ISOLATION, SCREENING AND RESPONSE OF MAIZE TO PLANT GROWTH PROMOTING *RHIZOBACTERIA INOCULANTS*

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The use of plant growth promoting rhizobacteria (PGPR) as biofertilizer is a relatively safer, environment friendly and cost effective. This work was designed to assess plant growth promoting abilities of rhizobacteria and evaluate their effect on germination and growth of maize. The bacteria were isolated and screened for plant growth promoting abilities using Pikovskaya agar, Aleksandrov agar and Jensen media. Twelve isolates that showed multiple attributes were further screened for indole acetic acid (IAA) and gibberellic acid (GA) production; best five isolates were selected for further studies. The results of IAA and GA production showed a considerable amount of IAA and GA produced by the isolates which ranged between 9–94 and 21–97 mg l^{-1}, respectively. The selected isolates identified as *Bacillusmojavensis*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Pseudomonas syringae* and *Bacillus cereus* showed a significant difference($P \leq 0.05$) in the amount of potassium(K) and phosphorus(P) solubilized at different source of K (KCl and mica powder) and P (Ca_2PO_4 and rock phosphate). The isolates also recorded significant level of nitrogen fixing ability with *Alcaligenes faecalis strain* P156 fixing the highest amount of nitrogen (11.4 mg N fixed per l) and least by *Bacillus mojavensis* strain NBSL51 (6.3 mg N fixed per l).The results of plant inoculation test showed that *Bacillus cereus strain* 20UPMNR significantly enhanced the root and shoot dry weight. All the selected isolates enhanced shoot and root length except *Bacillus mojavensis* which produced less effect on root length when compared to the control. These results have provided vital information for the development of a bio fertilizer for maize.

P-solubilization, N-fixation, K-solubilization,

sciendo

doi: 10.2478/sab-2019-0025
Received for publication on February 21, 2018
Accepted for publication on June 10, 2019

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are beneficial soil bacteria which inhabit the rhizosphere and roots of plants and enhance plant growth and protect against disease causing organisms (El-Mancy, Kotb, 2006;Van Loon, 2007; Myresiotis, Vryzas, 2012). These organisms promote plant growth through solubilizing some valuable nutrients in soil such as potassium and phosphorus and through synthesis of plant growth hormones thereby making them available to plant. Currently, there is an increasing interest in testing PGPR-based products in agricultural crop production systems. These products are mainly applied as seed treatment, soil amendment, or soil drench at the time of seeding or immediately after transplanting, to promote plant growth and effectively suppress several diseases in a number of crops (Kloepper et al., 2004).

Researchon PGPR with non-leguminous plants has shown beneficial effects throughN_2-fixation,increase droot growth with enhanced nutrient uptake (Saharan, Nehra, 2011).The exploitation of microbes for biofertilization can contributeto minimizing the use of expensive inorganic fertilizers which may negatively affects oil physiology (Sepril,2012) and reduce soil fertility. PGPR are the natural potential resources which colonize roots of plant sand stimulate growth and yield directly and indirectly (Afzal, Bano, 2008). The indirect plant growth promotion is associated with the production of antibiotics or siderophores by PGPR which decrease or prevent the deleterious

effects of plant pathogenic microorganisms (Sivan, Chet, 1992) whereas the direct promotion of growth by PGPR includes production of metabolites enhancing plant growth. Phosphate and potassium solubilizing bacteria have been shown to enhance solubilization of insoluble phosphate and potassium compounds through the release of organic acids and phosphatases (Sharma, 2005) and this makes K and P available to plants. A considerable number of bacterial species isolated from the rhizosphere have shown evidence of plant growth promotion (El-Hadad et al.,2010). However, the continuous use of synthetic fertilizers affects beneficial soil organisms and their vital function in the soil as well as soil chemical properties and fertility. As a result of this, the utilization of plant growth promoting bacteria in place of chemical fertilizers will not only reduce the over dependence on chemical fertilizers and restoration of soil fertility but will reduce the risk associated with it. This study therefore was designed to isolate and assess plant growth promoting abilities of native bacterial isolates from the rhizosphere of maize and evaluate their effect on the germination and growth of maize.

MATERIAL AND METHODS

Samples collection

Soil samples were collected from maize rhizosphere at different maize farms in the Institute of Agricultural Research and Training Moor Plantation Ibadan, Oyo State, Nigeria.The soil (loamy, pH 6.5–8.0) bound to the roots of maize was collected into sterile polythene bags placed on ice and taken immediately to the laboratory section of the institute for analysis.

Isolation of plant growth-promoting rhizobacteria

The PGPR were isolated by suspending 1g rhizosphere soil in 9ml of distilled water and serially diluted to 10^{-8}. An aliquot (1ml) of the sample diluted to 10^{-8} was inoculated on Pikovskaya agar, Aleksandrov agar and Jensen media by pour plate technique and incubated at 30°C for 72 h. The colonies showing phosphate, potassium, solubilizing zone around them were considered as P and K solubilizers a(Tan et al.,2014). Distinct colonies appearing on respective media were stored on agar slants for further studies.

