Research Article
Open Access
CrossMark

Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (*Lycopersicon esculentum Mill*) varieties

Abstract

Several PGPR have been reported to individually enhance growth, seed emergence and crop yield, and some have been commercialized. This present study investigated the growth promoting abilities of individual species of bacteria as compared to their consortium. Bacteria were isolated and identified from the rhizosphere, rhizoplane and non-rhizosphere of three varieties (Roma Vf, Beske and Ibadan local) of tomato plant. Four isolates (Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniaeand Citrobacter youngae) were outstanding in in-vitro assays for phosphate solubilisation, ammonia, hydrogen cyanide and indole acetic acid production. Results from the green house study revealed that tomato seed treated with these four isolates and their consortiumsignificantly enhanced the seedling height, stem girth, number of leaves and leaf area. However, tomato seed treated with the consortium showed significant growth above individual cultures. It could be concluded that the consortium of several effective strains for growth enhancement performed better than their individual culture.

Keywords: plant growth promoting rhizobacteria, consortium, rhizosphere, rhizoplane

Volume 5 Issue 1 - 2016

Taiwo Michael Oluwambe, Akintokun Aderonke Kofoworola

Department of Microbiology, Federal University of Agriculture, Nigeria

Correspondence: Taiwo Michael Oluwambe, Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, Tel 08060012150, Email taiwomikeo@gmail.com

Received: August 20, 2016 | **Published:** November 09, 2016

Abbreviations: PGPR, plant growth promoting Rhizobacteria; PGPB, plant growth promoting bacteria; FUNAAB, federal university of agriculture, Abeokuta; IAA, indole acetic acid; HCN, hydrogen cyanide; SNK, student-newman-keuls; ANOVA, analysed by analysis of variance

Introduction

Plant Growth Promoting rhizobacteria (PGPR) are free living, non pathogenic microorganisms which successfully colonize the soil and root region of host plant, compete with pathogenic organisms and suppress their growth, thereby acting as biofertilizer and/or antagonist (biopesticides) to pathogens.[1] Many of these bacteria had been classified into the genera *Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Paenibacillus, Klebsiella, Actinomyces, Clostridium, Enterobacter, Gluconacetobacter, Pseudomonas* and *Serratia,* among which *Pseudomonas and Bacillus* are the most comprehensively investigated genera.[2] Beneficial effects of PGPR on plant growth have been attributed to mechanisms such as production of phytohormones, nitrogen fixation, solubilization of phosphates, suppression of pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic activity.[1] The use of plant growth promoting bacteria (PGPB) has depicted potentials in developing sustainable agricultural systems for crop production and protection. PGPR agents isolated outside the region and imported to Africa have performed inconsistently under field conditions.[3] Therefore suggested that PGPR agents should be isolated from the soil locality where they are expected to function.[4] Most approaches for plant growth promotion have used single bacterial species as biofertilizers while few had used a consortium of selected bacterial species. A microbial consortium is the combination of two or more microbial species which act together as a community for a particular purpose. This study was therefore undertaken;[1] to isolate bacteria from the rhizosphere, rhizoplane and non-rhizosphere of three tomato varieties (Roma Vf, Beske and Ibadan local) in Abeokuta, Ogun State, Nigeria[2] to screen for indigenous bacteria that can serve as growth promoting agent[3] to prepare a consortium of the PGPR and compare with individual species[4] to evaluate the *in-vitro* and green house growth promoting activity of the isolates.

Materials and methods

Isolation and Identification of Indigenous Bacteria

Soil and root samples were collected aseptically from the rhizosphere, rhizoplane and non rhizosphere of three tomato plant varieties (Roma Vf, Beske and Ibadan local) at the farm site of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. Serial dilutions were done and 0.1ml of the 10^{-6} was placed on nutrient agar medium using pour plate method. The plates were sealed with parafilm, inverted and incubated at 28±2°C for 48hours. Isolates differing in morphological appearance on nutrient agar were selected and were streaked onto new plates until pure cultures were obtained. Pure cultures of bacterial isolates were maintained on slants and were stored at 4°C. The bacteria isolates were subjected to standard microbiological methods such as morphological characteristics of the colony, gram staining and biochemical tests according to the method of.[5,6]

Evaluation of plant growth-promoting characteristics of bacteria isolates

Indole acetic acid (IAA) production: The bacterial isolates were inoculated in triplicate to tryptophan nutrient broth (5grams of tryptophan per litre of nutrient broth) and incubated with shaking for 48hours at 28°C. Visually turbid cultures were observed and 5.0ml of each culture were transferred to a 10ml tube. They were centrifuged at 10,000rpm for 15minutes. Then 1.0ml of the supernatant was mixed with 2.0ml of Salkowsky reagent (50ml of 35% Perchloric acid, 1ml of 0.5 M $FeCl_3$ solution), and the mixture was then incubated at room temperature for 25minutes. Development of pink color after incubation at room temperature indicated IAA production. The absorption of positive reaction was determined at 530nm using a spectrophotometer. The colours produced by the respective strains were categorized into low, medium and high.[7]

