PLANT GROWTH PROMOTING RHIZOBACTERIA FROM LOCAL AND HYBRID MAIZE (Zea mays) VARIETIES

Chinakwe, Etienne[1], **Mberede**, Chidimma[1], **Ngumah**, Chima[1]

[1]Department of Microbiology, Federal University of Technology Owerri, P.M.B 1526 Owerri, Nigeria.
ccngumah@yahoo.com

ABSTRACT

The rhizobacteria of a local maize variety and a hybrid maize variety were analyzed. Pure isolates were enumerated, identified, and screened for five plant growth promoting traits – indole acetic acid (IAA) production, ammonia production, hydrogen cyanide production, siderophore production, and phosphate solubilization. The rhizobacteria isolated from the local maize were Enterobacter sp., Agrobacterium sp., and Pseudomonas sp., while Staphylococcus sp., Bacillus sp., Pseudomonas sp. and two Acinetobacter species were isolated from hybrid maize. Rhizobacteria from local maize exhibited four plant growth promoting traits, while those from hybrid maize exhibited three plant growth promoting traits.

Keywords: plant-associated bacteria, biofertilizer, plant growth promoters

Received: 11.03.2019 Reviewed: 30.05.2019 Accepted: 09.06.2019

INTRODUCTION

The root system of higher plants is not only associated with an inanimate environment composed of organic and inorganic substances, but also with a vast population of metabolically active microorganisms. Living plant roots create a unique subterranean habitat for microorganisms, thus the microflora that responds to the presence of plant roots is distinctly different from the characteristic soil population (Alexander, 1961). The thin layer of soil immediately surrounding the plant roots which is specifically influenced by the root system is called the *rhizosphere* (Saharan and Nehra, 2011). This plant root influence is called the *rhizosphere effect*. The rhizosphere is rich in nutrients when compared to non-rhizosphere zones due to the accumulation of a variety of plant exudates, such as amino acids and sugars, and sloughed off tissue which serve as sources of energy, carbon, nitrogen, or growth factors. This in turn causes a denser population of bacteria in the rhizosphere (10 to 100 times higher) than that in that in the adjoining bulk soil. The diverse groups of bacteria colonizing the rhizosphere are called *rhizobacteria* (Beneduzi *et al*., 2012).

According to Dobbelaere *et al*. (2003), plant-associated bacteria can be classified into beneficial, deleterious, and neutral groups on their basis of influence on plant growth. Beneficial free living soil bacteria that can directly or indirectly facilitate rooting or plant growth are referred to as plant growth-promoting rhizobacteria – PGPR (Glick, 1995; Mayak *et al*., 1999). Some PGPR that have been extensively studied belong to the genera *Acetobacter, Acinetobacter, Alcaligenes, Arthrobacter, Azoarcus, Azospirillium, Azotobacter, Bacillus, Beijerinkia, Burkholderia, Derxia, Enterobacter, Gluconoacetobacter, Herbaspirillium, Klebsiella, Ochrobactrum, Pantoae, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas*, and *Zoogloea* (Jha and Saraf, 2015). PGPR, according to their mode of action have been divided into two groups: biocontrol PGPR that indirectly benefit plant growth and PGPR that directly affect plant growth, seed emergence, or improve crop yields (Glick *et al*., 1999). Direct plant growth promotion mechanisms include: production of phytohormones (indole acetic acid, cytokinins, gibberellins, etc.), solubilization of phosphates, nitrogen fixation, siderophore production (increased uptake of iron), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production. Indirect mechanism include: antibiosis, production of lytic enzymes, siderophore production (inhibit root pathogens by creating iron limiting conditions in the rhizosphere via iron chelation), induced systemic resistance (ISR) and biofilm formation (exo-polysaccharides production) (Gupta *et al*., 2015).

This study will explore rhizobacteria associated with a local maize variety and a hybrid maize variety growing on the same farm; rhizobacteria will be screened for specific plant growth promoting traits.

MATERIALS AND METHODS

Collection of soil samples from rhizosphere

Fifteen rhizosphere soil samples were collected respectively from alocalmaize variety and a hybrid maize variety, cultivated on the same farm maintained by the Department of Crop Science and Technology, Federal University of Technology Owerri. These growing maize varieties were uprooted, and adhering soil was carefully brushed off from the roots.

