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Plant Growth-Promoting Potentials of Some Indigenous Bacterial Isolates

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Abstract: *Plant growth promoting rhizobacteria (PGPR) are the rhizosphere bacteria that increase plant growth and suppress plant diseases. The present study was conducted to evaluate some bacterial isolates with plant growth promoting potential obtained from the roots of maize plants (Zea mays L.). A total of twenty-eight (28) bacterial isolates were obtained and identified on the basis of cultural, morphological characteristics, Gram's reaction and biochemical tests. These isolates were characterized using Bergey's manual of systematic bacteriology, they included members of genera Bacillus (35.71%), Burkhoderia (7.14%), Enterobacter (7.14%), Lactobacillus (3.57%), Leuconostoc (7.14%), Micrococcus (14.29%), Pseudomonas (17.86%), Serratia (3.57%), and Streptococcus (3.57%). These isolates were tested for specific plant growth promoting activities such as ammonia production, indo-acetic acid (IAA) production, phosphate solubilization and hydrogen cyanide (HCN) production. All the Pseudomonas and Bacillus species were IAA positive. Also, all species were positive for phosphate solubility test except Lactobacillus and Streptococcus spp. For ammonium test, two species of Bacillus cereus, Streptococcus and Lactobacillus species were negative. Therefore, the present study underpins the potentials of these isolates as members of PGPR, which can be used as biofertilizer to improve the growth and production of maize plants.*

Keywords: *Plant growth promoting rhizobacteria; Maize plants; Biofertilizer; Indigenous bacterial isolates*

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I. Introduction

Maize (*Zea mays L.*) also known as corn was first domesticated in Southern Mexico/Central America and is one of the most important grain in the world. Nigeria produced 11.0 million metric tons of maize in 2018 [1,2], which is a good chunk of Africa's corn production but considerably small when compared to the world's production which was 1,122.17 million tons in 2018 [2]. Imagine if we could increase Maize production in Nigeria by another 10%. This would raise our production to about 12.1 million metric tons which would have a significant impact in the nations GDP and more employment opportunities will be created. Maize has various health benefits such as B-complex vitamins in maize are good for skin, hair, heart, brain, and proper digestion [3]. The presence of vitamins A, C, and K together with beta-carotene and selenium helps to improve the functioning of thyroid gland and immune system [4].

Rhizobacteria are root-inhabitants that form symbiotic associations with many plants. *Rhiza* in Greek means root. Rhizobacteria are important group of microorganisms that can be used for production of biofertilizer. The relationship between PGPRs and plants is species dependent. The two classes of rhizobacterial relationships identified and they are rhizospheric and endophytic. Rhizospheric relationships consist of rhizobacteria that colonize the surface of the root or intercellular spaces of plants [5,6,7] while for endophytic rhizobaterial relationships, the rhizobacteria grow within the space outside the host's plasma membrane. In both relationships, plants are able to benefit from the available nutrients provided by the rhizobacteria under good environmental conditions [8]. The moment PGPRs establish within the roots of appropriate plants, they help to increase the nutrient content of the soil through solubilization process [9].

Solubilization of unavailable forms of nutrient and production of siderophores are major ways through which PGPRs increase nutrient availability in soils. For example, phosphorus is usually found in insoluble forms [10]. Some researchers have suggested that PGPRs can help to reduce the harmful effects of heavy metal toxicity stresses in plants by inducing morphological and biochemical modifications [11,12]. The dominant species of rhizobacteria found in the soils include; *Azospirillum brasilense, Acinetobacter lwoffii, Bacillus pumilus, Chryseobacterium balustinum, Paenibacillus alvei, Pseudomonas fluorescens, Pseudomonas putida, Serratia marcescens*, and *Pseudomonas stutzeri* [13,14,15,16]. They colonize roots of plants such as vegetables, crops, and even trees [17].