Phosphate solubilisation: The potential of bacterial isolates to solubilize phosphate were carried out according to the method of.[1,8] The phosphate solubilizing medium was prepared according to the modified method of.[9] Five grams of $CaHPO_4$ was used as the source of phosphate in agar plates that also contained 2.5grams of glucose, 1.0gram of $MgSO_4 \cdot 7H_2O$, and 20.0grams of agar per litre (pH 6.8). Each isolate was spot-inoculated onto three replicate plates and incubated at 28°C for 48hours. The formation of halozone surrounding the colonies indicated positive result. The clear zones were then measured and recorded.

Hydrogen Cyanide Production: Bacterial isolates were grown in 10% trypone soy agar supplemented with glycine ($4.4gl^{-1}$). A Whatman filter paper No. 1 soaked in 2% Sodium carbonate and 0.5% picric acid solution was placed to the underside of the Petri dish lid. To avoid the escape of the gas, the plates were sealed with parafilm and incubated at 30°C for 5days. The production of HCN was determined by the change in colour of filter paper from yellow to red-brown.[10]

Production of ammonia: Freshly grown bacterial cultures were inoculated in 10ml nutrient broth and incubated at 30°C for 48hours in a rotator shaker. After incubation, 0.5ml of Nessler's reagent was added to each tube. The development of a yellow to brown colour indicated a positive reaction for ammonia production.[11]

Preparation of Bacterial Inocula and the Microbial Consortium

Bacterial inocula were prepared by incubating bacterial cultures for 24hours in a nutrient broth medium. The microbial consortium was prepared by inoculating 0.1ml of the 24hours old culture of selected isolates in a 20ml nutrient broth and incubated with shaking at 37°C for 24hours. They were all diluted with sterile distilled water to give a concentration of approximately 10^6 cells/ml (10^6 CFU/ml), adjusted with a haemocytometer.

Seed germination bioassay

In-vitro germination assay for plant growth promoting bacteria

The effect of each bacteria isolates on tomato seed germination was carried out in the laboratory using blotter techniques method according to ISTA (1999).[12] Tomato seeds (Beske variety) obtained from the Tissue Culture Laboratory of the Department of Crop Protection, FUNAAB were surface sterilized with 0.5% Sodium hypochlorite (NaOCl) for 2minutes, followed by 30seconds dip in 70% ethanol, two rinses in distilled water and then air dried at room temperature.[13] Fifteen sterilized seeds were then inoculated with each bacteria isolates and their consortium by soaking in a suspension of bacteria for 30min and then air dried at room temperature for 1 hour. The experiment was carried out in triplicates. The seeds used for control experiment were treated with sterile water. Seeds inoculated with each bacterium and their consortium were placed in 9cm diameter petri dishes lined with sterilized moistened cotton wool and incubated for 7days at 28±2°C. Germinated seeds were counted at day 7. The average radicle and plumule lengths for each petri dish were also recorded for calculation of the vigor index. The vigor index was calculated as (mean of plumule+radicle lengths)xgermination rate.

Green house assay for isolate effect on seed germination of tomato plant:

Method[9] was modified to determine the bacteria isolates that exhibited plant growth promoting characteristics in a conventional green house on tomato. Soil sample collected from the Federal University of Agriculture, Abeokuta (FUNAAB) Teaching and Research farm was passed through a 2mm sieve to remove extraneous materials. It was sterilized in an autoclave at 121°C for 15minutes after which it was allowed to cool and stabilized. Sterilized seeds were dipped into a 24-hour nutrient broth culture ($1x10^{-6}$cells/ml) of each bacterial cell and a consortium of the PGPR respectively for 1 hour and then allowed to air dry on a filter paper at room temperature. Five seeds were sown in each planting bags containing 1.5kg of sterilized soil. Each treatment was replicated three times in a completely randomized design. A control was equally set up in which the tomato seeds were soaked in a sterile water and allowed to air dry. Seedlings were observed after every 7days for 4 weeks to determine the number of germinated seed (%), seedling height (cm), stem girth (cm), number of leaves and leaf area (cm^2) for each pot. The percentage of germination was calculated as:

$$\textit{number of germinated seeds} \textit{total number of planted seeds} 1x100$$

Statistical analysis of data collected

Data were analysed using statistical package for social sciences (SPSS) version 16.0 for Windows (SPSS, Chicago IL, U.S.A). The means of the data obtained from the bacterial load were analysed and means were separated and compared with standard error using Turkey-Kramer HSD test at α=0.05. To determine the plant growth-promoting characteristics of the isolates, data obtained from plant height, stem girth, leaf number and leaf area for green house seed germination were analysed by analysis of variance (ANOVA), means were separated using Student-Newman-Keuls (SNK) test at α=0.05.