Screening of the isolates for plant growth promoting abilities

The N_2 fixation ability was tested by growing the isolates on Glucose Nitrogen Free Mineral medium (GNFM medium) and incubated for 3–5 days at 30°C. The ability of the isolates to fix N_2 was indicated by the colour change of the medium from green to yellow after the incubation period (Bashir et al., 2013). All the experiments were conducted in triplicates.

Phosphate solubilization test

The phosphate solubilization activity of the isolates was evaluated by growing them in Pikovskaya agar for 72 h at 30°C, the bacterial isolates were spot-inoculatedat the centre of the prepared plates (Tan et al., 2014). The zones of phosphate solubilization formed around colonies were recorded after 72 h. The solubilization indexes of the isolates were determined by dividing the total diameter of the halo zone (colony + halo zone) by the diameter of the colony.

Potassium solubilization ability

The selected isolates were grown on a modified Aleksandrov agar medium (Hu et al., 2006). To determine the ability to solubilize potassium, a loopful of bacterial suspension was streaked onto the medium and incubated at 30°C and inspected on the 5th day of incubation. The ability to solubilize muscovite mica as a source of insoluble form of potassium was manifested by the formation of a clear halo zone around the colony. The solubilization index of the isolates was determined by dividing the total diameter of the halo zone (colony + halo zone) by the diameter of the colony (Tan et al., 2014).

Indole acetic acid (IAA) production ability

Bacterial isolates were inoculated into 100 ml peptone water and shaken on an orbital shaker for 24 h. One ml of the bacterial culture was transferred into 100 ml of fresh peptone water with the addition of 5 ml of L-tryptophan as a precursor of indole acetic acid (Tan et al.,2014).Peptone water without bacterial inoculum served as control. A portion (5 ml) of the bacterial culture was transferred into a sterile tube and centrifuged at 7000 rpm for 7 min. The supernatant (1ml) was mixed with 2ml of Salkowsky reagent (2% of 0.5M $FeCl_3$ in 35% perchloric acid) (Gordon, Weber, 1951). The solution was allowed to stand for 25 min and the IAA production was indicated by a colour change into pink. The absorbance values were determined using a Jenway 6105 UV VIS spectrophotometer (Jenway UK) at 535 nm and compared to the standard curve to determine the IAA concentration. The IAA standard curve was prepared using pure IAA at 0, 5, 10, 15, 20, 25, 30 mg/ml of IAA.

Gibberellic acid (GA) production

The isolates were inoculated in Jenson broth and incubated on a shaker for 5 days. The culture medium was filtered to eliminate bacterial biomass, the

pH value of supernatant was adjusted to 2.5 using stock (37%) HCl. The supernatant was extracted using the liquid-liquid (ethylacetate/NaHCO3) extraction method (Umi et al., 2014).The amount of GA in the ethylacetate phase was measured by a Jenway 6105 UV VIS spectrophotometer (Jenway, UK) at 254 nm.

Identification and characterization of plant growth promoting bacteria

The bacteria isolates with multiple abilities (P and K solubilization, nitrogen fixing ability and phytohormone production) were identified using their morphological characteristics. Biochemical tests such as oxidase test, sucrose, lactose fermentation, starch hydrolysis, gelatin hydrolysis, nitrate reduction, etc. were carried out. The isolates were identified following Bergey's manual for systematic bacteriology methods(H o l t s ,1994).

Selection of isolates for further studies

The selection of five out of eleven isolates with multiple abilities was based on the potassium and phosphorus solubilization index and the amount of IAA acid and GA produced in the culture.

Isolation of bacteria genomic DNA of five selected PGPR

Bacterial genomic DNA was extracted using the protocol of T r i n d a d e et al. (2007). Single colonies of the selected isolates on nutrient agar medium were transferred to 1.5 ml ofluria broth and cultures were grown on a shaker for 48 h at 28°C. After incubation, the bacterialcultures were centrifuged at 4600 *g* for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0).15µl of 20% SDS and 3 µl of proteinase K (20 $mgml^{-1}$) were added. The mixture was incubated for 1 h at 37°C, then 100 µl of 5 M NaCl and 80 µl of a 10% CTAB solution in 0.7 M NaCl were added and mixed. The suspension was incubated for 10 min at 65°C and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 *g* for 20 min.The aqueous phase was transferred to a new tube, isopropanol (1: 0.6) was added and DNA was precipitated at –20°C for 16 h. The DNA was collected by centrifugation at 7200 *g* for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately 3 h and finally dissolved in 50 µl of TE buffer.

