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DISTRIBUTION AND PHENOTYPIC CHARACTERIZATION OF PLANT GROWTH PROMOTING *PSEUDOMONAS* SPECIES ISOLATED FROM SOILS OF FIELD GROWING RICE (*ORYZA SATIVA*) CROPS IN EBONYI STATE, NIGERIA

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ABSTRACT

The phenotypic characterization of plant growth promoting *Pseudomonas* species isolated from soils of field growing rice (*Oryza sativa*) crops in Ebonyi State South eastern Nigeria were carried out. Soil samples were collected from the rhizosphere of two months old rice seedlings using a quadrant simple sampling collection technique. Isolation and identification of *Pseudomonas* species were carried out using standard microbiological procedures. The isolated *Pseudomonas* species were assessed for their ability to produce plant growth promoting substances namely; siderosphore, phosphate solubilisation, ammonia and indole acetic acid. Also, the potential of the isolated *Pseudomonas* species as biofertilizer were assessed using *in vitro* pot experiment. The results revealed that 66 *Pseudomonas* species were isolated and its distribution showed that 7(10.6%) were isolated from Abakaliki LGA, 22(33.3%) from Afikpo North, 11(16.7%) from Ezza North, 7(10.6%) from Ezza South., 16(24.2%) from Ikwo and 3(4.6%) were isolated from Ohaukwu L.G.A. The result of the plant growth promoting characteristics of the pseudomonas species was presented with many of the *Pseudomonas* species producing high plant growth promoting substances. The pot experiment revealed that the organism significantly enhanced the growth of rice plants when compared with both the positive and negative controls. Therefore, the bacterial isolates have the potential for future applications as biofertilizers in rice farm in Ebonyi State, Nigeria.

Keywords: ***Pseudomonas spp, Rhizosphere , Oryza sativa, Nigeria***

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important food crops in the world, nourishing approximately 50% of the population and directly providing 20% of human calorie intake **[1]**. It is the most important staple food crop in many developing countries and one of the most important crops grown in Ebonyi State, Nigeria **[2].** Declining soil fertility as a result of continuous cropping without replenishing soil nutrients is a major problem in Nigeria **[2]**.

The rice plant presents a habitat for diverse microorganisms, those that colonize the aerial parts (Phyllosphere), the root surface (rhizoplant) as well as the zone around the root (rhizosphere) **[3]**. The phyllosphere comprises the aerial parts of plants and is dominated by the leaves. Most studies on the identity of microorganisms have focused on *Pseudomonas* and, to lesser extent, fungi **[4]**.

Rhizosphere is the root surface zone where microorganisms attach themselves using surface structure such as flagella, frimbriae or cell surface polysaccharides. The boundary between rhizoplane and rizosphere is very thin and therefore the habitat is largely considered as a continuum **[5]**. The rhizosphere is a thin layer of soil immediately surrounding plant roots. This is an extremely important and active area for root activity and metabolism. A large number of microorganisms such as bacteria, Fungi, Protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them **[6]**.

One of the most common strategies to increase agricultural production is through the improvement of soil fertility. Nitrogen (N) and Phosphorus (P) are the two most limiting nutrients in soil. Due to Phosphorous fixation and precipitation which occur in soil, the concentration of soluble phosphate in soil is usually very low **[7]**, especially in Sub-Saharan Africa. Soil microorganisms are known to be effective in releasing P from organic pools of total phosphate by mineralization and from organic complexes through solubilization **[2]**.

Pseudomonas species are utilized in agriculture for various purposes; as important components of organic amendments and composts, as inoculants for biological nitrogen fixation, phosphorous solubilization and to improve crop quality and yields **[8]**. *Pseudomonas* species with rice plants, such as plant growth promotion by bacteria, nitrogen fixation or plant hormone production have been studied **[3]**. Through the activities of this *Pseudomonas*, by exudating organic acids which directly dissolve the rock phosphate, or chelate calcium ions, many soil microorganisms are able to solubilize the unavailable phosphate and release it to the solution **[9]**.

The Knowledge through which these *pseudomonas* can biologically fix nitrogen,

solubilize phosphorous and induce substance that can contribute to the improvement of rice growth hence depending less on chemical fertilizers, is crucial for sustainable rice cultivation.