Results

Total bacterial counts of samples

There were significant differences in the bacterial counts in rhizosphere, rhizoplane and non rhizosphere soil of the three tomato varieties. Beske tomato variety had the highest bacterial count in the rhizosphere ($119.0x10^6$CFU/g), followed by the rhizoplane ($116.0x10^6$CFU/g). Ibadan local recorded the highest count in the rhizoplane ($94.3x10^6$CFU/g) followed by the rhizosphere soil ($90.0x10^6$CFU/g) while Roma VF recorded the highest count in the rhizosphere ($55.3x10^6$CFU/g) followed by the rhizoplane

Citation: Oluwambe TM, Kofoworola AA. Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (Lycopersicon esculentum Mill) varieties. *Adv Plants Agric Res.* 2016;5(1):448–455. DOI: 10.15406/apar.2016.05.00167

($44.3x10^6$CFU/g). The non-rhizosphere soil had the least bacterial count in all the three varieties (Table 1).

Distribution of different bacterial species

A total of one hundred and twenty four bacterial isolates were obtained from the rhizosphere, rhizoplane and non rhizosphere soil samples of the three variety of tomato (Roma Vf, Beske and Ibadan local). Nine different bacterial isolates identified were selected based on their cultural and morphological differences. Table 2 showed the distribution of different bacterial species from the rhizosphere, rhizoplane and non rhizosphere soil of three tomato varieties. *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Citrobacter youngae*, *Enterobacter cloacae* and *Klebsiella pneumonia* were the common bacterial species isolated from the rhizosphere, rhizoplane and non-rhizosphere of the three tomato varieties. *Staphylococcus aureus* and *Escherichia coli* were isolated only from the rhizosphere and the non-rhizosphere and not the rhizoplane while *Proteus mirabilis* and *Staphylococcus saprophyticus* were isolated only from the non rhizosphere.

Growth promoting characteristics of bacterial species

Nine bacterial species were identified and selected based on their cultural and morphological differences from the total bacterial isolates. All the nine isolates were screened for growth-promoting abilities. For hydrogen cyanide (HCN) production, only *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were positive, which is indicated by the development of red brown colouration on agar plate (Plate 1-4). All bacterial species except *Staphylococcus saprophyticus* were able to produce ammonia. However, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Citrobacter youngae*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia* were good ammonia producers while *Staphylococcus aureus* and *Proteus mirabilis* were medium and weak producers respectively. On the other hand, only *Bacillus subtilis* (0.54mm) and *Pseudomonas aeruginosa* (0.30mm) were able to solubilize phosphorus with the formation of clear zone around the bacteria colony (Plate 1). All bacterial species were able to produce indole acetic acid (IAA) in varying quantities. *Bacillus subtilis* (1.60mg/ml) was the highest producer, followed by *Pseudomonas aeruginosa* (0.92mg/ml) and *Citrobacter youngae* (0.78mg/ml) while *Staphylococcus aureus* (0.15 mg/ml) had the lowest (Table 3).

In-vitro germination assay of PGPR

According to the growth-promoting characteristics of all the nine bacterial species evaluated, four species (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Citrobacter youngae*) exhibited greatest and consistent growth promoting characteristics (Table 1) and were selected for further evaluation in association with tomato (Beske) germination assay. As shown in Table 4, there were no significant differences for percentage seed germination in all the bacterial species and their consortium. On the other hand, there were significant differences in radical length and plumule length between all the isolates. For radical length, the consortium (10.2cm) recorded the highest, followed by *Bacillus subtilis* (8.7cm) and then *Pseudomonas aeruginosa* (8.3cm) as against the control (5.4cm). Similar result was gotten for plumule length with the consortium (3.9cm) being the highest, followed by *Bacillus subtilis* (3.1cm) and then *Pseudomonas aeruginosa* (2.9cm) as against the control (0.8cm). There were significant differences in vigor index for all the bacterial species, with the consortium being the highest and the control being the least (Table 4).

Screen house assay of PGPR effect on seed germination of tomato plant

Results from the screen house showed a significant enhancement ($P<0.05$) in the growth parameters (seedling height, stem girth, number of leaves and leaf area) at different weeks after planting with seeds inoculated in each bacterial species and their consortium. The consortium recorded the highest seedlings height throughout the weeks of planting, followed by *Bacillus subtilis* and then *Pseudomonas aeruginosa* while the least was recorded in the control (Figure 1). Similar result was gotten for the stem height, number of leaves and the leaf area with the consortium inducing the highest effect throughout the weeks of planting, followed by *Bacillus subtilis* and then *Pseudomonas aeruginosa* while the least was recorded in the control for the three parameters (Figures 2-4).

