



Screening of endophytic plant growth-promoting bacteria isolated from two Nigerian rice varieties.

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Abstract

Probiotic bacteria, inhabiting the endosphere of plants, presents a major opportunity to develop cheap and eco-friendly alternatives to synthetic agrochemicals. Using standard microbiological procedures, culturable bacteria were isolated from the endosphere (root, stem and leaf) of two Nigerian rice varieties (Ofada and ITA 150). Assays including indole acetic acid (IAA) production, phosphate solubilization, seed germination assay, hydrogen cyanide (HCN) and ammonia production, and antifungal assay were conducted to identify the phytobeneficial bacterial strains. A total of 193 bacteria were isolated from all samples. The roots had the highest bacterial count of 45 and 61 x 10⁸ cfu/g in Ofada and ITA 150 respectively when compared with the stem and leaf samples. The bacterial genera isolated were *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Staphylococcus*, *Citrobacter* and *Escherichia*. Eight isolates produced IAA as well as ammonia. Phosphate solubilization was observed in five isolates while four isolates produced HCN. The germination rate of rice seeds coated with bacterial isolates ranged from 81.48% to 100%. Eight bacterial isolates inhibited the growth of *Fusarium oxysporum* and *Rhizoctonia solani*, but *Citrobacter freundii* and *Enterobacter cloacae* showed the highest antagonism. Thus, eight bacteria isolates showed multiple beneficial abilities and may be considered as promising microbial inoculants for rice plant growth and productivity.

Keywords: Bacteria, endosphere, plant growth, rice

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Introduction

The world's population has been projected to hit 9 billion by 2050 (Ronald, 2011), and most of this growth is expected to happen in sub-Saharan Africa, particularly in Nigeria. The challenge of feeding the world's poorest people in an environmentally sustainable manner will no doubt be immense. Rice consumption is expected to increase as well. Increasing rice production worldwide will help to feed the world. Despite the 6.74 million tonnes of rice produced in Nigeria in 2014 (FAOSTAT, 2015), Nigeria has remained one of the largest importers of rice, second to Indonesia. Its importation bill of rice has increased from \$1,000,000 in the 1970s to as high as \$800 million in 2005 (WARDA, 2007) and in 2014, Nigeria spent N1 billion daily importing rice (Osagie, 2014).

Among other factors, soil fertility

management and biotic stresses such as pest and disease outbreaks, are limiting factors of optimal rice production in Nigeria. The use of chemical fertilizers and pesticides to remedy the problem of low soil fertility and disease outbreak is currently limited due to rising costs and environmental concerns. In order to make rice cultivation more sustainable and less dependent on chemical nitrogen and phosphorus fertilizers and pesticides, the use of endophytic plant growth-promoting bacteria (PGPB) that could protect and promote rice growth would be an alternative for rice production. (Chinnusamy et al., 2006; Cong et al., 2009; Mäder et al., 2012).

Endophytic bacteria are generally referred to as microorganisms that colonize healthy plant tissue without causing obvious symptoms or produce obvious injuries to the host (Bacon and Hinton, 2006). They are also involved in the supply of biologically

fixed nitrogen to their host plant (Boddey et al., 2005). The beneficial effects of endophytic bacteria on plants include; the production of phytohormones, siderophores, ammonia, HCN, phosphate solubilization (Compant et al., 2005; Rajkumar et al., 2006), and the production of lytic enzymes (Nagarajkumar et al., 2004). Bacterial endophytes colonize the ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg et al., 2005).

The use of endophytic bacteria as biofertilizer in the enhancement of rice growth and yield has been reported by Rodrigues et al. (2008) in which the inoculation of *Azospirillum amazonense* increased dry matter accumulation of rice grain (7 to 11.6%), the number of panicles (3 to 18.6%) and nitrogen accumulation at grain maturation (3.5 to 18.5%). *Azospirillum brasilense* inoculum was reported to increase yield of rice up to 76% (Thakuria et al., 2004). Some of these bacteria have already been applied as biofertilizers and they are available to farmers in the developed world (Beatty and Good, 2011).