Isolation, maintenance, and identification of rhizobacteria

1g of rhizosphere soil was added to 9ml of sterile physiological water, mixed, and shaken at 140 rpm for 2 mins. Ten-fold serial dilutions were prepared from the supernatants, and 0.1ml spread on trypticase and King's B agar plates. The plates were incubated at 28^{o}C for 24h and 48h for trypticase and King's B respectively. Bacterial populations were determined as colony forming unit per gram (CFU/g) of soil sampled. Distinct colonies were counted, recorded and sub-cultured to get axenic cultures. Pure isolates were stocked on solid sterile trypticase agar slants. Stock cultures were stored at 4^{0}C until required. Identification of isolates was done using the schemes of Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

Screening of rhizobacteria for multiple plant growth promoting activities

Indole acetic acid (IAA) production

Luria Bertani broth medium (25ml) amended with 100µ/ml tryptophan was inoculated with bacteria isolate. They were incubated for 24 - 72 h at 27^{o}C. Fully grown cultures were centrifuged at 3000 rpm for 30 minutes. 2 ml of supernatant was mixed with two drops orthophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid + 1 ml, 0.5 M $FeCl_3$ solution) and incubated for 25 minutes at room temperature. The development of a pink colour indicates IAA production (Brick *et al*., 1991).

Production of ammonia

Bacteria isolates were tested for the production of ammonia in peptone water. Each freshly grown culture were inoculated in 10 ml peptone water and incubated at 27^{o}C for 48 – 72 h. 0.5 ml Nessler's reagent was added to each tube. Development of brown to yellow colour was indicative of ammonia production (Cappuccino and Sherman, 1992).

Production of HCN

Trypticase agar plates amended with 4.4g/L of glycine were streaked with bacteria isolates. Whatmann No.1 filter paper (soaked in 2% sodium carbonate in 0.5% picric acid) was placed in the lid of each Petri dish. The plates were then sealed airtight with parafilm and incubated at 27^{o}C for 2 – 4 days. A colour change of the filter paper from deep yellow to orange or reddish brown indicated HCN production (Bakker and Schipperes, 1987).

Siderophore production

Siderophore production was estimated qualitatively. 7 - 10 days broth culture was centrifuged at 3000 rpm for 30 minutes. 0.5 ml supernatant was added to 0.5 ml of 0.2% aqueous ferric chloride solution. The appearance of orange or reddish brown colour indicated the presence of siderophore (Chari *et al*., 2015).

Phosphate solubilization

Pikovskaya's agar was poured as a thin layer in sterile Petri plates and incubated at ambient room temperature for 24h. After which the Pikovskaya's plates were spot inoculated with test isolate and incubated at ambient room temperature for 4 – 5 days. Formation of clear zones around the colonies was considered positive result for phosphate solubilization (Chari *et al*., 2015).

Statistical analysis

Biodiversity was analyzed using Simpson's diversity index. Simpson's index of diversity (1-D) was derived using Al Young's online biodiversity calculator (https://www.alyoung.com/labs/biodiversity_calculator.html).

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

D = Simpson's index

n = the total number of organisms of a particular species

N = the total number of organisms of all species

RESULTS AND DISCUSSION

Table 1 reveals the rhizobacteria isolated from local breed and hybrid maize rhizosphere – their enumeration and some plant growth promoting factors. *Enterobacter* sp., *Agrobacterium* sp., *and Pseudomonas* sp. were isolated from local breed maize. All three bacterial isolates were able to produce indole acetic acid (IAA) and solubilize phosphate. Only *Agrobacterium* sp. and *Pseudomonas* sp. were capable of ammonia production, while only *Agrobacterium* sp. produced siderophores.

Table 1. Plant growth promoting traits of rhizobacteria isolated from local and hybrid maize

Organism	Population CFU/g	IAA production	Ammonia production	HCN production	Siderophore production	Phosphate Solubilization
			Local breed maize			
Enterobacter sp.	9.1×10^5	+	-	-	-	+
Agrobacterium sp.	75×10^5	+	+	-	+	+
Pseudomonas sp.	4.95×10^5	+	+	-	-	+
		Total culturable rhizobacteria count: 89.05×10^5				
			Hybrid			
Staphylococcus sp.	10.05×10^5	+	+	-	-	+
Acinetobacter sp.	109.5×10^5	+	+	-	-	+
Bacillus sp.	42.5×10^5	+	-	-	-	+
Acinetobacter sp.	21.5×10^5	+	+	-	-	+
Pseudomonas sp.	2.05×10^5	+	-	-	-	+
		Total culturable rhizobacteria count: 185.6×10^5				

The five rhizobacteria isolated from the hybrid maize rhizosphere were *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., and *Acinetobacter* sp. All these rhizobacteria were able to produce IAA and solubilize phosphates. All except *Bacillus* sp. and *Pseudomonas* sp. were able to produce ammonia. No rhizobacteria from either the local breed maize or the hybrid maize produced hydrogen cyanide.

Rhizobacteria from hybrid maize had a higher total population density (185.6×10^5 CFU/g) than that of the local maize breed (89.05×10^5 CFU/g). Simpson's diversity index (1 – D) showed that rhizobacteria from the hybrid maize also had a higher diversity index (0.58) than that of the local breed (0.27). On the other hand, rhizobacteria from the local maize breed displayed a higher plant growth promoting functional diversity than that of the hybrid maize. This is evident, since out of the five plant-growth promoting factors investigated, rhizobacteria from the local maize breed displayed four, while those of the hybrid maize displayed only three.