Table 1 Total bacterial counts of samples from the rhizosphere, rhizoplane and non rhizosphere of three tomato varieties

Variety	Rhizosphere (×106 CFU/g)	Rhizoplane (×106 CFU/g)	Non rhizosphere (×106CFU/g)
Roma Vf	55.33±3.5a	44.33±3.1a	31.33±4.7a
Beske	119.00±5.9c	116.00±4.0c	91.33±2.3c
Ibadan Local	90.00±2.3b	94.33±8.6b	62.33±3.6ab

Results are mean values ± standard error of mean for three replicates. Values followed by different letters within a column indicate significant differences according to Turkey-Kramer HSD test at $\alpha = 0.05$.

Table 2 Distribution of different bacterial species from the rhizosphere, rhizoplane and non rhizosphere of three tomato varieties

Isolation site	Bacterial species
Rhizosphere	*Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli, Staphylococcus aureus* and *Citrobacter youngae.*
Rhizoplane	*Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Citrobacter youngae, Enterobacter cloacae*
Non Rhizosphere	*Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter youngae, Escherichia coli, Staphylococcus aureus*, *Proteus mirabilis, Staphylococcus saprophyticus*

Citation: Oluwambe TM, Kofoworola AA. Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (Lycopersicon esculentum Mill) varieties. *Adv Plants Agric Res.* 2016;5(1):448–455. DOI: 10.15406/apar.2016.05.00167

Table 3 Growth promoting characteristics of bacterial species from the rhizosphere, rhizoplane and non rhizosphere of three tomato varieties

Isolates	HCN production	Ammonia PRODUCTION	Phosphate solubilization (mm)	IAA production (mg/ml)
*Klebsiella pneumoniae**	+	+++	-	0.72±0.15[d]
Escherichia coli	-	+++	-	0.30± 0.03[g]
Proteus mirabilis	-	+	-	0.31±0.03[g]
*Bacillus subtilis**	+	+++	0.54±0.21[a]	1.60±0.32[a]
Staphylococcus saprophyticus	-	-	-	0.56±0.13[e]
*Pseudomonas aeruginosa**	+	+++	0.35±0.13[b]	0.92±0.17[b]
*Citrobacter youngae**	-	+++	-	0.78±0.40[c]
Staphylococcus aureus	-	++	-	0.15±0.01[i]
Enterobacter cloacae	-	+	-	0.46±0.08[f]
Control	-	-	-	0.00±0.00[j]

- = No production; + = weak producer; ++ = medium producer; +++ = good producer. Values are means ± standard error of mean of three replicates Different letters within the column indicate statistically significant differences according to Student-Newman-Keuls multiple-range test (P<0.05).

Table 4 Effects of bacterial species on tomato seed germination and vigour index

Isolate	Percentage GERMINATION	Radicle length (cm)	Plumule length (cm)	Vigour index
Control	93.3 (0.0)[a]	5.4 (0.0)[d]	0.8 (0.0)[d]	578.3 (0.4)[f]
Klebsiella pneumoniae	93.3 (0.0)[a]	7.2 (0.2)[c]	1.4(0.4)[c]	802.3 (0.4)[e]
Bacillus subtilis	100.0 (0.0)[a]	8.7 (0.0)[b]	3.1(0.1)[b]	1180.2(0.2)[b]
Pseudomonas aeruginosa	100.0 (0.0)[a]	8.3 (0.4)[b]	2.9(0.1)[b]	1120.3(0.4)[c]
itrobacter youngae	93.3 (0.0)[a]	7.5 (0.0)[c]	1.5(0.0)[c]	839.4 (0.6)[d]
Consortium	93.3 (0.0)[a]	10.2 (0.0)[a]	3.9(0.1)[a]	1315.5(0.6)[a]

Results are mean values (standard deviations) for three replicates. Values followed by different letters within a column indicate significant differences according to the Student-Newman-Keuls multiple-range test (0.05).

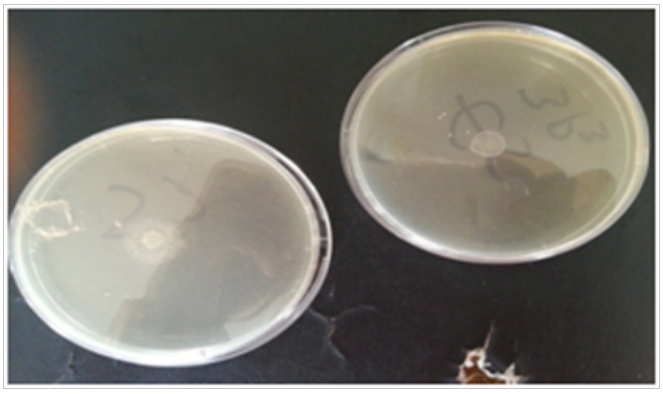

Plate 1 Formation of clear zone (A) as compared to control (B) indicating the solubilization of Phosphate.