Amplification of 16S rRNA

A polymerase chain reaction cocktail consisted of 10 µl of 5XColorless GoTaq®reaction buffer (Promoga, USA), 3 µl of MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol ofeach primer:27F 5'-AGA GTT TGA TCM TGG CTC AG-3' and 1525R, 5′-AAGGAGGTGATCCAGCC-3′, and 0.3 units of GOTaq® DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water and8µl DNA template. ThePCR was carried out using a GeneAmp® PCR System 9700 thermalcycler (Applied BiosystemsInc., USA) with an initial denaturation at 94°C for 5 min; 30 cyclesat 94°C for 30 s, at 50°C for 60 s and at 72°C for 90 s;and a final extension at 72°C for 10 min, chill at 4°C.

Purification of the amplified product

The amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of 3 M Na acetate and 240 µl of 95% ethanol were added to each 40µl PCR amplified product in a new sterile 1.5 µl Eppendorf tube, mixed thoroughly by vortexing, and kept at –20°C for at least 30 min. Then centrifugation for 10 min at 13000 *g* and 4°C was followed by the removal of supernatant (invert tube on trash once) after which the pellets were washed by adding 150 µl of 70% ethanol and mixed, then centrifuged again for 15 min at 7500 *g* and 4°C. Again all supernatant was removed (invert tube on trash) and the tube was inverted on paper tissue and allowed to dry in the fume hood at room temperature for 10–15 min. It was then resuspended with 20 µl of sterile distilled water and kept at –20°C prior to sequencing. The purified fragment was checked on 1.5% agarose gel run on a voltage of 110V for about 1h, so as to confirm the presence of the purified PCR product.

Sequencing of the amplified product

The amplified fragments were sequenced using a 3130xl Genetic Analyzer (Applied Biosystems) using manufacturers' manual while the sequencing kit used was that of BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA). BioEdit software and MEGA 6 were used for all genetic analysis. The DNA sequence was compared with the sequence available in NCBI database using BLAST.

Quantification of potassium and phosphorus solubilized by the selected isolates

Quantification of potassium. The method of T a n et al.(2014) was adopted in potassium quantification. One ml of an over-night culture of each isolate diluted to 0.5 McFarland standard was inoculated to 50ml of Aleksandrov broth and incubated for five days. At the end of the five days, the broth cultures were centrifuged at 10 000 rpm for 10 min to separate the supernatant from the bacterial cells and insoluble potassium. One ml of the supernatant was taken into a

Table 1. Biochemical characteristics of the plant growth promoting bacteria isolates

Biochemical test	Isolates code										
	MY2	MY20	MY9	MY25	MY19	MY17	MW10	MW18	MY15	MY16	MY4
G/stain	+	–	–	+	–	+	–	–	–	–	+
Motility	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	–	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	–	+	–	+	–
Indole test	–	–	–	–	–	–	–	–	–	–	–
Methyl Blue test	–	–	–	+	–	–	–	–	–	–	+
Malachite Green	–	–	–	+	–	–	–	–	–	–	+
Glucosefermentation	+A	+	+	+AG	–	+A	+G	+	+G	+	+A
Mannitol	+A	+	+	+AG	–	+A	–	+	+	+	+A
Lactose	+A	+A	+A	+AG	+	+A	–	+	+	+A	+A
Fructose	+A	+A	+A	+AG	–	+A	–	+	+	+A	+A
Arabinose	+A	+A	+A	+AG	–	+A	–	+	+	+A	+A
Xylose	+A	+A	+A	+AG	–	+A	+	+	+	+A	+A
Maltose	+A	+A	+A/G	+AG	+	+A	–	+	+	+A	+A
Urease	+	+	+	+	–	+	+	+	+	+	–
Starch hydrolysis	–	+	+	+	+	–	+	+	+	+	–
Citrate utilization	–	+	+	–	+	–	+	–	+	+	–
Nitrate reduction	–	–	–	–	–	–	+	–	+	–	–
Methyl Red	–	–	–	–	–	–	+	–	+	–	+
Vogesproskauertest	–	+	+	–	–	–	–	+	–	+	+

MY2 = *Micrococcus* sp., MY20 = *Pseudomonas* sp., MY9 = *P. fluorescence,* MY25 = *Bacillus cereus*,MY19 = *Alcaligenesfaecalis,* MY17 = *Micrococcus* sp.,MW10 = *Proteus mirabilis,* MW18 = *P. aeruginosa*, MY15 = *Citrobacterfreundii*,MY16 = *Pseudomonas* sp., MY4 = *Bacillus* sp.A-acid production, A/G-acid and gas production

50ml volumetric flask and the volume was made up to 50ml with distilled water and mixed thoroughly. The available K content in the solution was determined using flame photometer. Standard curve was prepared using various concentrations of KCl. The amount of potassium solubilized by the isolates was calculated from the standard curve.