Siderophores have been implicated in both direct and indirect enhancement of plant growth promoting rhizobacteria. The direct benefit of bacterial siderophères on plant growth include increased uptake of chelate iron across the plant cell membrane which leads to enhanced chlorophyll levels (Sharma *et al.*, 2003). On the other, the indirect benefit of bacterial siderophores to plants is by binding most of the ferrous iron (Fe^{3+}) in the rhizosphere, thus effectively preventing the proliferation of fungal pathogens by depriving them of available iron (Kloepper *et al.*, 1980). These benefits of bacterial siderophores

mentioned above are likely to give added immunity advantage to the local maize breed.
The higher densities of plant-growth promoting rhizobacteria of hybrid maize may contribute to its faster and bigger growth when compared to local maize variety. However, the siderophores production trait seen in the local maize variety but lacking in the hybrid maize may also confer on the local maize variety its peculiar comparative advantage. The ability of the local maize variety rhizobacteria to produce siderophores confer on the local maize variety a more efficient photosynthetic system (due to increased chlorophyll levels) and a more disease-resistant root system (Gamalero and Glick, 2011).
It should be stated, however, that the results obtained in this work are best analyzed alongside other considerations, as: crop yield, plant growth rate, fruiting time, fruit size, fruit sweetness, chlorophyll levels, disease resistance, and so on.

CONCLUSIONS

In this study, the rhizospheric soils of hybrid maize variety displayed a higher rhizobacteria density and diversity than that of the local maize variety. On the other hand, the rhizobacteria from the local maize breed rhizosphere soils demonstrated more plant growth promoting functional properties than those of the hybrid maize variety.

REFERENCES

[1] Alexander, M. (1961) Introduction to Soil Microbiology. First edition. John Wiley and Sons Incorporated, New York, US.

[2] Bakker, A. W. and Schipperes, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomoas spp*. - mediated plant growth stimulation. *Soil Biol. Biochem.* 19: 451 - 457.

[3] Brick, J. M., Bostock, R. M., and Silverstone, S. E. (1991). Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Applied Environment Microbiology*, 57: 535–538.

[4] Cappucino, J.C. and Sherman, N. (1992). *In*: Microbiology: A Laboratory Manual. Benjamin/Cummings, New York. 125 – 179.

[5] Chari, K.D., Reddy, R.S., and Trimurtulu, N. (2015). Screening and characterization of diazotrophic bacterial isolates for plant growth promoting properties. *Int. J. Curr, Microbiol. & Appl. Sci.*, *4*(9): 704 -710.

[6] Dobbelaere, S., Vanderleyden, J., and Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.*, 22: 107–149 (10.1080/713610853)

[7] Gamalero, E. and Glick, B.R. (2011). Mechanisms used by plant growth promoting bacteria. *In*: Bacteria in Agrobiology (Maheshwari, D.K Editor). Springer, New York. 17 – 46.

[8] Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, 41: 107 – 117.

[9] Glick, B.R., Patten, C.L., Holgin, G., and Penrose, D.M. (1999) Biochemical and Genetic Mechanisms used by Plant Growth Promoting Bacteria. Imperial College Press, London, 267.

[10] Holt, J.G., Kreig, N.R., Sneath, P.H., Staley, J.T., and Williams, S.T. (Eds.) (1994). Bergey's Manual of Determinative Bacteriology (ninth edition). Baltimore, USA: Williams & Wilkins.

[11] Kloepper, J.W., Ryu, C.M., and Zangh, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus spp. Phytopathology*, 94: 1259 – 1266.

[12] Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., and Singh, V. (2015). Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. *Microbial and Biochemical Technology*, *7*(2): 96 – 102.

[13] Mayak, S., Tirosh, T., Glick, B.R. (1999) Effect of wild type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. *J Plant Growth Regul*. 18:49–53.

[14] Jha, C.K. and Saraf, M. (2015). Plant growth promoting rhizobacteria (PGPR): A review. *J. Agricultural Res and Dev.*, *5*(2): 108 – 119.

[15] Saharan, B.S. and Nehra, V. (2011). Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medicine Research, 2011: 1 – 30.

[16] Sharma, A., Johria, B.N., Sharma, A.K., and Glick, B.R. (2003). Plant growth promoting bacterium *Pseudomonas sp*. Strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck).

[17] Beneduzi, A., Ambrosini, A., and Passaglia, L.M.P. (2012). Plant growth promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology, 35*(4): 1044 – 1051.

[18] https://www.alyoung.com/labs/biodiversity_calculator.html