Novel Research in Microbiology Journal (2024), 8(3): 2469-2490

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/

Research Article

DOI: 10.21608/NRMJ.2024.293974.1607

Plant growth-promoting rhizobacteria: selective screening and characterization of drought-tolerant bacteria isolated from drought-prone soils

Osama M. Ghanem[1]; Nehal Kamal[2]; Alaa F. Said[3*]

[1]Soil and Water Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt; [2]Department of Botany and Microbiology, Faculty of Science, Suez University, Suez 43512, Egypt; [3]Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt

[*]Corresponding author E-mail: alaa.fathalla@yahoo.com

Received: 26 May, 2024; Accepted: 23 June, 2024; Published online: 25 June, 2024

Abstract

Plant growth promoting rhizobacteria (PGPR) have evolved to thrive in challenging environments and provide the plants with defense against the harmful impacts of the environmental stressors. The present study aimed to isolate, screen, and identify PGPB obtained from the rhizosphere soil of some field crops at different sites in Ismailia, Egypt. Isolation process was carried out from drought-suffered locations to obtain efficient promising strains adapted to carry out vital processes under an irrigation water shortage. Eight bacterial isolates were selected and identified by phenotypic properties, and were subjected to screening procedures to assess their growth capabilities and evaluate their potential as PGPB. The screening process involved investigating various PGPB features, such as phosphate solubilization, formation of indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN) production. Among the eight isolates, two isolates only (MW3 and AB3) gave positive results for all the tested plant growth promoting traits. The promising isolates were identified by sequencing of their16S rRNA as *Arthrobacter globiformis* (MW3) and *Micrococcus luteus* (AB3). A field trial was conducted to evaluate the activity of the two PGPB and their mixture to act as biofertilizers for maize under deficit irrigation 0.75 from crop evapotranspiration (ETc). All tested inoculants significantly increased yield components of maize, NPK uptake by plants, availability of N and P in soil, activity of some soil enzymes, and total bacterial counts compared to the un-inoculated control. Utilization of stress adapting PGPR showed great potential in overcoming the challenges of sustainable agriculture under environmental stress conditions.

Keywords: Drought stress, PGPB, Maize, Soil enzymes, 16S rRNA sequencing

1. Introduction

Abiotic stresses, such as drought, salinity, severe temperatures, and pollutants such as pesticides and heavy metals, lead to substantial economic losses by significantly affecting the plant development and output. Drought is a main environmental issue that is currently posing a challenge to the majority of countries worldwide, as it reduces plant growth and yield, thus affecting the agricultural and food industries. Over the past 50 years, drought stress is predicted to have reduced grain production by 10 %, with further productivity losses predicted for over 50 % of arable land by 2050 (Akhtar *et al*., 2021; Koza *et al*., 2022). Water deficit occurs when evaporation from leaves surpasses root absorption, leaving the plant to suffer from shortage of water that it needs to survive. Many aspects of plant biology, including morphology, physiology, biochemistry, ecology, and molecular processes, are influenced by this issue. Water deficiency is a key source of plant stress, slowing plant growth in many ways, including photosynthesis, hormone production, membrane integrity, etc... (Azeem *et al*., 2022).

Therefore, it is imperative to explore several methods to mitigate this risk by improving plant development in drought-prone environments (Chukwuneme *et al*., 2020). In recent times, there has been a growing trend of the employment of environmentally beneficial techniques, such as plant growth promoting bacteria (PGPB) to enhance the agricultural sustainability (Chiaiese *et al*., 2018; Michavila *et al*., 2022). PGPR improve plant defenses and yield, enhance produce quality, and alleviate plant stress (Shukla *et al*., 2019; Almeida *et al*., 2024). However, stress tolerance is possibly the most important advantage of the PGPR (Paul *et al*., 2019; Ajijah *et al*., 2023).

There are numerous ways by which microorganisms can be used in agriculture, with the primary goal of replacing the nutrients and synthetic pesticides Enhancement of utilization of microorganisms as an integral component of the agricultural system can improve sustainable crop output by supporting the plant drought tolerance (Chieb and Gachomo, 2023; Kálmán *et al*., 2023). It has been demonstrated that inoculation with PGPB increases stress tolerance in plants growing under extreme stress conditions by lengthening the roots and improving the plants' ability to acquire water (Kang *et al*., 2014; Cohen *et al*., 2015). Several processes help bacteria resist drought damage, including generation of phytohormones such indole-3-acetic acid (IAA) and gibberellins (GA), acting as biosurfactants and siderophore's producers (Wang *et al*., 2014; Ferioun *et al*., 2023). Many distinct PGPB genera have been identified and characterized by their ability to promote plant development and reduce the negative effects of drought on plants such as *Pseudomonas* spp. from sunflower (Sandhya *et al*., 2009), *Bacillus* spp. and *Paenibacillus* spp. from finger millet, sunflower and maize (Vardharajula *et al*., 2011), and *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, and *Staphylococcus* from wheat (Verma *et al*., 2016). Our primary study objective was to explore how soil microbiotas can enhance plant drought resistance under drought stress conditions. The hypothesis suggests that soil from drought-suffered locations may have greater quantities of microorganisms that can adjust to the osmotic changes, thus potentially enhancing crop yield by mitigating the impact of drought stress. In this study, the plant growth-promoting properties of new bacterial strains were evaluated and their contribution to improving maize plant growth was investigated.

2. Materials and methods

2.1. Collection of soil samples

Several soil samples were obtained from the rhizosphere of several crops viz., wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and faba

bean (*Vicia faba* L.) growing at different locations in Ismailia Governorate, Egypt. All locations were suffering from irrigation water shortage and the plants showed symptoms of drought stress.

2.2. Estimation of bacteria from rhizosphere soils by serial dilution technique

Bacterial isolation was performed from drought-suffering locations to obtain efficient promising bacterial strains adapting to carry out vital processes under these stress conditions. The isolation of bacteria was processed by serial dilution method according to Johnston and Booth, (1983). In the beginning 10 g sample of soil collected from the rhizosphere of selected plants had been mixed with 100 ml of sterilized dist. water to obtain a 10^{-1} dilution. Then, 1ml of the 10^{-1} dilution was transferred to 9 ml of sterilized water to create a 10^{-2} dilution. This process was repeated until dilutions reached 10^{-6} - 10^{-7}. One ml aliquot from the required dilutions (10^{-6} and 10^{-7}) was inoculated into nutrient agar (NA) petri plates, spread using a sterile glass spreader, and the plates were incubated for 24-48 h at 28 °C. Isolates with discernible colony characteristics were selected from the total bacterial count plates and transferred to NA plates. The streak plate procedure was utilized to acquire pure cultures (Aneja, 2013).

2.3. Morphological and cultural identification of the bacterial isolates

Bergey's Manual of Determinative Bacteriology (Buchanan *et al*., 1974) was used to characterize the purified bacterial isolates according to their morphological and biochemical characteristics. A freshly prepared culture of bacteria was mixed with 25 % sterile glycerol in a 1.5 ml Eppendorf and stored at -80 °C.

2.4. Screening for bacterial plant growth-promoting traits *in vitro*

2.4.1. Phosphate solubilization

Evaluation of phosphate solubilization effectiveness of the bacterial isolates was performed using Pikovskaya agar media, comprising in g/ l: glucose: 10; $Ca_3(PO_4)_2$: 5; $(NH_4)_2SO_4$: 0.5; NaCl: 0.2; $MgSO_4.7H_2O$: 0.1; KCl: 0.2; $FeSO_4.7H_2O$: 0.002; yeast extract: 0,5; $MnSO_4.2H_2O$: 0.002; agar: 15; and dist. H_2O: 1 l). In brief, the selected bacterial isolates were spotted in the middle of a Pikovskaya agar plate and incubated at 35± 2 °C for 48 h. Isolates showing positive phosphate solubilizing were detected by measuring the diameter of the clear zone developing around each bacterial growth (Jasim *et al*., 2013). There were three replicates for each bacterial isolate. Quantitative analysis of phosphate solubilization was performed by the method suggested by Nautiyal, (1999). To perform quantitative investigation of phosphate solubilization, the selected isolates (MW3, MW9, AB1, AB3, AB4, AB7, QF1, and QF5) were grown in Pikovskaya's broth medium containing tricalcium phosphate. After shaker incubation for 72 h at 35± 2 °C and filtration using Whatman filter paper no. 1, the crude supernatant was centrifuged at 60000 rpm for of 20 min. to eliminate the inactive constituents. To detect phosphate solubilization, the optical density (OD) was measured using a spectrophotometer (Jenway 6105, UK) at 882 nm and compared to a standard curve of KH_2PO_4 solution.

