



## Assessment of Indigenous Rhizospheric Soil Microbes from *Zea mays* and *Manihot esculenta* for Plant Growth Promoting (PGP) Traits

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### ABSTRACT

Plant growth promoting Rhizobacteria and fungi (PGPR)/(PGPF) are microbes that colonize the root of plants to enhance plant growth and inhibit phytopathogens. Hence, this study was designed to isolate, screen and characterize bacteria and fungi present in the rhizosphere of cassava CV (*Manihot esculenta*) and maize MZ (*Zea mays*) plants within Iyamho community, Etsako West LGA, Edo state. Samples were collected using standard methods and total culturable heterotrophic bacteria and total fungi were enumerated on standard media for both plants. The bacterial isolates for both plants were observed to be higher with an order of magnitude of  $10^6$  compared to fungi  $10^4$ . A total of 10 bacterial and 10 fungal isolates exhibited at least one in vitro plant growth promoting trait and as such isolates were designated to be PGPR/PGPF, respectively. The screening result for both bacteria and fungi showed a similar trend for plant growth traits. No isolate produced hydrogen cyanide (HCN) while above 50% of the isolates produced catalase and indole acetic acid (IAA). For phosphate solubilisation on Pikovaskaya Agar, 2 bacterial isolates (CV1 and MZ4) and one fungal isolate (MZF3) tested positive. In addition, 7 fungal and 4 bacterial isolates produced Ammonia when grown on Urea broth. Gram negative bacteria dominated the bacterial group while *Aspergillus* was the dominant fungi. Generally, isolates from CV plant displayed more plant growth promoting traits than maize plant. However, both plants harboured PGPR/PGPF that may be applied as biostimulants to enhance crop yield and promote sustainable agriculture

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## 1. Introduction

The rhizospheric region of plants is a well-defined environment for microbes which comprises of a plant root system with very high bacterial population influenced by root exudates and surrounded by a large volume of soil (Goswami *et al.*, 2016; Sharma and Shrivastava, 2017).

Rhizospheric microbes that are responsible for the promotion of plant growth are referred to as plant growth promoting bacteria and fungi (PGPR/PGPF). The PGPR/PGPF is a class of microbes involved in the colonization of plant to stimulate plant growth and suppress disease and stress tolerance by several direct and indirect mechanisms. PGPR/PGPF are now known as biofertilizers, phytostimulators and bio control agents for promoting the growth of various plants and controlling soil borne pathogens. With regards to increasing interest for food and environmental sustainability globally, the application of PGPR/PGPF minimizing chemical inputs in the form of fertilizers in agricultural practices is a potentially essential issue (Ashish *et al.*, 2016).

Cassava (*Manihot esculenta* Crantz), grows in the tropics and sub-tropic regions of the world. It is characterised as a perennial edible root with woody shrubs. Cassava in the sub-Saharan African countries and other developing countries is very critical in the agricultural industry as a result of its ability to grow under low rainfall conditions and low nutrient availability. Also importantly as a perennial crop, harvest is done when required while maize is a cereal crop rich in vitamins, carbohydrate, protein and essential minerals. Nigeria is the largest cassava producer in the world with an estimate of 54 million metric tonnes of cassava produced annually (Onyewoke and Simonyan, 2014). Also, maize has a shallow structure, susceptible to drought, intolerant of nutrient deficiency and in Africa, growing maize is rain fed. Nigeria is the largest African producer of maize with about 8 million tons annually (GAIN, 2019). The growing application of chemical fertilizers and other agrochemicals in modern agriculture to enhance plant has resulted in excessive damages to the soil structure, plant and the environment. However, the application of PGPR as a sustainable and eco-friendly alternative to chemical fertilizer application in the growth of these very essential crops should be highly encouraged

