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Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize

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ABSTRACT

Drought is a major cause of the present decrease in crop yield and agricultural productivity around the globe. The disastrous effects of drought on plants call for a renewed concern on effective strategies to improve plant growth and yield under drought stress. This study was designed to evaluate the effectiveness of *Arthrobacter arilaitensis* and *Streptomyces pseudovenezuelae* to improve maize growth under drought stress at three soil moisture levels using two inoculation methods. Seven rhizosphere actinomycetes isolates were screened for the production of plant growth promoting (PGP) traits and it was observed that all isolates produced one or more PGP properties. The inoculated plants were not only protected from the deleterious effects of drought, but also showed significant increases in the physiological parameters measured. The findings of this study suggest that these isolates are important tools capable of being developed into bio-inoculants to effectively improve drought tolerance in plants.

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KEYWORDS
Actinomycetes; drought tolerance; maize; plant growth promoting traits; and rhizosphere

Introduction

A major environmental problem facing most countries of the world today, regarding agricultural productivity and food availability, is drought. Drought has been a subject of concern as it has led to reduced plant growth and yield. It is therefore very important to seek means of reducing this menace, to increase food availability and sustain food security. At present, strategies like breeding and genetic modifications are being used to manage this problem (Langridge and Reynolds 2015; Maazou et al. 2016). Agricultural practices including soil amelioration and mulching have also been used (Jongdee et al. 2006). However, these strategies are not very efficient as they are not only time consuming but labor and cost intensive (Ashraf 2010; Eisenstein 2013). Often times, some desirable plants traits in the host plant gene pool can be unintentionally lost in the process of breeding (Philippot et al. 2013). Moreover, plant breeding transfers benefit to single host specie and not to other crop systems, as it is usually difficult to identify the genetic component responsible for this improvement (Coleman-Derr and Tringe 2014). The drawbacks mentioned above have made these technologies highly unreliable, leading to a quest for better and more efficient means to tackle this problem.

In recent times, the use of beneficial microbial species with plant growth promoting capabilities to relieve plants of the adverse effects of drought has become more relevant in agriculture (Babalola and Glick 2012). Bacteria are important soil components, able to form mutualistic and beneficial associations with most plants (Ndeddy Aka and Babalola 2016). Symbiotic bacteria are capable of conferring stress tolerance to a wide variety of plant hosts through phytohormonal modifications, production of exopolysaccharides, accumulation of osmolytes and acting as defense against reactive oxygen species (Zhang et al. 2008; Coleman-Derr and Tringe 2014). These bacteria are also able to synthesize antibiotic substances, fix atmospheric nitrogen, produce soluble iron compounds (siderophore), and solubilize inorganic phosphates (Babalola 2010; Adegboye and Babalola 2013). In addition, they serve as plant growth regulators by producing the phytohormones indole-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC), cytokinins and gibberellins (GA) (Khantsi et al. 2013; Ndeddy Aka and Babalola 2017). These outstanding properties of the plant growth promoting bacteria (PGPB) facilitate the efficient stimulation of plant growth during unfavorable environmental conditions like drought (Yandigeri et al. 2012). Several studies have revealed the successful application of isolated PGPB on drought stress improvement in plants (Figueiredo et al. 2008; Yandigeri et al. 2012; Gusain et al. 2015). However, most of these studies have concentrated on certain groups of bacteria species, mostly *Pseudomonas and Bacillus*. The use of actinomycetes species to enhance stress tolerance in plants have received very little attention over the years. Actinomycetes, found mostly in soils, are widely known for their antibiotic and bioactive secondary metabolites production as well as their outstanding ability to survive in unfavorable environments (Adegboye and Babalola 2013; Adegboye and Babaloa 2015; Passari et al. 2015). Their ability to produce certain plant growth promoting properties has also been identified, but with little information on the extent of the properties produced (Ali et al. 2014; Sreevidya et al. 2016). Hence, this study was conducted to investigate the effects of actinomycetes inoculation on maize plant under well-watered, semi-watered and

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drought stressed conditions. It also examined the level of production of certain plant growth promoting (PGP) substances ACC, IAA, siderophore, phosphate and ammonia (NH_3) by the drought tolerant actinomycetes.

Materials and methods

Isolation and selection of drought tolerant bacteria

In a previous study (Chukwuneme 2018), seven drought tolerant bacteria were isolated from two maize plantations. These bacterial isolates were identified and deposited in the GenBank with the following names and accession numbers: *Streptomyces werraensis* MG547867, *Streptomyces luteogriseus* MG669347, *Streptomyces indiaensis* MG547868, *Arthrobacter arilaitensis* MG547869, *Streptomyces pseudovenezuelae* MG547870, *Microbacterium oxydans* MG640368, and *Streptomyces* sp. MG640369. The isolates were selected for this study based on their ability to withstand drought stress by growing at 20% concentrations of PEG 8000. In the present study, these bacteria were qualitatively and quantitatively screened for the presence of plant growth promoting traits. Due to their higher tolerance of higher concentrations of PEG 8000, *S. pseudovenezuelae* MG547870 and *A. arilaitensis* MG547869 were chosen for greenhouse studies, to assess the effect of their individual and combined inoculation on the growth of maize plants under drought stress.

Qualitative and quantitative assessment of plant growth promoting properties of bacterial isolates

Ammonia production

The production of ammonia by actinomycetes isolates was tested according to the protocol of Islam et al. (2009). 10 µl (0.2 OD) of freshly prepared actinomycetes cultures were inoculated into test tubes containing 10 ml of peptone water. The inoculated test tubes were incubated at 25°C for 7days after which 1 ml of Nessler's reagent was added to each test tube, and any color changes were observed. A change in the color of the media to yellow or brown specifies a positive result for ammonia production. The experiment was done in triplicate.

Phosphate solubilization activity

To evaluate the ability of actinomycetes isolates to solubilize phosphate, the method of Islam et al. (2009) was used. 10 µl of freshly prepared culture was spot inoculated on Pikovskaya's agar plates containing 2% tri-calcium phosphate. Inoculated plates were incubated at 37°C for 7 days, plates were observed for the appearance of a clear zone around the actinomycetes colonies.

Hydrogen cyanide activity

Hydrogen cyanide activity was determined according to the protocol of Bakker and Schippers (1987). Bacterial cultures were separately streaked on Luria Bertani (LB) agar amended with 0.4% (w/v) of glycine. A Whatman no. 1 filter paper soaked in 0.5% (w/v) picric acid in 2% (w/v) sodium carbonate was placed on the lid of the Petri dish. Thereafter, plates were properly sealed with parafilm and incubated for seven days. The change in color of the filter paper from yellow to deep orange when observed with the eyes indicates a positive result.

Indole-3-acetic acid production

The method of Ndeddy Aka and Babalola (2016) was used for qualitative determination of indole-3-acetic acid production by actinomycetes isolates. Freshly prepared bacterial cultures (20 µl) were inoculated in LB broth (20 ml) amended with 5 mmol L-tryptophan (Merck, SA) and incubated at 25°C for 7 days. After incubation, 1 ml of bacterial culture was transferred into sterile Eppendorf tubes and centrifuged at 5000 g for 15 min. The supernatant was collected in a 15 ml centrifuge tube. Subsequently, 2 ml of the supernatant and 2–3 drops of orthophosphoric acid was added to 4 ml of Salkowsky reagent (50 ml of 35% perchloric acid in 1 ml of 0.5 M $FeCl_3$). The contents in the tubes were incubated at room temperature under dark conditions for 20 min, the development of a pink color indicated IAA production. The absorbance of the pink color was read using a UV spectrophotometer (ThermoSpectronic, Merck chemical, SA) at 530 nm.

The amount of IAA produced by each bacterial isolate was determined by the generation of a standard curve. Standards were made in LB broth at 0,5,10, 20, 50 and 100 µg/l including a control consisting of LB broth only, 4 ml of Salkowsky reagent was added to 2 ml of each standard and incubated at room temperature for 20 min. Absorbance was read at 530 nm using a UV spectrophotometer (ThermoSpectronic, Merck chemical, SA).

