25	e
	C. firmus	128.13	d	28.20	c	124.64	d	2.75	c
	B. safensis + C. firmus	150.50	b	31.74	b	164.50	b	3.28	b
clay loamy	control	70.40	d	10.39	f	107.22	f	1.29	de
	B safensis	121.03	e	13.48	e	121.11	e	1.41	d
	C. firmus	134.50	c	32.21	b	145.26	c	3.20	b
	B. safensis + C. firmus	172.53	a	38.15	a	184.57	a	3.70	a
LSD 0.05		2.974		1.084		3.431		0.127	

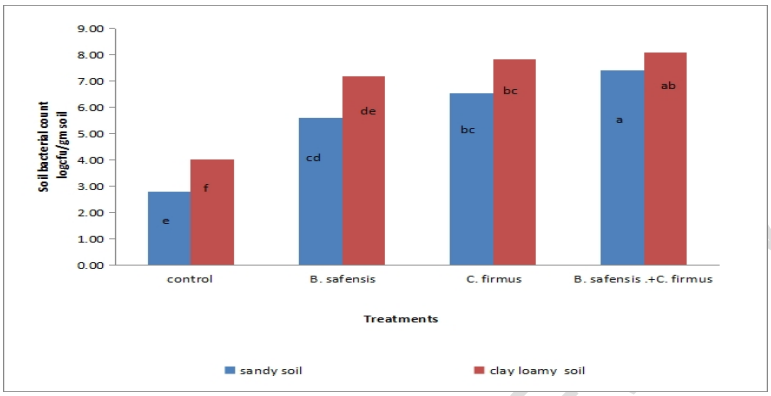
In contrast, excessive phosphorus levels may reduce the availability of zinc. P and Zn are both required nutrients for plant growth, but their combined effect may be antagonistic at some levels, particularly when soil-P is applied at a higher rate. [52]. Overall, our findings indicated that a comprehensive understanding of the effects of P administration on Zn absorption, translocation, and remobilization is currently lacking.

3.3.2. Bacterial count in soil after 30- days of planting in saline soils:

The reduction in microbial count with increasing salt concentration illustrated the possible impact of increased salt concentrations [53].

Figure (4) showed that after 30 days, total bacterial counts were considerably higher in clay loamy soil treatments than in sandy soil treatments. Furthermore, data revealed that the mixed inoculation treatment (*B. safensis + C. firmus*) had the highest total bacterial count in both sandy and clay loamy soils when compared to the control. The highest values of total bacterial counts were 7.40 and 8.10 Log cfu/g soil for sandy soil and clay loamy soil, respectively. Individual inoculation treatments yielded lower numbers than the combination treatment. Total bacterial counts increased considerably in the *(B. safensis)* and (*C. firmus*) treatments compared to the control treatment. These findings are consistent with those obtained by Plants treated with biofertilizers had higher total bacterial numbers and phosphate-solubilizing bacteria in their rhizospheres than the untreated control group. Increased bacterial populations may be attributed to nutrient availability in the rhizosphere of biofertilizer-treated plants, which provide the necessary energy for soil microbes to breakdown organic materials. Inoculation with biofertilizers boosted overall microbial densities and CO2 levels in the rhizosphere [54]. Additionally, these findings are consistent with a comparable study [55] that found biofertilizers enhanced bacterial numbers in wheat rhizospheres. The addition of PSB may enhanced the community function of rhizosphere bacteria, particularly phosphorus-cycling capabilities. This behavior may be attributed to enhanced nutrients provided by PSB inoculation, which in turn changed the rhizosphere bacterial population composition and function [56].

Fig. 4. Bacterial count in soil after 30-days of planting in saline soils



3.3.3. Barley photosynthetic pigment and proline content:

Chlorophyll content in Barley crop, a fundamental physiological marker of a plant's photosynthetic capability, was significantly altered by the two bacterial strains (*B. safensis*) and (*C. firmus*) inoculation. Figure (5) shows that the concentrations of parley chlorophyll and cartenoid increased significantly following HT-PGPR treatment. This effect, manifested as more chlorophyll in the end product, is most likely caused by increased nutrient intake and decreased Na concentrations in the plants. The data show that it was pre-inoculated with *Bacillus safensis* (*B safe*nsis) and *Cytobacillus firmus* (*C. firmus*). Clay loam soil contained more barley total chlorophyll and carotenoid concentrations than sandy soil.