Rhizobacteria are able to promote plant growth by colonizing the plant roots to enhance growth of plants and control pathogens through several mechanisms. These mechanisms can be direct or indirect. Direct mechanisms occur outside the plant and alter the equilibrium of regulators involved in plant growth (releasing growth regulators that are incorporated into the plant) (Glick, 2014) such as atmospheric nitrogen fixation, phosphate solubilisation, production of phytohormones and ammonia production (Siddikee *et al.*, 2010). On the other hand, indirect mechanisms is involved in biocontrol of phytopathogens which occurs inside the plant and requires the active involvement of the plants defensive mechanisms/metabolic processes which responds to the signal sent from the bacteria colonizing the plants (Aeron *et al.*, 2011). PGPF has received lesser attention over the years compared to PGPR. Recently, some researches conducted to determine the role of PGPF in the rhizosphere as well as mechanism of action have documented that both PGPR and PGPF have the same plant growth promoting traits and similar mechanism of action (Verma *et al.*, 2019). In addition, PGPF protects plants by induced systemic resistance (ISR) against phytopathogenic bacteria, fungi, viruses and nematodes (Verma *et al.*, 2019). As a result of the intense beneficial contributions of PGPF in agricultural practice, researches have now directed attention to the use of PGPF to induce plant tolerance to environmental stresses and plant growth enhancement by stimulating induced systemic resistance (ISR) in plants (Jogaiah *et al.*, 2013; Zhang *et al.*, 2018). Plant growth promoting fungi ISR by accumulating substances like phenols, lignin and callose which modifies the cell wall preventing entry, proliferation and growth of invading phytophthogens (Muslim *et al.*, 2019; Naziya *et al.*, 2020). This trait has also been found in several PGPR.

Based on numerous studies in the last few decades, myriad of bacterial and fungal species have been documented with plant growth promoting properties from the rhizosphere of different plants and different environments. Some of these species include *Streptomyces* spp (Suarez-Moreno, *et al.*, 2019), *Trichoderma* (Zhang *et al.*, 2016), *Aspergillus*, spp, *Fusarium* spp, *Penicillium* spp, (Lopez and Sword, 2015 ; Hossain *et al.*, 2017; Jaber and Enkerli, 2017), *Aeromonas*, *Pseudomonas* and *Enterobacter* (Aarab *et al.*, 2015), *Serratia*, *Bacillus*, *Azotobacter*, *Azospirillum* and *Rhizobium* (Sharma and Shrivasta, 2017). To date limited

studies have been conducted on PGPF colonizing the rhizosphere of different plants, even lesser studies on the characterisation of PGPR and PGPF colonizing the rhizosphere of plants. With the understanding that each microbial group have diverse roles to play in the rhizosphere of plants, this study was designed to ascertain the microbial communities present in the rhizosphere of two different plants, cassava and maize and to determine *in vitro* the presence of specific plant growth promoting traits.

## 2. Methodology

### Site description

Rhizospheric soil samples were obtained from a mixed farm where both cassava and maize is cultivated, located at Iyamho community, Etsako West LGA, Edo State, Nigeria. The community is a developing rural area where farmers and petty traders dominate the population.

### Sample collection

Soil samples were obtained from the rhizospheric region of two plants: Maize (*Zea mays*) and Cassava (*Manihot esculenta*). The uprooted plants with adhering soils were carefully removed aseptically from the roots into a sterile collection bag (Chinakwe et al., 2018). Samples were transported in an ice-packed cooler to the laboratory and stored at 4°C in refrigerator for further analysis

### Enumeration of total culturable heterotrophic bacteria and total culturable fungi

Ten grams (10 g) of rhizosphere soil sample was transferred into a conical flask (250 ml) and 90 ml of sterile normal saline was introduced to obtain stock culture by shaking the flask on a rotary shaker for 10 minutes. One millilitre (1 ml) of suspension was added to 9 ml vial and shaken for 2 minutes. Serial dilution was performed up to  $10^{-7}$  dilution to enumerate microbial population. An aliquot (0.1 ml) of diluents  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  were spread using a glass spreader on the plates of Luria Bertani (LB) agar medium and Nutrient agar plates in triplicates for bacterial isolation while Potato dextrose agar was inoculated for fungal isolation. Plates were incubated for 48 hours at 28°C and 72hours at 37°C for bacteria and fungi, respectively.

### In-vitro screening of PGP traits

The plant growth promoting (PGP) traits of the isolated organisms were screened for the following activities:

#### *Phosphate solubilisation*

Pikovaskaya agar was introduced as a thin film into a sterile petri dish. Afterwards, the Pikovaskaya's plates were spot inoculated with test isolates and incubated at 28°C for about 5 days. Production of clear halos around each colony was inferred as positive for phosphate solubilization (Chari et al., 2015).

#### *Ammonia production*

The process involves inoculation of 18h old bacterial culture into urea broth containing peptone and incubated at 37°C for 24 hrs. Afterwards, the isolate was centrifuged and 1 ml of Nessler's reagent was added to the supernatant. Formation of yellow to brown colour was inferred as positive result while no change in colour was designated as negative. Ammonia production is achieved by an increase in the pH of the urea broth medium as a result of production of urease which breaks urea into ammonia (Marques et al., 2010).