Siderophore production

The production of siderophore by bacterial isolates was assayed according to a modified protocol described by Schwyn and Neilands (1987) using an indicator dye, chrome azurol S (CAS from Merck, SA). Briefly, 60.5 mg of CAS was dissolved in 50 ml of distilled water and mixed with 10 ml of iron (III) solution (1 mM $FeCl_3$. $6H_20$ in 10 mMHCl). The mixture was slowly added while constantly stirring with a magnetic stirrer to 72.9 mg of hexadecyltrimethylammonium (HDTMA, Merck, SA) bromide dissolved in 40 ml distilled water and then autoclaved at 121°C for 15 min. The final mixture (100 ml) was added while stirring to 900 ml of sterilized LB broth adjusted to pH 6.8 and poured into Petri plates. Upon solidification, freshly prepared bacterial cultures were spot inoculated on the Petri plates and incubated at 25°C for 7 days. A yellowish – orange halo around the bacterial colonies was considered a positive result for siderophore production.

The amount of siderophore produced by each bacterial isolate was estimated following the protocol of Alexander and Zuberer (1991) using a modified CAS assay solution. Hexadecyltrimethylammonium (HDTMA, 21.9 mg) was dissolved in 25 ml of distilled water with constant stirring under low heat. In a 50 ml flask, 1.5 ml of 1 mM $FeCl_3$. $6H_20$ in 10 mMHCl was added to 7.5 ml of 2 mM CAS. This solution was slowly added to the HDTMA solution and the resultant mixture transferred to a 100 ml flask. A buffer solution was prepared by dissolving 9.76 g of 2-(N-morpholino) ethanesulfonic acid (MES, Merck, SA) in 50 ml distilled water and the pH adjusted to 5.6 with 50% KOH. This buffer solution was then added to the flask containing the dye solution while distilled water was added to get a final volume of 100 ml. A shuttle assay solution was prepared by

adding 87.3 mg of 5-sulfosalicyclic acid to the above solution before use. All seven isolates to be tested for siderophore were inoculated in 5 ml sterilized LB medium without added Fe and incubated at 25°C for five (5) days. Bacterial cells were pelleted by centrifugation at 3000 g for 10 min and the supernatant was collected in tubes. The concentration of siderophore in the supernatant was obtained by mixing 100 µl of CAS assay solution with 100 µl of supernatant and allowed to equilibrate for 3–4 h, the absorbance of the 200 µl mixture was measured at 630 nm using a UV spectrophotometer (ThermoSpectronic, Merck chemicals, SA). The percentage siderophore produced was calculated by the equation:

$$\% \text{ Siderophore units} = \frac{\text{Ar} - \text{As}}{\text{Ar}} \times 100.$$

ere Ar = Absorbance of reference at 630 nm (CAS reagent),
As = Absorbance of sample at 630 nm.

ACC deaminase activity

The seven drought tolerant bacterial isolates used in this study were screened for ACC deaminase activity based on their ability to utilize ACC as sole nitrogen source, following the method by Ali et al. (2014). All bacteria were first grown on 5 ml of tryptone-soy broth (TSB, rich medium, Merck, SA) and incubated at room temperature for 48 h. Bacterial cells were harvested by centrifugation at 5000 g for 5 min, washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5). Washed bacterial cells were spot inoculated on Petri plates containing modified Dworkin and Foster salts minimal medium (Dworkin and Foster 1958). Minimal salts medium was composed of 2 g glucose, 2 g gluconic acid, 2 g citric acid, 4 g KH_2PO_4, 6 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$ and 10 ml micro nutrient solution (200 mg $CaCl_2$, 200 mg $FeSO_4.7H_2O$, 15 mg H_3BO_3, 20 mg $ZnSO_4.7H_2O$, 10 mg Na_2MoO_4, 10 mg KI, 10 mg NaBr, 10 mg $MnCl_2$, 5 mg $COCl_2$, 5 mg $CuCl_2$, 2 mg $AlCl_3$, 2 mg $NiSO_4$ and 1000 ml distilled H_2O) in 990 ml distilled H_2O amended with 3 mM ACC as a sole nitrogen source. Negative control for this experiment was Petri plates containing only DF minimal salts medium without ACC while the positive control consisted of plates containing DF minimal salts medium +0.2% (w/v) $(NH_4)_2SO_4$. Inoculated plates were incubated at 30°C for 7 days. The growth of bacterial isolates on DF minimal plates containing ACC was used to compare those of the positive and negative controls. Petri plates were selected based on bacterial growth by utilizing ACC as sole source of nitrogen. The experiment was performed thrice.

The activity of ACC deaminase was measured by growing all 7 actinomycetes isolates on TSB medium at 30°C for 7 days. The induction of ACC deaminase activity was achieved by collecting bacterial cells by centrifugation at 5000 g for 5 min and washing with 0.1 M Tris-HCl (pH 7.5). Washed cells were resuspended in 2 ml of modified DF minimal medium containing 3 mM concentration of ACC, then incubated under shaking at 30°C for 7 days. ACC deaminase activity was determined by measuring the production of α-ketobutyrate and ammonia generated when ACC cleaved to ACC deaminase (Penrose and Glick 2003). Induced bacterial cells were harvested by centrifugation at 5000 g for 10 min, washed twice with 0.1 M Tris-HCl (pH 7.5) solution, then resuspended in 200 µl of 0.1 M Tris-HCl (pH 8.5). Toluene (5% v/v) was added to the cells to labilize and cells were vortexed at highest speed for 30 s. In sterile Eppendorf tubes, fifty (50) µl of labilized cells was collected and 5 µl of 3 mM ACC was added. Tubes were incubated at 30°C for 30 min. For this assay, the negative control consisted of 50 µl of labilized cell suspension without ACC while the blank consisted of 50 µl of 0.1 M Tris-HCl (pH 8.5) with 3 mM ACC. Samples were then thoroughly mixed with 500 µl of 0.56 N HCl by vortexing and cell pellets were removed by centrifugation at 10, 000 g for 10 min. The supernatant (500 µl) was transferred into test tubes and mixed with 400 µl of 0.56 N HCl and 150 µl of 2, 4 DNP solution (0.1 g 2, 4-dinitrophenylhydrazine in 100 ml of 2 N HCl). The mixture was incubated at 30°C for 30 min. One ml of 2 N NaOH was finally added to the samples after which their absorbances were measured at 540 nm using a UV spectrophotometer (ThermoSpectronic, Merck chemicals, SA).

Alpha-ketobutyrate concentration in each sample was determined by comparing with a standard curve generated as follows: alpha-ketobutyrate solutions (500 µl) of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mM were mixed each with 400 µl of 0.56 N HCl and 150 µl of 2,4-DNP solution. One (1) ml of 2 N NaOH was then added and mixed. The absorbance was measured at 540 nm and the values obtained for the absorbance against concentration (mM) were used to generate a standard curve (Renn 2013). Note: each standard was replicated 3 times.

Greenhouse experiments

Greenhouse experiments were conducted to: (i) evaluate the effect of bacterial inoculation on the growth of maize plant under three moisture levels [field capacity (FC, 100%), moderately wet (MW, 50%) and completely dry (D, 0%)], and (ii) evaluate the effect of the method of inoculation on drought stress tolerance in maize. Maize as a drought intolerant crop was chosen for this study based on its high nutritional and economic importance in South Africa. Knowledge gained from this study will help to find suitable means of improving maize growth and yield, despite the ravaging drought.

Actinomycetes inoculum preparation

The two actinomycetes isolates *A. arilaitensis* (MG547869) and *S. pseudovenezuelae* (MG547870) used in this experiment were based on their outstanding ability to resist drought stress *in-vitro* as well as their plant growth promoting potentials. The inocula were prepared by growing the bacterial strains in 250 ml conical flasks containing 100 ml of sterilized LB broth. Inoculated flasks were incubated at 30°C under constant shaking (120 rpm) for 7 days. Pellets were collected by centrifugation at 10000 rpm for 20 min and washed twice with sterile distilled water. Pelleted cells were resuspended in 0.01M phosphate buffer at pH 7 and adjusted to an absorbance of 1.2 at 600 nm with a UV spectrophotometer (Thermospectronic, Merck, SA) (Ndeddy Aka and Babalola 2016).

Soil collection and preparation for pot experiments

Soil for the trial was collected from the farm behind the Animal Health Center of the North-West University, Mafikeng Campus at 25°S Latitude, 25°E Longitude and elevation of 1278.3 m. The soil was collected at the surface 0–20 cm depth, oven dried at 70°C for 48 h, passed through a 2 mm sieve and autoclaved at 121°C for 15 min. Sterilized soil was allowed to cool for 2 days after which 10 kg of soil was aseptically transferred into plastic pots.

