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Screening of Endophytic Bacteria towards the Development of Cottage Industry: An *in Vitro* Study

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ABSTRACT Discovery of novel technology which use beneficial endophytic bacteria associated with the root of *Sorghum bicolor*, *Spinacia oleracea*, and *Zea mays* was researched. Total of 23 endophytic bacteria were characterized on the basis biochemical analysis and plant growth-promoting traits. Results showed that Gram-negative (60.8%) were isolated more frequently than Gram-positive bacteria (39.1%). Approximately 65.2% were motile and the remaining 34.7% were non-motile. Eleven isolates were able to produce indole acetic (IAA) (0.15-2.84 mgl^{-1}). Eleven isolates showed the ability to produce ammonium. Hydrogen cyanide (HCN) production was observed in 10 isolates. It was observed that 16 isolates solubilized insoluble phosphates in Pikovskya plates (8-60.5%). All the isolates tested were active against *Fusarium oxysporum*. Therefore, following these tests it can be concluded that 11 isolates exhibited differences and they were subjected to partial 16S-rDNA gene sequencing using polymerase chain reaction for phylogenetic analysis. MEGA 5.10 package was used to identify the following isolated bacteria: *Pseudomonas* sp. (KC010520), *Ochrobactrum intermedium* (KC010521), *Ochrobactrum intermedium* (KC010522), *Ochrobactrum anthropi* (KC010523), *Ochrobactrum anthropi* (KC010524) *Ochrobactrum* sp TOA62, and *Ochrobactrum* sp TOA64. Inoculation of *Zea mays* seeds with the identified bacterial showed a good level of germination (66%) compared to the control (44%).

INTRODUCTION

Over the past decades, agriculture production was regarded as the main target for human foods (Phat et al. 2012). Currently the population of the world is expected to reach 8 billion by the year 2025. As the world population increases, the problem of food security arises. This means that the increase of agriculture production has to meet the human need of the fast-growing population (Roger et al. 1994). The challenges faced by the world are how to feed the increasing populations where there is little food. To solve the problem, farmers have to apply natural fertilizers to agriculture land. The objective is to increase the annual productivity and yield in farmers' agricultural land (Elizabeth et al. 2004; Crawford et al. 2006). The insufficient food level of population has driven farmers to change their agricultural behavior which leads to the use of chemical fertilizer in order to meet the human demand. The purpose for applying chemical fertilizer to agriculture land was to promote high-yield of crop production (Crawford et al. 2006; Liu et al. 2009; Seng 2010). Over 15 million tons of P fertilizer is applied worldwide every year, of which up to 80% is lost into insoluble forms with Fe-,Al-, Ca- and Mg-ions, that is, forms unavailable to plants (Zhao et al. 2014). This application has led to serious environmental problems such as depletion of soil quality and health, rivers and ground water pollution, and emergence of resistant pathogens (Delmer 2005; Sununtar 2006). There is an increasing demand by governments today for safe chemical fertilizers with low toxicity, short term persistence, and low mobility in the soil to avoid ground-water contamination and limited effects on organisms (Sununtar 2006). These concerns about environmental health and safety have led to increased restrictions on a variety of chemical fertilizers including those used to suppress plant diseases (Josephine 2005). In addition, the growing cost of biofertilizers, particularly has led to a search for substitutes for these products (Josephine 2005). Knowing and understanding the negative effects of chemical fertilizers in agriculture, novel technology using the application of endophytic bacteria associated with plants, also called biofertilizers may help to sustain productivity and improve plant health (OECD 2004).

This group of bacteria is considered as an environmentally friendly alternative solution of

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reducing the use of chemicals fertilizers in agriculture sector (Maheshwari 2011; Gagne-Bourgue et al. 2012; Goswami et al. 2014). Endophytic bacteria can be defined as a group of beneficial free-living soil bacteria that colonize the inside root cells of plant without showing any external sign of infection on their host (Babalola 2010; Ahemad and Kibret 2014; Arora et al. 2014). Many species of plant that exist in the world, host one or more endophytic microorganisms which few of them have not been studied on basis to their relation with the host plant (Strobel 2004; Ryan et al. 2007). Therefore, the opportunity to find new and beneficial endophytic species in ecosystems is considerable (Ryan et al. 2007). Like rhizosphere bacteria, endophytic bacteria have been shown to have the ability to control plant disease, which include the capacity to colonize plant roots surfaces closely adhering to soil interface, increase mineral nutrient solubilization and nitrogen fixation (Ngoma et al. 2012; Ji et al. 2014; Goswami et al. 2014). Furthermore, they have been shown to improve plant health during early stages of growth due to the synthesis of 1- aminocyclopropane-1-carboxylate (ACC) deaminase which modulates the level of ethylene by hydrolyzing ACC, a precursor of ethylene, in ammonia and a-ketobutyrate (biofertilizers) (Glick 2014). Lastly, they increase yield and suppress plant diseases by the production of siderophores, antibiotics, and competition for nutrients (bio-protectants) (Haas and Défago 2005; Ngoma et al. 2013; Ji et al. 2014). The bacteria are not only important from an agricultural point of view, but may also play a vital role in soil bioremediation strategies (Whiting et al. 2001; Marques et al. 2010). In recent years, some of the endophytic bacteria such as *Bacillus, Streptomyces, Pseudomonas, Burkholderia,* and *Agrobacterium*, have been genetically engineered to enhance plant growth and improve stress tolerance for commercial uses (Suryanarayanan et al. 2009; Mei and Flinn 2010; Gagne-Bourgue et al. 2012; Kang et al. 2014). The best known and studied beneficial plant-associated bacteria are rhizobia for the reason that they are able to fix nitrogen during the Rhizobium-legume symbiosis (Ahemad and Kibret 2014). This is a novel solution that provides incentives and less expensive mechanisms in plant science which may add benefits to poor farmers. Development and propagation of low-cost technologies would certainly help in the improvement of the farmers economic situations.

Current research on the discovery of new microorganism species in Africa has focused on their efficacy under laboratory and field conditions (Ngoma et al. 2013). Substantial efforts have been made in endophytic bacteria research to isolate and identify indigenous biofertilizers for agricultural production. Application of exotic biofertilizers will negatively affect native populations in the ecosystem (Campos-Herrera et al. 2011). In this respect, the isolation of local endophytic bacteria will value the specie, not only from a biodiversity perception but also from a more applicable standpoint (Ngoma et al. 2013). It is possible that the findings may add to much needed knowledge of Mafikeng for biofertilizers and biodegradation of heavy metals remains. However, very little research work has been done on isolation, characterization of indigenous endophytic bacteria in Mafikeng, North West province. Hence, the present study was taken up to isolate and characterize such environmental friendly and effective indigenous endophytic bacteria which have the ability to colonize the roots and improve plant health. In order to achieve the above objectives, a series of endophytic bacteria species associated to root of: *Sorghum bicolor*, *Zea mays* and *Spinacia oleracea*, cultivars were isolated and identified by using morphological and biochemical techniques. The suspect endophytic bacteria were screened for their plant growth promoting activities (IAA, HCN, ammonia production, and antifungal activities). Molecular-based techniques using 16S rDNA sequence analysis was used for their identification at species level. Selected endophytic bacteria were further tested with *Zea mays* seed in order to evaluate their effect on their germination and seedling traits.