One of the major macronutrients needed by plants is phosphorus (P). Phosphorus is often applied to soil in form of chemical fertilizers. Microorganisms with phosphate solubilizing potentials increase the

availability of soluble phosphates and can also enhance plant growth by increasing the availability of trace elements such as iron and zinc by production of plant growth promoting regulators [18]. However, phosphate solubilization is a complex phenomenon, which depends on many factors such as physiological, nutritional, and growth conditions of the microbial culture. It has been observed by many investigators that a high proportion of phosphate solubilizing microorganisms (PSMs) especially bacteria, fungi and actinomycetes reside in the rhizosphere of plants and play an important role in plant nutrition as they enhance phosphate availability to roots through converting the insoluble phosphates into soluble ions [19]. Increasing crop yield through the use of PGPR as microbial inoculants is now the method of choice by most people because of increased demand for food and sustainable environment [20]. Hence, the focus of this study is to isolate, identify indigenous bacterial isolates and to determine their suitability as plant growth-promoting rhizobacteria.

II. Materials And Methods

Collection of the Sample

The roots of maize (*Zea mays L.*) and soil sample from the rhizosphere of the root of the maize used for this study was collected from Adekunle Ajasin University's Research Farm, Akungba-Akoko, Ondo State, Nigeria. Samples were collected under aseptic condition and taken to the Microbiology laboratory Adekunle Ajasin University, Akungba-Akoko and maintained at 4^{o}C throughout the study period.

Preparation of Sample

The field-moist soil were passed through a 4 mm sieve to eliminate coarse rock and plant material, thoroughly mixed to ensure uniformity and stored at 4^{o}C prior for use. A sub-sample of about 0.5 kg of sample was air-dried and passed through 2 mm sieve which was used to determination the physical and chemical characteristics.

Determination of Physiochemical Properties of Samples

The pH value of the soil in the root nodules was determined using a pH meter; the pH meter's electrode was washed with distilled water and inserted into a portion of the soil. The temperature was recorded by dipping mercury thermometer into the portion of the soil.

Isolation of Bacterial species

Rhizosphere-associated bacteria were isolated by taking 1 g of roots with tightly adhering soil using serial dilution plating technique on nutrient agar; the suspension was spread on the nutrient agar and incubated at 37^{o}C for 24 h till the appearance of bacterial colonies. Individual colonies were picked and streaked on nutrient agar plate for further purification.

Bacterial Count

The bacterial population was estimated by most probable numbers (PMN) count according to method described by Mishra *et al.* [21].

Determination of Morphological and Cultural characteristics

Microscopic characteristics of the microbial growth on various media were observed and recorded. Cultural characteristics investigated includes: elevation, edge, pigmentation and shape of the colonies. Bacterial isolates were microscopically investigated by Grams staining. Distinct colony was purified by streak plate and pure colonies were stored in agar slant.

Determination of Biochemical characteristics

The following tests were conducted gram staining, sugar fermentation, catalase, coagulase, motility, caseinase, citrate utilization, lipase, oxidase methyl red, Voges-Proskauer's, starch hydrolysis, and hydrogen sulphide (H_2S) [22].

Congo red Agar (CRA) Test

The medium composed of Brain heart in fusion broth (37 g/L), sucrose (5 g/L), agar number 1 (10 gm/L) and Congo red dye (0.8 g/L). Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121^{o}C for 15 min. Then it was added to autoclaved Brain heart in fusion agar with sucrose at 55^{o}C. Plates were inoculated with test organism and incubated at 37^{o}C for 24 to 48 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production; weak producer's usually remained pink, though occasional darkening at the center of colonies was observed.

Indole Acetic Acid (IAA) Test

Three millimeters of 1% nutrient broth was placed into different tubes leaving out one to serve as controlled experiment in which a loop full of bacterial isolate were inoculated. The test tubes were inoculated at 37^{o}C for 48 h. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently afterwards it was allowed to rest for 20 min to allow the reagent to mix homogenously and also to rise to the surface layer of the mixture in the test tube. A red coloration indicates a positive result while a yellow color gives rise to a negative result.

Phosphate Solubilization Test

Phosphate solublization was performed by inoculating individual bacterial isolates on Pikovskayas medium [23]. The plates were incubated 4-5 days at 37°C. A clear zone around the bacterial colony was considered as a positive which indicate phosphate solublization.

Ammonia Production Test

For the production of ammonia, the isolate was grown in Peptone broth 10 mL and incubated at 37°C for 48 to 72 h. After incubation, 0.5 mL of Nessler's Reagent was added to bacterial suspension. The development of brown to yellow color indicated [24].