Seed viability test in petri plates

Seed germination tests were conducted to evaluate the effect of bacterial inoculation on the germination of the test seeds in the presence of 5% polyethylene glycol (PEG) 8000 (Rincón et al. 2008). Prior to the test, drought sensitive maize seeds of the variety S0/8/W/ I137TNW//CML550 obtained from the Agricultural Research Council (ARC), South Africa were first washed with tap water, then sterilized using 70% ethanol for 5 min follwed by 2% sodium hypochlorite ($NaClO_2$) solution for 15 min and severally rinsed with sterile distilled water to remove the remains of the disinfectant (Madhaiyan et al. 2007). Thereafter, 4 clean Petri plates (replicated three times) were prepared by placing two filter papers at the bottom of each plate and subsequently 10 ml of each bacterial suspension in 5% PEG 8000 or 10 ml of sterile tap water (in the case of the control) was pipetted in each Petri plate. Sterile seeds were immersed in 10 ml of bacterial suspension containing 5% PEG 8000 for 5 h in a rotary shaker at 150 rpm after which 20 seeds were placed in each petri plate and incubated at 25°C for 10 days. Germinated seeds in each Petri plate were counted and 5 seedlings per plate were randomly selected for growth parameter measurements (shoot length, root length and dry seedling weight). Percentage germination and vigor index were estimated according to the method of Ghorbanpour and Hatami (2014) as follows:

$$\text{Germination rate}\,(\%) = \frac{n}{\mathrm{N}} \times 100.$$

Where n is the number of germinated seeds after 7 days and N is the total number of seeds

Vigor index = % germination × total length of seedling (shoot length + root length)

Preparation of maize seeds for greenhouse study

Drought susceptible maize seeds of the cultivar S0/8/W/ I137TNW//CML550 were firstly immersed in 70% ethanol for 15 min and washed three times with sterile distilled water. Thereafter, the seeds were soaked in 2% sodium hypochlorite ($NaClO_2$) solution for 10 min, and then thoroughly rinsed twice with sterile distilled water.

Seed inoculation with bacterial isolates

In this study, two methods of inoculation were employed to enable the bacterial isolates adhere to the surface sterilized maize seeds. This was done to determine the effect of mode of inoculation on drought stress amelioration in maize plants. Firstly, surface sterilized maize seeds were inoculated by direct immersion in bacterial cultures (1.5 OD_{600}/ ml) or 0.01M phosphate buffer (pH 7) for the control treatment for 12 h. Secondly, maize seeds were immersed in bacterial cultures (1.5 OD_{600}/ ml) or 0.01M phosphate buffer (pH 7) in the case of the control for 12 h. Following immersion, seeds were resuspended in 1% carboxylmethyl cellulose (CMC, binder) in a 500 ml conical flask and finely ground and sterilized vermiculite was spread all over the seeds until they were completely coated. Both the directly inoculated and coated seeds were left to dry overnight in a sterile laminar flow chamber prior to being sown in the greenhouse.

Greenhouse evaluation of bacterial induced tolerance to drought stress

In the 2 × 3 × 4 factorial greenhouse experiment, a total of seventy-two (72) pots (23-cm diameter) were used, representing twenty-four (24) treatment combinations based on three (3) experimental factors (seed treatments, bacterial types and soil moisture levels). The three experimental factors used in the present study were:

(i) Two seed treatments (inoculation method): directly inoculated seeds and vermiculite coated seeds as described earlier
(ii) Four types of seed inoculation: without bacteria isolate (control), with *S. pseudovenezuelae*, with *A. arilaitensis* and combination of both bacterial isolates, and
(iii) Three moisture levels: Field capacity (FC, 100%), moderately wet (MW, 50%) and completely dry (DS, 0%).

All experimental pots containing treatments were arranged in a completely randomized design (CRD) with three replicates. Soil sterilization was performed by autoclaving at a temperature of 121°C for 30 min. Four seeds were sown per plastic pot containing 10 kg of sterilized soil at a depth of 5 cm. Ten days after germination, each pot was thinned and only one seedling was left in each pot. Bacterial suspension of 50 ml (1.5 OD per ml) was added near the plant root zone in each pot and this was done every 2 weeks till the end of the experiment. Pots were watered daily with 200 ml of water for the first 15 days after seed germination before drought stress was induced and lasted till the 35th day. During the period of drought stress, the treatments at field capacity received 200 ml of water daily. For moderately wet treatments, plants received 100 ml of water while completely dry treatments, plants received no water till the 35th day. All plants were watered again for 2 days after the 35th day and above ground data (chlorophyll content index, shoot length, number of plant leaves and leaf area) were collected before the plants were carefully uprooted from the pots. After uprooting, plants were washed with distilled water to remove adhering soil and root lengths were measured for each plant. Plant shoot and roots were packaged in aluminum foil papers and dried in an oven at 68°C for three days. Dry root and shoot weights were collected using a weighing balance. Thereafter, plant samples were stored in polyethylene bags at −4°C for further analysis. The greenhouse experiments were conducted twice.

Data analysis

Data obtained from this study were analyzed by One-way analysis of variance (ANOVA) using the Statistical analysis software (SAS), version 9.4 (SAS 2014). For each treatment, generated data were presented as arithmetic means ± S.E. Significantly different means were separated using New Duncan Multiple Range Test (DMRT) at 5% level of significance.

Results

Drought tolerance by actinomycetes isolates

The actinomycetes strains used in this study were those isolated and identified in our previous study due to their high

Table 1. Qualitative plant growth promoting abilities of bacterial isolates.

PGP traits	S4	S7	S11	S12	R11	R15	S20
Indole-3-acetic acid (IAA)	+	+	+	+	+	+	+
ACC deaminase activity	+	+	+	+	+	+	+
Siderophore	+	+	+	+	+	+	+
Ammonia production	+	+	+	+	+	+	+
Phosphate solubilization	+	–	–	+	+	+	+
Hydrogen cyanide	–	–	–	+	–	–	–

S and R stand for bacterial isolates codes.

tolerance to higher concentration of PEG 8000. *S. pseudovenezuelae* (MG547870) and *A. arilaitensis* (MG547869), chosen for greenhouse studies grew well at the highest PEG 8000 concentration of 20% with a growth of 0.786 ± 0.076 (OD_{600}) for *S. pseudovenezuelae* MG547870 and 1.379 ± 0.134 for *A. arilaitensis* MG547869.

Plant growth promoting characteristics of bacterial isolates

All tested isolates produced multiple plant growth promoting characteristics. The results of the qualitative plant growth promoting tests conducted are presented in Table 1 Results revealed that all seven isolates tested were positive for ammonia, Indole-3-acetic acid, siderophore and ACC deaminase activity, five isolates [*S. werraensis* (S4), *Streptomyces* spp. (S12), *S. indiaensis* (R11), *A. arilaitensis* (R15) and *S. pseudovenezuelae* (S20) tested positive for phosphate solubilization by showing clear zones around colonies on petri dishes while only *Streptomyces* spp. (S12) was positive for hydrogen cyanide activity.

Indole-3-acetic acid production by bacterial isolates

IAA production by the tested bacterial isolates varied (Figure 1(a)). *Streptomyces werraensis* showed maximum indole-3-acetic acid production (10.12 ± 0.02 µg/ml) followed by *A. arilaitensis* (9.44 ± 0.01 µg/ml), *S. pseudovenezuelae* (8.96 ± 0.03 µg/ml), *S. luteogriseus* (8.68 ± 0.01 µg/ml), *S. indiaensis* (7.46 ± 0.02 µg/ml), *Streptomyces* spp. (6.98 ± 0.02 µg/ml), and *M. oxydans* (5.03 ± 0.01 µg/ml).

Phosphate solubilization by bacterial isolates

From the results in Table 1, five out of the seven tested bacterial isolates solubilized phosphate by showing clear zones around colonies on agar plates. These bacterial isolates include S20, R15, S12, R11 and S4 (*S. pseudovenezuelae*, *A. arilaitensis*, *Streptomyces* spp., *S. indiaensis* and *S. werraensis* respectively).