MATERIAL AND METHODS

Collection and Isolation of Endophytic Bacteria

The samples were collected at Molelwane Farm, North West province, South Africa. The bacteria were isolated from the roots of *Sorghum bicolor*, *Spinacia oleracea* and *Zea mays*, plants grown in the farm field of North-West University in February and March 2013. Intact root systems for each plant were uprooted, collected, placed immediately in sterile polyethylene bags which were labelled according to plant names. The bags were placed on a dry cool box to avoid

moisture accumulation or excessive drying. The boxes were carefully taken immediately to the laboratory and stored at 4°C. To isolate endophytic bacteria from the samples, the roots were first washed with sterile distilled water and soaked in 70% ethanol for 5 min, in 6.25% sodium hypochlorite for 10 min followed by several rinses with sterile distilled water so as to remove the traces of chemical substances. Root system were then suspended in 0.05 M Phosphate buffered saline (PBS) and crushed with a sterilized mortar and pestle. A milky bacterial suspension obtained was serially diluted ten-fold using PBS prior to spreading on plate count agar (Sigma Aldrich, South Africa) (Piromyou et al. 2010). The inoculated plates were incubated for 24 hours at 37°C and observed suspect colonies showing morphological difference were selected and streaked plated on freshly prepared agar media to obtain pure colonies. All the isolates were preserved at -80°C in 30% glycerol (Kumar et al. 2012).

Phenotypic Identification

In morphological analysis, macroscopic (form, elevation, margin, diameter, surface, opacity, texture, motility), and microscopic (Gram reaction) features of selected bacteria were studied. Additionally, an array of biochemical tests such as Catalase activity, Oxidase activity, Citrate test, Indole test, Methyl red test, Voges-Proskauer test, Motility test, Triple sugar ion test, and Nitrate reduction test was performed following Bergeys Manual of Systematic Bacteriology (Kreig and Holf 1984).

Detection of PGP Traits

Indol Acetic Acid (IAA) Production

Colonies of endophytic bacterial obtained earlier from *Zea mays*, *Sorghum bicolour* and *Spinacia oleracea*, were grown in 50 ml of Nutrient broth (NB) supplemented with L-tryptophan to a concentration of 100 µg/ml. The test tubes were incubated at 28°C on a rotator shaker (SI-600, LAB Companion, Korea) for 48 hours at 200 rpm. The broth was centrifuged at 10,000 rpm for 15 min. Two-millilitre culture supernatant was transferred to a fresh tube and mixed with 2 drops of ortho-phosphoric acid. The aliquots were shaken and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid and 1 ml 0.5M $FeCl_3$ solution) was added and vortex thoroughly. The mixture was then incubated at room temperature for 25 min, and the development of pink color indicated IAA production, optical density was read at 530 nM after 30 min in a UV-VIS Spectrophotometer (Shimadzu UV-1700 Pharmaspec, Japan). The experiment was performed thrice with three replicates for each bacterial strain. The compound quantification value was recorded by extrapolating calibration curve made by using pure IAA as standard (10-100 Ag/ml) (Gordon and Weber 1951).

Hydrogen Cyanide Production (HCN)

Screening of bacterial isolates for HCN production was done by using Castric's method (Castric 1975). All suspected isolates were grown in 10% trypone soy agar supplemented with glycine (4.4 gl^{-1}) (Sigma, South Africa). A Whatman filter paper No. 1 was soaked in 2% sodium carbonate and 0.5% Picric Acid solution and fitted to the underside of the plate lids. To avoid the escape of the gas, the plates were sealed with parafilm and the reaction mixture was incubated at 30°C for 5 days to allow color development from yellow to red-brown

Phosphate Solubilization

Phosphate solubilization ability of isolates was determined by spotting 10 ìL of cultures separately on Pikovskya's agar plates. Plates were then incubating at 30°C for one week (Nautiyal 1999). The appearance of clear halo zone around the colonies indicated solubilization of inorganic phosphate by bacteria (Pikovskya 1948). The halo size produced by the respective bacterial was calculated according to the formula below (Nguyen et al. 1992):

$$\text{Solubilization efficiency} = \frac{\text{Solubilization diameter}}{\text{Growthdiameter}} \times 100$$

The experiment was performed thrice with three replicates for each bacterial isolates.

Production of Ammonia

Bacterial isolates were tested for the ability to produce ammonia in nutrient broth. Freshly grown bacterial cultures were inoculated in 10 ml nutrient broth in each tube and incubated at

30°C for 48 hours in a rotator shaker (SI-600, LAB Companion, Korea) at 200 rpm. Thereafter incubation, 0.5 ml of Nessler's reagent was added and mixed thoroughly in each tube. The development of a yellow to brown colour indicated a positive reaction for ammonia production (Cappuccino and Sherman 1992).

Antifungal Activity

The bacterial isolates were screened for their ability to suppress growth of *F. oxysporum* fungal (Davies Diagnostic, South Africa). Potato Dextrose Agar was used to study the antagonistic activity since both types of microorganisms (fungi and bacteria) can grow on this medium. Overnight grown bacterial cultures were individually streaked 2 cm from the mycelia plug at four opposite locations around the periphery of plate and the mycelia of fungal species were placed in the middle of them and the plates were incubated at 28°C. The width of cleared zones of antagonism (distances between the bacterial and fungal growth) were measured after 10 days. Each experiment was repeated four times in triplicate (Boruah and Kumar 2002). The percentages of radial growth inhibition were recorded by the following formula:

$$\%\ \text{Inhibition in radial growth} = \frac{R1 - R2}{R1} \times 100$$

Where R1 is the radial growth of *F. oxysporum* in control plate and R2 is the radial growth of *F. oxysporum* interacting with antagonistic bacteria (Noori and Saud 2012).

Genotypic Identification

Isolation of Genomic DNA

Bacterial isolates were grown in 10 ml of nutrient broth at 30°C for 48 hours. Genomic DNA was extracted using Zymo-research kit Fungal/ Bacterial DNA following the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

Amplification of 16S rDNA gene was carried out by polymerase chain reaction using an Engine DYAD Peltier thermal cycler (BioRad, USA). Reaction volume of 50 ìL, containing: 25 ìL PCR Master Mix, 2 ìL template DNA, 19 ìL nuclease free water and 2 ìL of each oligonucleotide primer (25 ìM) was prepared and mixed in the PCR tubes (Ntougias et al. 2004). PCR of endophytic bacteria was done by using the universal primers: forward 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse 1492R (5'-TGA CTG ACT GAG GCT ACC TG-3'). These primers were commercially synthesised by Inqaba Biotechnical Industrial (Pty) Ltd. (Pretoria, South Africa). The thermo cycling conditions consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 5 min, followed by single final extension step at 72°C for 7 min and incubated at 4°C forever. Amplified fragments of DNA were fractionated on a 1% w/v agarose gel during 100 min at constant voltage of 80 V in 0.5×TAE (Tris-Acetat EDTA). A 10-kb reference marker (Sigma, D7058) was used to allow standardization. Following staining with ethidium bromide (10 ìg ml^{-1}), the gel was visualized using Syngene Ingenius Bioimager (UK) under UV light to confirm the expected size of the product.

DNA Sequencing

Purified PCR fragment of the 16S rDNA of the strains were analyzed for nucleotide sequence determination by using ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems) at Inqaba Biotechnical Industrial (Pty) Ltd. (Pretoria, South Africa). The acquired sequences were aligned against GenBank data base using Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST) from the National Center for Biotechnology Information (NCBI) to identify sequences with high similarity (Altschul et al. 1990).