2.4.2. Screening for Indole-3-acetic acid (IAA)

Production of IAA by the bacterial isolates was screened by Salkowski reagent method (Patten and Glick, 2002). The isolates were cultured overnight in nutrient broth (NB) medium supplemented with L-tryptophan (0.1 %) and without L-tryptophan, incubated at 30 °C for 3 d before being centrifuged at 10,000 rpm for 10 min. Two ml of the supernatant were mixed with 4 ml of Salkowski's reagent for 10 min. (Patten and Glick, 2002). Appearance of a pinkish color indicated the successful production of IAA. For quantitative estimation of IAA production, the OD was measured using a spectrophotometer (Jenway 6105, UK) at 535 nm and compared to a standard curve.

2.4.3. Siderophore production

For qualitative detection of siderophore production by the bacterial isolates, Chromo Azurol-S (CAS) medium was used in reference to the assay conducted by Alexander and Zuberer, (1991). A fresh bacterial culture was spotted on CAS agar and incubated at 28 °C. After incubation for 72 h, positive siderophore production was indicated by the formation of an orange halo zone around the bacterial isolate, measured using a calibrated ruler. Observations were recorded on the basis of a 0-3 rating scale as follows: 0 = no zone; 1 = zone less than 1 mm; 2 = zone of 1−5 mm; and 3 = zone of 6 mm and above (Gopalakrishnan *et al*., 2015).

2.4.4. Hydrogen cyanide (HCN) production

The selected bacterial isolates were cultured incubated at 28± 2 °C for 2 d onto King's B agar medium supplemented with 4.4 g/ l glycine to detect their ability to produce HCN (Geetha *et al*., 2014). After incubation, Whatman no. 1 filter paper was immersed in a solution of 2 % sodium carbonate and 0.05 % picric acid and placed on the lid of the inoculated king's B petri plate. The two plate parts were wrapped using parafilm and incubated for 48 h at 28± 2 °C. Transformation of the filter paper's color from deep yellow to reddish-brown indicated a successful HCN production. The intensity of developing yellow color by the HCN producing bacteria had been classified into three categories; mainly: 3, high production (dark brown); 2, moderate production (moderate brown); 1, low production (light brown) (Devarajan *et al*., 2022).

2.5. Molecular identification of the selected bacteria

The bacteria that showed positive results for all in vitro tested plant growth-promoting properties were selected and identified based on their 16S rRNA genes sequence. Extraction of the genomic DNA was performed following the modified technique reported by Miller *et al*., (1999). In summary, a distinct bacterial colony was selected using a sterile toothpick and then placed in 50 µl of sterilized and deionized water. The cell suspension was incubated for 10 min. in a water bath at 97 °C, and centrifuged for 10 min. at 15000 rpm in order to extract the upper layer containing DNA. The DNA concentrated in the collected layer was determined by measuring its absorbance at 260 nm using a UV-spectrophotometer. The DNA was amplified using two universal 16S rRNA primers; 27F: 50-AGAGTTTGGATCMTGGCTCAG-30 and 1492R: 50-CGGTTACCTTGTTACGACTT-30. The PCR tube contained PCR buffer (1 x), $MgCl_2$ (0.5 mM), Tag DNA polymerase (2.5 U, QIAGEN Inc.), deoxynucleoside triphosphate (dNTP, 0.25 mM), universal primer (0.5 µM), and bacterial DNA (5 ng/ µl). The PCR cycling conditions consisted of an initial denaturation step at 94 °C for 3 min., followed by 30 cycles of denaturation at 94 °C for 30 sec., annealing at 55 °C for 30 sec., extension at 72 °C for 60 sec, and a final extension step at 72 °C for 10 min. The resulting PCR products were sequenced and BLAST was used to match the nucleotide sequences to the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Field experiment

2.6.1. Prior preparation for cultivation of maize

A field trial was carried out in the summer season of 2023 at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, to evaluate the effect of inoculation of rhizobacteria on maize plant yield, nutrient contents, and some soil enzyme activities. The experiment was laid out in a randomized complete block design with three replications for each treatment. Each plot consisted of three rows of 21 maize plants, with a spacing of 30 cm among them within each row, and a distance of 70 cm among the rows. The maize seeds (*Zea mays var*. Pioneer 3080) were cultivated in accordance with the Ministry of Agriculture's prescribed agricultural procedures. Compost was added to all treatments at a rate of 50 m^3/ ha. Superphosphate (50 kg P_2O_5/ ha) was well-mixed in each plot before planting. Potassium sulfate (50 % K_2SO_4) was added at a rate of 120 K_2SO_4 kg/ ha at two equal doses after 30 and 55 d

from cultivation. Ammonium sulfate (20.5 % N) was applied at three doses (20, 30 and 50 % of the total amounts, *i.e*. 285 kg N/ ha) after 20, 30 and 55 d from planting, respectively. The experiment was irrigated with Ismailia canal water (0.39 dSm). It included five treatments; mainly full irrigation water quantity (1.0 from crop evapotranspiration, ETc) without bacterial inoculants, 75 % of full irrigation water requirement (0.75 ETc) without bacterial inoculation, and under deficit irrigation (0.75 ETc) with three bacterial inoculants (*i.e*., *Arthrobacter globiformis* MW3, *Micrococcus luteus* AB3, and a mixture of both). Prior to planting, a comprehensive analysis was conducted on a composite soil sample and compost, to determine various qualities (Gee and Bauder, 1986; Sparks, 1996).

2.6.2. Irrigation schedule

Two levels of irrigation water quantities (1.0 and 0.75 from crop evapotranspiration, ETc) were used. The full irrigation water quantity was used without bacterial inoculation to represent the regular farming practices. Deficit irrigation (0.75 ETc) was used as a control without bacterial inoculants and in combination with three bacterial inoculants used to assess the ability of the tested rhizobacteria to help the maize plant resist irrigation water lack. Local potential evapotranspiration ETo was calculated using Penman-Monteith equation (Allen *et al*., 1998). The meteorological data utilized in this study were obtained from the CLIMWAT program (version 2.0). These data were subsequently integrated into the CROPWAT software (version 8.0), and used to estimate the crop water requirements (ETc) for maize cultivation in the region of Ismailia, Egypt, in reference to Clarke and Fryer, (1998); Muñoz and Grieser, (2006).

2.6.3. Inoculants preparation and seed inoculation for cultivation

Rhizobacterial inoculants were cultivated in 400 ml of sterilized Nutrient agar (NA) medium. The inoculant for each strain was prepared by taking a little amount from the stock culture followed by incubation at 28 $^{\circ}$C for 72 h. Currently, the viable cell counts in cell suspensions of all the rhizobacterial strains ranged from 10^7-10^8 cfu/ ml. In order to initiate inoculation, the seeds of maize (*Zea mays cv*. Pioneer 3080) undergone a surface sterilization process by immersing in a solution of 95 % ethanol for a 5 min., and rinsed three times with sterilized water (Jacobson *et al*., 1994). The surface sterilized seeds were soaked in 400 ml cell suspension of each bacterial strain for 1 h prior to planting. In the experimental setup involving the un-inoculated control, the sterilized seeds were immersed in 400 ml of NB medium.

2.6.4. Samples collection

Three plant samples from each treatment were collected for laboratory analyses, dried at 65 °C and analyzed for total NPK. Also, three soil samples for each treatment were collected after 30 d (vegetative stage), 50 d (flowering stage), and 95 d (ripening stage) from sowing and analyzed for enzymatic activities (*i.e*., urease, and acid and alkaline phosphatases) and total bacterial counts. Soil pH and available NPK were determined in the soil after plant harvest.

2.6.5. Plant analyses

Grain and stover yields were assessed during the harvest period. The quantification of total nitrogen (N) in plant samples was conducted using the Kjeldahl method described by Bremner, (1996). On the other hand, determination of phosphorus (P) and potassium (K) contents involved a wet digestion process utilizing a mixture of nitric acid (HNO_3), sulfuric acid (H_2SO_4), and perchloric acid ($HClO_4$) in a volumetric ratio of 4:1:8. The spectrophotometric measurement of P was conducted using the molybdenum-blue method conducted by Jackson, (1973), whereas the flame photometric approach was employed to measure K content.

2.6.6. Soil chemical analyses

The soil pH values were determined by measuring soil-water suspensions (1: 2.5) using a pH meter (Jenway 3510, UK). Extraction of available inorganic nitrogen was conducted using a 2.0 M potassium chloride solution, and its quantification was performed following the Kjeldahl method described by Bremner, (1996). The analysis was performed on a 0.5 M $NaHCO_3$-soil extract using the Olsen method described by Kuo and Morgan, (1996). The exchangeable K^+ was extracted using 1 N ammonium acetate and measured using a flame photometrically (Jenway PFP7, UK).