ACC deaminase activity of bacterial isolates

In the present study, the ACC deaminase activity of all tested isolates on agar plates containing ACC as the sole nitrogen source was positive (Table 1) However, in the quantitative assay, variations were observed in the amount of ACC deaminase activity produced among tested bacterial isolates (Figure 1(b)). *Streptomyces pseudovenezuelae* produced the highest amount of ACC deaminase activity (0.903 ± 0.024 µmol αKB mg^{-1} h^{-1}) followed by *A. arilaitensis* which produced 0.899 ± 0.023 µmol αKB mg^{-1} h^{-1}. *Streptomyces* indiaensis, *M. oxydans, Streptomyces* spp., *S. werraensis* and *S. luteogriseus* respectively produced 0.696 ± 0.028, 0.713 ± 0.003, 0.850 ± 0.032, 0.741 ± 0.004 and 0.671 ± 0.027 µmol αKB mg^{-1} h^{-1} of ACC deaminase activity. In our previous work, PCR amplification of ACC gene revealed that the tested isolates amplified the *accd* gene when run on agarose gel indicating a possible presence of the gene.

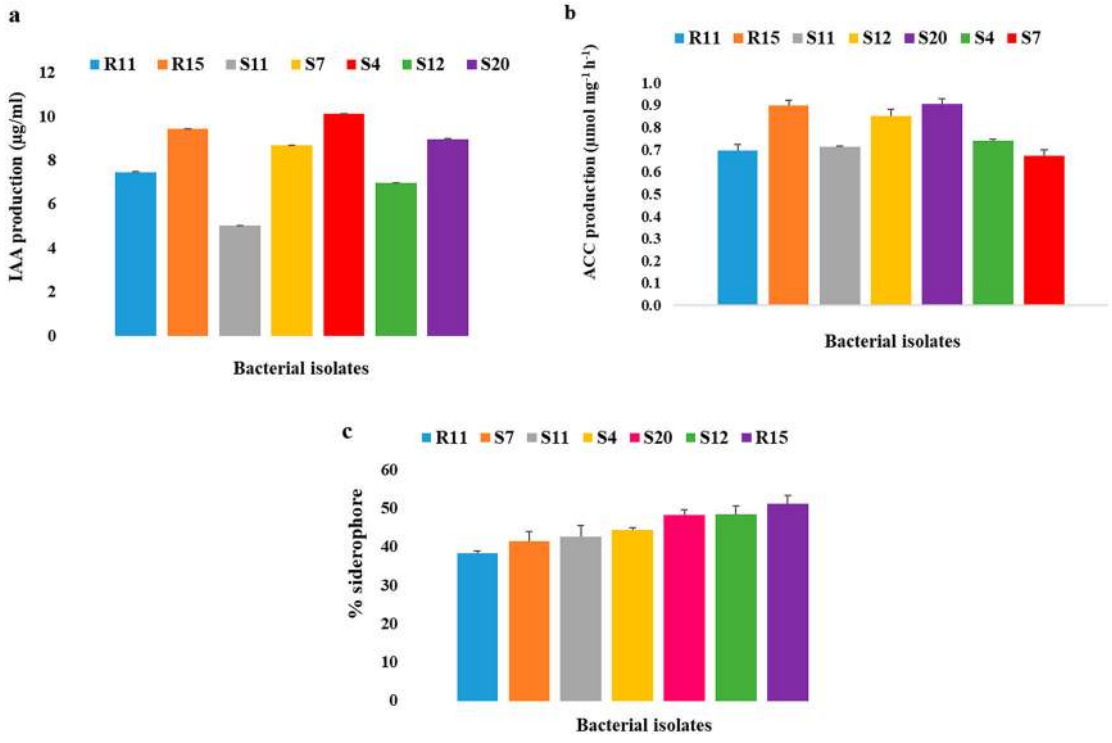

Figure 1. (a) IAA production by bacterial isolates, (b) ACC deaminase activity of bacterial isolates, and (c) Percentage siderophore production by bacterial isolates. All values are means of triplicate determinations ± S.E.

Table 2. Seed germination test.

Treatment	S. length (cm)	R. length (cm)	Germ %	V. index
C1	4.8 ± 0.53^{cd}	5.23 ± 0.57^{de}	66.67 ± 3.21^{d}	675.56 ± 102.16^{d}
C2	2.5 ± 0.21^{f}	3.17 ± 0.24^{f}	42.59 ± 3.70^{g}	243.7 ± 31.73^{f}
M1	9.27 ± 0.99^{a}	10.17 ± 0.84^{a}	94.44 ± 3.21^{a}	1825.93 ± 133.74^{a}
M2	5.3 ± 0.66^{c}	5.83 ± 0.80^{cd}	61.11 ± 3.21^{de}	678.33 ± 88.06^{d}
R 15	$5.5 \pm 0.80b^{c}$	6.23 ± 0.82^{bc}	75.92 ± 1.85^{c}	888.33 ± 118.10^{c}
R15+PEG	3.73 ± 0.61^{e}	4.57 ± 0.32^{e}	50 ± 3.21^{f}	417.41 ± 65.00^{e}
S20	6.23 ± 0.72^{b}	6.9 ± 0.85^{b}	83.33 ± 3.12^{b}	1086.3 ± 105.60^{b}
S20+PEG	4.13 ± 0.61^{de}	4.7 ± 0.56^{e}	55.56 ± 3.21^{ef}	492.78 ± 80.20^{e}

where: C1 = un-inoculated seeds; C2 = un-inoculated seeds + PEG 8000; M1 = seeds + S20 + R15 (S20 = *S. pseudovenezuelae* and R15 = *A. arilaitensis*); M2 = seeds + bacterial isolates S20 and R15 + PEG 8000; S20 = seeds + bacterial isolate S20, S20 + PEG = seeds + bacterial isolate S20 + PEG 8000; R15 = seeds + bacterial isolate R15 and R15 + PEG = seeds + bacterial isolate R15 + PEG 8000, S. length = shoot length and R. length = root length. All values are means of triplicate determinations ± S.E. Means followed by the same letters are not significantly different at $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

Ammonia, siderophore and hydrogen cyanide production by bacterial isolates

In the present study, all tested bacterial isolates produced ammonia. Siderophore production by bacterial isolates on CAS agar plates was positive for all the tested isolates (Figure 1 (c)). In the quantitative siderophore tests, siderophore production was significantly highest ($p < 0.05$) in *A. arilaitensis* (51.3 ± 2.11%), followed by *Streptomyces* spp. (48.6 ± 2.04%), *S. pseudovenezuelae* (48.3 ± 1.41%), *S. werraensis* (44.5 ± 0.48%), *M. oxydans* (42.7 ± 2.97%), *S. luteogriseus* (41.4 ± 2.57%) while *S. indiaensis* had the least production (38.3 ± 0.58%).

Seed germination tests

The results of the seed germination tests by *S. pseudovenezuelae* (MG547870) and *A. arilaitensis* (MG547869) are presented in Table 2. Shoot length, root length, germination % and vigor index was significantly highest in treatment M1 (9.27 ± 0.99 cm,10.17 ± 0.84 cm, 94.44 ± 3.21% and 1825.93 ± 133.74) respectively, compared to the other treatments. This was followed closely by treatment S20 with Shoot length, root length, germination % and vigor index of 6.23 ± 0.72 cm, 6.9 ± 0.85 cm, 83.33 ± 3.12% and 1086.3 ± 105.60 respectively. However, treatment C2 which was un-inoculated with PEG 8000 had the least Shoot length, root length, germination % and vigor index (2.5 ± 0.21 cm, 3.17 ± 0.24 cm, 42.59 ± 3.70% and 243.7 ± 31.73) respectively.

Effect of bacterial inoculation on drought tolerance in maize

The study showed that the plants inoculated with both bacterial isolates (*S. pseudovenezuelae* and *A. arilaitensis*) had significantly higher ($p < 0.05$) chlorophyll content index (CCI) of 10.85 ± 0.87 µ/g compared to the un-inoculated plants (8.17 ± 0.52 µ/g) at field capacity. For the plants that received moderate water, the inoculated plants also performed better than the un-inoculated ones as the highest CCI (8.27 ± 0.35 µ/g) at moderately wet category was observed in the plants co-inoculated with the two bacterial isolates while the lowest (7.37 ± 0.38 µ/g) was seen in the un-inoculated plants (Table 3). The results obtained for CCI on the completely drought stressed plants revealed that better CCI values were obtained by the inoculated plants than the un-inoculated plants as the co-inoculated plants produced CCI values of 7.13 ± 0.19 µ/g, the singly inoculated plants (BS and BR) produced CCI values of 6.72 ± 0.19 µ/g

Table 3. Effect of bacterial inoculation on growth parameter measurement of well-watered (FC), moderately watered (MW) and drought stressed (DS) maize plants.