Phylogenetic Analysis

The sequences were analyzed and edited using Bio Edit Sequence Alignment Editor (Hall 1999). Multiple alignments of the sequences were carried out by Mafft program 6.864 against corresponding nucleotide sequences retrieved from Gen-Bank. Evolutionary distance matrices were generated as described by Jukes and Cantor (Jukes and Cantor 1969). The aligned 16S rDNA gene sequences were used to construct a phylogenetic tree as implemented in the MEGA 5.10 package (Tamura et al. 2011) and the neigh-

bor-joining (NJ) method (Saitou and Nei 1987); minimum evolution; maximum likelihood (Fitch 1986); UPGMA and maximum parsimony (Rzhetsky and Nei 1992). A bootstrap confidence analysis was performed with 1,000 replicates. Putative chimeric sequences were identified using the Chimera Buster 1.0 software. Manipulation and tree editing were carried out using Tree View (Page 1996). The sequences have been lodge with Gen-Bank database for accession numbers.

Seed Germination Test

Bacterial isolates were designated for testing plant growth promotion. Each isolates was grown in 150 ml flask containing 60 ml nutrient broth and incubated at 37°C on a rotator shaker (SI-600, LAB Companion, Korea) for 48 hours at 200 rpm. After incubation bacterial cells were harvested by centrifuging at 10,000 rpm/min for 20 min. Pellets were suspended and washed in 1ml distilled water in the test tubes and centrifuged at 10.000 rpm for 5 min in at least 3 times and the supernatant discarded. Then the pellet of bacterial cells was adjusted to $1x10^8$ cfu/ml using UV spectrophotometer, obtained by adjusting 0.5 OD at 535 nm (Gholami et al. 2009). Seeds of *Zea mays* were obtained from the agriculture shop in Mafikeng, North West province, South Africa. Healthy seeds were selected and washed with sterile distilled water and soaked in 70% ethanol for 5 min, in 6.25% sodium hypochlorite for 10 min and rinsed six times with sterile distilled water so as to remove the traces of chemical substances. After that, the seeds were soaked in various endophytic bacterial cultures for 30 min. As outlined in the experimental design, 18 seeds of both inoculated and controls were put in sterilized Petri dishes containing filter paper (Whatman # 102, Sigma, South Africa) considered as experimental arena. Four petri dishes were used for each isolated bacterial and were kept in plant growth chamber (LABCON growth chamber, Germany) at 30°C and they were watered daily for one week. The seedling percent emergence was calculated with the following formula:

$$\% \text{ Emergence} = \frac{\textit{Number of emerged seedling}}{\textit{Number of seeds sown}} \times 100$$

The experiment was setup in 3 replications with 6 treatments. Seedling height and root length of each plant were recorded in centimeter. Then plants were dried in an oven at 65°C for 3 days. Then the shoot and root dry weights were recorded in gram.

Determination of Metals Concentrations in Soils

Soils specimens were microwave digested for detection of metals such as Fe, K, Ca, Mn, Zn, and Cu. After digestion, the aliquot was quantitatively transferred in a 100 ml beaker and diluted with 50 ml deionized water. The mixed samples were analyzed for metal contents by using Energy-Dispersive X-ray Fluorescence Spectrometry (EDX 720/800HS, Shimadzu, South Africa) equipped with a liquid nitrogen cooled Si (Li) detector (Yi et al. 2007).

RESULTS

Isolation and Phenotypic Characterization of Bacterial Endophytes

In this study, 23 endophytic bacterial isolates were selected for further biochemical and morphological characterization. They were designated as MMC43, MMC46, MA39, MA49, MC30, SOC3, SOC1, SOA10, SOA6, SOMC14, SA37, SA38, SC26, SMC31, SC42, PM20, PC23,

Table 1: Bacterial endophytes isolated from plants of *Zea mays, sorghum bicolor,* and *Spinacia oleracea,* at Molelwane farm

Sample	*Root variety in the field*	*No. of isolate*	*Names of isolates*
1	*Mays* L	8	MMC43 MMC46 MA39 MA49 MC30 PM20 PC16 PA19
2	*Sorghum* L	7	SOC3 SOC1 SOA10 SOA6 SOMC14 TOC69 PC23
3	*Spinacia* L	8	SA37 SA38 SC26 SC42 SMC31 TEC57 TOA62 TOA64

PC16, PA19, TOC69, TOA62, TOA64, and TEC57 which 8 were recovered from *Zea mays*; 7 were isolated from *sorghum bicolor*; and 8 were from *Spinacia oleracea* (Table 1).

Morphological observations of these bacteria showed that Gram-negative (60.8%) were isolated more frequently than Gram-positive bacteria (39.1%) and most of them were fast growers. While in case of motility 65.2% were motile and the remaining 34.7% were non-motile. A total of 14 of these endophytic bacteria such as MMC43, MMC46, MA39, SOA10, SOA6, SA37, SA38, SC 42, PM20, PC23, TOA 62, TOA64, TOC69 and TEC57 were confirmed to be Gram negative, straight to slightly curved rods shaped, occurring singly or in pairs, 0.5-1.2x2.6-4.0 mm, low convex, smooth, with entire edges and crescent. Out of 14 Gram negative, 4 were from *Zea Mays* (MMC43, MMC46, PM20 and MA39); 4 from *Sorghum bicolor* (SOA10 TOC69, PC23 and SOA6); 6 from *Spinacia oleracea* (SA37, SA38, SC42, TOA62, TOA64 and TEC57) (Table 2). The remaining nine were Gram reaction positive, they differed in colour according to media used (Cemetride, McConkey and/or Aeromonas agar), and all were odourless. Among them, 2 (TEC57 and TOC69) did not produce pigments on plates. The isolates MA39 and SOA10 produced pigment on Cetrimede agar while the remaining on MacConkey. Their diameter varied from 0.2 to 2 mm (Table 2).

Biochemical characteristics like nitrate, ammonium, indole, H_2S production, catalase oxi-

Table 2: Morphological and physiological characteristics of 3 days old colony, potential endophyte bacterial isolates

Sample No.	*Size*	*Shape*	*Margin*	*Elevation*	*Texture*	*Opacity*	*Pigmentation*	*Gram's reaction*	*Motility*
MMC43	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	+
MMC46	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	+
MA39	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (-) rod	+
MA49	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (+) rod	+
MC30	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (+) rod	-
SOC3	Small	Irregular	Irregular	Flat	Smooth	Opaque	Withe	Gram (+) rod	-
SOC1	Small	Irregular	Irregular	Flat	Smooth	Opaque	White	Gram (+) rod	-
SOA10	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	+
SOA6	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	+
SOMC14	Medium	Round	Entire	Flat	Smooth	Trans-parent	Yellow	Gram (+) cocci	+
SA37	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (-) rod	+
SA38	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (-) rod	+
SC26	Medium	Round	Entire	Flat	Rough	Trans-parent	Yellow	Gram (+) cocci	-
SC 42	Medium	Round	Entire	Raised	Smooth	Trans-parent	Yellow	Gram (-) cocci	-
SMC31	Small	Irregular	Entire	Slightly raised	Rough parent	Trans Yellow	Gram (+) cocci		+
PM20	Small	Irregular	Entire	Slightly raised	Rough	Trans parent	Golden yellow	Gram (-) bacilli	+
PC23	Small	Irregular	Irregular	Slightly raised	Smooth	Trans parent	Golden yellow	Gram (-) rod	+
PC16	Small	Irregular	Entire	Slightly raised	Rough	Trans-parent	Golden yellow	Gram (+) rod	-
PA19	Medium	Round	Irregular	Flat	Rough	Trans-parent	Golden yellow	Gram (+) rod	-
TOC69	Medium	Round	Entire	Raised	Smooth	Opaque	No	Gram (-) cocci	+
TOA62	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	-
TOA64	Medium	Round	Entire	Raised	Smooth	Opaque	White	Gram (-) rod	+
TEC57	Small	Irregular	Irregular	Raised	Rough	Opaque	No	Gram (-) cocci	+

+ Positive - Negative

dase, citrate, triple sugar iron, methyl red, and Voges-proskauer test were tested and shown in Table 3.