2.6.6. Soil biological analyses

Some soil enzyme activities were detected as integrative indicators for soil health that could reflect the stimulated plant growth and increase in its resistance to various abiotic stresses. Urease activity (mg $NH4^+$-N released/ g of soil/ 2 h) was assayed according to Tabatabai, (1994) by measuring the concentration of $NH4^+$ after adding urea substrate to the soil before incubation at 37 $^{\circ}$C. Control samples were prepared without substrate to determine $NH4^+$ produced without adding urea. Acid and alkaline phosphatases (µg *p*-nitrophenol released/ g soil/ h) were assayed by determining P concentration in soil after incubation with *p*-nitrophenyl phosphate substrate at 37 $^{\circ}$C as described by Tabatabai, (1994). Total count of bacteria was quantified using the dilution plate technique involving Tryptone Soy Agar (TSA) medium (Starr et al., 1981).

2.7. Statistical analysis

All obtained data were subjected to analysis of variance (ANOVA). The least significant difference test (LSD) was applied to conduct comparisons among the means, with a significance level of $P< 0.05$. Correlations were computed using the SPSS Program Version 22.0.

3. Results

3.1. Isolation of the bacterial rhizosphere isolates

Eight bacterial isolates were designated symbols based on (i) the location of samples: El-Manayef (M), Abu-Khalifa (A), and El-Qantara el-sharqiya (Q); (ii) host plants: wheat (W), barley (B), and faba bean (F) as shown in Table 1.

3.2. Morphological and cultural identification

The morphology of the isolates was examined microscopically using Gram staining technique. The results revealed that three isolates were Gram-negative rods, two isolates were Gram-positive coccobacilli, a single isolate was Gram-positive cocci, and two isolates were Gram-positive rods. The isolates were subjected to characterization based on their biochemical properties, including oxidase activity, citrate assimilation, catalase activity, sugar fermentation, and nitrate reduction. The isolates were identified as following: MW3 and QF1 were identified as *Arthrobacter* sp., AB1and AB7 as *Bacillus* sp., AB3 as *Micrococcus* sp., MW9 and AB4 as *Pseudomonas* sp., and QF5 as *Serratia* sp. The obtained identification results are presented in Table (2).

3.3. PGP characteristics of the drought-tolerant bacterial isolates

The bacterial isolates displayed plant growth promoting traits that differed from each other. In the present investigation, qualitative determination of phosphate-solubilizing capacity of the tested isolates showed that seven isolates were able to solubilize $Ca_3(PO_4)_2$ (Fig. 1). Phosphate solubilization varied from 55.2 to 224 mg P / l and the optimal soluble P concentration released was recorded by the isolate MW3, while isolate AB7 possesses the lowest phosphate solubilization value. All selected isolates except for isolate QF5 synthetized IAA, with the highest amount produced by the isolate AB7 (Fig. 2). Maximum IAA production was recorded for isolate AB7 (8.77 and 14. 3 mg/ l) followed by isolate MW3 (7.06 and 13. 8 mg/ l) in the absence or presence of L-tryptophan, respectively. Isolate MW9 showed the lowest amounts (2.77 and 4. 83 mg/ l) in the absence or presence of L-tryptophan, respectively. Production

of HCN was assayed qualitatively. Our results showed that six isolates from eight produced HCN. In addition, results showed that six isolates produced siderophores measured by Chrome Azurol Sulphonate (CAS) assay. Among the isolates, the intensity of orange halo and its diameter showed a wide variation (Fig. 2). MW3 isolate had the largest orange halos, which was strongly correlated with siderophore production.

Table 1: Sample locations, cover vegetation, and bacterial isolate symbols of the rhizobacterial isolates

Location no.	Location name	Cover vegetation	Bacterial isolate symbol
I	El Manayef (M)	Wheat (W)	MW3, MW9
II	Abu-Khalifa (A)	Barley (B)	AB1, AB3, AB4, AB7
III	El-Qantara el-sharqiya (Q)	Faba bean (F)	QF1, QF5

Table 2: Morphological and biochemical characterization of the rhizobacterial isolates

Character	Location I (2 isolates)		Location II (4 isolates)				Location III (2 isolates)	
	Isolate MW3	Isolate MW9	Isolate AB1	Isolate AB3	Isolate AB4	Isolate AB7	Isolate QF1	Isolate QF5
Gram test	+	-	+	+	-	+	+	-
Shape	rod–coccus	rods	long rods	cocci	rods	medium rods	rod–coccus	rods
Endospore position	-	-	central	-	-	central	-	-
Aerobic	+	+	+	+	+	+	+	
Anaerobic	-	+	+	-	-	+	-	
Catalase	+	-	+	+	-	+	+	+
Oxidase	-	+	+	+	+	+	-	-
Nitrate reduction	-	+	-	-	+	-	-	+
Starch hydrolysis	-	-	+	+	-	+	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+
Lactose fermentation	-	-	-	-	-	-	-	-
Mannitol fermentation	+	-	-	+	-	-	+	+
Citrate assimilation	-	-	-	-	+	-	-	+
Urease	-	-	-	+	-	-	-	-
Methyl red	+	+	+	-	+	-	-	-
Voges-Proskauer	+	+	+	-	+	+	+	+
H_2S production	-	+	-	-	+	-	-	-
Possible genus	*Arthrobacter* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Micrococcus* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Arthrobacter* sp.	*Serratia* sp.

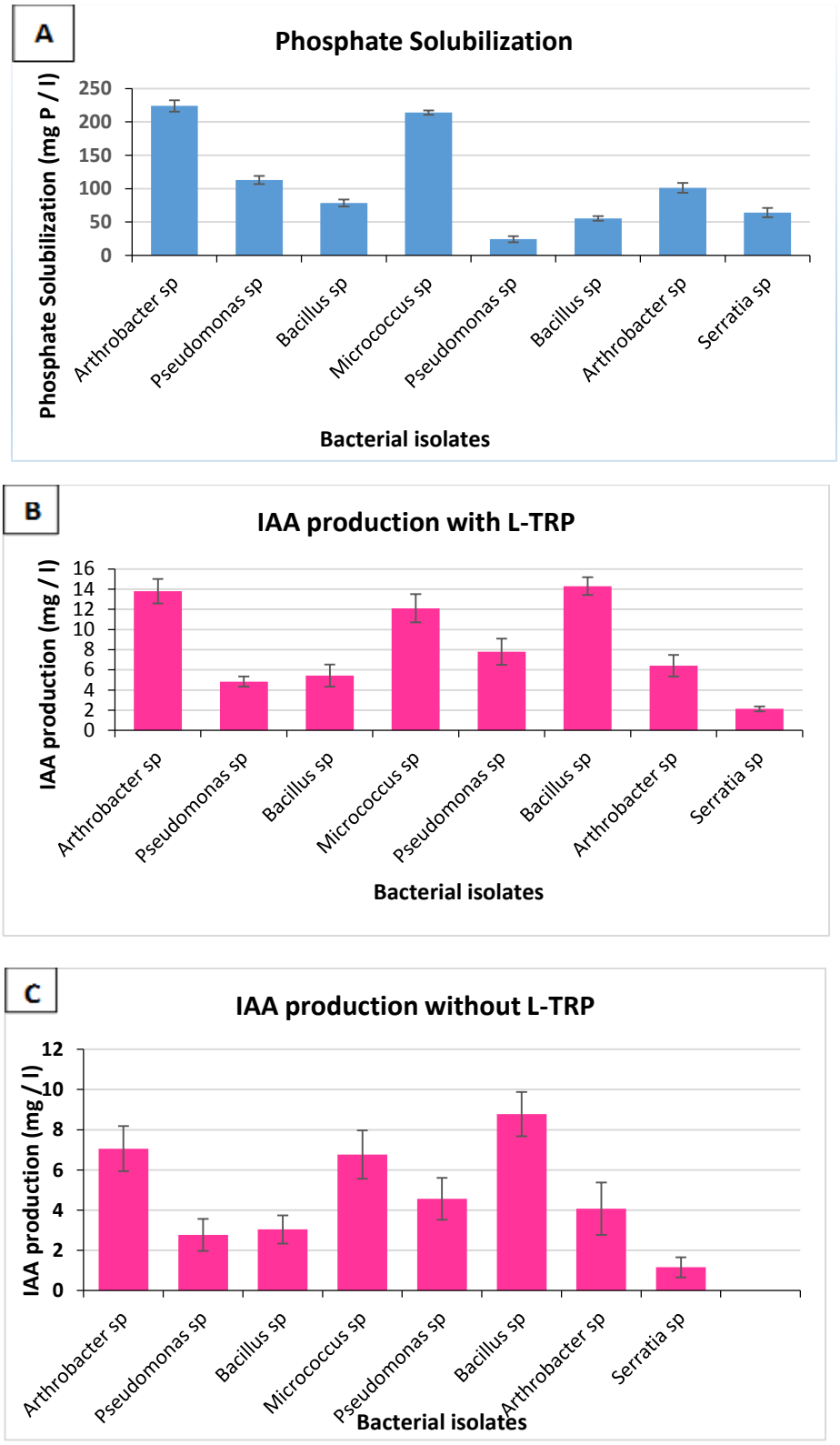

Fig. 1. Plant growth promoting activities of the selected bacterial isolates. (A) Tri-calcium phosphate solubilization of selected strains in Pikoskvaya medium after 48 h incubation, and (B and C) Indole acetic acid production by the selected isolates in NB medium with or without L-tryptophan (L-TRP). All tests were performed in duplicate with three replicates for each treatment