Plate 2 Development of a yellow colour in control (B) to brown colour (A) indicating a positive reaction for ammonia production.

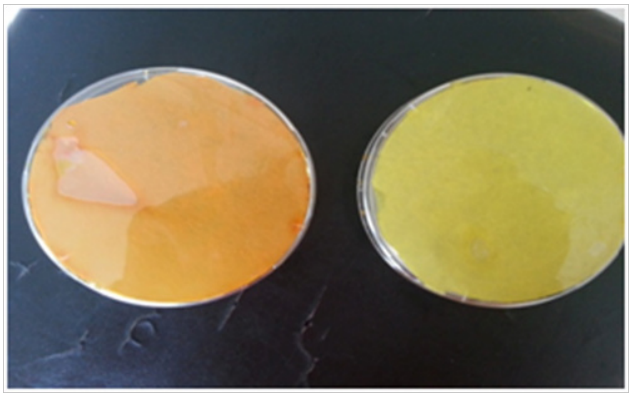

Plate 3 Formation of red-brown colour of filter paper (A) compare to yellow colour in control (B) indicating the detection of HCN production.

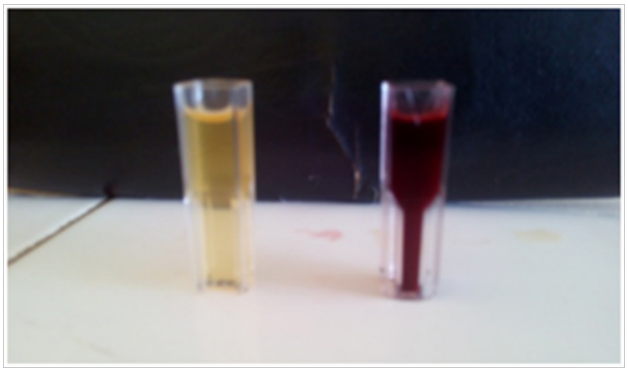

Plate 4 Development of pink colour (B) as compared to yellow colour in control (A) indicating IAA production.

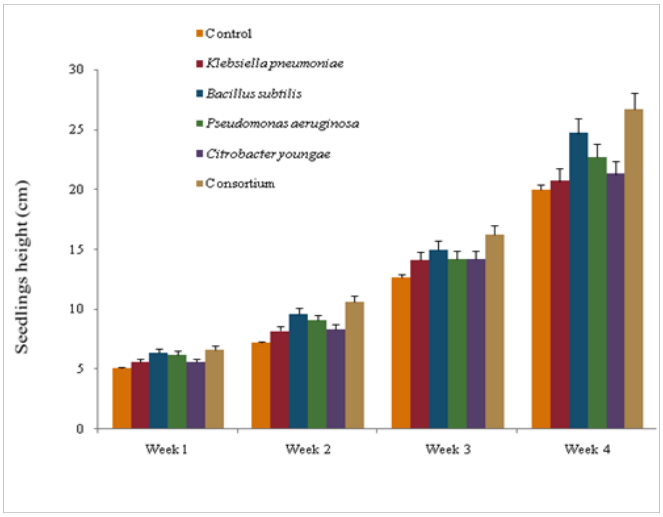

Figure 1 Effect of bacterial species on seedlings height of tomato plant at different weeks after planting.

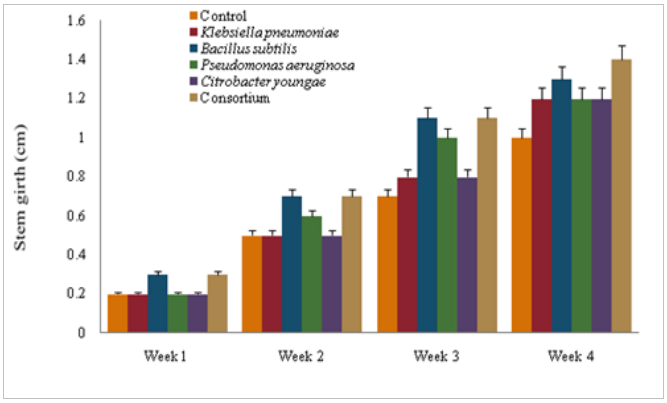

Figure 2 Effect of bacterial species on stem girth of tomato plant at different weeks after planting.

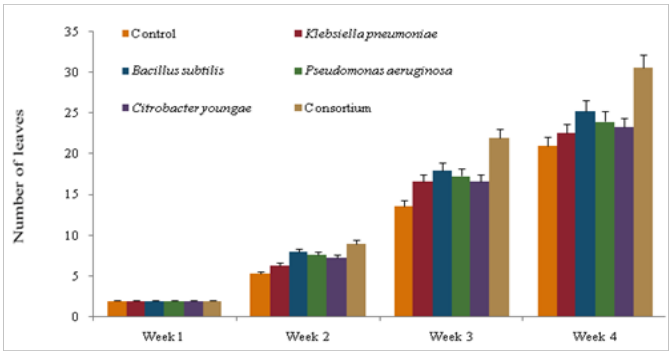

Figure 3 Effect of bacterial species on the number of leaves of tomato plant at different weeks after planting.