Determination of Plant Growth Promoting Rhizobacteria (PGPR) Traits

The results of plant growth promoting activities are shown in Table 4. Endophytic bacterial isolates were analyzed for the quantitative of IAA in presence or absence of tryptophan compound. As mentioned in the literature, this IAA biosynthesis is significantly influenced by the presence of L-TRP precursor. L-TRP is the principal precursor for the formation of IAA, in several microorganisms. In this study IAA production was increased on increasing the concentration of tryptophan compound in nutrient broth. Bacterial strain growth in free tryptophan nutrient broth produced very low IAA as compared to others bacterial strains growth in nutrient broth supplemented with 1 mg/ml^{-1} (Table 4). Bacterial isolates MA39 showed significant concentration of IAA (2.2-2.84) followed by SOA6 (2.05-2.67), TOA64 (1.9 -2.53), MMC43 (1.91-2.48), TOA62 (1.89-2.25) and MMC46 (1.69-2.03) at 0 to 1 mg/ ml^{-1} tryptophan in broth after 48 hours incubation. The remaining endophytic bacteria showed either very low trend or negative reaction of IAA production. Studies on phosphate solubilizing bacterial revealed the formation of transparent halo zones around the bacterial colony growth on Pikovskya's agar plates. The results showed that out of 23 isolated bacteria, 11 isolates showed the development of maximum sharp phosphate solubilization zones, ranging from 50% to 60% while 5 showed less halo zone around the colony ranging from 8%- 13%. The isolates MA49, SOC3, SA38, PM20, PA19, TOC69 and TEC57 were negative (Table 4 and Fig. 1). The production of HCN was detected in 10 isolates (Table 4 and Fig. 2).

The production of ammonia is another characteristic of PGPR that indirectly influence development of plant. Eleven bacterial isolates were able to produce ammonia while 12 isolates were unable to do so. The number of positive and negative bacterial isolates for each plant growth promoting activity was observed and given in Table 4. All the bacterial isolates exhibited antifungal activity against *F. oxysporum* pathogen in culture assay; the activity produced by the respective isolates was expressed in percentage on the basis of inhibition zone. Elev-

Table 3: Identification of potential indigenous bacterial endophytes based on biochemical tests

Samples No.	*Catalase test*	*Oxidase test*	*Citrate test*	*Methyl Red test*	*Voges-Proskauer test*	*Nitrate Reduction test*	*H_2S production*
MMC43	+	+	+	-	-	+	-
MMC46	+	+	+	-	-	+	-
MA39	+	+	+	-	-	-	-
MA49	+	-	+	-	-	+	-
MC30	+	+	+	-	-	+	-
SOC3	+	+	+	-	-	-	-
SOC1	+	+	+	-	-	-	-
SOA10	+	+	+	-	-	+	-
SOA6	+	+	+	-	-	+	-
SOMC14	+	-	+	+	+	+	+
SA37	+	+	+	-	-	-	-
SA38	+	+	+	-	-	-	-
SC26	+	+	+	+	-	-	-
SC 42	+	+	+	+	-	-	-
SMC31	+	-	-	-	+	+	+
PM20	+	-	+	-	-	+	+
PC23	+	-	+	+	+	+	+
PC16	+	-	+	-	-	+	-
PA19	+	+	+	-	-	-	-
TOC69	+	+	+	-	-	+	-
TOA62	+	+	+	-	-	+	-
TOA64	+	+	+	-	-	+	+
TEC57	+	+	+	-	-	-	-

+: Activity; No activity: -

Fig. 1. Halo formation on Pikovskya's agar plates (A) as compared to the control (B).

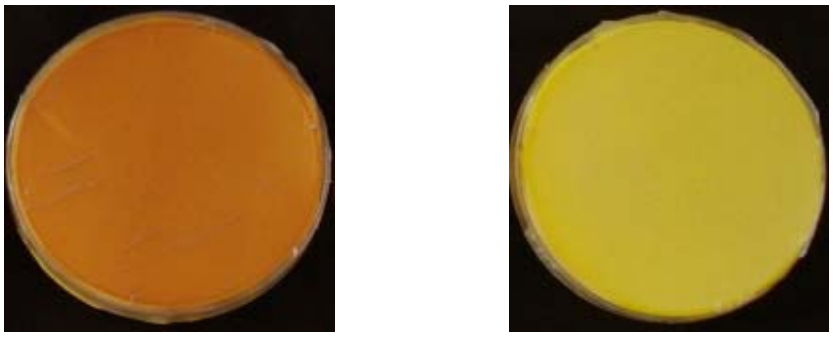

Fig. 2. Detection of HCN production (A) control (B).

Table 4: Assessment of the IAA, HCN, Ammonium production, Phosphate solubilization and Antifungal activities

Samples No.	*IAA Production (mg/ ml⁻¹)*		*HCN production*	*Phosphate solubilization (%)*	*Ammonium production*	*Antifungal activities (%)*
	No trypto-phan	*1 mg/ml - tryptophan*				
MMC43	1.91	2.48	+	54.5	+	75
MMC46	1.69	2.03	+	60.5	+	84.6
MA39	2.2	2.84	+	54.5	+	71.1
MA49	-	-	-	-	-	8.44
MC30	0.16	0.21	-	50.2	+	50
SOC3	-	-	-	-	-	17.5
SOC1	-	-	-	10	-	6.7
SOA10	1.77	2.02	+	50	+	72.3
SOA6	2.05	2.67	+	50	+	71.1
SOMC14	-	-	-	12.4	-	14.9
SA37	0.15	0.21	+	52	+	50.5
SA38	-	-	-	-	-	12.65
SC26	-	-	-	13	-	10.9
SC 42	0.16	0.22	+	50	+	54.5
SMC31	-	-	-	9	-	32.6
PM20	-	-	-	-	-	22.7
PC23	0.18	0.21	+	51	+	56.3
PC16	-	-	-	8	-	18.7
PA19	-	-	-	-	-	15.7
TOC69	-	-	-	-	-	24.6
TOA62	1.89	2.25	+	53.2	+	84.6
TOA64	1.9	2.53	+	60	+	84.6
TEC57	-	-	-	-	-	8.8

+: Activity; No activity: -

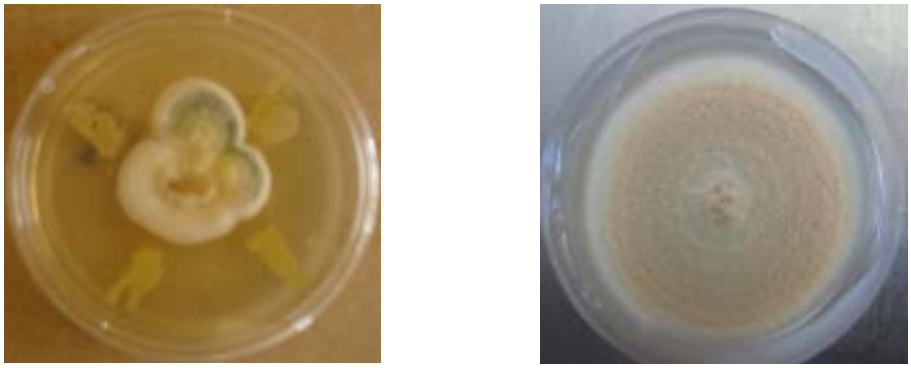

Fig. 3. Bacterial antifungal activity in the potato dextrose agar *F. oxysporum* plug growth was completely inhibited in the presence of the endophytic bacteria streaked in the plates (A), as compared to the control (B), which had no bacteria.

en isolates such as MMC43, MMC46, MA39, MC30, SOA10, SOA6, SA37, SC42, PC23, TOA62 and TOA64 showed the maximum percent inhibition rate range from 50% to 84.6%. The remaining isolates revealed less reduction in radial growth range from 8% to 32.6% (Table 4 and Fig. 3).