Quantification of phosphorus. The phosphorus quantification was carried out using the vanadomolybdophosphoric acid method (Ribeiro, Cardoso, 2012). One ml of an over-night culture of each isolate diluted to 0.5 McFarland standard was inoculated to 50 ml of Pikovskaya broth and incubated for five days. At the end of the five days, the broth cultures were centrifuged at 10 000rpm for 10min to separate the supernatant from the bacterial cells and insoluble phosphorus, 2.5 ml of the supernatant were taken in a 25ml volumetric flask and 20ml of distilled water were added, this was followed by the addition of 2.5ml of Bartons reagent and was left for colour development for 10 min. The absorbance value was determined using a spectrophotometer at 430 nm. Standard curve was prepared using a stock solution of KH_2PO_4 at different concentrations. The amount of phosphorus solubilized by the isolates was calculated from the standard curve.

Nitrogen fixation

The efficiency of nitrogen fixing abilities by the selected isolates was determined usinga semi-solid Nfb medium containing 0.05% of malate as a carbon source. The isolates were inoculated in the Nfb medium and incubated at 30°C for 10days. After 10 days of incubation, the total amount of nitrogen fixed was determined using a Micro-Kjeldahl apparatus (Kanimozhi,Panneerselvam,2010). Using this method, the media containing the isolates were digested in a 100ml Micro-Kjeldahl flask by adding the salt mixture (50:10:1 ratio of K_2S0_4, $CuSO_4$ and metallic selenium) and 3ml of concentrated H_2SO_4. After digestion, 100 ml of distilled water was added and cooled. The digested samples were poured into the Micro-Kjeldahl apparatus. For quick delivery, 10ml of 40% NaOH were added into the distillation apparatus. In a 20 ml Erlenmeyer flask, 10ml of 4% boric acid reagent and 3 drops of mixed indicator were added. The flask was placed under the condenser of the distillation apparatus and the tip of the condenser outlet was beneath the surface of the solution in the flask. The solution, boric acid mixed with indicator containing ammonia was titrated against standard HCl.

Table 2. Plant growth promoting abilities of the isolates

Isolates	P solubilizer	K solubilizer	Nitrogen fixing ability	Indole acetic acid production
MY2	+	+	+	pink
MY4	+	+	+	slightly pink
MY9	+	+	+	nc
MY6	+	+	_	pink
MY25	+	+	+	pink
My19	+	+	+	pink
MW10	+	+	+	nc
MY20	+	+	+	pink
MY15	+	+	+	nc
MY16	+	+	+	pink
MY17	+	+	+	nc
MW18	+	+	+	pink

pink and (+) = a positive result,nc= no colour change,(–) = negative result

Plant inoculation test

The five selected isolates that were able to fix nitrogen, solubilize potassium and phosphate, as well as produce phytohormones were used for the plant inoculation test. Seeds of maize(ART/98SWI YELLOW) were surface sterilized with 0.1% aqueous solution of mercuric chloride. The seeds were placed in a suspension of bacterial cells diluted to 0.5 McFarland standard for 2 h. Thereafter, they were placed in a sterile Petri dish lined with filter paper moistened with double distilled water. The seeds were grown in a controlled chamber for 7 days. The length of root and shoot was measured after 7days and compared with control. The root system was separated from shoots and both were oven dried at 65°C and dry weights were recorded against the control.

Statistical analysis

Data collected from the experiment were subjected to the analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

Isolation of plant growth promoting bacteria

A total of 63 bacterial strains were isolated from the rhizosphere of white (BR9943DMR-SR-W) and yellow maize (SW1 yellow) using Pikovskaya agar, Aleksandrov agar and Jensen media. Eleven out of the 63 isolates were selected based on their ability to show multiple growth promoting abilities (P and K solubilization, N fixation) and were identified using a biochemical test (Table1). The selected isolates and their respective abilities were presented in Table 2, while their phosphate and potassium solubilization indexes were presented in Fig.1.Significantly the highest phosphate solubilization index showed *Bacillus cereus* (MY25), followed by*Pseudomonas aeruginosa* (MW18), when compared to other isolates. The isolates

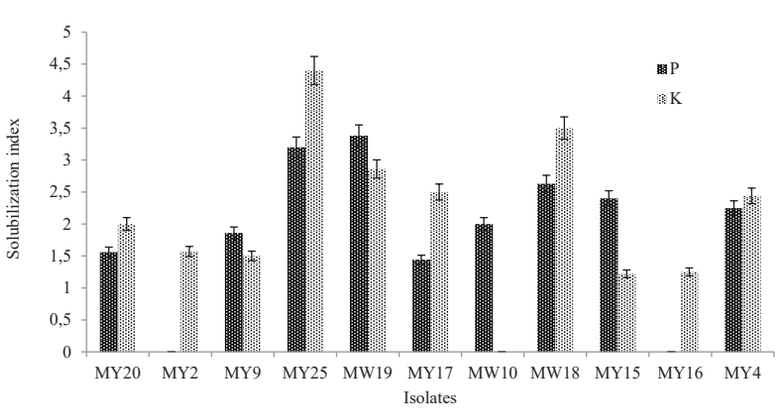


Fig. 1. Phosphate and potassium solubilizing index of the isolates

Table 3. Mean indole acetic acid (IAA) and gibberellic acid (GA) production by the isolates