The use of endophytic bacteria as biofertilizers and biopesticides to improve rice production in Nigeria remains largely untapped despite the potential benefits of using rhizobacteria in crop production and protection. According to Abiala et al. (2015), the development of indigenous biofertilizer and biocontrol strains that suit local environments may help enhance competitiveness with in situ microorganisms and effectiveness at suppressing local pathogen strains. Also, Howell (2003) suggested that biocontrol agents should be isolated from the soil locality where they are expected to function in disease control. Thus, the present study was undertaken to isolate, identify and screen bacteria present in the endosphere of two Nigerian rice cultivars (Ofada and ITA 150) for their multiple phyto-beneficial effects.

MATERIALS AND METHODS

Collection of samples and bacteria isolation

Leaf, stem and root samples of two Nigerian rice cultivars (OFADA and ITA 150) were randomly collected from the FADAMA farm of the Federal University of Agriculture, Abeokuta in triplicates. The samples collected were separately washed with tap water, followed by surface sterilization using 70% ethanol for 30 seconds, 2% Sodium hypochlorite (NaOCl) for 5 minutes, 3% Hydrogen peroxide for 30 seconds and then rinsed five times with distilled water. Ten grams of these samples were cut to 2-3 cm pieces and macerated using sterilized mortar and pestle with 12.5 mM potassium phosphate buffer (pH 7.1), followed by a 10-fold serial dilution where aliquots (100 μ L) of the 10^{-6} and 10^{-8} dilutions were plated using the pour plate method on Nutrient Agar

supplemented with cycloheximide (100 μ g/mL) to inhibit fungal growth. Inoculated Petri plates were incubated at 28°C for 48 hours. After the incubation time, the colony forming units (CFU) for each plate was estimated.

Isolates differing in morphological appearance were selected and were streaked onto new plates until pure cultures were obtained. Pure cultures of bacterial isolates were maintained on NA slants and were stored at 4°C.

Cultural characterization of bacterial isolates

The bacterial isolates were characterized based on their morphological and biochemical characteristics (Cappucino and Sherman, 2002) and examined according to the Bergey's Manual of Determinative Bacteriology (Bergey et al., 1994). Biochemical tests including catalase, citrate utilization, capsule staining, Voges-Proskauer, Methyl red and sugar fermentation tests were carried out on the isolates.

Assays for phyto-beneficial abilities of isolates Indole Acetic Acid Production

Detection of IAA production was done as described by Sharma et al. (2012). Pure bacterial isolates were inoculated in nutrient broth enriched with tryptophan (200 μ g/mL), followed by incubation at 28°C for 48 h in a shaker incubator. 10 ml of the resultant culture was centrifuged at 4°C for 10 min at 15,000 revolutions per minutes (rpm). Two millilitres of the supernatant was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski's reagent (50 ml, 35 % of perchloric acid, 1 ml 0.5M $FeCl_3$ solution). This mixture was kept in a dark room for 20 min, afterwards the light absorption of the mixture was estimated at 540 nm using a spectrophotometer. The light absorption estimates were compared to a standard curve to determine quantity of IAA produced by each isolate in μ g/ml This experiment was carried out in triplicates for each bacterial isolate.

Phosphate solubilization

The bacterial isolates were spot-inoculated on the Pikovskaya media containing tricalcium phosphate in a plate and incubated at 28°C for 72 h (Sharma et al., 2007). Plates were then observed for the presence of halo zones (clear zones) around the spotted colonies. The halo zone surrounding the colonies were measured, and the phosphate solubilization efficiency (PSE), expressed as a percentage according to Sharma et al. (2007).