Molecular Characterization of Isolates

Based on morphological, biochemical and characterization and PGPR traits, all the suspected endophytic bacterial were selected and analyzed by PCR profile to confirm their strain identification as shown in Figure 4.

Out of 23 isolates, only eleven such as MMC43, MMC46, SC42, MA39, SOA10, PC23, SOA6, TOA62, SA37, MC30 and TOA64 presented the PGPR traits and they were qualified for their identification at species level. Therefore, 7 unique band positions such as MMC43, MMC46, MA39, SOA10, SOA6, TOA62 and TOA64 were identified on UV and sequenced. They were identified as *Pseudomonas* sp (MA39), *Ochrobactrum intermedium* (MMC43) *O. intermedium* (MMC46) *O. anthropi* (SOA6) *O. anthropi* strain (SOA10), *Ochrobactrum* sp (TOA62), and *Ochrobactrum* sp (TOA64). When using BLAST n for nucleotides similarities search, problem was detected in the following sequences: TOA62 and TOA64, as a result, they were not assigned accession number. The remaining 5 were deposited in GenBank and assigned accession numbers indicated in parentheses: *Pseudomonas* sp. (KC010520) (MA39),

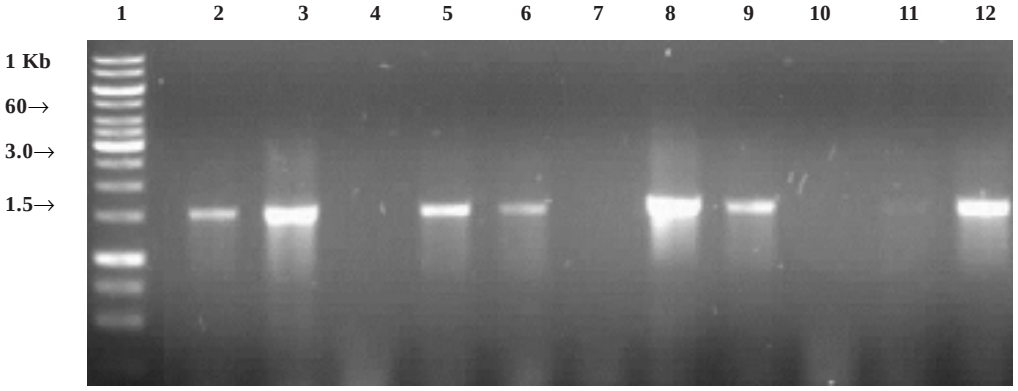

Fig. 4. PCR profiles of 165 rDNA fragments amplified from endophyte bacterial isolates. From left to right 1 Kb marker (1), MMC43(2), MMC46(3) sc42 (4), MA39 (5), SOA10(6), PC23 (7) SOA6(8), TOA62 (9), SA37 (10), MC30 (11) and TOA64 (12).

Table 5: Results of 16S rDNA sequence similarities of endophytic bacteria isolates and GenBank accession numbers using BLASTN Algorithm

Isolate code	*Sequence length (bp)*	*Closest related in database* (*Accession number*)		*Similarity (%)*	*E-value*
MMC 43	1267	*O. intermedium*	(KC146415.1)	100	0
MMC46	1272	*O. intermedium*	(KC146415.1)	100	0
MA39	838	*Pseudomonas* sp	(KC898257.1)	100	0
SOA6	1311	*O. anthropi*	(JN248785.1)	100	0
SOA10	1305	*O. anthropi*	(JN248785.1)	100	0
TOA62	1270	*Ochrobactrum* sp.	(AF515675.1)	100	0
TOA64	1264	*Ochrobactrum* sp.	(AF515675.1)	100	0

O. intermedium (KC010521) (MMC46), *O. intermedium* (KC010522) (MMC43), *O. anthropi* (KC010523) (SOA6) *O. anthropi* strain (KC010524) (SOA10). Results of their closest relatives are shown in Table 5. The phylogenetic tree shows a better picture of the relationships among them (Fig. 5).

Germination Parameters

This was done to assess the effectiveness of PGPR isolates on germination, root length; seedling height and dry weight of plant which reflects the quality of seeds. It was evident that seedling emergence was influenced by different types of PGPR inoculation. Initially, the treatments did not show any differences in emergence. However, one week after inoculation, seeds inoculated with *O. anthropi* KC010523 and *O. anthropi* KC010524 showed higher percent germination (66%) compared to others (55%) and the control (44%) (Table 6). Treated *Zea mays* seedlings were found to be comparably different from the untreated in morphologi-

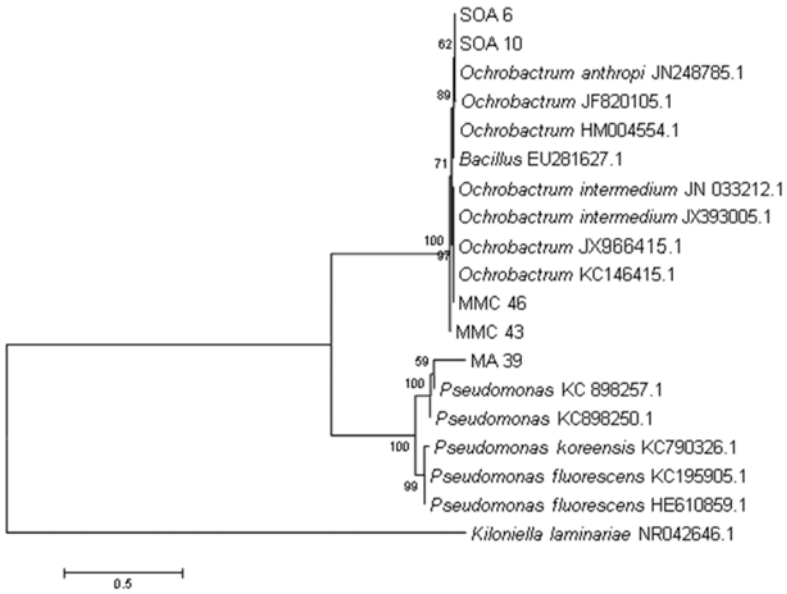

Fig. 5. Phylogenetic tree expressing the relationships of identified bacterial endophytes (bold font) to taxonomically similar bacteria based on the 165 rDNA sequences. The tree was clustered with the neighbour-joining method using MEGA 4.1 package. Bootstrap values based on 1000 replications were listed as percentages at the nodes. The scale-bar indicated 0.05 substitutions per nucleotide position. The GenBank accession number is given in parentheses for each organism. The 165 rDNA sequence of *Kiloniella laminariae* NR042646. 1 was utilized as an out group.