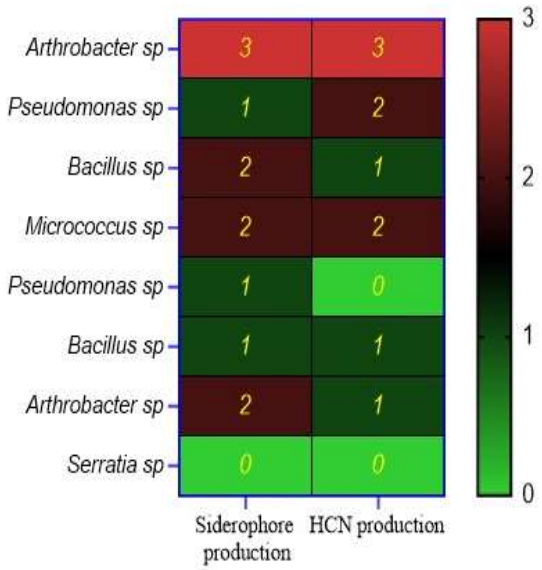

Fig. 2. Heat map of *in vitro* siderophore and HCN production assays of the selected bacterial isolates. Where; the green color (0) indicates no production, the green color (1) indicates low production, the red color (2) indicates moderate production, and the red color (3) indicates high production. The assays were repeated twice and carried out in triplicates

3.4. Molecular characterization of the selected isolates

Two bacterial isolates (MW3 and AB3) that gave positive results for all the studied plant growth promoting traits were selected for molecular identification by sequencing of their 16S rRNA. PCR amplification showed that the two investigated isolates have the amplified gene with an appropriate amount (Fig. 3). The isolate MW3 had 95 % similarity with *Arthrobacter globiformis* and assigned an accession number of NBRC 12137, while isolate AB3 had the closest genetic relationship 97 % with *Micrococcus luteus* with an accession number of CP033200.

3.5. Field experiment

3.5.1. Soil properties

Before planting, several physical and chemical analyses were conducted for the initial soil and compost used in this study as shown in Table 3.

3.5.2. Maize yield

Maize response to inoculation with both bacterial strains (*i.e*., *Arthrobacter globiformis* MW3 and *Micrococcus luteus* AB3) and a mixture of both under drought stress was evaluated in a field experiment. The maximum grain yield was attained under the treatment of 0.75 ETc+MW3 and AB3 where the increment over the corresponding un-inoculated control was 25.5 % and over the full irrigation treatment (1.0 ETc) alone was 3.9 %. Similarly, the maximum stover yield was obtained under the same above-mentioned treatments where the increment over the respective un-inoculated control was 29.4 % and over the full irrigation treatment alone was 3.95 %. Similarly, the highest biological yield (grain plus stover yields) was observed under the treatment 0.75 ETc+MW3&AB3 where the increase over the corresponding un-inoculated control was 28.01 % (Table 4).

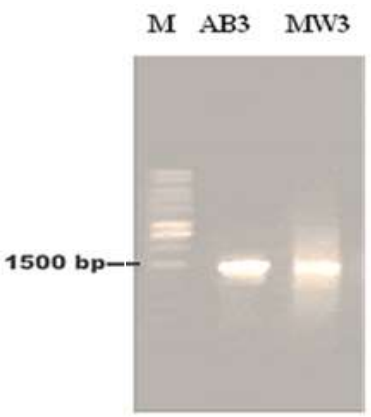

Fig. 3. Analysis of PCR products using agarose gel electrophoresis. Where; M: marker; AB3: *Micrococcus luteus*, and MW3: and *Arthrobacter globiformis*. AB3 strain had a single band at 1450 (bp) and MW3 had a band at1400 bp

Table 3. Physio-chemical properties of the soil and compost used in this study

Properties	**Soil**	**Compost**
Particle size distribution (%)		
Sand	95.05	-
Silt	3.35	-
Clay	1.60	-
Textural class	Sand	-
Bulk density (g/ cm^{-3})	1.56	-
Soil order	Aridisols	-
Field Capacity (%)	17.20	-
pH	8.13*	7.40**
EC (dS/ m) ***	1.35	9.55
Soluble cations (meq/ l) ***		
Ca^{2+}	6.92	23.4
Mg^{2+}	4.13	10.2
Na^{+}	2.45	29.4
K^{+}	0.31	32.5
Soluble anions (meq/ l) ***		
CO_3^{2-}	0.00	0.00
HCO_3^{-}	1.51	30.2
Cl^{-}	6.21	55.7
SO_4^{2-}	5.78	9.60
Organic C (g/ kg)	1.65	196
Total N (g/ kg)	0.106	18.1
Available N (mg/ kg)	5.49	182
Available P (mg/ kg)	8.16	155

Where: *pH value measured in soil-water suspension (1:2.5); **pH value measured in compost-water suspension (1:5); ***Electric conductivity (EC) and soluble cations and anions values determined in the soil or compost saturated paste extracts

Table 4: Effect of irrigation water quantities and PGPR inoculants on grain, stover, and biological yields (Mg/ ha) of maize plants

Treatments		Grain yield	Stover yield	Biological yield
Water requirement	**Biofertilizer**			
1.0 ETc	Un-inoculated	8.34	17.71	26.05
	non inoculated	6.91	14.23	21.14
0.75 ETc	MW3	8.56	17.42	25.98
	AB3	8.28	16.86	25.14
	MW3+AB3	8.67	18.41	27.08
L.S.D. $_{0.05}$		1.32	2.56	2.69

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference

3.5.3. Nutrient content in maize plants

Regarding the impact of bacterial inoculants on the nitrogen (N), phosphorus (P), and potassium (K) concentrations in maize plants, the findings are presented in Tables 5 and 6, which demonstrate that there were no significant differences in nutritional concentrations of both of the grains and stover as a result of bacterial inoculation. However, it was observed that the uptake of the three nutrients by grains and stover was greatly increased by bacterial inoculation compared to the control group that was not infected. Application of the rhizobacterial inoculants and deficit irrigation treatments resulted in a considerable increase in the absorption of NPK nutrients by both the grains and stover, compared to the use of deficit irrigation alone at 0.75 ETc. However, no significant difference was recorded between the three bacterial inocula treatments and the full irrigation treatment alone (Tables 4 and 5).

3.5.4. Soil pH and available NPK

In general, it was observed that the pH values were significantly decreased as a result of seed inoculation with the investigated rhizobacterial strains and/ or their combination, compared to the control group that was not inoculated (Table 7). The pH values were at their lowest values of 7.59 and 7.66, for the treatment 0.75 ETc+MW3 & AB3 and 0.75 ETc+MW3, respectively. Results presented in Table (3) show that comparing to the initial soil pH value of 8.13, the reductions in pH values in the above-mentioned treatments were of 0.54 and 0.47, respectively.

Regarding the impact of bacterial strains on availability of nitrogen in the soil, it can be noted from Table 7 that all strains exhibited a considerable increase in the levels of available nitrogen compared to the non-inoculated control group. However, no significant distinction was observed between the various bacterial inoculants employed. This study detected a considerable rise in soil phosphorus (P) levels due to the application of bacterial inoculants compared to the control group. However, the variations between the different bacterial strains did not always provide statistically significant changes. According to Table (7), inoculation of the bacterial strains did not result in a significant increase in soil potassium (K) levels compared to the non-inoculated control group. The most significant levels of soil N were found in the treatments of 0.75 ETc+ MW3&AB3 and 0.75 ETc+MW3. The increase in soil N compared to the un-inoculated control was 18.3 % and 15.1 %, respectively. Similarly, the maximum levels of available P in the soil were recorded under the treatments 0.75 ETc+MW3& AB3 and 0.75 ETc+MW3, where the increase over the un-inoculated corresponding controls were 33.9 % and 28.7 %, respectively (Table 7).