Seed Germination Bioassay

Seedling bioassay was conducted based on the method described by Dey et al. (2004). Bacterial inocula were prepared by incubating bacterial

cultures for 24h and were diluted with sterile distilled water to give a concentration of approximately 10^6 cells/mL (10^6 CFU/mL) adjusted with a spectrophotometer (Jenway, UK). Rice seeds (Ofada variety) were surface sterilized with 0.5% NaOCl for 2 min, followed by 30 seconds in 70% ethanol and two rinses in distilled water, followed by air drying. Seeds were coated with each bacterial isolate by immersion in a suspension of bacteria containing 10^6 CFU/mL for 30 mins and were then dried in a laminar flow cabinet for 1 to 2 h. Nine seeds inoculated with each bacterium were placed in 9-cm-diameter petri dishes lined with sterilized moistened filter paper and were incubated for 7 days at 28°C. Germinated seeds were counted at day 7. Germination rate, average plumule and radical lengths as well as vigour index were calculated using the method described by the International Seed Testing Agency (1999).

Ammonia Production

The ability of each bacterial isolate to produce ammonia was assayed according to Cappucino and Sherman (1992). Bacterial isolates were tested for the production of ammonia as follows: 24 h old bacterial cultures were inoculated in 10 ml nutrient broth and incubated at 30°C for 48 h in a rotator shaker. After incubation, 0.5 ml of Nessler's reagent was added to each tube. The development of a yellow to brown colour indicated a positive reaction for ammonia production.

Hydrogen Cyanide (HCN) Production

Production of HCN by the isolates was detected according to the method of Ngoma et al. (2013). Nutrient broth was amended with 4.4 g/L glycine, followed by autoclaving and pouring in Petri dishes. Each bacterial isolate was streaked on the modified agar. A Whatman filter paper soaked in 2% Sodium carbonate in 0.5% picric acid solution was placed directly on each of the streaked agar plate. Plates were then sealed with parafilm and incubated at 28°C for four days. Development of orange to red

colour was the indicator of HCN production.

Antagonism assay against phytopathogenic fungi

The antifungal activity of the bacterial isolates was assayed according to Noori and Saud (2012). Potato Dextrose Agar (PDA) was prepared, autoclaved and poured in sterile petridishes. A 5 mm mycelia mat of *Fusarium oxysporum* and *Rhizoctonia solani* was placed in the center of each plate. Each bacterial isolate was streaked 3 cm away from the fungi on both sides of the fungus and the plates incubated for 5-7 days at 28°C. The zone of inhibition was observed around the fungus used and the inhibition index was estimated using the formular:

$$\text{Inhibition index (\%)} = \frac{(R1-R2) \times 100}{R1}$$

Where R1=Radial growth of *F. oxysporum* in control plate and R2=radial growth of *F. oxysporum* interacting with antagonistic bacteria. The control was a plate with the phytopathogenic fungi only. All experiments were done in triplicates.

Statistical analysis

Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows (SPSS, Chicago IL, USA). Data obtained were analyzed using descriptive statistics (mean and standard deviation) and Analysis of Variance (one-way) followed by *post hoc* pairwise comparisons using the Student-Newman-Keuls multiple-range test.

Results

Enumeration of bacterial isolates

A total of 193 bacterial strains were isolated from the endosphere of the two rice cultivars examined at 10^8 CFU/g (Plate 1). The endophytic bacterial population of the roots in both rice varieties (61.00 ± 2.44 , 45.00 ± 2.44) CFU/g were significantly higher than those of the stems (29.33 ± 2.44 , 32.00 ± 2.44) CFU/g and leaves (15.67 ± 2.44 , 9.67 ± 2.44) in both varieties at dilution 10^8 (Figure 1).