HCN Determination

A modified method of Bakker and Schippers was used for the production of HCN by bacterial isolates. Nutrient agar medium (NAM) plates enriched with 4.4 g glycine and Whatman no. 1 paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the upper lids of Petri plates along with uninoculated control were used for the detection of HCN production. Parafilm-sealed Petri plates were incubated at 37^{o}C until light, moderate or dark brown which indicated the production of HCN [24].

III. Results

Thirty bacterial isolates were obtained from soil samples collected from root of maize. Four *Pseudomonas species* (*Pseudomonas tolaasii*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens, Pseudomonas putida),* five *Bacillus* species (*Bacillus* sp, *Bacillus mycoides*, *Bacillus megaterium*, *Bacillus cereus*), *Serratia marcescens*, *Leuconostoc* spp, *Micrococcus luteus*, *Enterobacter* sp, and *Burkholderia* spp were identified as plant growth promoting rhizobacteria (Table 1 and 2). The present study encompassed the isolation and identification of plant growth promoting rhizobacteria from root of maize. Plant rhizosphere is known to be preferred ecological niche for soil microorganisms due to nutrient availability.

Physiochemical Characteristics

The pH for the 10 soil samples were determined and were found to be between the range of 6.4 to 6.8 (Table 1). The critical pH for crop production is 4.5. In this study, the pH of the soils from the selected sample was above the critical level and could therefore consider moderately fertile.

Table 1: Physiochemical Properties of Soil

SAMPLES	TEMPERATURE (^{o}C)	pH
1	27.50	6.6
2	26.50	6.6
3	27.50	6.7
4	26.50	6.6
5	27.00	6.6
6	28.00	6.8
7	27.50	6.7
8	28.50	6.6
9	29.00	6.7
10	28.00	6.4

Percentage Occurrence of Bacterial Species

The highest percentage occurrence of the maize rhizosphere bacterial isolates include the following genera *Bacillus* (35.71%), *Burkholderia* (7.14%), *Enterobacter* (7.14%), *Lactobacillus* (3.57%), *Leuconostoc* (7.14%), *Micrococcus* (14.29%), *Pseudomonas* (17.86%), *Serratia* (3.57%), and *Streptococcus* (3.57%) (Table 2).

Table 2: Percentage of occurrence of species

Isolate	Frequency of Occurrence	Percentage of occurrence
Bacillus cereus	3	10.71
Bacillus megaterium	1	3.57
Bacillus mycoides	2	7.14
Bacillus subtilis	1	3.57
Bacillus sp	3	10.71
Burkholderia sp	2	7.14
Enterobacter sp	2	7.14
Lactobacillus sp	1	3.57
Leuconostoc sp	2	7.14
Micrococcus luteus	4	14.29
Pseudomonas aeruginosa	1	3.57
Pseudomonas fluorescens	1	3.57
Pseudomonas putida	1	3.57
Pseudomonas tolaasii	2	7.14
Serratia marcescens	1	3.57
Streptococcus mutans	1	3.57
Total	28	100

Percentage of occurrence = $\frac{Fc}{N} \times \frac{100}{1}$

KEY: Fc = Frequency of occurrence of each isolate N = Total number of isolates

Screening for plant growth promoting rhizobacteria

To identify plant growth promoting rhizobacteria isolates, indole acetic acid test, hydrogen cyanide test, phosphate solubility test and ammonium test were carried out. All the *Pseudomonas* and *Bacillus* spp were IAA positive and all species were positive except *Leuconostoc*, *Lactobacillus* and *Streptococcus* spp for phosphate solubility test. For ammonium test, two species of *Bacillus cereus* (BJT2 and ABT2), *Leuconostoc*, *Streptococcus* and *Lactobacillus* spp were negative (Table 3).