Table 5: Effect of irrigation water quantities and rhizobacterial inoculants on the concentrations (g/ kg) and uptake (g/ plant) of NPK in maize grains

Treatments		N		P		K	
Water requirement	**Biofertilizer**	**conc.**	**uptake**	**conc.**	**uptake**	**conc.**	**uptake**
1.0 ETc	non -inoculated	21.2	3.65	4.23	0.908	17.8	2.76
0.75 ETc	non -inoculated	20.9	2.81	4.08	0.621	17.3	1.96
	MW3	21.4	3.89	3.97	0.891	16.6	3.11
	AB3	20.8	3.76	4.12	0.922	17.5	2.62
	MW3+AB3	20.9	4.07	4.11	1.11	16.9	3.05
L.S.D. $_{0.05}$		ns	0.717	ns	0.226	ns	0.532

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference; ns: non-significant

Table 6: Effect of irrigation water quantities and rhizobacterial inoculants on the concentrations (g/ kg) and uptake (g/ plant) of NPK in maize stover

Treatments		N		P		K	
Water requirement	**Biofertilizer**	**conc.**	**uptake**	**conc.**	**uptake**	**conc.**	**uptake**
1.0 ETc	non inoculated	33.5	13.7	3.21	0.754	12.2	4.65
0.75 ETc	non inoculated	33.7	11.3	2.91	0.396	12.7	3.18
	MW3	33.2	14.1	3.41	0866	11.9	4.88
	AB3	33.5	13.4	3.09	0.769	13.4	4.12
	MW3+AB3	33.9	14.5	2.80	0.859	12.8	4.93
L.S.D. $_{0.05}$		ns	1.85	ns	0.289	ns	0.693

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference; ns: non-significant

Table 7: Effect of irrigation water quantities and rhizobacterial inoculants on soil pH and NPK availability (mg/ kg) in soil

Treatments		Soil pH	Available nutrients		
Water requirement	**Biofertilizer**		**N**	**P**	**K**
1.0 ETc	non inoculated	7.91	9.12	12.8	188
0.75 ETc	non inoculated	7.95	9.38	11.5	197
	MW3	7.66	10.8	14.8	201
	AB3	7.72	10.3	13.6	207
	MW3+EW3	7.59	11.1	15.4	199
L.S.D. $_{0.05}$		0.302	1.03	1.18	ns

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference; ns: non-significant

3.5.5. Enzyme activities in the soil

In the current study, the activities of urease, acid and alkaline phosphatases were determined for their crucial roles in N and P cycles in the soil The activity of the three enzymes progressively rose over time, peaking after 50 d of maize culture and subsequently declined by 95 d. Results presented in Tables (8-10) show that all bacterial inoculants significantly improved the activities of the three enzymes compared to the un-inoculated controls; however, the differences among the three bacterial inoculants were not always significant. Table 8 shows that the activity of urease reached its maximum levels in the soil after 30, 50 and 95 d from maize sowing under the treatment 0.75 ETc+MW3, where the increases over the un-inoculated corresponding control were 21.3, 26.4 and 24.6 %, respectively. According to the data presented in Tables (9, 10), the alkaline phosphatase consistently exhibited higher levels of activity compared to the acid phosphatase across all soil samples. Under the treatment of 0.75 ETc+MW3 at 30, 50, and 95 d after maize sowing, higher activities of alkaline phosphatases were recorded in the soil up to 170, 207 and 112 µg ρNP/ g soil/ h, respectively. However, no statistically significant difference was observed between this treatment and the 0.75 ETc+MW3 and AB3 treatment.

3.5.6. Total bacterial counts in the soil

The results shown in Table 11 display the recorded quantities of bacteria found in the rhizosphere soil samples of maize plants at 30, 50, and 95 d following the first sowing. In general, the total bacterial counts in all the inoculated rhizosphere soil samples showed an increase compared to the un-inoculated control. Furthermore, the bacterial counts were consistently higher after 50 d of maize planting compared to the counts at 30 or 95 d. The highest bacterial count was recorded in the soil after 50 d from sowing under the treatment of 0.75 ETc+MW3 and AB3, where the increase over the un-inoculated corresponding control was 32.8 %.

4. Discussion

Drought is a significant environmental challenge that has the capacity to diminish plant growth and agricultural yields (Bhanbhro *et al*., 2024). Similar decreases in plant growth and photosynthetic pigments due to drought have been previously reported in various crops, including soybean (Nguyen *et al*., 2023), and maize (Jing *et al*., 2023). Several ecofriendly strategies have been employed to promote agricultural sustainability (Chiaiese *et al*., 2018), including biostimulants. Biostimulants promote plant defense, yield, fruit quality, and stress resistance (Msimbira and Smith, 2020). Plant growth-promoting bacteria (PGPB) inoculation increases stress tolerance, promotes plant growth, and mitigates the negative effects of drought (Enebe and Babalola, 2018; Xiong *et al*., 2021; Bouremani *et al*., 2023). Water stress exerts a direct impact on the soil microbiota by promoting the proliferation of stress-tolerant microbial species. Furthermore, plants have the ability to impact the composition of their rhizosphere microbiota under stressful conditions through alterations in the profile of compounds released from their roots (Naylor and Coleman-Derr, 2018). Application of PGPB to agricultural crops has been found to be a highly efficacious strategy for augmenting the plant stress tolerance. The rapid onset and recurrent occurrence of water stress during crop production provide significant challenges for plants in terms of their adaptation to the stress and sustaining optimal quality. Hence, the capacity of PGPB to establish colonization of the plant roots and subsequently induce plant stress responses is an advantageous approach for pre-conditioning the crops in an anticipation of stress initiation. The utilization of stress-tolerant bacteria and their capacity to impart water stress resistance to plants has been firmly demonstrated (Hussain *et al*., 2014). This inoculation would be most effective in the drought-stressed soils if the bacteria were isolated from arid soils or drought-tolerant plants (Niu *et al*., 2018; Astorga-Eló *et al*., 2021).

Table 8: Effect of irrigation water quantities and rhizobacterial inoculants on the activity of urease in the soil after 30, 50 and 95 d from maize cultivation

Treatments		**Urease (mg NH_4^+ -N released/ g of soil/ 2h)**		
Water requirement	**Biofertilizer**	**30 d**	**50 d**	**95 d**
1.0 ETc	non inoculated	307	314	243
0.75 ETc	non inoculated	301	299	236
	MW3	365	378	294
	AB3	348	362	279
	MW3+AB3	362	374	292
L.S.D. $_{0.05}$		16.74	17.09	22.92

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference

Table 9: Effect of irrigation water quantities and rhizobacterial inoculants on the activity of acid phosphatase in the soil after 30, 50 and 95 d from maize cultivation

Treatments		**Acid phosphatase, (µg *ρ*NP/ g soil/ h)**		
Water requirement	**Biofertilizer**	**30 d**	**50 d**	**95 d**
1.0 ETc	non inoculated	60.6	80.1	51.3
0.75 ETc	non inoculated	53.6	64.9	42.0
	MW3	98.3	118	67.2
	AB3	84.5	98.7	57.6
	MW3+AB3	95.9	114	65.1
L.S.D. $_{0.05}$		11.21	16.44	9.74

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference

Table 10: Effect of irrigation water quantities and rhizobacterial inoculants on the activity of alkaline phosphatase in the soil after 30, 50 and 95 d from maize cultivation

Treatments		**Alkaline phosphatase, (µg *ρ*NP/ g soil/ h)**		
Water requirement	**Biofertilizer**	**30 d**	**50 d**	**95 d**
1.0 ETc	non inoculated	116	143	82.1
0.75 ETc	non inoculated	97.7	114	72.8
	MW3	170	207	112
	AB3	148	173	97.3
	MW3+AB3	169	204	109
L.S.D. $_{0.05}$		19.69	25.11	13.25

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference

Table 11: Counts of total bacteria (cfu/ g soil) in the rhizosphere of maize after 30, 50 and 95 d from sowing

Treatments		**Total bacteria (cfu/ g soil)**		
Water requirement	**Biofertilizer**	**30 d** **× 10^6**	**50 d** **× 10^6**	**95 d** **× 10^5**
1.0 ETc	non inoculated	5.8	6.9	5.1
0.75 ETc	non inoculated	5.4	6.1	4.2
	MW3	6.7	8.1	6.6
	AB3	6.1	7.9	6.2
	MW3+AB3	6.9	8.2	6.9
L.S.D. $_{0.05}$		0.654	0.773	0.721

Where; ETc: Crop Evapotranspiration; L.S.D: least significant difference

The most frequently mentioned PGPB strains of diverse genera, include *Pseudomonas*, *Klebsiella*, *Bacillus*, *Azotobacter*, and many *Pseudomonas* species, which exhibit a very high diversity of growth-promoting traits (Abdelaal *et al*., 2021). Therefore, many scientific laboratories are searching for such valuable drought-resistant isolates (Sarma and Saikia, 2014; Kumar *et al*., 2016).

Several bacterial and fungal species possess the capability to release inorganic phosphorus forms as a result of their creation of organic acids that facilitates the transfer of inorganic phosphates into a solution (Haney *et al*., 2018). Under drought stress, Ma *et al*., (2019) reported increased phosphatase activity in *Bacillus megatherium* and inorganic phosphate solubilization in *B. saryghattati* strains. The currently tested bacteria possessed the ability to dissolve the insoluble phosphates forms. Utilization of phosphate dissolving bacteria as biofertilizers has been reported to increase the availability of P, Fe, Mn, Zn, and Cu for plants and consequently increase the crop yield (Mahanta *et al*., 2014; Mukhtar *et al*., 2017).