Treatment	Chlorophyll content index (CCI) (µ/g)	Plant height (cm)	Root length (cm)	Number of leaves per plant	Leaf area (cm^2)	Dry shoot weight (g)	Dry root weight (g)
S (FC)	$8.17 \pm 0.52^{d-h}$	71.00 ± 2.64^{cde}	$43.00 \pm 7.63^{c-f}$	$11 \pm 0.34^{a-d}$	2887.2 ± 422.10^{de}	5.92 ± 0.47^{de}	$4.67 \pm 0.54^{d-g}$
S (Mw)	6.98 ± 0.38^{ijk}	58.80 ± 2.99^{ij}	$34.86 \pm 5.44^{f-i}$	10 ± 0.34^{fg}	2108.3 ± 323^{ghi}	$4.23 \pm 0.37^{g-k}$	$3.05 \pm 0.61^{g-k}$
S (DS)	5.65 ± 0.29^{l}	46.95 ± 2.52^{k}	19.43 ± 1.69^{jk}	8 ± 0.31^{i}	1012 ± 218.23^{k}	1.67 ± 0.16^{m}	1.15 ± 0.19^{m}
S + V (FC)	$8.45 \pm 0.38^{c-f}$	71.5 ± 2.02^{cde}	45.77 ± 7.29^{cde}	11 ± 0.17^{abc}	2952.3 ± 409.34^{cd}	6.12 ± 0.44^{de}	4.75 ± 0.53^{def}
S + V (Mw)	$7.37 \pm 0.18^{f-j}$	67.12 ± 2.47^{efg}	$37.98 \pm 4.92^{e-h}$	10 ± 0.17^{def}	2132.6 ± 399.87^{ghi}	$4.47 \pm 0.40^{f-j}$	$3.38 \pm 0.59^{f-k}$
S + V (DS)	6.03 ± 0.30^{kl}	54.88 ± 2.80^{j}	22.43 ± 1.49^{jk}	9 ± 0.21^{h}	1183.0 ± 235.03^{k}	2.4 ± 0.33^{lm}	1.57 ± 0.20^{lm}
S + BS (FC)	$8.77 \pm 0.59^{b-e}$	74.72 ± 2.52^{bc}	51.37 ± 9.49^{bcd}	11 ± 0.17^{abc}	3346.2 ± 350.36^{bc}	7.18 ± 0.42^{cd}	5.57 ± 1.03^{cde}
S + BS (Mw)	$7.5 \pm 0.43^{f-i}$	$66.4 \pm 2.05^{e-h}$	39.03 ± 6.46^{efg}	10 ± 0.17^{def}	244.3 ± 332.45^{efg}	$4.83 \pm 0.46^{e-i}$	$3.62 \pm 0.70^{f-j}$
S + BS (DS)	6.29 ± 0.32^{jkl}	60.27 ± 2.95^{hij}	26.05 ± 3.42^{ijk}	9 ± 0.21^{gh}	1710.2 ± 213.60^{ij}	3.0 ± 0.42^{kl}	$1.87 \pm 0.32^{k-m}$
S + BS + V (FC)	9.48 ± 0.50^{bc}	78.88 ± 2.31^{ab}	58.43 ± 9.84^{ab}	11 ± 0.17^{abc}	3613.6 ± 447.49^{ab}	8.05 ± 0.44^{bc}	6.78 ± 1.07^{bc}
S + BS + V (Mw)	$8.12 \pm 0.34^{d-h}$	$69.93 \pm 2.0^{c-f}$	41.42 ± 5.95^{def}	$10 \pm 0.17^{c-f}$	2646.3 ± 282.95^{def}	5.38 ± 0.42^{efg}	$3.98 \pm 0.64^{f-i}$
S + BS + V (DS)	6.72 ± 0.19^{ijk}	63.07 ± 2.44^{ghi}	$30.28 \pm 3.52^{g-i}$	10 ± 0.21^{fg}	1983.6 ± 196.65^{hi}	$3.55 \pm 0.32^{i-l}$	$2.45 \pm 0.36^{i-m}$
S + BR (FC)	$8.47 \pm 0.65^{c-f}$	73.98 ± 5.18^{bcd}	47.07 ± 8.88^{cde}	$11 \pm 0.50^{a-d}$	2.989 ± 532.92^{cd}	6.18 ± 0.77^{de}	4.80 ± 0.81^{def}
S + BR (Mw)	$7.32 \pm 0.44^{g-j}$	63.10 ± 4.04^{ghi}	$37.55 \pm 6.15^{e-h}$	10 ± 0.33^{efg}	2265.6 ± 417.39^{fgh}	$4.35 \pm 0.62^{g-j}$	$3.40 \pm 0.67^{f-k}$
S + BR (DS)	6.10 ± 0.38^{kl}	55.28 ± 4.18^{j}	23.10 ± 2.01^{jk}	9 ± 0.33^{h}	1378 ± 241.38^{jk}	$2.43l \pm 0.43^{m}$	$1.63 \pm 0.31l^{m}$
S + BR + V (FC)	8.92 ± 0.66^{bcd}	75.30 ± 3.06^{bc}	52.27 ± 9.37^{bc}	11 ± 0.17^{abc}	3433.1 ± 446.95^{b}	7.1 ± 0.74^{cd}	5.93 ± 1.03^{bcd}
S + BR + V (Mw)	$7.68 \pm 0.43^{e-h}$	$67.65 \pm 2.26^{d-g}$	40.15 ± 6.62^{efg}	10 ± 0.26^{def}	2476.6 ± 349.10^{efg}	$5.13 \pm 0.45^{e-h}$	$3.75 \pm 0.70^{f-i}$
S + BR + V (DS)	6.37 ± 0.33^{jkl}	62.80 ± 2.79^{ghi}	$28.60 \pm 3.53^{h-k}$	9 ± 0.26^{gh}	1771.7 ± 271.21^{ij}	3.3 ± 0.35^{jkl}	$2.05 \pm 0.36^{j-m}$
S + BR + BS (FC)	9.77 ± 0.60^{b}	79.60 ± 2.30^{ab}	62.02 ± 9.97^{a}	11 ± 0.17^{ab}	3761.8 ± 521.12^{ab}	8.47 ± 1.22^{b}	7.37 ± 1.02^{b}
S + BR + BS (Mw)	$8.17 \pm 0.36^{d-h}$	$70.40 \pm 2.20^{c-f}$	$42.20 \pm 6.46^{c-f}$	$10 \pm 0.26^{c-f}$	2669.2 ± 326.88^{def}	5.72 ± 0.66^{ef}	$4.38 \pm 0.60^{d-h}$
S + BR + BS (DS)	6.83 ± 0.24^{ijk}	$63.70 \pm 2.09^{f-i}$	$33.43 \pm 4.38^{f-i}$	10 ± 0.22^{fg}	1986.7 ± 246.42^{hi}	$3.62 \pm 0.50^{i-l}$	$2.82 \pm 0.32^{h-k}$
S + BR + BS + V (FC)	10.85 ± 0.87^{a}	83.23 ± 2.37^{a}	65.67 ± 11.12^{a}	11 ± 0.33^{a}	3991.3 ± 491.67^{a}	10.77 ± 0.67^{a}	9.027 ± 1.99^{a}
S + BR + BS + V (Mw)	$8.27 \pm 0.35^{d-g}$	70.87 ± 1.96^{cde}	$43.13 \pm 7.16^{c-f}$	$10 \pm 0.33^{b-e}$	2801.6 ± 362.73^{de}	6.13 ± 0.41^{de}	$4.33 \pm 0.65^{f-i}$
S + BR + BS + V (DS)	7.13 ± 0.19^{ijk}	$64.10 \pm 2.40^{f-i}$	$34.42 \pm 4.86^{f-i}$	10 ± 0.22^{efg}	2074.7 ± 258.17^{ghi}	$4.0 \pm 0.31^{h-k}$	$2.75 \pm 0.35^{h-k}$

S = Maize seed, V = vermiculite coated, BS = bacteria S20 (*S. pseudovenezuelae*), BR = bacteria R15 (*A. arilaitensis*), FC = field capacity, Mw = moderately wet and DS = drought stressed (completely dry). All values are means of triplicate determinations ± S.E. Means followed by the same letters are not significantly different at $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

Table 4. Effect of inoculation method on growth parameter measurements of maize plants.