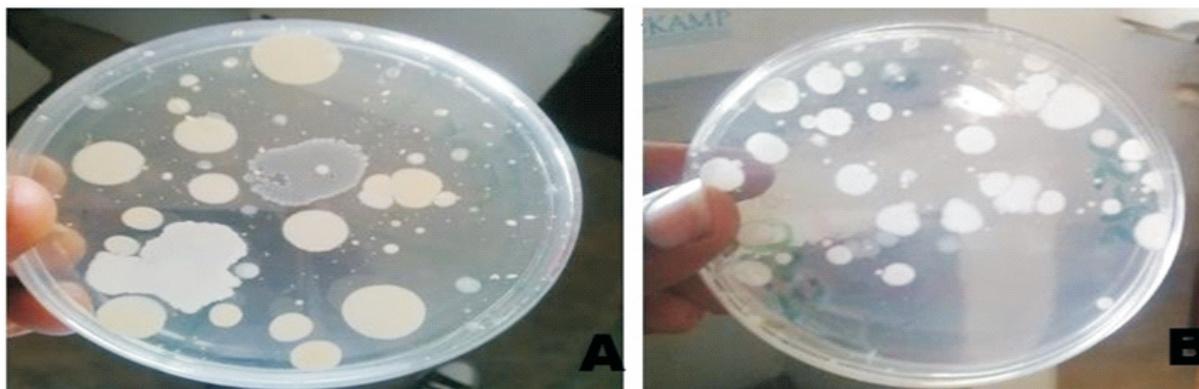


Plate 1: Bacterial colonies isolated from rice stem tissues at 10^8 dilution (A=ITA 150 variety, B=Ofada variety).

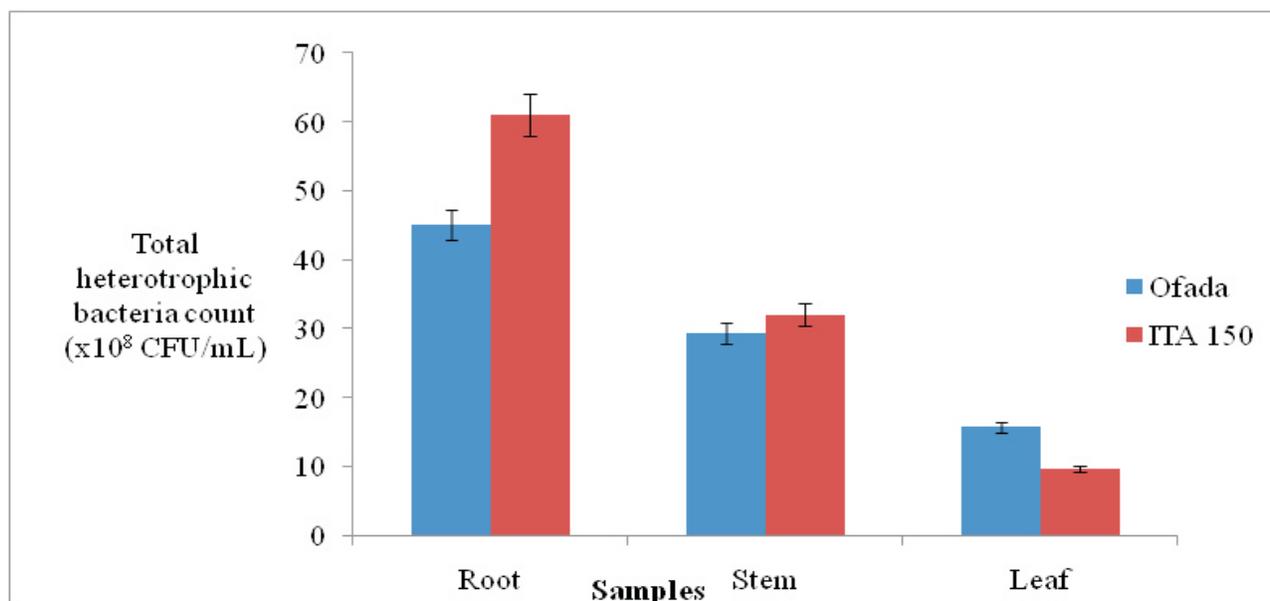


Figure 1: Comparison of the total heterotrophic bacterial count of the different samples in two different rice varieties (ITA 150 and Ofada).