The vital roles of IAA in plants include cell division, extension, promotion of seed germination, improving xylem and root development, and control of vegetative growth processes, which are proved to improve plant yield (Ahemad and Kibret, 2014; Kumar *et al*., 2022). Phytohormones play a crucial role in enabling plants to mitigate or endure abiotic stress under challenging ecological conditions (Andreozzi *et al*., 2019; Borah *et al*., 2019). *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Micrococcus*, and *Mycobacterium* strains are some of the best-known families of rhizobacteria, which produce IAA (Bharucha *et al*., 2013). Beneficial bacteria can help plants deal with drought stress by giving them IAA, which is a key growth inhibitor for plants. About 80 % of the rhizobacteria can control how much IAA plants make. The roots of plants release L-tryptophan, which is a major building block for IAA (Spaepen and Vanderleyden, 2011; Ikiz *et al*., 2024). In the rhizosphere, PGPR changes tryptophan into IAA, which the plants take up (Glick, 2012; Ikiz *et al*., 2024). PGPR can also make their own IAA that plant cells take up, which activates the auxin signal transduction pathway in plants, and triggers the growth of new plant cells (Glick, 2012).

Siderophores play a crucial role in the survival of microbes as they facilitate the formation of complexes with iron, hence increasing its solubility and absorption in iron-deficient environments (Rajkumar *et al*., 2017). Siderophores possess high Fe^{3+} affinity and are iron-transporting agents. They have the ability to establish stable complexes with heavy metals that are of significant environmental importance, including but not limited to aluminium (Al), cadmium (Cd), copper (Cu), gallium (Ga), and indium (In). Therefore, utilization of bacterial siderophores serves to mitigate

the adverse effects exerted on plants due to elevated concentrations of heavy metals in the soil (Kumar *et al*., 2017). In their study, Arzanesh *et al*., (2011) conducted an investigation on siderophores and their correlation with drought resistance. They observed that the strain exhibiting a greater production of siderophores has shown a positive association with enhanced drought resistance in its host plant.

In this study, the significant increases in maize yield and nutrients uptake due to seed inoculation with tested rhizobacterial strains could be attributed to the ability of these bacteria to possess several plant enhancing traits including production of indole-3-acetic acid (IAA), HCN, siderophores and solubilization of insoluble inorganic phosphate (Meena *et al*., 2020; Kumar *et al*., 2022) . Moreover, improving nutrient uptake by biofertilizers could be attributed to the fact that inoculated plants have larger root surface area and interact with the surrounding soil and microorganisms. Larger roots are expected to exude much organic acids that alter soil pH in the rhizosphere and act as chelating agents to deliver more nutrients to the plant root in an available form (Dal Cortivo *et al*., 2017).

Recent studies have shown that the notable reductions in soil pH values can be ascribed to several factors, e.g., presence of citric, tartaric, oxalic and succinic acids, nitrification of the added ammonium N, and/ or possible increase in partial pressure of carbon dioxide in the soil atmosphere due to increased activity of the native and applied microorganisms (Kumar *et al*., 2022). Reduction of values of the soil pH due to biofertilization have been reported by several studies (Singh *et al*., 2019; Ghanem and El-Kharbotly, 2020).

The observed rise in soil available phosphorus resulting from addition of the rhizobacterial strains to seeds may be attributed to influence of the added rhizobacteria on the overall bacterial population in the rhizosphere. This, in turn, leads to an increase in phosphatase activity and the production of organic acids, ultimately resulting in notable decreases in soil pH values. The crucial involvement of soil phosphatases in organic phosphorus compound mineralization and solubilization of insoluble mineral phosphates in soil has been widely recognized. Polysaccharides secreted by soil bacteria act as a grateful surfactant that affect the stability of soil aggregates and facilitate the diffusion of phosphate ions from bulk soil through the rhizosphere (Bhattacharyya and Jha, 2012).

Soil enzyme activities are higher at the middle crop stage than the early and later stages, which can be explained by several aspects that influence secretion rate of the extracellular enzymes. Soil microorganisms seem to be the rationale source for supplying most of soil enzymatic activities due to their high metabolic activities, large biomass, and short lifetimes that allow them to release relatively large amounts of extracellular enzymes (Gianfreda, 2015; Singh *et al*., 2019).

Values of alkaline phosphatase activity proved to be higher than those of acid phosphatase activity. This observed phenomenon can be ascribed to the presence of alkaline soil conditions, which promote the prevalence of alkaline phosphatase. According to Tabatabai, (1994), acid phosphatase is the primary enzyme responsible for microbial activity in acid soils, whereas in neutral to alkaline soils, both the acid and alkaline phosphatases are active, with alkaline phosphatase being the predominant enzyme.

Increased bacterial counts in inoculated treatments relative to the control may be attributed to the applied bacteria in the rhizosphere. This indicates that the analyzed bacterial strains are able to proliferate in the soil without interference from native microorganisms. According to Kloepper, (1994), PGPB must be able to colonize the root surface, survive, multiply, and compete with the other microorganisms for at least the period necessary to exert their plant growth-promoting effects. The observed decline in the overall bacterial population at 95 d compared to the populations at 30 or 50 d can potentially be ascribed to the un-favorable

soil moisture conditions resulting from desiccation of the soil prior to the maize harvest.

Conclusion

The current study illustrated that the tested bacterial inoculants caused significant increases in maize yield, NPK uptake by plants, availability of N and P in soil, activities of the three studied soil enzymes and total bacterial counts relative to the un-inoculated control. Thus, no significant difference was observed in maize yield between 1.0 and 0.75 ETc when the latter was combined with any of the tested bacterial inoculants, indicating that the irrigation water quantity for maize in the Egyptian sandy soils may be reduced by 25 % in the presence of one of these biofertilizers without reduction in maize yield. These increments can be attributed to increase in soil enzyme activities, significant reductions in soil pH, increase of the availability of some plant nutrients such as N, P, K, Fe, Mn, Cu, and Zn, and production of phytohormones; mainly auxins and cytokinin.

Acknowledgement

The authors acknowledge the technical staff of Soil and Water Department, and Agricultural Botany Department, Faculty of Agriculture, Suez Canal University, for their help.

Conflict of interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Funding

No fund was received.

Ethical approval

Not applicable.

Author's Contributions

Conceptualization, O.M.G. and A.F.S.; Roles/Writing-original draft, O.M.G. and A.F.S.; Data curation, O.M.G., N.K. and A.F.S.; Formal analysis, N.K. and A.F.S.; Investigation, O.M.G., N.K. and A.F.S.; Methodology, O.M.G. and A.F.S.; Software, N.K. and A.F.S. Supervision, O.M.G. and N.K. and A.F.S.; Visualization, O.M.G., and N.K.; Validation, O.M.G., N.K. and A.F.S.

5. References

Abdelaal, K.; AlKahtani, M.; Attia, K.; Hafez, Y.; Király, L. and Künstler, A. (2021). The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. Biology. 10(6): 520. https://doi.org/10.3390/biology10060520

Ahemad, M. and Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University–Science. 26: 1-20. https://doi.org/10.1016/j.jksus.2013.05.001

Ajijah, N.; Fiodor, A.; Pandey, A.K.; Rana, A. and Pranaw, K. (2023). Plant Growth-Promoting Bacteria (PGPB) with Biofilm-Forming Ability: A Multifaceted Agent for Sustainable Agriculture. Diversity. 15(1): 112. https://doi.org/10.3390/d15010112

Akhtar, N.; Ilyas, N.; Mashwani, Z.U.; Hayat, R.; Yasmin, H.; Noureldeen, A. et al. (2021). Synergistic effects of plant growth promoting rhizobacteria and silicon dioxide nano-particles for amelioration of drought stress in wheat. Plant Physiology and Biochemistry. 166: 160–176. https://doi.org/10.1016/j.plaphy.2021.05.039.

Alexander, D.B. and Zuberer, D.A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of Soils. 12: 39-45. https://doi.org/10.1007/BF00369386.

Allen, R.G.; Pereira, L.S.; Raes, D. and Smith, M. (1998). Crop evapotranspiration-Guidelines for computing crop water requirements. FAO Irrigation

and drainage. 300(9): D05109. https://doi.org/10.3178/jjshwr.16.589.

Almeida, L.C.O.; Santos, H.L.; Nogueira, C.H.deC.; Carnietto, M.R.A.; Silva, G.F.; Boaro, C.S.F. et al. (2024). Plant Growth-Promoting Bacteria Enhance Survival, Growth, and Nutritional Content of Sugarcane Propagated through Pre-Sprouted Seedlings under Water Deficit. Agriculture. 14(2): 189. https://doi.org/10.3390/agriculture14020189.

Andreozzi, A.; Prieto, P.; Mercado-Blanco, J.; Monaco, S.; Zampieri, E.; Romano, S. et al. (2019). Efficient colonization of the endophytes *Herbaspirillum huttiense* RCA24 and *Enterobacter cloacae* RCA25 influences the physiological parameters of *Oryza sativa* L. *cv*. Baldo rice. Environmental Microbiology. 21(9): 3489-3504. https://doi.org/10.1111/1462-2920.14688.