Treatments	Chlorophyll content (CCI) (μ/g)	Plant height (cm)	Root length (cm)	No. of leaves/ plant	Leaf area (cm^2)	Dry shoot weight (g)	Dry root weight (g)
S	6.93 ± 0.52^{e}	60.31 ± 2.63^{e}	32.37 ± 7.62^{d}	9 ± 0.36^{e}	2002.5 ± 403.2^{d}	3.94 ± 0.42^{e}	2.96 ± 0.49^{e}
S + V	7.28 ± 0.38^{de}	62.78 ± 2.84^{de}	35.39 ± 5.46^{cd}	10 ± 0.34^{d}	2101.5 ± 325^{d}	4.30 ± 0.33^{ed}	3.25 ± 0.21^{de}
S + BS	7.52 ± 0.29^{de}	66.64 ± 2.48^{c}	$38.82 + 1.56^{bc}$	10 ± 0.29^{bc}	2500 ± 215.26^{c}	5.01 ± 0.19^{cd}	3.68 ± 0.57^{cde}
S + BS + V	8.11 ± 0.34^{bc}	70.78 ± 2.03^{ab}	43.38 ± 7.19^{ab}	10 ± 0.15^{ab}	2748.9 ± 387.82^{ab}	5.77 ± 0.42^{b}	4.41 ± 0.41^{bc}
S + BR	7.2 ± 0.19^{de}	65.46 ± 2.43^{cd}	35.91 ± 1.93^{cd}	10 ± 0.16^{cd}	2198.7 ± 230.64^{d}	4.35 ± 0.4^{ed}	3.26 ± 0.26^{de}
S + BR + V	7.66 ± 0.28^{cd}	67.74 ± 2.82^{bc}	40.34 ± 9.25^{bc}	10 ± 0.17^{cd}	2560.5 ± 355.1^{bc}	5.18 ± 0.31^{bc}	3.91 ± 0.32^{cd}
S + BR + BS	8.26 ± 0.55^{ab}	71.33 ± 2.29^{a}	45.88 ± 6.42^{ab}	10 ± 0.21^{abc}	2804.9 ± 227.2^{a}	5.82 ± 0.42^{b}	4.8 ± 0.37^{ab}
S + BR + BS + V	8.75 ± 0.41^{a}	72.81 ± 2.91^{a}	47.74 ± 3.42^{a}	10 ± 0.33^{a}	2955.8 ± 217.69^{a}	7.0 ± 0.46^{a}	5.36 ± 0.54^{a}

S = maize seed, V = vermiculite coated, BS = bacteria S20 (*S. pseudovenezuelae*), BR = bacteria R15(*A. arilaitensis*). All values are means of triplicate determinations. Means followed by the same letters are not significantly different at $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

and 6.37 ± 0.33 μ/g respectively while the un-inoculated plants produced a CCI of 5.65 ± 0.29 μ/g.

From Table 3, the greatest increases in plant heights (relative to the control) were observed in plants that received water at field capacity, followed by the moderately wet plants while the lowest increase was observed in plants that did not receive water at all (completely dry). At field capacity, the highest height of 83.23 ± 2.37 cm was observed in the plants inoculated with the two bacterial strains while the lowest height of 71 ± 2.64 cm was observed in the un-inoculated plants at field capacity. For the moderately wet category, the highest height of 70.87 ± 1.96 cm was observed in the co-inoculated plants followed by the individually inoculated plants whose heights were 69.93 ± 2.0 cm for plants inoculated with *S. pseudovenezuelae* and 67.65 ± 2.26 cm for plants inoculated with *A. arilaitensis* while the lowest height of 58.80 ± 2.99 cm was observed in the un-inoculated plants at this water level. Results at the no water level revealed that better plant height of 64.10 ± 2.40 cm was observed in plants whose seeds were co-inoculated with the two bacterial strains followed by plants whose seeds were singly inoculated by either *S. pseudovenezuelae* (63.07 ± 2.44 cm) or *A. arilaitensis* (62.80 ± 2.79 cm) while the lowest heights of 46.95 ± 2.52 cm was observed in the plants whose seeds were un-inoculated.

Results on the root lengths followed the same trend, as the longest root was observed in plants whose seeds were co-inoculated with *S. pseudovenezuelae* and *A. arilaitensis* with root length of 65.67 ± 11.12 cm at field capacity, 43.13 ± 7.16 cm at moderately wet level and 34.42 ± 4.86 cm at the completely dry level. On the other hand, lower root lengths were observed in the un-inoculated plants (43 ± 7.63 cm) at field capacity, 34.86 ± 5.44 cm at moderately wet and 19.43 ± 1.69 cm at the completely dry level.

The number of leaves on each plant also varied according to treatment as more leaves were observed in the inoculated plants than in the un-inoculated ones. Plants inoculated with the combination of the two bacterial isolates produced approximately 11 ± 0.33 leaves per plant at field capacity, 10 ± 0.33 leaves per plant at moderately wet capacity and 10 ± 0.22 at drought stress capacity. On the other hand, significantly lower leaf number were observed per plant in the un-inoculated plants at moderately wet (10 ± 0.34) and drought stressed (8 ± 0.31) levels. However, there was no significant difference in the number of leaves obtained at field capacity for both inoculated and un-inoculated plants, as approximately 11 leaves per plant were observed at this water level for both treatments. Leaf area of 3991.3 ± 491.67 cm^2 was observed in the co-inoculated plants at field capacity, 2801.6 ± 362.7 cm^2 at moderately wet capacity and 2074.7 ± 258.17 cm^2 was observed in these plants when there was no water application at all. For the un-inoculated plants, total leaf area per plant was 2887.2 ± 422.10 cm^2 at field capacity, 2108.3 ± 323 cm^2 at moderately wet capacity and 1012 ± 218.23 cm^2 at zero water capacity.

Significant differences in dry shoot weights were observed in this study (Table 3). Co-inoculated plants produced significantly higher ($p < 0.05$) shoot weights of 10. 77 ± 0.67 g at field capacity, 6.13 ± 0.41 g at moderate water application and 4.0 ± 0.31 g at zero water application while lower shoot weights were observed in un-inoculated plants as weights of 5.92 ± 0.47 g were obtained at field capacity, 4.23 ± 0.37 g at moderately wet and 1.67 ± 0.16 g at zero water level. Higher dry root weights were also observed in the inoculated plants than the un-inoculated plants as the highest weight of 9.027 ± 1.99 g was observed in the co-inoculated plants at field capacity level while the lowest weights of 4.67 ± 0.54 g were observed in the un-inoculated plants at this capacity. At moderately wet capacity, 4.38 ± 0.60 g was observed in co-inoculated plants while 3.05 ± 0.61 g was observed in un-inoculated plants and at zero water level, the highest dry root weight was observed in co-inoculated plants as 2.75 ± 0.35 g while the lowest was observed in the un-inoculated plants as 1.15 ± 0.19 g.

Effect of inoculation method on growth parameter measurements of maize plants

The mean data on the effect of inoculation method on the growth of maize are presented in Table 4. The effect of the two inoculation methods used in the study showed that greater growth parameters were observed in plants whose seeds were bound with carboxylmethyl cellulose and coated with vermiculite than the directly inoculated plants. From the results, treatment S + BR + BS + V produced significantly higher ($p < 0.05$) CCI value (μ/g) of 8.75 compared to 8.26 μ/g obtained in treatment S + BR + BS. Similarly, treatment S + BS + V gave a CCI of 8.1 μ/g while treatment S + BS gave a CCI value of 7.52 μ/g. Improved CCI was also observed in treatment S + BR + V (7.66 μ/g) compared to treatment S + BR (7.2 μ/g). For the control seeds, results showed that better CCI value was observed in treatment S + V (7.28 μ/g) than in treatment S (6.93 μ/g). The data on plant height, root length, number of leaves per plant, leaf area, dry shoot and root weight also revealed that for all the treatments, plants with seeds immersed in 1% CMC and coated with vermiculite were better in terms of growth and all the parameters measured than the plants with seeds were either directly inoculated with bacteria or distilled water.