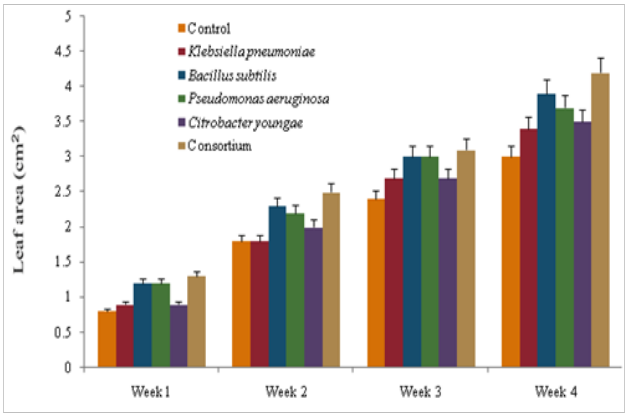

Figure 4 Effect of bacterial species on leaf area of tomato plant at different weeks after planting.

Citation: Oluwambe TM, Kofoworola AA. Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (Lycopersicon esculentum Mill) varieties. *Adv Plants Agric Res.* 2016;5(1):448–455. DOI: 10.15406/apar.2016.05.00167

Discussion

In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue. In this study, there were significant differences in the bacterial counts in rhizosphere, rhizoplane and non rhizosphere soil of the three tomato varieties. Beske and Roma VF variety had higher bacterial count in the rhizosphere ($1.19x10^8$CFU/g, $5.53x10^7$CFU/g), than the rhizoplane ($1.16x10^8$CFU/g, $4.43x10^7$CFU/g) and the non rhizhosphere ($9.1x10^7$CFU/g, $9.1x10^7$CFU/g). This is in agreement with[14] who also reported higher bacteria count in the rhizosphere than other regions of red pepper plant. In contrary, Ibadan local had higher count in the rhizoplane ($9.43x10^7$CFU/g) than the rhizosphere ($9x10^7$CFU/g) and non-rhizosphere soil ($6.23x10^7$CFU/g). This might be because the bacteria in the root zone (rhizoplane) through competition for nutrient and niches, take advantage of the nutrient that a plant provides.[14,15]

However, all the nine bacterial species identified based on their cultural and morphological differences were able to penetrate to either the rhizosphere or the root region except *Proteus mirabilis*and*Staphylococcus saprophyticus*. These two isolatesshowed no potential for plant growth-promotion and biocontrol in *in-vitro* and *in-vivo* assays. They were found to be at the non-rhizosphere soils and unable to penetrate to the rhizosphere and rhizoplane soils. This might be due to what is reported by,[1,16] that in order for some microorganisms (especially the genera Bacillus and Pseudomonas) to survive in the soil, they produce antagonistic or toxic substances such as antibiotics, siderophore and lytic enzymes against other microorganisms.

An integrated approach, similar to that of[1,9,10] was taken, testing isolates for a variety of plant growth promoting characteristics. This provides insight into the functional differences between isolates and is necessary for careful selection of beneficial indigenous isolates. Some of the isolated rhizobacteria exhibited more than one plant growth-promoting trait, which is expected to be advantageous for seedling growth under multiple types of adverse conditions. All the isolates tested produced IAA and consequently, are considered as IAA producing rhizobacteria. Recent studies have shown that IAA biosynthesis is greatly influenced by L-tryptophan which is believed to be the primary precursor for the formation of IAA in several microorganisms.[17] This phytohormone affects many physiological activities of plant such as cell enlargement, cell division, root initiation, and growth rate. In this study, the range of IAA production was low (0.15 to 1.60mgl^{-1}) as compared to previous reports of[18] (3.3 and 6.2mgl^{-1}); and[19] (2.13 and 3.6mgl^{-1}).[20] revealed that IAA production by PGPR could vary among different species and strains of rhizobacteria, culture and medium conditions. However,[21] reported that a low level of IAA produced by rhizobacteria promotes primary root elongation, whereas a high level increases lateral and adventitious root formation but inhibits the primary root growth. Such type of clarification suggested that even at low concentration, these isolates may be able to stimulate the development of the tomato plant.