Isolates	IAA(mg/l)	GA(mg/l)
MY2	55.73 ± 0.00^{a}	59.55 ± 0.07^{a}
MY20	64.51± 0.01^{b}	89.20 ± 0.42^{b}
MY9	73.94 ± 0.91^{c}	58.61± 0.01^{a}
MY4	68.30 ± 0.28^{c}	75.31± 0.00^{c}
MW10	68.40± 0.00^{c}	36.89 ± 0.92^{d}
MY25	80.96± 0.03^{d}	82.02 ± 0.82^{e}
MY19	93.68± 0.03^{a}	97.40± 0.00^{e}
MW15	42.66± 0.76^{d}	46.98± 0.04^{f}
MY18	14.06± 0.04^{e}	60.20± 0.00^{a}
MY16	14.06± 0.04^{e}	28.55± 0.07^{ab}
MY17	9.77± 0.00^{f}	21.15± 0.35^{bc}

means with the same letters within a column are not significantly different($P \leq 0.05$)

with multiple abilities(P and K solubilization, N fixation) were further tested for IAA and GA production and it has been shown that a considerable amount of IAA and GA was produced by the isolates which ranged between 9–94 and 21–97 mg l^{-1}, respectively (Table 3).

Molecular identification of the selected isolates

The molecular identification of the selected isolates showed the closest relatives of the isolates to include *Bacillus mojavensis* strain NBSL51 (MY4), *Pseudomonas aeruginosa* strain ZSL-2(MW18), *Alcaligenes faecalis* strain P156(MY19), *Pseudomonas syringae pv.syringae* strainHS191(MY20) and *Bacillus cereus* strain 20UPMNR (MY20).The result of the DNA quantification and the Accession numbers of the identified isolates are presented in Table 4.

Qualification of P and K solubilizing and N_2-fixing ability by selected isolates

The results of the amount of P and K solubilized by the selected isolates were presented in Tables 5, 6. The isolates were able to solubilize P and K at different degrees. The highest amount of K was found to be solubilized by *Bacillus cereus* strain 20UPMNR (1279 ± 0.5mg l^{-1}),followed by *Pseudomonas aeruginosa* strain ZSL-2 (mean K solubilized 1262 ±0.438mg l^{-1}) and the least by *Pseudomonas syringae pv.syringae* strainHS191 (611mg l^{-1})when KCl was used as a source of K, while *Pseudomonas aeruginosa* strain ZSL-2 recorded the highest amount of K solubilized (60 mg l^{-1}) and *Pseudomonas syringae pv.syringae* strain HS191 the least (34 mg l^{-1}) when mica powder was used as a source of potassium (K). The amounts of K solubilized by the isolates were significantly different ($P \leq 0.05$) when compared to control.

The results of the amount of phosphorus solubilized by the isolates using Ca_2PO_4 and rock phosphate as a source of Pare presented in Table 5. *Pseudomonas aeruginosa* strain ZSL-2 showed the highest amount of P solubilized with mean phosphate solubilization of 850 mg l^{-1} when Ca_2PO_4 was used as a source of P.. The results revealed that Ca_2PO_4 are more readily solubilized when compared to rock phosphate. The results also showed a significant difference ($P \leq 0.05$) in the amount of Psolubilized when Ca_2PO_4 and rock phosphate were compared. The nitrogen fixing abilities by the selected isolates ranged from 5.8 to 11.4 mg Ng^{-1}. The highestnitrogen fixing ability was recorded by *Alcaligenes faecalis* strain P156 (11.4 mg Ng^{-1}) and the least by *Bacillus mojavensis* strain*NBSL51* (5.8 mg Ng^{-1}) (Fig.2). *Pseudomonas syringae pv.syringae* strain HS191, *Bacillus cereus* strain 20UPMNR, and *Pseudomonas aeruginosa* strain ZSL-2 recorded 6.3, 8.2, and 9.4mg Ng^{-1}, respectively. The results are presented in Fig. 3.