Discussion

Plants are continuously exposed to abiotic stresses such as drought, salinity and cold (Bardi and Malusà 2012). Drought, being one of the most serious environmental problems affecting the growth and development of plants and subsequently agricultural yields and food supply, has gained research attention over the years. The ravaging effects of drought on plants can be reduced by the action of PGPB with PGP traits. These bacteria are capable of tolerating and surviving under harsh environments through the regulation of phytohormones, production of ACC deaminase activity, accumulation of osmolytes, production of volatile compounds and antioxidant defense (Vurukonda et al. 2016).

Plants' developmental processes are being regulated by the production of phytohormones in their various parts. The phytohormone, indole-3-acetic acid, plays a major role in plant development and its supply is capable of supporting its host during stress conditions like drought and pathogenic attacks (Bardi and Malusà 2012; Sathya et al. 2017). It also improves seedling growth, and cell differentiation, as well as enhancing both elongation and development of lateral roots in plants (Vurukonda et al. 2016; Sathya et al. 2017).

Several rhizospheric bacteria have been documented for their ability to produce IAA as well as their different biosynthetic pathways of IAA production (Cassán et al. 2014; Duca et al. 2014; Vijayabharathi et al. 2016). Results from the present study revealed that all tested bacterial isolates produced indole-3-acetic acid. However, the amount of this acid produced varied in bacterial isolates tested. The highest IAA production was obtained in *S. werraensis* (10.12 ± 0.02 µg/ml) while the least (5.03 ± 0.01 µg/ml of IAA) was produced by *M. oxydans*. The increased amount of IAA produced by these bacteria in the medium used was due to the presence of L-tryptophan, as corroborated by Idris et al. (2007) who revealed that the secretion of IAA can be increased by the addition of tryptophan in medium. In this regard, inoculating maize plants with IAA producing bacteria can improve the growth and development of the plant under drought stress. The IAA result of this study is in agreement with previous reports of IAA production by bacteria. Studies have shown that the endophytic IAA producing *Streptomyces* species (*astrovirens, olivaceoviridis, rimosus, rochei and viridis*) improved seed germination, growth and root elongation in plants (El-Tarabily 2008; Khamna et al. 2010). Matsukawa et al. (2007) also reported that IAA triggered cell differentiation, sporulation and hyphal elongation in *Streptomyces atroolivaceus*.

Solubilization of phosphate is an important mechanism of plant growth promotion (Richardson 2001). Bacteria are capable of increasing the availability of phosphorus (P) to plants through mechanisms such as the secretion of phosphatase to free P bound in organic matter and the production of organic acids / chelating substances that helps to decrease rhizosphere pH (Rashid et al. 2004; Chen et al. 2006). Several bacterial species including *Pseudomonas* and *Bacillus* have also been reported to solubilize inorganic phosphates. In a study by Chabot et al. (1993), growth of lettuce and maize were enhanced by certain microorganisms capable of solubilizing mineral phosphate. Rodríguez and Fraga (1999) also reported phosphate solubilization in *Pseudomonas striata* and *Bacillus polymyxa*. In this regard, the result from the present study conforms to other previous studies on phosphate solubilization.

The introduction of drought tolerant ACC deaminase producing bacteria to drought stressed soils helps to improve stress tolerance in plants by lowering the production of stress induced ethylene. Several studies have reported the production of ACC deaminase activity bacteria (Glick et al. 2007; Rashid et al. 2012; Glick 2014). Drought tolerant bacteria are capable of surviving in dry environments by adhering to the roots of developing seedlings or on seed coats of plants causing the deamination of ACC (the immediate precursor of ethylene in plants) by ACC deaminase which decreases the level of plant ethylene and consequently enhances plant growth and development (Glick 2005; Ali et al. 2014). The mechanism of action of ACC deaminase producing bacteria in the improvement of both biotic and abiotic stresses is by the reduction of stress ethylene level through the activity of the enzyme ACC deaminase which breaks down ACC into α-ketobutyrate and ammonia instead of ethylene (Arshad et al. 2008). Bacteria producing ACC deaminase activity are known to improve the growth of a wide range of plants under stressful conditions like drought, salinity, heavy metals and flooding (Belimov et al. 2009; Shakir et al. 2012; Ali et al. 2014; Vurukonda et al. 2016). They also play major roles in plant nodulation processes in different leguminous plant species (Belimov et al. 2009).

The methods used by bacteria to inhibit pathogenic growth may include the secretion of volatile compounds like ammonia and other antifungal enzymes, the production of HCN, competitive secretion and the production of siderophores (Brimecombe et al. 2000). The production of low molecular weight metal chelators (siderophore) by the tested bacterial isolates offers them a competitive advantage to be used as biocontrol agents and to contribute to disease suppression in plants due to insufficient supply of essential trace minerals in natural environments (Laslo et al. 2012). A stimulated biosynthesis may cause these tested bacterial isolates to directly secrete antimicrobial compounds. In antagonism effect development, siderophore production and antifungal effect play major roles, although antifungal effects encompass other features (Selvakumar et al. 2008). Results obtained from the present study concur with the work of Laslo et al. (2012) who reported that 36.2% of tested isolates produced siderophore. Quan et al. (2010) detected different types of siderophores in *Pseudomonas* species. Furthermore, Plant growth promoting bacteria are able to produce ammonia as secondary metabolite, also playing a major role in antagonistic effects (Compant et al. 2005). Among all tested bacterial isolates for HCN activity, only *Streptomyces* spp. produced HCN, indicating its potential for use as a biocontrol agent.

Plant growth promoting rhizobacteria capable of colonizing both the surface and inner parts of plant roots play essential roles that directly or indirectly influence plant growth and development (Gerhardt et al. 2009). In this study, maize seed treatment with the two selected bacterial strains *S. pseudovenezuelae* and *A. arilaitensis* significantly improved the emergence and growth of the seedlings. Different mechanisms have been proposed for the promotion of plant growth by PGPB, which include the indirect enhancement of seed germination and vigor index by reduction in the incidence of seed mycoflora that can negatively affect plant growth (Begum et al. 2012). In a study by Duarah et al.

(2011), amylase activity was increased during rice and legume germination after treatment with PGPB. Starch is hydrolyzed by amylase to metabolizable sugars to provide the roots and shoots of germinating seeds with the energy to grow. The production of phytohormones such as IAA is another commonly reported mechanism of plant growth promotion (Patten and Glick 2002). In this study, all tested isolates produced IAA. Studies have shown that several PGPB produce IAA (Ng et al. 2012; Zahid 2015). IAA promotes root development and nutrient uptake making it a very important mechanism of plant growth promotion.

Drought stress is a serious environmental problem in agriculture as it causes severe loss in plant yield, depending on its severity (Farooq et al. 2009). In this study, maize survival and growth was affected when drought stress was introduced. However, under drought stress conditions, better growth was observed in bacterial inoculated maize plants than the un-inoculated plants as better survival, dry root and shoot weight, root and shoot length and chlorophyll content were observed. Over the years, PGPB have been used mostly to promote plant growth because of their ability to stimulate plant growth through certain mechanisms such as the production of plant growth regulators and fixation of nitrogen (Lucy et al. 2004). Studies have demonstrated other beneficial effects of PGPB on plants including their ability to enhance tolerance toward several abiotic stresses such as drought (Yang et al. 2009; Wang et al. 2012; Yandigeri et al. 2012; Vurukonda et al. 2016). A study by Creus et al. (2004) demonstrated that there was significant increase in growth, water content, water potential, relative water content and apoplastic water function in roots and shoots of wheat plants primed with *Azospirillum brasilense* Sp245 compared to un-primed plants. In addition, Pereyra et al. (2012) reported that inoculation of *Azospirillum* on maize seedlings under osmotic stress enhanced better water status of the seedlings indicated by the morphological modifications of the coleoptile xylem architecture. Their results were also attributed to *Azospirillum* ability to produce IAA and improved bacterial IAA synthesis.