Auxin is the first phytohormones to be identified among plant hormones which play an important role in root system development and plant yield. Indole acetic acid is the common natural auxin that shows all auxin activity and extensively affects plants physiology **[10]**. Reports have indicated the ability of many bacteria to produce phyto hormone that can enhance the plant root contact surface with uptake via root elongation. Due to this ability, microbial inoculants can be used as a substitute for chemical fertilizer in partially fertile soils and or at least as a supplement for chemical fertilizers in partially fertile soils and/ or at least as a supplement for chemical fertilizers in infertile soils.

Having rice as one of the major sources of carbohydrate food consumed in Ebonyi State and Nigeria, including most Sub-Saharan African countries; there is urgent need to focus on the various ways in which this food product can stand in space of time and give high yield for high product. The use of chemical fertilizers has been considered as threat as it can cause leaching, erosion and depreciation of soil nutrients **[2]**.

The depreciation of soil nutrient by the activities of various inorganic fertilizer used in the soil as a method of nutrient replacement has not only helped in the growth of plants, but has also caused the depreciation of the vital components of the soil.

The negative effect of chemical fertilizer on soil cannot be overemphasised; hence, this calls for urgent need for its substitution with a bio-fertilizer. Since bio fertilizer has been proven without reasonable doubt as a better substitute for chemical fertilizer **[11]**. The aim is of this research was to determine the distribution and phenotypic characteristics of plant growth promoting *Pseudomonas* species from field-growing rice crops in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Site and Description of Study area

Ebonyi is one of the states in south-east Nigeria. It lies approximately within longitude $7^{0}30^{1}$ and 7^{0}E and, latitude $5^{0}40^{1}$ and $6^{0}45^{1}$ N. It has a population of 149,683 people, and a land mass of about 5,935 square kilometres **(Fig. 1)**. The State shares border with Benue State to the North, Enugu State to the west, Imo and Abia States to the south and Cross River State to the east. Abakaliki the State capital has tropical climate with an average relative humidity of 75% and may reach 80% during rainy season. The vegetation characteristics are predominantly rainforest with atmospheric temperature of 30°C.