Aneja, K.R. (2013). Experiments in Microbiology Plant Pathology and Biotechnology. New Age International Publishers. http://macl-ustm.digitallibrary.co.in/handle/123456789/5543.

Arzanesh, M.H.; Alikhani, H.A.; Khavazi, K.; Rahimian, H.A. and Miransari, M. (2011). Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. Under drought stress. World Journal of Microbiology and Biotechnology. 27(2). https://doi.org/10.1007/s11274-010-0444-1.

Astorga-Eló, M.; Gonzalez, S.; Acuña, J.J.; Sadowsky, M.J. and Jorquera, M. A. (2021). Rhizobacteria from 'flowering desert' events contribute to the mitigation of water scarcity stress during tomato seedling germination and growth. Scientific Reports. 11: 13745. https://doi.org/10.1038/s41598-021-93303-8.

Azeem, M.; Haider, M.Z.; Javed, S.; Saleem, M.H. and Alatawi, A. (2022). Drought Stress Amelioration in Maize (*Zea mays* L.) by Inoculation of *Bacillus* spp. Strains under Sterile Soil Conditions. Agriculture. 12(1): 1-21. https://doi.org/10.3390/agriculture12010050.

Bhanbhro, N.; Wang, H.J.; Yang, H.; Xu, X.J.; Jakhar, A.M.; Jakhro, M.I. et al. (2024). Revisiting the molecular mechanisms and adaptive strategies associated with drought stress tolerance in common wheat (*Triticum aestivum* L.). Plant Stress. 11: 100298. https://doi.org/10.1016/j.stress.2023.100298.

Bharucha, U.; Patel, K. and Trivedi, U.B. (2013). Optimization of Indole Acetic Acid Production by *Pseudomonas putida* UB1 and its Effect as Plant Growth-Promoting Rhizobacteria on Mustard (*Brassica nigra*). Agricultural Research. 2(3): 215-221. https://doi.org/10.1007/s40003-013-0065-7.

Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology. 28: 1327-1350. https://doi.org/10.1007/s11274-011-0979-9.

Borah, A.; Das, R.; Mazumdar, R. and Thakur, D. (2019). Culturable endophytic bacteria of *Camellia* species endowed with plant growth promoting characteristics. Journal of Applied Microbiology. 127(3): 825–844. https://doi.org/10.1111/jam.14356.

Bouremani, N.; Cherif-Silini, H.; Silini, A.; Bouket, A.C.; Luptakova, L.; Alenezi, F. N. et al. (2023). Plant growth-promoting rhizobacteria (PGPR): A rampart against the adverse effects of drought stress. Water. 15(3): 418. https://doi.org/10.3390/w15030418.

Bremner, J.M. (1996). Nitrogen-total. Methods of Soil Analysis: Part 3 Chemical Methods. 5: 1085-1121. https://doi.org/10.2136/sssabookser5.3.c37.

Buchanan, R.E.; Gibbons, N.E. and Cowan, S.T. (1974). Bergey's manual of determinative bacteriology. Lippincott Williams and Wilkins. https://doi.org/10.5962/bhl.title.10728.

Chiaiese, P.; Corrado, G.; Colla, G.; Kyriacou, M.C. and Rouphael, Y. (2018). Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop performance. Frontiers in Plant

Science. 9: 1782. https://doi.org/10.3389/fpls.2018.01782.

Chieb, M. and Gachomo, E.W. (2023). The role of plant growth promoting rhizobacteria in plant drought stress responses. BMC Plant Biology. 23(1): 407. https://doi.org/10.1186/s12870-023-04403-8.

Chukwuneme, C.F.; Babalola, O.O.; Kutu, F.R. and Omena, B.O. (2020). Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. Journal of Plant Interactions. 15(1): 93-105. https://doi.org/10.1080/17429145.2020.1752833.

Clarke, T.A. and Fryer, J.G. (1998). The development of camera calibration methods and models. The Photogrammetric Record. 16(91): 51-66. https://doi.org/10.1111/0031-868X.00113.

Cohen, A.C.; Bottini, R.; Pontin, M.; Berli, F.J.; Moreno, D.; Boccanlandro, H. et al. (2015). *Azospirillum brasilense* ameliorates the response of Arabidopsis thaliana to drought mainly *via* enhancement of ABA levels. Physiologia Plantarum. 153(1): 79-90. https://doi.org/10.1111/ppl.12221.

Dal Cortivo, C.; Barion, G.; Visioli, G.; Mattarozzi, M.; Mosca, G. and Vamerali, T. (2017). Increased root growth and nitrogen accumulation in common wheat following PGPR inoculation: Assessment of plant-microbe interactions by ESEM. Agriculture, Ecosystems and Environment. 247: 396-408. https://doi.org/10.1016/j.agee.2017.07.006.

Devarajan; A.K.; Truu, M.; Gopalasubramaniam, S.K.; Muthukrishanan, G. and Truu, J. (2022). Application of data integration for rice bacterial strain selection by combining their osmotic stress response and plant growth-promoting traits. Frontiers in Microbiology. 13: 1058772. https://doi.org/10.3389/fmicb.2022.1058772.

Enebe, M.C. and Babalola, O.O. (2018). The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: A survival strategy. Applied Microbiology and Biotechnology. 102(18): 7821–7836. https://doi.org/10.1007/s00253-018-9214-z.

Ferioun, M.; Srhiouar, N.; Tirry, N.; Belahcen, D.; Siang, T.C.; Louahlia, S. et al. (2023). Optimized drought tolerance in barley (*Hordeum vulgare* L.) using plant growth-promoting rhizobacteria (PGPR). Biocatalysis and Agricultural Biotechnology. 50: 102691. https://doi.org/10.1016/j.bcab.2023.102691.

Gee, G.W. and Bauder, J.W. (1986). Particle-size analysis. Methods of Soil Analysis: Part 1. Physical and Mineralogical Methods. 5: 383–411. https://doi.org/10.2136/sssabookser5.1.2ed.c15.

Geetha, K.; Venkatesham, E.; Hindumathi, A. and Bhadraiah, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on *Vigna radita* (L.) R. Wilczek. International Journal of Current Microbiology and Applied Sciences. 3(6): 799–809. http://www.ijcmas.com/vol-3-6/K.Geetha,%20et%20al.pdf.

Ghanem, O.M. and El-Kharbotly, A.A. (2020). Improving Wheat Growth and Yield through Application of Compost and Plant Growth Promoting Rhizobacteria under Deficit Irrigation in a Sandy Soil. Journal of Soil and Water Sciences. 5(1): 21-30. http://dx.doi.org/10.21608/JSWS.2020.156304.

Gianfreda, L. (2015). Enzymes of importance to rhizosphere processes. Journal of Soil Science and Plant Nutrition. 15(2): 283-306. http://dx.doi.org/10.4067/S0718-95162015005000022.

Glick, B.R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 963401-963401. https://doi.org/10.6064/2012/963401.

Gopalakrishnan, S.; Srinivas, V.; Prakash, B.; Sathya, A. and Vijayabharathi, R. (2015). Plant growth-promoting traits of *Pseudomonas geniculata* isolated from chickpea nodules. 3 Biotech. 5(5): 653-

661. http://link.springer.com/article/10.1007%2Fs13205-014-0263-4.

Haney, R.L.; Haney, E.B.; Smith, D.R.; Harmel, R.D. and White, M.J. (2018). The soil health tool-theory and initial broad-scale application. Applied Soil Ecology. 125: 162-168. https://doi.org/10.1016/j.apsoil.2017.07.035.

Hussain, M.B.; Zahir, Z.A.; Asghar, H.N. and Asgher, M. (2014). Can Catalase and Exopolysaccharides Producing Rhizobia Ameliorate Drought Stress in Wheat?. International Journal of Agriculture & Biology. 16(1). http://www.fspublishers.org/published_papers/87766_pdf.

Ikiz, B.; Dasgan, H.Y. and Gruda, N.S. (2024). Utilizing the power of plant growth promoting rhizobacteria on reducing mineral fertilizer, improved yield, and nutritional quality of Batavia lettuce in a floating culture. Scientific Reports. 14: 1616. https://doi.org/10.1038/s41598-024-51818-w.

Jackson, M.L. (1973). Soil Chemical Analysis,(2nd Indian Print) Prentice-Hall of India Pvt. Ltd. New Delhi. 38: 336.

Jacobson, C.B.; Pasternak, J.J. and Glick, B.R. (1994). Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Canadian Journal of Microbiology. 40(12): 1019-1025. https://doi.org/10.1139/m94-162.

Jasim, B.; John Jimtha, C.; Jyothis, M. and Radhakrishnan, E.K. (2013). Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. Plant Growth Regulation. 71: 1-11. https://doi.org/10.1007/s10725-013-9802-y.