Morphological and Biochemical Characteristics of Bacterial isolates

The colonial morphology of isolates was significantly diverse. All the isolates produced round shaped colonies, the elevation was either flat or raised, had smooth or undulate margin with the

colony colour ranging from cream to green. Motility was observed in most isolates examined and the cell shape was mostly rod (Table 1). All the isolates were positive for Catalase and were Glucose fermenters. Majority of the isolates were Gram-negative in reaction (Table 2).

Table 1: Morphological characteristics of bacterial isolates

| Isolate code | Rice Variety | Rice plant tissue | Shape | Motility | Colour | Edge | Elevation | Opacity | Size |
|--------------|---------------|-------------------|-------|------------|--------------|----------|-----------|-------------|-------|
| OP1 | Ofada, ITA150 | Leaf Root | Cocci | Motile | Cream | Undulate | Flat | Opaque | Small |
| OP2 | Ofada, ITA150 | Stem Root | Rod | Motile | Cream | Undulate | Raised | Opaque | Small |
| OP3 | Ofada, ITA150 | Root Root | Cocci | Non-motile | Cream | Entire | Flat | Transparent | Big |
| OP4 | Ofada, ITA150 | RSL RSL | Rod | Motile | Light yellow | Entire | Flat | Opaque | Small |
| OP5 | Ofada, ITA150 | - Root | Rod | Non-motile | Cream | Entire | Flat | Opaque | Big |
| OP6 | Ofada, ITA150 | RS RS | Rod | Non-motile | White | Undulate | Flat | Transparent | Small |
| OP7 | Ofada, ITA150 | RSL Stem | Rod | Motile | Cream | Undulate | Flat | Transparent | Big |
| OP8 | Ofada, ITA150 | Leaf RS | Rod | Motile | Yellow | Entire | Flat | Opaque | Small |

KEY: RSL-Root, Stem and Leaf, RS-Root and Stem

Table 2: Biochemical characteristics of bacterial isolates from rice varieties

| ID | GR | CA | CP | CO | MO | IN | CI | UR | MR | VP | G | L | M | Suspected bacteria |
|-----|----|----|----|----|----|----|----|----|----|----|---|---|---|-------------------------------------|
| Op1 | + | + | - | - | - | - | - | - | - | + | A | - | - | <i>Staphylococcus saprophyticus</i> |
| OP2 | - | + | - | - | + | - | - | + | + | - | A | A | A | <i>Escherichia coli</i> |
| OP3 | + | + | - | + | - | - | - | - | - | + | A | A | A | <i>Staphylococcus aureus</i> |
| OP4 | + | + | + | - | + | - | + | - | - | + | A | - | A | <i>Bacillus subtilis</i> |
| OP5 | - | + | - | - | + | - | + | + | + | - | - | - | - | <i>Pseudomonas aeruginosa</i> |
| OP6 | - | + | + | - | - | - | + | + | - | + | A | A | A | <i>Klebsiella sp</i> |
| OP7 | - | + | - | - | + | - | + | + | + | + | A | A | A | <i>Enterobacter sp</i> |
| OP8 | - | + | - | - | + | + | + | - | + | - | A | A | A | <i>Citrobacter sp</i> |

KEY: GR= Gram stain, CA= Catalase, CP= Capsule stain, CO= Coagulase, MO= Motility, IN= Indole, CI= Citrate, UR= Urease, MR= Methyl-red, VP= Voges proskeur, G= Glucose, L= Lactose, M= Mannitol, A= Acid production, + = POSITIVE, - = NEGATIVE

Assays for phytobeneficial abilities of isolates

IAA production ability

This assay was carried out on each of the 193 bacterial isolates, out of which eight isolates were positive for IAA production. A standard curve was prepared using different concentrations of pure IAA and this curve was used to compute for IAA produced by the bacterial isolates assayed. All of the eight isolates examined produced significant quantities of IAA ranging from 1 µg/mL produced by isolate OP8 to 32 µg/mL produced by isolate OP 2. Isolates OP8 and OP4 produced the largest quantities of IAA at 32 µg/mL and 31 µg/mL respectively while isolates OP1 and OP2 produced the least quantities of IAA at 12 µg/mL and 1 µg/mL respectively (Table 3).