Table 6: Effect of different endophytic bacteria on seed germination of *Zea Mays* after one week

Sample N°	*No. of seeds used*	*No. of seeds germinated*	*No. of seeds ungermi-nated*	*% of germi-nation*	*MSL (cm)*	*MSH (cm)*	*Dry weight (mg/plant)*
Control	18	8	10	44	5	3.5	2.4
Pseudomonas. KC010520	18	10	9	55	6	5	3
O.intermedium KC010521	18	10	9	55	5	4	3
O.intermedium KC010522	18	10	9	55	8	4	3.6
O. anthropi KC010523	18	12	8	66	7	5	3.6
O.anthropi KC010524	18	12	8	66	7	4	3.6

MSL -Mean Root Length; MSH-Mean Seedling height

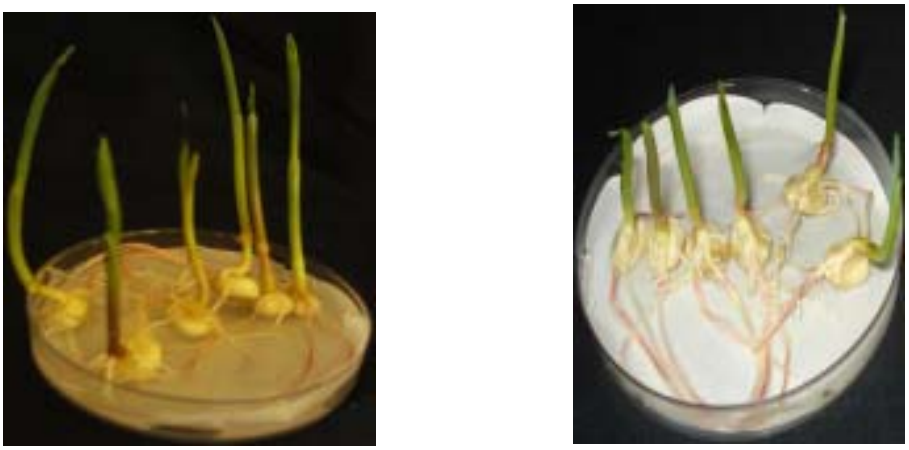

Fig. 6. Effect of Selected endophytic bacteria on the height of *Zea mays* seeds (A) compared to the control (B)

Fig. 7. Effect of selected endophytic bacteria on the length of root of *Zea mays* seeds (1) *O. anthropi* KC010523, (2) *O. intermedium* KC010521, (3) *O. anthropi* KC010524, (4), *Pseudomonas*, KC010520 (5) *intermedium* kc010522, (6) Control

cal parameters like plant height and root length (Table 6). The inoculation of the *Zea mays* seeds with these endophytic bacterial excluding *O .intermedium* KC010521 had a significant effect on fresh roots length ranging from 6 to 7 cm as compared with the control (Figs. 6 and 7). The plate dishes experiment showed also the beneficial effects of these strain on dry weight ~3 and ~3.6 mg over the control (Fig. 7). The highest dry matter was recorded in isolates *O. intermedium* KC010522 (3.6 mg) which was similar to *O. anthropi* KC010523 (3.6 mg) and *O. intermedium* KC010522 (3.6 mg) followed by *Pseudomonas* KC010520 and *O. intermedium* KC010521. The isolate *O. anthropi* KC010523 produced the highest Seedling height (5 cm plant), in comparison to other isolates growth (Table 6).

Metals Concentration in Soil

Metals, such as Ca, Cu, Mg, Mn, Zn, and Fe, are essential nutrients for plants. Their con-

Table 7: *Zea mays* quantitative result

Analyte	*Result*	*(Std.Dev.)*	*Proc.-Calc. Line*	*Int.(cps/uA)*
Fe	7.158 %	(0.014) Quan-FP	FeKa	62.9257
K	4.657 %	(0.053) Quan-FP	K Ka	2.3975
Ca	0.887 %	(0.021) Quan-FP	CaKa	0.8886
Mn	0.137 %	(0.003) Quan-FP	MnKa	0.8876
Zn	0.127 %	(0.001) Quan-FP	ZrKa	7.4830
Cu	0.019 %	(0.001) Quan-FP	CuKa	0.2979

Table 8: *Sorghum bicolor* quantitative result

Analyte	*Result*	*(Std.Dev.)*	*Proc.-Calc. Line*	*Int.(cps/uA)*
Fe	6.405 %	(0.013) Quan-FP	FeKa	58.1320
K	4.161 %	(0.053) Quan-FP	K Ka	2.1393
Ca	0.358 %	(0.020) Quan-FP	CaKa	0.3620
Mn	0.113 %	(0.003) Quan-FP	MnKa	0.7537
Zn	0.112 %	(0.001) Quan-FP	ZrKa	7.0052
Cu	0.021 %	(0.001) Quan-FP	CuKa	0.3512

Table 9: *Spinacia oleracea* quantitative result

Analyte	*Result*	*(Std.Dev.)*	*Proc.-Calc. Line*	*Int.(cps/uA)*
Fe	7.894 %	(0.015) Quan-FP	FeKa	64.9120
K	4.327 %	(0.054) Quan-FP	K Ka	2.1382
Ca	2.071 %	(0.028) Quan-FP	CaKa	1.9951
Mn	0.158 %	(0.004) Quan-FP	MnKa	0.9611
Zn	0.036 %	(0.001) Quan-FP	ZnKa	0.6237
Cu	0.022 %	(0.001) Quan-FP	CuKa	0.3189

centrations in the soil showed variable values and this variability depended upon the sampling sites. The soil samples which were collected in *Spinacia oleracea* agriculture land, exhibited higher concentration of Fe, Cu, Mn, Fe and Ca, in comparison with the other sites (Tables 7, 8, 9).

DISCUSSION

Endophytic bacteria have been variously defined as bacteria which establish symbiotic relationship with healthy plant tissues leading to the formation of root nodules where they fix atmospheric nitrogen (Quang Hung and Annapurna 2004; Dubey et al. 2010). This investigation was to describe indigenous bacterial endophytes associated to the root of *Sorghum bicolor*, *Zea mays* and *Spinacia oleracea*, and their effect in *Zea mays* germination. Morphological observations of the indigenous isolates showed that Gram negative bacteria (60.8%) were predominant in the root of the plants than Gram-positive. These results are similar to those demonstrated by some scientists (Stoltzfus et al. 1997; Elbeltagy et al. 2000). However, in some literature the percentage of Gram negative and Gram positive bacteria are equals (Zinniel et al. 2002). The identification of the bacterial isolates to the species level is vital since this provides informative insight about the microorganism if it is novel or not. The identification of the endophytic bacteria at specie level was done by standard PCR of 16S rDNA that readily amplified large amounts of DNA about 1.3-1.4 kb in size. Sequence analysis of the ubiquitous 16S rDNA has become the ideal tool for classification, detection, and evaluation of the microbial evolutionary relatedness (Ngoma et al. 2013). The advantage of the 16S rDNA gene sequence allows for a better identification of poorly described and rarely isolated strains (Clarridge 2004). This molecular technique is routinely used in the food industry (Handschur et al. 2005), clinical studies (Knapp 2005), microbial ecological studies (Muyzer and Smalla 1998), for the identification of novel pathogens and uncultured microbes (Ercolini 2004). Based on NCBI database, results showed that endophytic bacterial were identified as *Pseudomonas* sp. (KC010520), *O. inter-*

medium (KC010521), *O intermedium* (KC010522), *O. anthropi* (KC010523) and *O. anthropi* (KC010524) respectively. The similarity level of *Pseudomonas* sp. (KC010520) with *Pseudomonas* sp KC898257.1 was 100%. The isolated bacteria *O. intermedium* (KC010521) and *O intermedium* (KC010522) showed 100% similarity with *O. intermedium* KC146415.1 while *O. anthropi* (KC010523) and *O. anthropi* (KC010524) showed 100% similarity with *O. anthropi* JN248785.1. Endophytes bacterial such as *Ochrobactrum* sp TOA62, and *Ochrobactrum* sp TOA64 were not assigned accession number due to a number of factors including: low quality sequencing, misassembled of the sequence reads, vector contamination or PCR artefact.