Effect of selected isolates on maize germination and growth

The results of the effect of the selected isolates on the germination of maize are presented in Table 7. The results show a significant mean percentage germination of 92% when the seeds were inoculated with *Bacillus mojavensis* strain NBSL5 and *Alcaligenes faecalis* strain P156 while the least effect was noticed when *Pseudomonas syringae pv.syringae* was used (mean % germination of 62%). *Bacillus cereus* and *Bacillus mojavensis* strains had the greatest effect on

Table 4. Molecular identification of the isolates

Isolate code	Accession No.	Identity	Organism
MY4	JN624928.1	89	*Bacillus mojavensis* strain *NBSL51*
MW18	FJ853495.1	96	*Pseudomonas aeruginosa* strain ZSL-2
MY19	CP021079.1	97	*Alcaligenes faecalis* strain P156
MY20	CP006256.1	90	*Pseudomonas syringae pv.syringae* strainHS191
MY25	KJ729602	95	*Bacillus cereus* strain 20UPMNR

Table 5.Mean amount of potassium (K) solubilized by selected isolates using different sources

Isolates	Sources of K Kcl (mg/l)	Mica powder
Bacillus cereus strain 20UPMNR	1279 ±0.53[a]	59 ±0.53[a]
Alcaligenesfaecalis strain P156	1262±0.44[b]	49.35±1.20[b]
Pseudomonas aeruginosa strain ZSL-2	1224±1.26[c]	60.155±1.40[c]
Bacillus mojavensis strainNBSL5	1084±0.15[d]	52.55±0.77[d]
Pseudomonas syringae strain HS191	611±1.27[e]	33.66±0.19[e]
Uninoculated	293±0.84[f]	18.05±0.61[f]

Means with the same letters within a column are not significantly different($P \leq 0.05$)

Table 6. Mean amount of phosphorus solubilizationby the selected isolates

Isolates	Ca_2PO_4 solubilized (mg l^{-1})	Rock phosphate(mg l^{-1})
Pseudomonas aeruginosa strain ZSL-2	815±5.86[a]	70.16±1.47[a]
Bacillus mojavensis strainNBSL5	712±0.63[b]	62.05±0.77[b]
Alcaligenes faecalis strain P156	531±1.83[c]	109.35±1.20[c]
Pseudomonas syringae strain HS191	510±0.80[c]	63.66±0.19[d]
Bacillus cereus strain 20UPMNR	405 ±0.02[d]	115.09±0.57[e]
Uninoculated	156 ±1.20[e]	07.08±0.66[f]

Means with the same letters within a column are not significantly different($P \leq 0.05$)

Table 7. Effects of the isolates on maize germination

Isolates code	3rd day	5th day	7th day	No. of leaves
Bacillus mojavensis strain NBSL5	91.65± 2.81[a]	91.65 ± 2.81[a]	91.65 ± 2.81[a]	2.0 ± 00[b]
Alcaligenes faecalis strain P156	88.3A ± 0.57[a]	91.65 ± 0.58[a]	91.65± 0.57[a]	2.3± 0.56[b]
Bacillus cereus strain 20UPMNR	73.3 ± 3.06[ab]	78.3 ± 2.08[ab]	78.3 ± 2.08[ab]	2.7± 0.53[ab]
Pseudomonas aeruginosa strain ZSL-2	83.3 ± 2.52[c]	83.3 ± 2.52[b]	83.3 ± 2.52[b]	2.3± ٠,٥٣[ab]
Pseudomonas syringae pv.syringae strain HS191	61.65 ± 2.08[c]	61.65 ± 2.08[b]	61.65± 2.08[b]	3.0 ± 0.00[a]
Control	73.3± 0.58[ab]	73.3 ± 0.58[c]	73.3± 0.57[c]	2.00± 0.00[b]

Means with the same letter within a column are not significantly different ($P \leq 0.05$)

*Bacillus mojavensis*strain NBSL5 (MY4), *Pseudomonas aeruginosa* strain ZSL-2(MW18),*Alcaligenesfaecalis*strain P156 (MY19), *Pseudomonas syringaepv.syringae*strain HS191(MY20), *Bacillus cereus* strain 20UPMNR(MY25), Control = uninoculated