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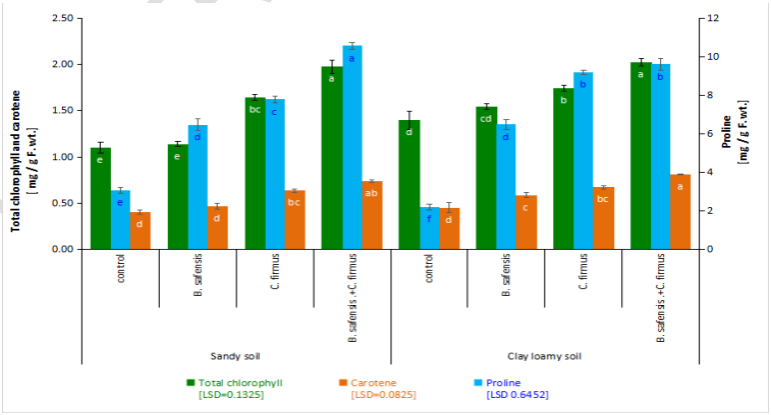
Fig. 5. Photosynthetic pigments and Proline contents of Barley plant after 30 days of planting in saline soils (sandy and clay loamy).



Furthermore, *C. firmus* produced better outcomes than *B. safensis* alone, whereas the combination outperformed both treatments. This is consistent with [36]. Microbial phytases are regarded to be a precise means of increasing plant growth and productivity on a worldwide scale. HT-PGPR are recognized as highly effective alternatives to synthetic fertilizers because to their simplicity, non-toxicity, environmental friendliness, and cost-effectiveness. [57] observed that the chlorophyll content of infected and non-inoculated plants at 0 mM NaCl was not significantly different. When the NaCl concentration grew in the bacterially infected plant, the chlorophyll content decreased, making it less than that of the non-inoculated plant. Previous investigations revealed similar findings [58].

Proline is well-known for its roles as an osmoprotectant, ROS scavenger, and plant cell subcellular structural stabilizer. Plant proline levels were also examined to determine the extent of cell osmotic adjustment in leaves caused by salt. However, as the plants experienced salt stress, their proline levels climbed. *Bacillus safensis* and *Cytobacillus firmus* inoculation increased proline levels in sandy and clay loamy soils compared to the control group. Plant stress tolerance can be increased through endogenous proline supplementation, changes in proline levels caused by salt stress. Plants can protect themselves against free radical damage by storing proline in salty conditions, where it worked as an osmolyte, regulating osmotic equilibrium, stabilizing proteins and membranes, and preventing cell death [59]. Our findings showed that two mixed inoculations in sandy and clay soils significantly altered leaf proline contents (10.56, 9.60 mg proline/g F.wt.) (Fig. 5). Finally, the co-inoculation of *Bacillus safensis* and *Cytobacillus firmus* resulted in higher proline concentrations than individual or non-inoculated (control) plants.

Examination of plant health in terms of photosynthetic pigment content (chlorophyll and carotenoids), and proline content, supporting the effect of the plant growth-promoting activities seen in each of the halotolerant PGPB strains. [38] While the presence of PGPB promoted higher levels of chlorophyll and carotenoid in the plants, the levels in the salinity stressed conditions were lower than those of non-inoculated plants, likely reflecting sodium accumulation, which decreases chlorophyll and reduces uptake of essential elements [60].]

3.3.4. Macronutrients total content in plant after 30- days of planting in saline soils:

Salinity is a big issue since agriculture with irrigation provide a considerable amount of food to the world [61]. Previous research had indicated that agricultural products of the most economical crops, such as rice, corn, wheat, and barley, are reduced by 70% due to salt stress [62]. The use of HT-PGPR has recently emerged as a "green" option for problems linked with rising soil salinity in agriculture [63].

[45] stated that PSB produce indole acetic acids (IAA), which IAA boosted growth parameters photosynthesis and NPK concentration in soil, affecting the total growth of the plant.

PSB's availability of Phosphorus made it vital for plant processes involving energy transfer such as photosynthesis, sugar transformation, and nutrient movement [38].