The identified isolates were tested *in vitro* for their plant growth promoting activities. The most common, best characterized and physiologically most active auxin in plant is IAA. The capacity to synthesize IAA is widespread among soil and plant-associated bacteria. Plant roots excrete L-TRP which can then be utilized by the rhizobacteria for IAA biosynthesis (Frankenberger and Arshad 1995; Yasmin et al. 2009). In the present study the indigenous endophytes bacterial strains isolated were able to synthesize IAA. This biosynthesis has been reported to play a major role in the growth and development of the plant including cell division, differentiation and vascular bundle formation, these three processes are also essential for nodule formation. Hence, it seems likely that auxin levels in the host legume plants are necessary for nodule formation (Gravel 2007; Ahemad and Kibret 2014). Out of 23 isolates tested 11 produced IAA and consequently, are considered as IAA producing rhizobacteria. The range of production was very low (0.15-2.84 mgl^{-1}) as compared to those presented in other reports (11.07 mgl^{-1} for *Erwinia cypripedii*) (Yasmin et al. 2009), (69.4 mgl^{-1} for *Pseudomonas*) (Maa et al. 2011) (15.9 mgl^{-1} for *Pseudomonas aeruginosac* (Ji et al. 2014). The isolates such as *Klebsiella* UPMSP9, *Pseudomonas* UPMSP2 and *Pseudomonas* UPMSP13 showed 9, 7 and 6-fold increases respectively in IAA production when grown in media with L-TRP (Frankenberger and Arshad 1995). Other studies showed the ability of *Pseudomonas* and *Acinotobacter* species isolated from wheat and rye rhizosphere to produce lower IAA ranging from 0.01-3.98 mg L^{-1} (Leinho and Vacek 1994). *Pseudomonas* (KC010520) (MA39) strains produced 2.2 mg/ml^{-1} of IAA, with the highest amount of 2.84 mg/ml^{-1} when grown in media with 1 mg L-TRP followed by TOA 64 (1.9-2.53 mg/ml^{-1}) and *O. intermedium* (KC010521) (MMC46) (1.91-2.48 mg/ml^{-1}). The other bacteria isolates yielded lower amounts at rates of 0.15 to 1.89 mg/ml^{-1} and 0.21-2.25 mg/ml^{-1} when grown in media with 1 mg L-TRP. It has been shown, that IAA production by PGPR may differ among species and strains of rhizobacteria, culture and medium conditions (Kumar et al. 2012). He further showed that IAA production could also be influenced by stage of the plant and sustenance availability. These findings are further strengthened by the low levels of IAA obtained in this study. Others researchers reported that low quantity of IAA production by rhizobacteria promotes primary root elongation, whereas high amount increases lateral and adventitious root formation but inhibits the primary root growth (Xie et al. 1996). Such type of explanation suggested that even at low concentration, these isolates probably synthesized IAA through TRP pathways and may be able to stimulate the growth of plant. The endophytic bacterial possess several traits to influences plant growth, one of the trait is phosphorus which is the second important plant growth-limiting nutrient after nitrogen (Usha-Rani et al. 2012; Ahemad and Kibret 2014). This element in the soil is usually present in the forms of insoluble phosphates and large portion of this phosphorus, is unavailable to plants (Zaidi et al. 2009; Glick 2012; Usha-Rani et al. 2012). Some endophytic bacteria possess the capacity to solubilize this unavailable fraction of soil phosphorus and these are generally termed as phosphorus solubilizing bacteria (Baig et al. 2010). These types of bacterial are particularly of great interest to agricultural land as it can improve the availability of phosphorus and iron for plant growth (Ngoma et al. 2012; Ji et al. 2014). In this study, it was interesting to note that 16 endophytic isolates, showed phosphate solubilization activity and their phosphate solubilizing efficiency has been calculated in percentage. Such organisms play a major role in plant growth promotion. Another PGPR trait is the production of HCN, a gas known to plays a role in the biological control of several soil-borne pathogenic fungi (Ramette et al. 2003). Some PGPR are known to have the ability to produce and excrete this substance into the rhizosphere. The release of HCN

into rhizosphere may affect negatively subterranean animals (Gallagher and Manoil 2001). Bacteria such *pseudomonas aeruginosa* has been shown to have lethal effects on nematodes (Gallagher and Manoil 2001). Studies suggest that the root colonizing *P. fluorescens* strain CHA0 suppress tobacco black root rot from *Thielaviopsis basicola* (Voisard et al. 1994) and weed seedling growth by *Pseudomonas* sp. 437 (Gallagher and Manoil 2001). Kumari and khanna (2014) reported HCN production by 12 isolates from chickpea rhizosphere, of which seven belonged to *Pseudomonas* spp and were found to be strong producers of causing color change from yellow to reddish brown. The remaining five belonged to *Bacillus* spp and were moderate producers indicated by orange brown color. Hydrogen Cyanide has been found to be very effective against wood- infecting termites, and as such, the HCN- producing bacteria can cause mortality among termites by the release of the substance into the substratum (Gallagher and Manoil 2001). From the previous studies it is evident that cyanogenic FPs could be potential antagonists against many plant pathogens (Voisard et al. 1994). In other hand some authors showed that the release of these volatile metabolites (HCN) can negatively affect root metabolism and root growth (Kremer and Souissi 2001). They further proved that *pseudomonads* isolated from *abutilon theophrasti* roots were able to reduce their viability and emergence significantly (Begonia and Kremer 1994). Inhibition of bean growth by *pseudomonas fluorescent* cyanide production has been previously reported by researchers (Alstrom and Burns 1989). About 50% of rhizobacteria isolated from *Solanum tuberosum* roots produced volatile metabolite which was implicated in measurable inhibition of potato growth (Bakker and Schippers 1987). Therefore, no specific role has been assigned to cyanide of fungal or bacterial origin (Blumer and Haas 2000; Devi et al. 2007). The results of HCN production showed that 10 isolated bacteria were capable of producing HCN (about 43.4%) as compared to the control. Ammonia can be produced by several processes such as: nitrite ammonification (Simon 2002), degradation of various amino acids (Kanapka 1983), decarboxylation of amino acids to produce biogenic amines as well as ammonia (Ozugul and Ozugul 2007), deamination, and the urease-mediated hydrolytic degradation of urea (Kleiner et al. 1998). This form of ammonia cannot be assimilated by plants but may be available through biological nitrogen fixation process that only prokaryotic cells have developed, including some eubacteria, cyanobacteria, and actinomycetes (Gnanamanickam 2006; Babalola 2010; Ngoma et al. 2012). Mostly, they are free-living soil organisms (Azotobacter), but some of them have developed an association with plant for nutrient in return , fix nitrogen which can be used by the plant for growth, for example, *Azospirillum*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Gluconacetobacter*, *Herbaspirillum*, and *Burkholderia* (Ngoma et al. 2012). These bacteria are valued for their importance in agricultural fertility. Rhizobium (*Azospirillum* spp) is the most well-known bacterial species that acts as the primary symbiotic fixer of nitrogen (Bashan and Levanony 1990; Glick 2012). In this regard, its influence is positive. Excess amounts of ammonia, however, or too much ammonia applied to the plant can damage or even kill the plant. In this study all seven identified by molecular technique (*Pseudomonas* sp. (KC010520) (MA39), *Ochrobactrum intermedium* (KC010521) (MMC46), *Ochrobactrum intermedium* (KC010522) (MMC43), *Ochrobactrum anthropi* (KC010523) (SOA6), *Ochrobactrum anthropi* (KC010524) (SOA10), *Ochrobactrum* sp TOA62, and *Ochrobactrum* sp TOA64 were able to produce ammonia while 12 isolates were unable to do so. When testing for their antifungal activity, the result revealed that all the isolates suppressed the mycelial growth of *F.oxysporum* in varying degree ranging from 6.7-84.6%. Similar antifungal activity exhibited by *pseudomonas* has been reported (Saravanan et al. 2013) and (Kumari and Khanna 2014). It has been reported that *O. anthropi*, either in aqueous suspension or as talc formulation induced growth of tea plants and suppressed brown root rot disease (Chakraborty et al. 2009). Similar result showed the antagonistic effect of the newly isolated PGPR *Bacillus* JUBM5 on *F.* oxysporum (Shobha and Kumudini 2012). Effectively this bacterium reduced the growth of *F. oxysporum* and the degree of inhibition was 3.25, 0.22 and 0.21 cm respectively. Available report showed that PGPR such as *Serratia* sp.J2, *Pseudomonas fluorescent* J3 and BB11 suppress wilt disease in tomato and increased the yield (Guo et al. 2004). It has been reported also that the bacteria belonging to *Pseudomonas*, which colonize roots of a wide range of crop plants, to