Phosphate solubilization

Five isolates of the 193 endophytic bacteria were able to solubilize tricalcium phosphate with efficiencies ranging from 200% to 128%, while three isolates did not solubilize the inorganic phosphate. Isolates OP4 and OP7 produced the highest phosphate solubilization efficiency of 170% as compared to isolate OP6 with 98% solubilization efficiency. Isolates OP1, OP2 and OP3 did not produce any halo zone on the Pikovskaya medium, and thus showed no phosphate solubilizing ability (Table 3).

HCN and Ammonia production

Only four isolates produced HCN of the 193 strains in vitro. However, the degree of HCN production ranged from high intensity to weak intensity. Eight bacterial isolates were positive for

ammonia production (Table 3).

Antagonism assay against phytopathogenic fungus

Eight isolates inhibited *Fusarium oxysporum* and *Rhizoctonia solani*. The highest inhibition index for *F. oxysporum* was observed in isolate OP7 (80% inhibition index), followed by isolate OP6 with 77.22% while isolate OP4 had the lowest inhibition index of 49.44%. For *R. solani*, isolate OP8 had the greatest antifungal effect of 86.36% inhibition index, while isolate OP7 showed the least inhibition index of 48.18% (Table 3).

Seed Germination Bioassay

All isolates were examined for their effect on Ofada rice seed germination in vitro. These isolates had varying degrees of influence on the germination rate of the Ofada rice variety used. While the untreated seeds of Ofada variety (Control) had the lowest germination rate of 70.37%, seeds coated with isolates OP1, OP5 and OP8 had the least germination rate of 81.48%, while seeds treated with isolate OP3 had the highest germination rate of 100%. The vigour index for each isolate was also computed from data obtained on the plumule and radicle lengths as well as the germination rate. It was observed that isolate OP3 had the highest vigor index of 682, while isolate OP4 had the least vigor index of 241.09. All 8 isolates assayed had significantly higher vigor index values than the control (Table 4).

Table 3: Determination of characteristics associated with plant growth promotion of bacterial strains isolated from rice rhizosphere.

| Isolate | IAA ($\mu\text{g/mL}$) | Phosphate solubilization efficiency (%) | HCN production | Ammonia production | F.oxysporum inhibition index (%) | R. solani inhibition index (%) |
|---------|-------------------------------|---|----------------|--------------------|----------------------------------|--------------------------------|
| OP1 | 12.00 \pm 0.58 ^c | - | - | ++ | 68.89 \pm 0.52 ^d | 66.36 \pm 0.52 ^b |
| OP2 | 1.00 \pm 0.58 ^d | - | ++ | ++ | 60.55 \pm 0.52 ^e | 64.55 \pm 0.52 ^c |
| OP3 | 20.00 \pm 0.58 ^b | - | - | + | 66.11 \pm 0.52 ^d | 59.09 \pm 0.52 ^d |
| OP4 | 31.00 \pm 0.58 ^a | 170.00 \pm 15.00 ^a | - | +++ | 49.44 \pm 0.52 ^f | 64.55 \pm 0.52 ^c |
| OP5 | 19.00 \pm 0.58 ^e | 127.10 \pm 44.80 ^c | + | ++ | 71.67 \pm 0.52 ^c | 62.73 \pm 0.52 ^c |
| OP6 | 22.00 \pm 0.58 ^b | 98.60 \pm 3.50 ^d | +++ | ++ | 77.22 \pm 0.52 ^b | 60.91 \pm 0.52 ^d |
| OP7 | 17.00 \pm 0.58 ^c | 170.00 \pm 10.00 ^a | - | + | 80.00 \pm 0.52 ^a | 48.18 \pm 0.52 ^e |
| OP8 | 32.00 \pm 0.58 ^a | 153.30 \pm 27.80 ^b | +++ | + | 63.33 \pm 0.52 ^e | 86.36 \pm 0.52 ^a |