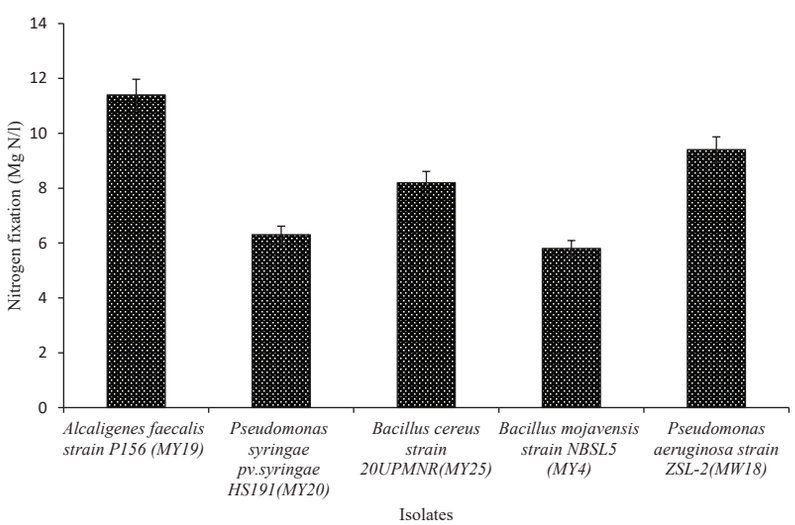
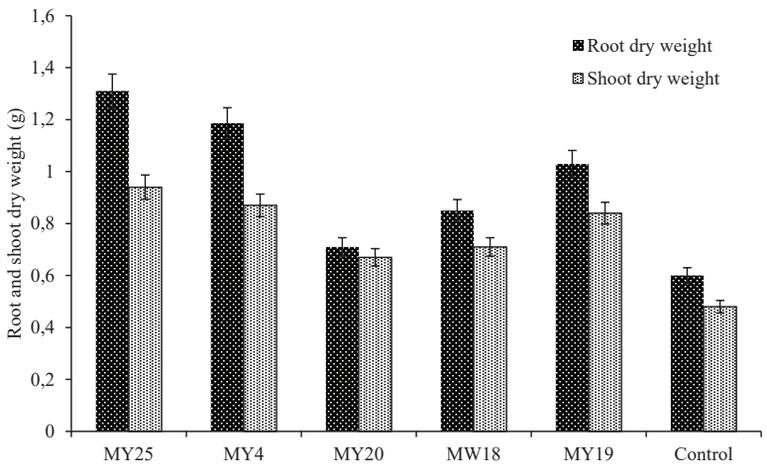


Fig. 2. Nitrogen fixing abilities of the isolates

Fig.3. Effects of the isolates on root and shoot dry weight

Bacillus mojavensisstrain NBSL5 (MY4), *Pseudomonas aeruginosa* strain ZSL-2(MW18),*Alcaligenes faecalis* strain P156 (MY19), *Pseudomonas syringae pv.syringae* strain HS191(MY20), B*acillus cereus* strain 20UPMNR(MY25)



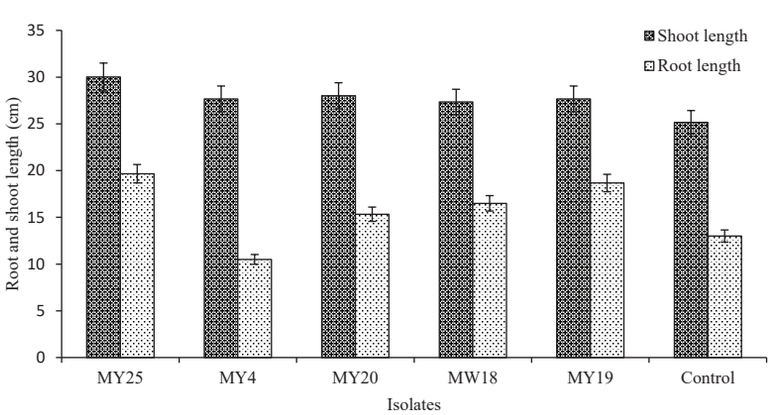
the root and shoot dry weight, with a mean shoot dry weightof 1.38 and 1.25 g, respectively, and mean root dry weight of 0.94 and 0.83 g, respectively. The least shoot and root dry weight was recorded by the control with the mean shoot and root dry weight of 0.48 and 0.6 g, respectively. The results are presented in Fig.3. The results of the effects of the isolates on the shoot and root length of maize is presented in Fig.4, the results were not significantly different ($P \leq 0.05$) when compared with the control. Bacillus cereus displayed the longest shoot and root (30.01 and 16.7 cm) and the shortest (25.2 and 13 cm) were recorded by the control.

DISCUSSION

The plant growth promoting abilities of bacteria isolates from the rhizosphere of yellow and white maize were examined. The isolates showed varying degrees of plant growth promoting abilities. Twenty-eight out of 63 bacteria isolates exhibited at least one plant growth promoting ability (P and K solubilization, nitrogen fixation, IAA or GA production). *In vitro* screening for beneficial characteristics of the isolates showed that twelve of the isolates have multiple abilities. The N_2-fixing ability was the first screening criterion of the isolates since this characteristic is very important for plant as a potential alternative to reduce the impact of chemical fertilizer application. Totally 41 and 25% of the isolates from Yellow and White maize, respectively, showed evidence of nitrogen fixation. T a n et al. *(*2014) isolated rhizobacteria with multiple abilities in the rhizosphere of rice. Five out of the twelve isolates with multiple abilities were selected based on their P and K solubilization index as well as the IAA production. These five isolates were identified as *Bacillus mojavensis*, *Pseudomonas aeruginosa*, *Alcaligenes feacalis*, *Bacillus cereus* and *Pseudomonas syringae* using 16SrRNA. These isolates exhibited varying degrees of P and K solubilization, IAA and GA production, as well as nitrogen fixation. The P and K solubilization index showed the *Bacillus cereus* strain 20UMNR to have the highest K solubilization followed by *Pseudomonas aeruginosa* strain