Jing, L.; Weng, B.; Yan, D.; Yuan, F.; Zhang, S.; Bi, W. et al. (2023). Assessment of resilience in maize suitable planting areas under drought stress. Agricultural Water Management. 277: 108096. https://doi.org/10.1016/j.agwat.2022.108096.

Johnston, A. and Booth, C. (1983). Plant pathologist's pocketbook. https://www.cabidigitallibrary.org/doi/full/10.5555/19831389731.

Kálmán, C.D.; Nagy, Z., Berényi, A., Kiss, E. and Posta, K. (2023). Investigating PGPR bacteria for their competence to protect hybrid maize from the factor drought stress. Cereal Research Communications. https://doi.org/10.1007/s42976-023-00388-0.

Kang, S.M.; Khan, A.L.; Waqas, M.; You, Y.H.; Kim, J.H.; Kim, J. G. et al. (2014). Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. Journal of Plant Interactions. 9(1): 673–682. https://doi.org/10.1080/17429145.2014.894587.

Kloepper, J.W. (1994). Plant growth-promoting rhizobacteria (other systems). *Azospirillum*/Plant Associations. 187: 137–166.

Koza, N.A.; Adedayo, A.A.; Babalola, O.O. and Kappo, A.P. (2022). Microorganisms in plant growth and development: Roles in abiotic stress tolerance and secondary metabolites secretion. Microorganisms. 10(8): 1528. https://doi.org/10.3390/microorganisms10081528.

Kumar, M.; Ahmad, S. and Singh, R.P. (2022). Plant growth promoting microbes: Diverse roles for sustainable and ecofriendly agriculture. Energy Nexus. 100133. https://doi.org/10.1016/j.nexus.2022.100133.

Kumar, V.; Menon, S.; Agarwal, H. and Gopalakrishnan, D. (2017). Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. Resource-Efficient Technologies. 3(4):434–439. https://doi.org/10.1016/j.reffit.2017.04.004.

Kumar, M.; Mishra, S.; Dixit, V.; Kumar, M.; Agarwal, L.; Chauhan, P. S. et al. (2016). Synergistic effect of *Pseudomonas putida* and *Bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signaling & Behavior. 11(1): e1071004. https://doi.org/10.1080/15592324.2015.1071004.

Kuo, S.M. and Morgan, D.R. (1996). Active noise control systems (Vol. 4). New York: Wiley. https://lccn.loc.gov/95038339.

Ma, Y.; Vosátka, M. and Freitas, H. (2019). Beneficial Microbes Alleviate Climatic Stresses in Plants. Frontiers in Plant Science. 10: 595–595. https://doi.org/10.3389/fpls.2019.00595.

Mahanta, D.; Rai, R.K.; Mishra, S.D.; Raja, A.; Purakayastha, T.J. and Varghese, E. (2014). Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. Field Crops Research. 166: 1–9. https://doi.org/10.1016/j.fcr.2014.06.016.

Meena, R.S.; Kumar, S.; Datta, R.; Lal, R.; Vijayakumar, V.; Brtnicky, M. et al. (2020). Impact of agrochemicals on soil microbiota and management: A review. Land. 9(2): 34. https://doi.org/10.3390/land9020034.

Michavila, G.; Alibrandi, P.; Cinà, P.; Welin, B.; Castagnaro, A.P.; Chalfoun, N.R. et al. (2022). Plant growth-promoting bacteria isolated from sugarcane improve the survival of micropropagated plants during acclimatisation. Italian Journal of Agronomy. 17(2). https://doi.org/10.4081/ija.2022.2006.

Miller, D.; Bryant, J.; Madsen, T. and Ghiorse, W. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Applied and Environmental Microbiology. 65(11): 47154724. https://doi.org/10.1128/AEM.65.11.4715-4724.1999.

Msimbira, L.A. and Smith, D.L. (2020). The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. Frontiers in Sustainable Food Systems. 4: 106. https://doi.org/10.3389/fsufs.2020.00106.

Mukhtar, S.; Shahid, I.; Mehnaz, S. and Malik, K.A. (2017). Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum* L.). Microbiological Research. 205: 107–117. https://doi.org/10.1016/j.micres.2017.08.011.

Muñoz, G. and Grieser, J. (2006). Water resources-development and management service, environment and natural resources service. In CLIMWAT 2.0 for CROPWATt. Food and Agriculture Organization of the UN.

Naylor, D. and Coleman-Derr, D. (2018). Drought stress and root-associated bacterial communities. Frontiers in Plant Science. 8: 2223. https://doi.org/10.3389/fpls.2017.02223.

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS microbiology Letters. 170(1): 265-270. https://doi.org/10.1016/S0378-1097(98)00555-2.

Nguyen, H., Thompson, A. and Costello, C. (2023). Impacts of historical droughts on maize and soybean production in the southeastern United States. Agricultural Water Management. 281: 108237. https://doi.org/10.1016/j.agwat.2023.108237.

Niu, X.; Song, L.; Xiao, Y. and Ge, W. (2018). Drought-Tolerant Plant Growth-Promoting Rhizobacteria Associated with Foxtail Millet in a Semi-arid Agroecosystem and Their Potential in Alleviating Drought Stress. Frontiers in Microbiology. 8: 2580. https://doi.org/10.3389/fmicb.2017.02580.

Patten, C.L. and Glick, B.R. (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Applied

and Environmental Microbiology. 68(8): 3795-3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002.

Paul, K.; Sorrentino, M.; Lucini, L.; Rouphael, Y.; Cardarelli, M.; Bonini, P. et al. (2019). Understanding the biostimulant action of vegetal-derived protein hydrolysates by high-throughput plant phenotyping and metabolomics: A case study on tomato. Frontiers in Plant Science. 10: 47. https://doi.org/10.3389/fpls.2019.00047.

Rajkumar, M.; Bruno, L.B. and Banu, J.R. (2017). Alleviation of environmental stress in plants: The role of beneficial *Pseudomonas* spp. Critical Reviews in Environmental Science and Technology. 47(6): 372-407. https://doi.org/10.1080/10643389.2017.1318619.

Sandhya, V.; Ali SK. Z.; Grover, M.; Reddy, G. and Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biology and Fertility of Soils. 46: 17–26. https://doi.org/10.1007/s00374-009-0401-z9.

Sarma, R.K. and Saikia, R. (2014). Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. Plant and Soil. 377: 111-126.

Shukla, P.S.; Mantin, E.G.; Adil, M.; Bajpai, S.; Critchley, A.T. and Prithiviraj, B. (2019). *Ascophyllum nodosum*-based biostimulants: Sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. Frontiers in Plant Science. 10: 655. https://doi.org/10.3389/fpls.2019.00655.

Singh, M.; Singh, D.; Gupta, A.; Pandey, K.D.; Singh, P.K. and Kumar, A. (2019). Plant growth promoting rhizobacteria: Application in biofertilizers and biocontrol of phytopathogens. In PGPR amelioration in sustainable agriculture (pp. 41–66). Elsevier. https://doi.org/10.1016/B978-0-12-815879-1.00003-3.

Spaepen, S. and Vanderleyden, J. (2011). Auxin and plant-microbe interactions. Cold Spring Harbor Perspectives in Biology. 3(4): a001438. https://doi.org/10.1101/cshperspect.a001438.

Sparks, D. (1996). Methods of Soil Analysis. Chemical Methods. 3: 1125-1131. https://doi.org/10.1016/j.geoderma.2016.01.021.

Starr, M.P.; Stolp, H.; Truper, H.G.; Balows, A.; Schlegel, H.G.; Lechevalier, H. A. et al. (1981). The prokaryotes. A handbook on habits, isolation, and identification of bacteria.

Tabatabai, M.A. (1994). Soil enzymes. Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. 5: 775-833. . https://doi.org/10.2136/sssabookser5.2.c37.

Vardharajula, S.; Zulfikar Ali, S.; Grover, M.; Reddy, G. and Bandi, V. (2011). Drought-tolerant plant growth promoting *Bacillus* spp.: Effect on growth, osmolytes, and antioxidant status of maize under drought stress. Journal of Plant Interactions. 6(1): 1–14. https://doi.org/10.1080/17429145.2010.535178.

Verma, P.; Yadav, A.N.; Khannam, K.S.; Kumar, S.; Saxena, A.K. and Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. Journal of Basic Microbiology. 56(1): 44-58 https://doi.org/10.1002/jobm.201500459.

Wang, S.; Ouyang, L.; Ju, X.; Zhang, L.; Zhang, Q. and Li, Y. (2014). Survey of Plant Drought-Resistance Promoting Bacteria from *Populus euphratica* Tree Living in Arid Area. Indian Journal of Microbiology. 54(4): 419. https://doi.org/10.1007/s12088-014-0479-3.

Xiong, Q.; Hu, J.; Wei, H.; Zhang, H. and Zhu, J. (2021). Relationship between plant roots, rhizosphere microorganisms, and nitrogen and its special focus on rice. Agriculture. 11(3): 234. https://doi.org/10.3390/agriculture11030234.