#### *Indole acetic acid (IAA) production*

Bacterial cultures were grown on Luria bertani agar supplemented with tryptophan. The plates were incubated for 3 days overlaid with Whatman filter paper. The filter paper was subsequently treated with 1ml of Salkowsky's reagent for 60 minutes at room temperature. The presence of red/pinkish halos on the paper around colonies indicated positive result. (Tahir et al, 2015).

### Hydrogen cyanide (HCN) production

Hydrogen cyanide production was determined by using Nutrient agar supplemented with glycine (4.4 g l<sup>-1</sup>). Filter papers were soaked with 2% Sodium carbonate and 0.5% picric acid, positioned on the lid of the petri dish and incubated at 28°C for 3 days. Decolouration of paper from yellow to brown indicated a positive result (Chinakwe *et al.*, 2019).

### Catalase activity

The screening of bacterial isolates for catalase activity involved the introduction of 1 ml of bacterial isolate into test tubes and few drops of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added. The production of oxygen evidenced by bubbles formation indicated catalase production

## Morphological and Biochemical identification

### Colonial morphology

Typical bacterial colonies were observed over the streak. Morphological characterizations of each bacterial isolate was examined on a 24 hr old nutrient agar plate to determine the colony margin, elevation, size, color, surface and shape. Gram stain test was conducted to determine the shape and reaction of the test isolates as described by Arora, 2003. Standard biochemical tests were conducted to determine the biochemical reaction for isolate identification (Kushawa *et al.*, 2013).

### Identification of Fungal isolates

Fungal isolates were identified by macroscopic and microscopic observations. For macroscopic observation of the fungal isolates, the growth rate of the colony, shape, colour and also the hyphae were observed and documented while wet mount method was adopted for microscopic characterization (Prescott *et al.*, 2008).

## 3. Results and Discussion

### Total culturable heterotrophic bacteria and total fungal counts

The bacteria colonies isolated from both *Z mays* and *M esculenta* on Luria Bertani recorded more growth with a mean value of 2.14 x 10<sup>6</sup> CFU/g and 3.54 x 10<sup>6</sup> CFU/g respectively than that of nutrient agar with a mean value of 1.3 x 10<sup>6</sup> CFU/g and 1.2 x 10<sup>6</sup> CFU/g respectively (Table 1). The total fungal counts from both *Z mays* and *M esculenta* on potato dextrose agar (7.82x 10<sup>4</sup> CFU/g and 9.1 x 10<sup>4</sup>CFU/g) is also presented in Table 1.

Table 1: Total culturable heterotrophic bacteria and total fungal counts

Isolates	Mean value of THB in L.B agar (CFU/g)	Mean value of THB in N.A agar (CFU/g)	Mean value of TCF (CFU/g)
Cassava (CV)	2.14 x 10 <sup>6</sup>	1.3 x 10 <sup>6</sup>	7.82 x 10 <sup>4</sup> 9.1 x 10 <sup>4</sup>
Maize (MZ)	3.54 x 10 <sup>6</sup>	1.2 x 10 <sup>6</sup>	

THB: Total heterotrophic bacteria, TFC: Total fungal count

This study employed culture dependent approaches to highlight the available microorganisms present in the rhizospheric soils of Maize (*Zea mays*) and Cassava (*Manihot esculenta*) and the plant growth promoting traits present in each of the isolated organisms. Generally, the total culturable heterotrophic bacteria count

obtained revealed a significant population for both samples and agar medium. However, bacterial count of cassava was higher than maize. Similarly, counts on Luria Bertani were appreciably higher than that of Nutrient agar.

**In-vitro screening of PGP traits**

**Plant growth promoting Rhizobacteria (PGPR)**

The bacterial isolates were screened for plant growth promoting traits. All isolates showed at least one trait as observed from the results

The plant growth promoting properties of the test bacterial isolates are presented in Table 2. *Proteus*, *Bacillus*, *Enterobacter*, *Staphylococcus*, *Serratia*, and *Pseudomonas* induced IAA production. *Proteus spp.*, from cassava (CV1) and *Serratia spp.* from maize (MZ4) isolates showed the ability to solubilize the phosphorus from tri-calcium phosphate by producing halo zones around each colony.