A number of abiotic stresses are associated with ROS species accumulation in plant cells. Reactive oxygen species react with DNA, membrane lipids and proteins and are capable of causing severe oxidative damage to plant tissues (Reddy et al. 2004). For plants to be able to survive under drought stress, they need to avoid oxidative damage. These species can be removed by certain enzymes: catalase and peroxidases such as glutathione peroxidase (GPX) and ascorbate peroxidase (APX) (Gong et al. 2005). In our previous study, *S. pseudovenezuelae* and *A. arilaitensis* showed the presence of glutathione peroxidase, indicating their capability of withstanding drought stress by the avoidance of oxidative damage. This could also be the reason behind the greener leaves of the inoculated plants over the un-inoculated ones. We also observed that the severity of drought stress showed more on the un-inoculated plants than the inoculated plants as the un-inoculated plants showed more signs of wilting than the inoculated plants. The increase in the severity of drought stress causes increase in enzyme (GPX and APX) activity. Koussevitzky et al. (2008) demonstrated that APX activity and APXI transcript levels were increased in *Arabidopsis thaliana* plants after their exposure to drought stress. They concluded that APX is necessary to protect plant chloroplast from increased levels of ROS during drought as APX helps in scavenging ROS. Omar et al. (2009) also recorded lower peroxidase and catalase activities in the leaves of barley plants primed with *Azospirillum brasilense* under salinity stress.

Damage to plant proteins often results from stress exposure, therefore it is necessary to maintain proteins in their functional forms to enable plants to survive under stress conditions (Wang and Huang 2004). Plant proteins like the Heat-shock proteins (HSP), Malic-enzyme proteins (ME), glycine-rich RNA binding proteins (GRP) and desiccation protectant proteins are often synthesized during stress conditions and are recognized as mechanisms of stress tolerance in plants (Wahid et al. 2007). They play major roles in translocation, protein folding, degradation and assembly in several cellular processes. They can also assist in stabilizing and refolding of proteins under different conditions of stress (Wang and Huang 2004). From our previous study on drought tolerant genes, *S. pseudovenezuelae* and *A. arilaitensis* were observed to possess the proteins glycine-rich RNA binding protein, and Malic-enzyme and this could have also contributed to their better survival under drought stress.

The growth of plants depends highly on differentiation, enlargement and cell division. Also, drought stress affects the physiological, morphological, ecological and genetic processes of plant growth (Taiz and Zeiger 2002; Farooq et al. 2009; Vurukonda et al. 2016). According to Farooq et al. (2009), severe water limitation causes an inhibition in cell elongation as a result of water flow interruption from the xylem to surrounding elongation cells leading to cell mitosis and cell expansion; and finally resulting in reduced plant growth. The growth and formation of lateral roots may be stimulated by PGPB, thereby increasing the capacity of water uptake of inoculated plants. Studies have described the roles of PGPB in modifying plant metabolism under normal and abiotic stress conditions by mechanisms including indole-3-acetic acid production, ACC deaminase activity, nitrogen fixation and antioxidant production (Dimkpa et al. 2009; Bashan and De-Bashan 2010). Plant growth promoting bacteria are also capable of producing compatible solutes (glycine-betaine and proline) that assist in the processes of osmoregulation (Dimkpa et al. 2009). In the present study, better tolerance to drought stress conditions was observed in bacterial inoculated plants than un-inoculated plants. This could be as a result of production of IAA, ACC deaminase and glycine-rich protein by these bacterial isolates.

Besides the inoculation of plants with single strains of PGPB, co-inoculation or combination of two or more strains also induces drought stress tolerance in plants to an even greater extent (Wang et al. 2012). From the results obtained in the present study as shown in Table 3, better tolerance was observed in the co-inoculation of *S. pseudovenezuelae* (MG547870) and *A. arilaitensis* (MG547869) in maize plants as better shoot and root lengths, dry shoot and root weights, chlorophyll content and numbers of leaves were observed in the plants. Moreover, wilting of leaves was observed to be lower in the co-inoculated plants than those inoculated with either *S. pseudovenezuelae* or *A. arilaitensis*, which were much better than the control. The results from this study are in agreement with the study of Wang et al. (2012), who observed enhanced drought tolerance in cucumber plants when the seeds were inoculated with a Microbial consortia consisting of the PGPB *Bacillus subtilis* SM21,

Bacillus cereus AR156 and *Serratia sp*. XY21 (BBS). According to Wang et al. (2012), darker green leaves, lighter wilting symptoms, relative electrical conductance, increased leaf proline and chlorophyll content and intension of root recovery were observed in BBS treated plants after water was withheld for 13 days. In a similar study, exopolysaccharide producing bacterial strains *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2) and *Alcaligenes faecalis* (AF3) exhibited better tolerance to drought stress in maize compared to individual PGPB strains (Naseem and Bano 2014). Results obtained from the study showed that maize physiological parameters were enhanced by the inoculation with both bacterial isolates, as well as with individual isolates, as better growths were observed in inoculated maize plants than the un-inoculated ones. This confirms the effectiveness of bacteria inoculation on growth and drought tolerance in plants.

The effect of inoculation method on the growth and drought tolerance potential of *S. pseudovenezuelae* (MG547870) and *A. arilaitensis* (MG547869) in maize seeds revealed that plants whose seeds were co-inoculated with *S. pseudovenezuelae* and *A. arilaitensis* and coated with vermiculite showed better increase in growth parameter measurements compared to the plants whose seeds were directly co-inoculated with the bacterial isolates. Similarly, better growth was observed when seeds were un-inoculated but coated with vermiculite compared to the plants with seeds merely immersed in distilled water. In all, seed inoculation with the combination of the two bacterial strains was more effective than inoculation with individual bacterial strains. The better results obtained from the growth parameter measurements of the vermiculite coated seeds when compared to the uncoated seeds of both inoculated and un-inoculated seeds on the effect of inoculation method used in this study, could have been due to the presence of 1% carboxylmethyl cellulose (CMC), as this adhesive may have facilitated the better binding of the bacterial isolates to the seeds. Carboxymethyl cellulose is an adhesive that has also been used in drug and food industries (Williams and Phillips 2004; Delcour and Poutanen 2013; Ibarra et al. 2016). It plays major roles in binding inoculants to seeds and also protects the seeds from desiccation. It may also provide nourishment for the inoculated plants (Elegba and Rennie 1984). On the other hand, vermiculite has a neutral pH with a good buffering capacity. It does not produce organic by-products and does not undergo any change in structure when sterilized. At extreme temperature, it exfoliates and kills contaminants as its mineral nature does not support the growth of microbes. During fermentation, vermiculite provides space for microbial growth, good aeration and quick temperature equilibration. It has good sticking properties which makes it widely used as seed coats. Therefore, the coating of the seeds with vermiculite in this study may have helped to protect the seeds from possible insect and pathogen attacks. From the results obtained in the present study, it is encouraged that for efficient tolerance to drought stress, inoculated seeds should be bound as well as coated with good binding and coating agents as this will reduce pest attacks, preserve seeds for longer periods and enhance easy delivery.

Conclusion

Changes in climatic conditions can bring about undesirable environmental conditions, including drought which is responsible for several physiological and morphological changes in plants leading to mass decrease in agricultural productivity. Soil harbors numerous bacteria that can be beneficial in agriculture to facilitate growth and abiotic stress tolerances in plants. Plant growth promoting bacteria facilitate plants' growth and help them to resist and adapt to harsh and dry environmental conditions (drought), and could also play a major role in solving the problem of global food insecurity. Tolerance to drought stress by PGPB can be enhanced through a variety of mechanisms ranging from phytohormonal modifications, ACC deaminase activity, to alteration in root morphology and molecular techniques. Drought stress tolerance by bacteria is an emerging technology that is cost effective and efficient to help solve the problem of low crop productivity and yield. This study has shown that the inoculation of drought tolerant *S. pseudovenezuelae* (MG547870) and *A. arilaitensis* (MG547869) strains increases plant growth as well as reduces the undesirable effect of drought stress when used as bioinoculants on maize plant. *Streptomyces pseudovenezuelae* and *Arthrobacter arilaitensis* with high IAA and ACC production will be beneficial isolates for biofertilization of crops especially under drought stress conditions, as they mitigated the impact of drought on the maize plants and increased plant biomass and physiological parameters.

However, for efficient results, it is highly encouraged that inoculated seeds are bound as well as coated as this will ensure that bacteria are well adhered to the seeds and also protected from insect attacks. Further studies are required to understand the exact molecular mechanisms of plant-bacterial interactions in the rhizosphere for plant growth and drought tolerance, as understanding of these mechanisms is necessary for efficient elicitation of drought stress in plants by soil bacteria.

Author contribution statement

All authors contributed in data collection, wet laboratory, analyses, and drafting of the manuscript for publication.

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