These halotolerant PGPR reduce salinity stress by a variety of mechanisms, including improved water absorption capacity, osmolyte buildup (proline and soluble sugars), and higher antioxidant levels [64]. By solubilizing phosphorus and fixing nitrogen, they can directly improve plant nutrient intake from their surroundings and crop productivity [5] in soils with low fertility and high salinity (9). They also created phytohormones such as indole acetic acid (IAA), which regulate plant growth.

Each treatment is assessed using many characteristics, including nitrogen (N%), phosphorus (P%), potassium (K%), and zinc (Zn mg/kg).

The provided table compared various inoculations on sandy and clay loamy saline soil. Several data were used to evaluate each treatment, including nitrogen (N%), phosphorus (P%), and potassium (K%).

The Salt stress lowers available phosphorus absorption, resulting in nutritional imbalance. Although barley is a salt-tolerant plant, some microorganisms should be supplied as adjuvants at the rhizosphere to increase productivity and improve barley macronutrient absorption, such as phosphorus. In this regard, it is useful to introduce salt-tolerant (PSB) that can excrete acid phosphatases and phytases, which are beneficial for converting phosphate from an insoluble form to a soluble one that may be absorbed by roots. [18]

The P-values for all parameters are less than 0.001, indicating that the differences between treatments are significant. This implied that the treatments had a significant effect on the measured parameters in saline soil.

Table (6) demonstrated that in sandy and clay loam soil, dual inoculation (*B. safensis* + *C. firmus*) (2.51, 3.50%) has the maximum nitrogen content, which is much greater than individual inoculation. While the control (1.10, 1.44) has the lowest value. As expected, the p and k analyses of the treatment (*B. safensis* + *C. firmus*) yield the greatest values (0.55,0.59,1.99, and 3.41%) for sandy and clay loamy soils, respectively. Individual inoculation (*C. firmus*) is then performed, yielding (0.512, 0.527, 2.2, and 3.27%), respectively.

These findings are consistent with [65], who found that phosphate solubilizing bacteria (PSB) can improve P management under salinity stress in terms of plant components as well as N, P, and K uptake in barley.

Table 6. Macronutrients total content (NPK %) of barley plant after 30- days of planting in saline soils.

Soil type	Treatment	N %	P %	K %
Sandy Soil	**control**	1.10 f	0.219 d	1.67 e
	B. safensis	1.83 d	0.460 c	2.1 d
	C. firmus	2.09 c	0.512 bc	2.25 c
	B. safensis .+C. firmus	2.51 b	0.555 ab	2.99 b
Clay loamy Soil	**control**	1.44 e	0.269 d	2.03 d
	B. safensis	2.40 b	0.476 bc	3 b
	C. firmus	3.37 a	0.527 a-c	3.27 a
	B. safensis .+C. firmus	3.50 a	0.594 a	3.41 a
LSD 0.05		0.169	0.059	0.144

[66] revealed that the mix. inoculation consistently exhibited the highest values across all parameters in both situations, indicating overall higher performance. The control treatment had the lowest values for these parameters , especially in the saline soil. These findings are comparable to those of [38], who observed that plants inoculated with each of the strains demonstrated increased growth compared to non-inoculated plants under saline conditions.

Our data supported the validity of the results, as *B. safensis* and *C. firmus* proved their ability to fix nitrogen, solubilize phosphorus, and produce phytase. [67] This contributes to enhanced phytate-P consumption by HT-PSB, lowering P resource inputs and pollution concerns to the surrounding environment [68]. Furthermore, the positive effects of microbial symbiosis on barley root growth, as well as water and nutrient absorption [18].

CONCLUSION

Bacillus safensis [OR341132] and *Cytobacillus firmus [OR431197]* are the most potent indigenous microbial strains isolated from Mangrove soil, demonstrating their superiority as halotolerant plant growth promoting rhizobacteria [HT-PGPR] and halotolerant phosphate solubilizing bacteria [HT-PSB] due to their ability to tolerate salt concentrations up to 10%, fix nitrogen, solubilize phosphorous, hydrolyze phytate, and produce IAA. These strains can be employed as PGPB biofertilizers to increase bacterial count in soil, photosynthesis, and NPK concentration in soil and plant, all of which affect the overall growth of barley [genotype Giza123] under salt stress. These strains enhance P management in barley under salinity stress. Another advantage of bio-inoculators is that they are highly efficient and may be used multiple times.

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