be antagonistic to soilborne plant pathogens (Ehteshamul-Haque et al. 2007; Tariq et al. 2009). Several works reported the positive effect of *Pseudomonas putida* in the root and shoot weight of corn plants and also possess antagonistic activity against *Fusarium* (Mehnaz and Lazarovits 2006; Saravanan et al. 2013). In the present study, maximum inhibition was shown by *Ochrobactrum intermedium* KC010522, *Ochrobactrum* sp TOA62, *Ochrobactrum* sp TOA64, *Ochrobactrum intermedium* KC010521, *Ochrobactrum anthropi* KC010524, *Ochrobactrum anthropi* KC010523 and *Pseudomonas* KC010520. This inhibition process observed *in vitro* may be due to the secretion of fungicidal metabolites or might be attributed to the secretion of antibiotics by the fungi or others inhibitory substances released by the antagonists in the PDA agar by the bacteria (Kumari and Khanna 2014).

Seed germination index was higher in bacterized seeds (66%), with all the strains in comparison to control (44%), whereas, the shoot length was increased in *O.intermedium* KC010522 (8 cm) *O. anthropi* KC01052 (7 cm), *O.anthropi* KC0105243 (7 cm), and *Pseudomonas* KC010520 (6 cm). The highest sseedling height was recorded in *O. anthropi* KC010523 which was similar to *Pseudomonas* KC010520. The plate dishes study results proved that the endophytic bacterial isolates such as *Ochrobactrum intermedium* KC010522, *Ochrobactrum* sp TOA62, *Ochrobactrum* sp TOA64, *Ochrobactrum intermedium* KC010521,*Ochrobactrum anthropi* KC010524, *Ochrobactrum anthropi* KC010523 and *Pseudomonas* KC010520 can significantly induce seed germination and improve root development. Therefore they can be considered as an effective biocontrol and plant growth promoters. Many researchers described the bacteria to have direct influence on root length, number of secondary roots, increase the elongation zone, root volume, and dry weight due to the production of IAA (Yadav et al. 2010). Study proved that bacteria such as *Rhizobium leguminosarum* bv. Trifolli strain E-11 was reported to have a good effect on rice due to the production of IAA (Baset Mia et al. 2012). Recent study has shown the effectiveness of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* on the quality of sorghum seed (Prathibha and Siddalingeshwara 2013). The main reason that attracts high concentration of bacteria around plant root is the presence of root exudates which contain free amino acids, proteins, carbohydrates, alcohols, vitamins, and hormones including micronutrients, important sources of their nutrition (Tak et al. 2013).

However micronutrient such as Fe is a constituent of haemoproteins (cytochromes, catalase) and Fe-S proteins (ferredoxin). haemoproteins are involved in electron transfer and the globins that bind oxygen. Cu is an important element of electron transfer proteins in photosynthesis (plastocyanin) and respiration (cytochrome *c* oxidase) processes, whereas Mn can fully play a role in plant metabolism and development and these happens in oxidation states II, III, and IV in 35 enzymes of a plant cell (Tak et al. 2013). Zn has a vital structural and catalytic role in many proteins and enzymes (Yan-de et al. 2007). Irrespective of whether they are vital or not, those metals at high concentration inhibit plant growth and microbial population by inhibiting its various metabolic activities (Yan-de et al. 2007). At elevated concentration these organisms may increase the opportunity for plants that grow in contaminated site to sequester heavy metals and to recycle nutrients, conserve soil structure, decontaminate chemicals pollutant, and control diseases (Tak et al. 2013). Generally, the metals have low mobility in soil and are not easily absorbed by plant roots. Several researchers have shown the positive impact of endophytic bacteria which may decrease the toxicity of metals by changing their bioavailability to the plants through release of chelating agents, uptake of certain nutrients such as nitrogen, phosphorus, sulfur, magnesium, and calcium (Abou-Shanab et al. 2003) therefore favoring plant yield (Yan-de et al. 2007). It has been reported that *bacillus edaphicus* could increase K contents in plant and that accomplishment could be attributed to the production of plant growth regulators, which stimulated root development and resulted in better absorption of water and nutrients from the soil (Sheng 2005). This aptitude exists in many rhizo and endospheric microorganisms (Yan-de et al. 2007). The results of soil analysis revealed tolerable concentration of metals. However this suggests that these endophytic bacteria isolates may be employed to increase the nutritional efficiency of various plants.

CONCLUSION

The results of the experiments revealed that most of the bacteria isolates, especially *Ochro-*

bactrum intermedium KC010522, *Ochrobactrum* sp TOA62, *Ochrobactrum* sp TOA64, *Ochrobactrum intermedium* KC010521, *Ochrobactrum anthropi* KC010524, *Ochrobactrum anthropi* KC010523 and *Pseudomonas* KC010520 were able to produce IAA, HCN and ammonia. In addition, the strains possess the capacity to solubilize the unavailable fraction of phosphorus on Pikovskya's agar plates and strong antagonism against *F. oxysporum*. All this suggested that the isolates were good candidates to be applied as a biofertilizer and a biocontrol agent. Their effectiveness is also observed in their ability to promote growth in plants and soilborne disease due to fact that both occupy the same ecological niche and have close contact.

RECOMMENDATIONS

Further studies on small-scale field trial are needed to clarify the role of these endophytic bacterial isolates as biofertilizers. If effective PGPR inoculum is developed, larger scale field trials using bioreactor for mass production will be designed. The development and propagation of these low-cost technologies will help farmer economic situations and thereby eliminate the cycle of poverty through collaborative efforts with the government. Their success in agriculture will help reduced farmer's dependency on agro-chemical and potential for commercialization through involvement of agricultural companies.

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