Table: 3: Screening for plant growth promoting rhizobacteria

S/N	Organism	Indole Acetic Acid	Phosphate Solubility	Ammonium	Congo Red	Hydrogen Cyanide
	Bacillus cereus	+	+	-	-	+
	Bacillus cereus	+	+	+	-	-
	Bacillus cereus	+	+	-	-	+
	Bacillus megaterium	+	+	+	-	+
	Bacillus mycoides	+	+	+	-	+
	Bacillus mycoides	+	+	+	-	+
	Bacillus sp	+	+	+	-	+
	Bacillus sp	+	+	+	-	-
	Bacillus sp	+	+	+	-	+
	Bacillus subtilis	+	+	+	-	+
	Burkholderia sp	-	+	+	-	+
	Burkholderia sp	-	+	+	-	-
	Enterobacter sp	-	+	+	-	-
	Enterobacter sp	-	+	+	-	+
	Lactobacillus sp	-	-	-	-	-
	Leuconostoc sp	-	-	-	-	-
	Leuconostoc sp	-	-	-	-	-
	Micrococcus luteus	-	+	+	-	+
	Micrococcus luteus	-	+	+	-	-
	Micrococcus luteus	-	+	+	-	-
	Micrococcus luteus	-	+	+	-	+
	Pseudomonas aeruginosa	+	+	+	-	+
	Pseudomonas fluorescens	+	+	+	-	+
	Pseudomonas putida	+	+	+	-	+
	Pseudomonas tolaasii	+	+	+	-	-
	Pseudomonas tolaasii	+	+	+	-	-
	Serratia marcescens	-	+	+	-	+
	Streptococcus mutans	-	-	-	-	-

KEY: + = positive test, - = negative test and S/N = serial number

IV. Discussion

Plant growth promoting rhizobacteria have a high diversity in soils and considered important for maintaining the sustainability of agriculture production in any kind of soil [25]. Rhizobacteria have the ability to fix nitrogen to the plant which is one of the main concerns of this study. It has been reported by some

researchers that most of PGPR are from the genus of *Bacillus* and *Pseudomonas* [26, 27]. Twenty-four isolates out of twenty-eight isolates were plant growth promoting rhizobacteria when tested for PGPR, the three other isolate are *Lactobacillus* sp and *Streptococcus* sp. Indole acetic acid (IAA) is one of the most important phytohormones which function as important signal molecules in the regulation of plant growth, uptake of nutrient. The amino acid, tryptophan played a major role in the production of IAA by rhizobacteria [28]. A 50% of the isolates were IAA producers. Similar studies have shown that IAA is very common among plant growth promoting rhizobacteria [26]. It was noted that not all phosphate solubilizing rhizobacteria were IAA producers. This information indicated that plant growth promotion in the environment is not driven by a single species but may be due to a composite effect of features present in several symbiotic bacteria.

Hydrogen cyanide is known to be intricately related to antifungal activity and the production of HCN in excess may play a critical role in the control of fungal diseases. The study revealed that 60% of isolates were positive for hydrogen cyanide test; the species can act as an inducer of plant resistance. This agrees with the report of Devi and Thakur [29] who stated that glycine has been found to be the direct precursor of microbial cyanide production and it has been found in root. In Congo red test, the appearance of the pink colour is due to poor absorption of dye present in the medium. This gave further evidence of plant growth promoting rhizobacteria isolate [30]. Lipase production from a variety of bacteria can produce different hydrolytic enzymes that are effective against soil fungi has been reported in several works, which were isolated mainly from soil. This findings showed that 67% of the isolates are lipase producers. Similar results were obtained by Mobarak-Qamsari *et al*. [31] and Prasanna *et al*. [32]. Also, about 60% of the isolates can hydrolyze starch and PGPR that produce on or more of these lytic enzymes have been found to have biocontrol ability against a range of plant pathogenic fungi and bacteria and enhance crop yield.

Soil is a store house of several forms of phosphate and ammonium including inorganic an organic phosphate, mineralization of most organic phosphorus compounds is carried out by means of phosphate enzymes. The ability of rhizobacteria to solublize insoluble phosphate has been of interest to agriculture microbiologist as it can enhance the availability of phosphorus for the plant to improve plant growth and yield [33]. Moreover, it has been reported that higher concentration of phosphate–solubilizing bacteria are commonly found in the rhizosphere as compared to bulk soil [34]. In this study, all isolates showed phosphate-solubilizing activity except isolate *Lactobacillus* sp. (ABO3) and *Streptococcus* sp. (TBA1). A study conducted by Woyessa and Assefa [35] reported that three isolate out of four showed phosphate-solubilization in soil. This confirms the solubility of plant growth promoting rhizobacteria. Production of ammonia is another important trait of PGPR, which indirectly influences the plant growth all of these isolates.

In conclusion, all PGPRs isolated are potential sources of bio-inoculants which can be used to reduce or replace the usage of chemical fertilizers and pesticide for sustainable cultivation of maize and related crops.

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