KEY:- = not detected. + = low. ++ = medium. +++ = high. Values are mean \pm standard error of mean. Values followed by different letters within a column indicates significant differences according to the Student-Newman-Keuls multiple-range test ($\alpha = 0.05$)

Table 4: Beneficial effects of identified bacterial isolates on Ofada seed germination and vigor index

| Isolates | Germination Rate (cm) | Mean Plumule length (cm) | Mean Radicle length (cm) | Vigor Index |
|----------|-------------------------------|------------------------------|------------------------------|---------------------------------|
| Control | 70.37 \pm 1.67 ^d | 1.1 \pm 0.55 ^d | 0.9 \pm 0.57 ^e | 140.74 \pm 27.35 ^e |
| OP1 | 81.48 \pm 2.94 ^c | 1.63 \pm 0.12 ^c | 1.45 \pm 0.13 ^d | 250.96 \pm 10.84 ^d |
| OP2 | 96.29 \pm 4.00 ^b | 2.3 \pm 0.85 ^b | 2.61 \pm 0.18 ^b | 472.78 \pm 41.31 ^c |
| OP3 | 100 \pm 5.00 ^a | 3.91 \pm 0.20 ^a | 2.91 \pm 0.51 ^b | 682.00 \pm 71.53 ^a |
| OP4 | 85.19 \pm 8.01 ^c | 1.38 \pm 0.16 ^d | 1.45 \pm 0.00 ^d | 241.09 \pm 4.81 ^d |
| OP5 | 81.48 \pm 2.94 ^c | 1.72 \pm 0.04 ^c | 1.58 \pm 0.16 ^d | 268.88 \pm 8.03 ^d |
| OP6 | 96.29 \pm 4.00 ^b | 2.34 \pm 0.09 ^b | 2.61 \pm 0.18 ^b | 476.64 \pm 30.51 ^c |
| OP7 | 96.29 \pm 4.00 ^b | 2.63 \pm 0.04 ^b | 3.78 \pm 0.38 ^a | 617.22 \pm 22.13 ^b |
| OP8 | 81.48 \pm 2.94 ^c | 1.8 \pm 0.30 ^c | 1.85 \pm 0.85 ^c | 297.40 \pm 49.37 ^d |

Values are mean \pm standard error of mean. Values followed by different letters within a column indicates significant differences according to the Student-Newman-Keuls multiple-range test ($\alpha = 0.05$)

Discussion

The use of microorganisms and the exploitation of beneficial plant-microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture worldwide (Berg, 2009). Various endophytic bacteria that inhabit plant tissues can aid in sustainable agriculture.

In this study, the endophytic bacterial population was observed to be highest in the roots, followed by the stems and lowest in the leaves of both rice cultivars examined. This can be attributed to the greater concentration of nutrients (exudates) present in the root region as compared to the phyllosphere. According to Bacilio-Jiménez et al. (2003), the root exudates produced by rice plants promote the interaction between endophytic bacteria and root tissues. These results are similar to endophytic bacteria isolated from rice landraces (Rangjaroen et al., 2015), the cultivated rice cultivar Khao Dawk Mali 105 (Prakamhang et al., 2009) and wild rice cultivars *Oryza rufipogon*, *O. nivara* and *O. granulata* (Koomnok et al., 2007).

Based on the morphology and biochemical characteristics of the isolated bacterial species, 8 distinct bacterial morphotypes were tentatively identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella sp*, *Enterobacter sp*, *Citrobacter sp*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Escherichia coli*. The species identified in this study are in correlation with other studies on rice endosphere microbiome (Mbai et al., 2013, Mwajita et al., 2013) who both implicated a wide variety of bacteria.