Only bacterial isolates; *Bacillus subtilis and Pseudomonas aeruginosa* showed the ability to solubilize complex calcium phosphate. Similar research reports have been documented by Rodriguez et al. [22] that bacterial strains belonging to the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, and *Erwinia* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite, and rock phosphate. Another important trait of PGPR is the production of hydrogen cyanide (HCN) which plays an important role in the biological control of several soil-borne pathogenic fungi.[23] Three isolates; *Bacillus subtilis*, *Pseudomonas aeruginosa and Klebsiella pneumonia* were able to produce HCN. The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules.[19] Cyanide is a dreaded chemical produced by them as it has toxic properties. Although cyanide acts as a general metabolic inhibitor, it is synthesized, excreted and metabolized by hundreds of organisms, including bacteria, algae, fungi, plants, and insects, as a mean to avoid predation or competition. However, the degree of ammonia production ranged from good producer (*Bacillus subtilis*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae*and*Citrobacter youngae*) to medium (*Staphylococcus aureus*), to weak producer (*Proteus mirabilis*), to no production (*Staphylococcus saprophyticus*). Inoculation with good ammonia producing bacteria may enhance the plant growth as a result of their ability to fix Nitrogen (N_2) to Ammonia (NH_3) making it an available nutrient for plant growth.[24]

The general concept of the success of PGPR was attributed to the inhibitory effects of antagonistic organisms.[25] All nine bacterial isolates were found to be antagonistic at varying degree to the soil borne fungal pathogens (*Fusarium oxysporum and Rhizoctonia solani*). The inhibition process observed *in vitro* may suggests the secretion of fungicidal metabolites by the bacteria.[26] All four of the isolates selected demonstrated growth promoting potentials in both *in vitro* and soil-based assays. Tomato inoculated with the four isolates and their consortium had higher germination rates than the control in *in-vitro* germination experiment, a result similar to findings for pearl millet[27] and maize.[9] Although suppression of seed pathogens could be involved in this improvement in seed germination, these findings may also be due to the synthesis of hormones such as IAA by the isolates in this study. IAA can trigger the activity of specific enzymes that promote early germination and increased plumule and radicle length,[28] and seed inoculation with IAA producing rhizobacteria has been shown to enhance early seedling establishment.[29] Particular isolates may also have been involved in the production and metabolism of auxin, which is responsible for cellular elongation,[30] or cytokinin, which stimulates cellular division.[31]

The significant enhancement in tomato growth parameters (height, girth, and leaf number) by the four isolates especially *Bacillus subtilis*, *Pseudomona aeruginosa* and the consortium on screen house could result from biological activity of the isolates such as antagonizing plant pathogens, synthesizing phytohormones, and increasing the availability and uptake of nutrients. The phosphate solubilisation ability of *Bacillus subtilis* and *Pseudomona aeruginosa* are similar to the outstanding performance of *Bacillus* strain BPR7 reported by Kumar et al.[32] However, the consortium was outstanding in all the growth parameters evaluated, this is in agreement with the reports of[33] who evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micro propagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries.

Conclusion and recommendation

Four isolates, *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Citrobacter youngae* were the most effective and consistent PGPR identified. The consortium of these PGPR performed better than when used singly. Further study with

this microbial consortium is now needed in field trials, under different local environmental conditions.

Acknowledgements

We are grateful to the Laboratory managements of the Department of Crop Protection, Department of Chemistry and that of Toxicology, Federal University of Agriculture Abeokuta, for providing some of the materials used for this study.

Conflict of interest

The author declares no conflict of interest.