*Proteus*, *Pseudomonas* and *Bacillus spp.* induced weak ammonia production, while *Staphylococcus spp.* induced a very strong Ammonia production, using Nessler’s reagent as the colorimetric reagent. All isolates except *Proteus spp.* and *Serratia spp.* isolates from maize produced gas bubbles indicating catalase activity.

Table 2: Plant growth promoting traits of Rhizobacterial isolates

Isolates	Genera	Phosphate Solubilization	HCN production	Ammonia production	Catalase activity	IAA production
CV1	<i>Proteus spp.</i>	+	-	++	+	+
CV2	<i>Pseudomonas spp.</i>	-	-	++	+	+
CV3	<i>Staphylococcus spp.</i>	-	-	+++	+	+
CV4	<i>Bacillus spp.</i>	-	-	-	+	-
CV5	<i>Staphylococcus spp.</i>	-	-	-	+	-
MZ1	<i>Proteus spp.</i>	-	-	-	-	+
MZ2	<i>Pseudomonas spp.</i>	-	-	-	+	+
MZ3	<i>Enterobacter spp.</i>	-	-	-	+	-
MZ4	<i>Serratia spp.</i>	+	-	-	-	-
MZ5	<i>Bacillus spp.</i>	-	-	++	+	+

+ = positive; - = negative; ++ = weak; +++ = strong

The rhizospheric soil is a multifaceted environment with diverse bacterial populations with significant benefit to host plants. Rhizobacteria is critical in the improvement of plant growth. Several genera of rhizobacteria have been isolated from plants reported to show plant growth promoting activity in many previous studies (Zhang et al., 2012 and Arruda et al., 2014). In this paper, we assessed the plant growth promoting traits of rhizobacteria and fungi from indigenous soil of *Z mays* and *M esculenta*. **Indole acetic acid (IAA)** production is referred to as an important phytohormones responsible for plant growth. Thus, an indicator for PGPR. Accordingly, rhizobacteria have been screened for the production of IAA till date in order to explore PGPR. Here, four bacterial isolates capable of producing IAA were isolated from cassava

(CV1, CV2, CV3) and maize (MZ5) rhizosphere classified as *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Bacillus* spp., *Enterobacter* sp. This corroborates with studies by Mahwish *et al.* (2015) with reported IAA production by *Pseudomonas* spp. and *Bacillus* spp. genera isolated from *Zea mays* in Patiskan.

Phosphorus is among the essential nutritional requirements for plant growth and improvement. The ability of PGPR to solubilize insoluble phosphate have attracted attention owing to their possible application in agriculture as bio fertilizers. Previous studies have described phosphate solubilising as a resultant effect of the ability of bacteria to synthesize certain organic acids (Tahir *et al.*, 2013). Contrary, the above-mentioned author reported the demonstration of phosphate solubilisation by one genera (*Pseudomonas*). Meanwhile in our study, phosphate solubilisation traits was demonstrated by *Proteus* spp. (CV1) and *Serratia* spp. (MZ5). The discrepancies in the result could be in the location and number of sites sampled although similar phosphate solubilisation methodology was employed.

Apart from their IAA production and phosphorus solubilising ability, four tested PGPR strains (*Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Bacillus* spp.) in this study demonstrated ammonia production, which is well supported by Nadège *et al.*, (2015) study which elucidated the ability of characterized PGPR (*Bacillus* spp. and *Pseudomonas* spp.) to produce ammonia from *Z mays* in Benin republic and demonstrated the beneficial effects of the plant under controlled environment. Additionally, in our current study, among all the 5 PGPR isolates studied from maize (*Z mays*); the isolates MZ1 (*Proteus* spp) and MZ4 (*Serratia* spp.) lacked catalase activity while all the isolates from cassava (*Proteus* spp., *Pseudomonas* spp., *Bacillus* spp. and *Staphylococcus* spp.) demonstrated catalase activity. Further assessment of the PGPR strains from both plants revealed that 0% lacked hydrogen cyanide (HCN) production. This findings obtained in this study is in consonance with the results from Chinakwe *et al.*, (2019) who worked on hybrid and local maize varieties in Owerri, Nigeria. But not in agreement with earlier studies which demonstrated that 100% of PGPR strains isolated from *Z mays* in 5 agro-ecological zones of central and northern Benin (Nadège *et al.*, 2015) and 2% of PGPR strains isolated from five varieties of cassava in Thiruvananthapuram and Kollam districts of Kerala (Suja *et al.*, 2014). Moreover, these authors applied similar methodology for hydrogen cyanide production; however, our study employed similar methodology but with significant modification.