Fig.4. Effects of the isolates on shoot and root length of maize

Bacillus mojavensis strain NBSL5 (MY4), *Pseudomonas aeruginosa* strain ZSL-2(MW18),*Alcaligenes faecalis* strain P156 (MY19), *Pseudomonas syringae pv.syringae* strain HS191(MY20), *Bacillus cereus* strain 20UPMNR(MY25)



ZSL-2 and *Alcaligenes faecalis* strain P156, while the P solubilization indexes of the two latter did not significantly differ. This is in line with the results of Sanjotha, Sudheer (2016) who reported the efficiency of *Bacillus* and *Pseudomonas* in phosphate solubilization. It is also in line with the findings of Priyanka, Sindhu (2013).

Meanwhile, the quantification of P solubilized by the isolates using inorganic source of phosphate (Ca_2PO_4) and organic source (rock phosphate) showed that the isolates can be of help in making phosphate available to plant through solubilization of the insoluble form of phosphate in the soil. This solubilization may result from the release of metabolite such as organic acid by the isolates (Panhwar et al., 2012) which reduced the media pH and consequently solubilized the insoluble tricalcium phosphate. The use of these isolates is necessary to minimize the excessive use of inorganic phosphate fertilizers as earlier stated by Sitepu et al.(2006). Tan et al. (2014) reported that the inorganic phosphatesolubilization was directly related to the decline in pH and this has been reported to be due to the production of organic acids and acid phosphatase by the microbes. Some analyses have shown that P released from insoluble form of phosphate was negatively correlated with the solution pH.

The isolates were also able to solubilize the insoluble source of potassium such as KCl and mica powder. *Bacillus cereus* strain 20UPMNR showed the highest evidence of solubilization when KCl was used as a source of K while *Pseudomonas aeruginosa* strain ZSL-2 recorded the highest value when mica powder was used. The least amount of solubilized Kwas observed in the control. The ability of these isolates to solubilize K might result from the production of acids,alkalis or chelators which enhance the release of elements from K being minerals such as mica powder (Tan et al., 2014).A greater K solubilization was observed in KCl than in muscovite mica. This agrees with the results of Priyanka, Sindhu (2013) who reported maximum solubilization when mica powder was replaced with KCl and K_2SO_4.The IAA production by the isolates was within the range of 65–94 mgl^{-1}. This result is in agreement with the findings of Umi et al.(2014). The results of IAA production by the isolates were also similar with that of selected isolates of *Pseudomonas* sp., *Bacillus* and *Azobacter* from rhizosphere soil of different crops and root nodules of chick pea (Ahmad et al., 2008).The IAA production by the isolates is believed to be very crucial for enhancing root elongation and root hair development. Duangpaeng et al.(2012) reported that selecting endophytic bacteria based on their IAA producing bacteria increased shoot and root length of rice while producing the highest number of shoots per plant and paper germination on Petri dish. The results of this study showed *Alcaligenes faecalis* strain P156 having the highest amount of IAA (94 mgl^{-1}) and the least was recorded by *Bacillus* sp.(MW2 – 3.29mgl^{-1}). The production of GA by the isolates was also evaluated. The results for GA production were not significantly different ($P \leq 0.05$) when the amount produced by different isolates was compared. *Alcaligenes faecalis* (MY19) recorded the highest amount of GA followed by *Pseudomonas syringae pv.syringae* (MY20), while the least was recorded by *Micrococcus* sp.(MY17). Tandya, Desai (2014) reported GA production in the range of 7.5–93.3 $mgml^{-1}$ by bacteria isolates from a rice field. Results of an *invitro* plant inoculation test showed a higher germination percentage in some of the seeds inoculated with the isolates. The results also showed more leaves in plants inoculated with these isolates.

Pandya, Desai (2014) also reported that bacterial isolates significantly enhanced the growth of chan and wheat, and also seed germination; root and shoot length was also significantly promoted if compared to the punitive control. The result of this study showed a significant difference ($P \leq 0.05$) in the root and shoot dry weights when compared with the control. The same is applicable to the root and shoot length. All the plants inoculated with the isolates had longer shoot when compared with the control.

CONCLUSION

The bacterial isolates in this study have shown efficacy in plant growth promotion through K and P solubilization, nitrogen fixation as well as IAA and GA production. These native isolates, which belong to the genera Bacillus, Pseudomonads and Alcaligenes, could be useful for the development of a biofertilizer for maize and other non-leguminous crops. This would be potentially cost effective and ecofriendly if compared to the use of chemical fertilizers.Further screen house and field trials are needed toverify the exploitation of these isolates in maize growth.

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