References

1. Sharma T, Navin K, Nishant R. Isolation, screening and characterization of PGPR isolates from rhizosphere of rice plants in Kashipur region (Tarai region). *Biotechnology International*. 2012;5(3):69–84.
2. Hariprasad P, Venkateswaran G, Niranjana SR. Diversity of cultivable rhizobacteria across tomato growing regions of Karnataka. *Biological Control*. 2014;72:9–16.
3. Debananda SN, Suchitra S, Salam N. Screening of actinomycete isolates from niche habitats in Manipur for antibiotic activity. *American Journal of Biochemistry and Biotechnology*. 2009;5(4):221–225.
4. Howell CR. Mechanisms employed by Trichoderma spp. in the biological control of plant diseases:the history and evolution of current concepts. *J Plant Dis*. 2003;87(1):4–10.
5. Fawole MO, Oso BA. Laboratory Manual of Microbiology. Ibadan, Nigeria: Spectrum Book; 1998. p. 1–55.
6. Cheesbrough M. *District Laboratory Practice in Tropical Countries: Part one*. 2nd ed. UK: Cambridge University Press; 2006. p. 143–157.
7. Patten CL, Glick BR. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Applied Environ Microbiol*. 2002;68(8):3795–3801.
8. Patel HA, Patel RK, Khristi SM, et al. Isolation and characterization of bacterial endophytes from Lycopersicon esculentum plant and their plant growth promoting characteristics. *Nepal Journal of Biotechnology*. 2012;2(1):37–52.
9. Abiala MA, Odebode AC, Hsu SF, et al. Phytobeneficial properties of bacteria isolated from the rhizosphere of maize in southwestern Nigerian soils. *Appl Environ Microbiol*. 2015;81(14):4736–4743.
10. Ngoma L, Boipelo E, Olubukola OB. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *African Journal of Biotechnology*. 2013;12(26):4105–4114.
11. Cappucino JC, Sherman N. *Microbiolgy: A laboratory manual*. New York, USA: Benjamin/Cummings Publishing Company; 1992. p. 125–179.
12. Institute of seed science and technology (ISTA). International rules for seed testing. *Seed Science Technology*. 1999;27 Supplememt.
13. Zinniel DK, Lambrecht P, Harris NB, et al. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *J Appl Env Microbiol*. 2002;68(5):2198–2208.
14. Oyeyiola GP, Ajokpaniovo H, Aliyu MB. Bacterial flora of the rhizosphere of Capsicum frutescens. *World Journal of Biological Research*. 2012;2(5):1994–5108.
15. Aliyu MB, Oyeyiola GP. Utilization of petroleum hydrocarbon invitro by rhizosphere bacterial isolates of groundnut (*Arachis hypogeae*). *Journal of Asian Scientific Research*. 2011;2(2):53–61.
16. Glick BR. The enhancement of plant growth by free–living bacteria. *Canadian Journal of Microbiology*. 1995;41(2):107–117.
17. Ahmad F, Khan MS. Assessment of plant growth promoting activities Rhizobacterium *Pseudomonas putida* under insecticide–stress. *Microbiology Journal*. 2011;1(2):54–64.
18. Gravel V, Antoun H, Tweddell RJ. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride:possible role of indole acetic acid (IAA). *Soil Biology & Biochemistry*. 2007;39:1968–1977.
19. Ahmad F, Ahmad I, Khan MS. Screening of free–living rhizospheric bacteria for their multiple plant growth promoting activities. *J Microbiol Res*. 2008;163(2):173–181.
20. Kumar P, Dubey RC, Maheshwari DK. Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol Res*. 2012;167(8):493–499.
21. Xie H, Pasternak JJ, Glick BR. Isolation and characterization of mutants of the plant growth–promoting rhizobacterium *Pseudomonas putida* GR12–2 that over produce indole acetic acid. *Current Microbiology*. 1996;32(2):67–71.
22. Rodriguez H, Fraga R, Gonzalez T, et al. Genetic of phosphate solubilisation and its potential applications for improving plant growth–promoting bacteria. *J Plant Soil*. 2006;287(1):15–21.
23. Ramette A, Moënne LY, Défago G. Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. *FEMS J Microbiology Ecology*. 2003;44(1):35–43.
24. Hayat R, Ali S, Amara U, et al. Soil beneficial bacteria and their role in plant growth promotion:a review. *Annals of Microbiology*. 2010;60(4):579–598.
25. Yildiz HN, Handan HA, Dikilitas M. Screening of rhizobacteria against *Fusarium oxysporum f.* sp. *melongenae*, the causal agent of wilt disease of eggplant. *African Journal of Microbiology Research*. 2012;6(15):3700–3706.
26. Figueiredo MVB, Seldin L, Araujo FF, et al. Plant growth promoting rhizobacteria:Fundamentals and applications. *Plant growth & health promoting bacteria*. 2010;18:21–43.
27. Niranjan SR, Deepak SA, Basavaraju P, et al. Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet. *Crop Protection*. 2003;22(4):579–588.
28. Kaufman PB, Wu LL, Brock TG, et al. Hormones and the orientation

of growth, In: Davies PJ editor. *Plant hormones:physiology, biochemistry, and molecular biology*. Dordrecht, Netherlands: Kluwer Academic; 1995. p. 547–570.

29. Khalid A, Arshad M, Zahir ZA. Screening plant growth–promoting rhizobacteria for improving growth and yield of wheat. *J Appl Microbiol*. 2004;96(3):473–480.
30. Bharathi R, Vivekananthan R, Harish S, et al. Rhizobacteria–based bio–formulations for the management of fruit rot infection in chillies. *J Crop Protection*. 2004;23(9):835–843.
31. Anzala FJ. Screening for the germination rate of Maize (*Zea mays* L.). *J Agric biotech*. 2006;5(6):16–23
32. Kumar A, Amit kumar, Devi S, et al. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities:an in vitro study. *Rec Res of Sci Tech*. 2012;4(1):1–5.
33. Jaizme Vega MDC, Rodríguez Romero AS, Guerra MSP. Potential use of rhizobacteria from the Bacillus genus to stimulate the plant growth of micro propagated bananas fruits; 2004. p. 83–90.

Citation: Oluwambe TM, Kofoworola AA. Comparison of single culture and the consortium of growth-promoting rhizobacteria from three tomato (Lycopersicon esculentum Mill) varieties. *Adv Plants Agric Res.* 2016;5(1):448–455. DOI: 10.15406/apar.2016.05.00167