The plant growth-promoting abilities of the isolated endophytic bacteria were assayed with varying results observed. Endophytes have been shown to promote plant growth by producing IAA (Mendes et al., 2007). Indole acetic acid (IAA) is a key phytohormone known for its ability to elongate and enlarge plant root cells in order to permit greater nutrient and water absorption. In this study, eight out of 193 endophytic bacterial isolates produced IAA in concentrations. The range of IAA produced in this study is similar to a report by Ji et al. (2014) who observed an IAA range of 3.1-24.6 µg/mL while lower range of IAA produced was reported by Rangjaroen et al. (2015).

Phosphorus, an essential plant macronutrient, although present in significant quantities in soils is not readily available for plant uptake due to its insoluble forms being dominant. Of the 193 strains, only 5 isolates solubilized inorganic phosphate by producing halo zones on the Pikovskaya medium. The phosphate solubilization efficiency

(PSE) recorded ranged from 98% to 128%. The range of PSE recorded is similar to a report by Kaushal and Kaushal (2011). The ability of bacteria to solubilize insoluble phosphates has been attributed to their capacity to reduce pH by the excretion of organic acids during the assimilation of ammonia (Gyaneshwar et al., 1999).

The production of hydrogen cyanide (HCN) and ammonia, secondary metabolites by certain plant-associated bacteria is another beneficial trait of a PGPB. HCN produced can inhibit the growth and metabolism of potential plant pathogen and thus protect the plant from harm as well as strengthen the host's disease resistance mechanism (Whipps, 2001). Ammonia can also have inhibitory effects on certain phytopathogens such as *Fusarium oxysporum*, *F. oxysporum* f. sp. *melongenae* and *Macrophomina phaseolina* (Yildiz et al., 2012). In this study, only four out of the 193 bacterial strains were able to produce HCN as *Citrobacter sp* and *Klebsiella sp* produced the highest colour intensities and thus the highest HCN while eight endophytic bacteria produced NH₃, as observed by the change in colouration of the broth medium.

This study investigated the effectiveness of bacterial isolates whether they could increase the seed germination rate as well as other plant growth parameters of the seedlings in vitro (plumule and radicle lengths). Eight isolates significantly increased the growth parameters of the inoculated rice seeds as compared to the uninoculated control. This could be attributed to the suppression of seed pathogens (Abiala et al., 2015) as well as the synthesis of hormones such as IAA by the isolates in this study (Ng et al., 2012). Indole acetic acid (IAA) can trigger the activity of specific enzymes that promote early germination and increased plumule and radicle length and seed inoculation with IAA producing rhizobacteria has been shown to enhance early seedling establishment (Khalid et al., 2004).

The unique ability of certain plant associated bacteria to control and inhibit the growth of plant pathogens is of utmost importance in selecting for potentially plant beneficial bacteria. In this study, eight bacteria were able to inhibit the growth of two soil-borne fungal pathogens *Rhizoctonia solani* and *Fusarium oxysporum*. *Enterobacter sp* (86.36%) showed the highest antagonism to *Rhizoctonia solani*. *Fusarium oxysporum* was also highly inhibited by *Citrobacter sp* (80%). The differential rates of fungal inhibition by the bacteria species suggests that the mode of action and types of antifungal metabolites produced vary from one bacterium to another (Williams and Asher, 1996). The reduction of fungal growth by certain plant beneficial bacteria and the in vitro inhibition zones may be probably due to

antifungal substances (such as Zwittermycine-A, kanosmine, lipopeptides) and/or lytic enzymes released by these isolates (Noumavo et al., 2015).

The results obtained from the various assays indicates that endophytic bacterial isolates OP4, OP7 and OP8 corresponding to *Bacillus subtilis*, *Enterobacter* sp and *Citrobacter* sp showed the best abilities to promote plant growth in vitro as seen by the results of the various assays conducted.

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