### **Plant growth promoting Fungi (PGPF)**

The PGPF from both plants in this study were mostly dominated by the genera *Aspergillus*. Among the plant growth promoting traits, phosphate solubilization was exhibited by 2 isolates from both plants; CVF3 (*Aspergillus niger*) and MZF4 (*Aspergillus flavus*). Contrary to PGPR, all PGPF isolated from both plants displayed IAA production. Ammonia production was displayed with varying magnitude. *Aspergillus flavus*, *Blastomyces dermatitidis* and *Aphanoascus flavescens* displayed weak production whereas *Aspergillus terreus* showed strong production. Furthermore, none of the isolates screened induced hydrogen cyanide production as observed among PGPR (Table 3).

In our study, the appearance of the zone of hydrolysis of tri-calcium phosphate on PKA indicated the Phosphate solubilisation potential of 2 isolates of *Aspergillus* spp. (*niger* and *flavus*) from both plants. Similarly, study by Jain *et al.* (2011) have reported phosphate solubilisation trait among *Aspergillus* spp. isolated from the rhizospheric soil. Furthermore, more recently, studies by Saxena *et al.* (2016) also showed phosphate solubilisation by *Aspergillus* spp. and plant growth enhancement following inoculation of Maize and Cassava seeds with *Aspergillus* sp.

Table 3: Plant growth promoting traits of Fungal isolates

Isolates	Fungal species	Phosphate Solubilization	HCN production	Ammonia production	Catalase activity	IAA production
CVF1	<i>Aspergillus flavus</i>	-	-	++	+	+
CVF2	<i>Aspergillus terreus</i>	-	-	-	-	+
CVF3	<i>Aspergillus niger</i>	++	-	+	+	+
CVF4	<i>Aspergillus nidulans</i>	-	-	-	+	+
CVF5	<i>Blastomyces dermatitidis</i>	-	-	++	+	+
MZF1	<i>Aspergillus fumigatus</i>	-	-	+	-	+
MZF2	<i>Aspergillus lentulus</i>	-	-	+	+	+
MZF3	<i>Aspergillus terreus</i>	-	-	+++	-	+
MZF4	<i>Aspergillus flavus</i>	+	-	-	-	+
MZF5	<i>Aphanoascus flavescens</i>	-	-	++	+	+

+ = positive; - = negative; ++ = weak; +++ = strong

Plant growth promotion revealed by enhanced uptake of phosphorus in shoots and roots of Maize plants after the application with *Aspergillus turingensis* and *A. niger* has been documented (Gurdeep and Reddy, 2017). In a similar study, inoculation with *Aspergillus* sp. significantly increased soil fertility

Indole acetic acid (IAA) was produced by all isolates including all species of *Aspergillus* identified in our study. These findings support that of Waqas *et al.* (2014) with reported production of IAA by *Aspergillus* sp. Likewise, Yadav *et al.* (2011) also documented the production of IAA by *Aspergillus niger*. We reported production of ammonia among *Aspergillus fumigatus* isolated from maize and other fungi isolated from the maize as well as cassava. This study also highlighted the production of ammonia and IAA in *Aspergillus* sp. obtained from the rhizosphere of cassava. However, the yield of ammonia and IAA produced by our isolates varied comparatively with reports from other related researches (Lubna *et al.*, 2018). This variation could be linked to the difference in study locations. In catalase activity, fewer isolates (*Aspergillus lentulus* and *Aphanoascus flavescens*) from maize showed positive reaction when compared to cassava. Generally, several researchers have reported considerable variations in the production of PGP traits by *Aspergillus* sp. and other fungi isolates associated with the rhizosphere of different plants (Yadav *et al.* 2011, Haas 2014, Pandya *et al.*, 2018).

#### 4. Conclusion

The results suggest that all ten bacterial and fungal isolates possessed at least one plant growth promoting trait although isolates from cassava displayed more activities than maize. The exhibition of these traits shows that the rhizosphere of these test plants harbors PGPR and PGPF which could be contributing significantly to the growth and development of the plants. It was also observed that the dominant PGPR group were gram negative motile organisms and *Aspergillus* sp. for fungi. Therefore, these isolates may be further explored and applied as biostimulants to enhance crop yield and promote sustainable agriculture.

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