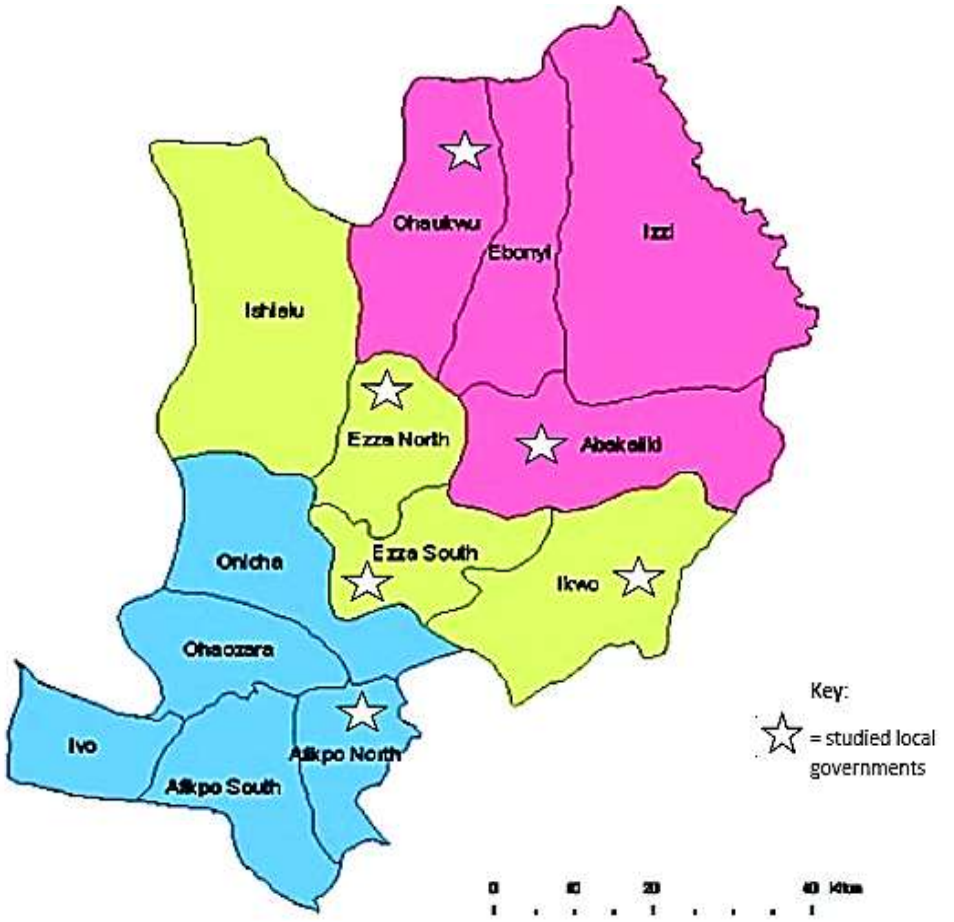

Figure 1: Map of Ebonyi State, showing six (6) study areas

Collection of samples:

Quadrat-simple sampling collection technique was used during the sample collection. Ten (10) soil samples were collected from the rhizosphere of 2 months old rice plants at 3 inches depth in each of the six selected local government areas in Ebonyi State, using soil auger. The samples were placed in plastic bags and transported to Applied Microbiology Laboratory, Ebonyi State University for analysis.

Sample procession and Isolation of *Pseudomonas* species

The soil samples were properly homogenised and sieved to remove impurities. One (1g) gram was weighed into a 20 mL of test tube, and 10 mL of sterile water was added. The tube was agitated for 2 minutes. Serial dilutions were performed up to 10^{-6} dilution. About 1 mL of the diluted sample was pipetted into sterile Petri-dish and 20 mL of sterile Pseudomonas Agar was poured into the Petri-dish, swirled gently and allowed to solidify. It was then incubated for forty eight hours at 30^0C according to **[12]**.

Morphological and Biochemical Characterisation

After incubation, the pigments were observed, colony morphology were identified, the pattern of arrangement were observed, Grams reaction was done, the shape was observed under microscope. The

Pseudomonas isolates were characterised by their morphological (shape, size, elevation, margin, colour, pigmentation and Gram's reaction) and some biochemical (Sucrose, Dextrose, Mannitol, Citrate utilization, starch and catalase test) characteristics using standard methods **[13]**.

Screening for plant growth promoting substances by the isolated *Pseudomonas* species

Phosphate Solubilisation: The plates were prepared with Pikovskaya's medium. The *Pseudomonas* isolates were streaked on the surface of Pikovskaya's agar plate and the phosphate solubilizing activity was estimated after 4 days of incubation at 28^{o}C. The phosphate solubilisation activity was determined by the development of the clear zone around the bacteria colonies **[14]**.

Production of Ammonia

The *Pseudomonas* isolates were tested for the production of ammonia in the peptone water. A 24 hours culture of the *Pseudomonas* isolates was inoculated each in 10 ml of peptone water and incubated for four days at 30^{o}C. I ml of Nessler reagent was added in each test tube. The development of brown to yellow colour was a positive test for ammonia production **[13]**.

Siderophore Production

The *Pseudomonas* isolates were tested for siderophore production on the chomazurol agar medium **[15]**. *Pseudomonas* was inoculated in chomazurol agar plates and incubated at 30^{o}C for 72 hours. Siderophore production was indicated by orange halos around the colonies after incubation.

Assay for indole acetic acid (IAA) production

Production of IAA was colorimetrically determined according to **[16]**. Briefly, bacterial cultures were grown in tryptone soy broth (TSB) for 24 hours at 28 ºC and 1 ml of each culture was centrifuged at 13,000 rpm. The supernatant was transferred to a clean 10 ml tube, mixed with 4 volumes of Salkowski reagent (250 ml, 150 ml H_2SO_4 96%, 7.5 ml 0.5M $FeCl_3$ solution), and then colour changes were monitored. Pure indole-3-acetic acid (Sigma, USA) was used as standard **[17]**.

Evaluation of the Potential of the isolated *Pseudomonas* species as a biofertilizer.

Determination of rice grains variability

The rice grain was immersed in a beaker containing distilled water, stirred and allowed to settle down for 3 minutes. The floating rice grains or less dense seedling was sieved out because it is regarded as having lesser nutrient and lacks the ability to germinate at due time. The seedlings at the bottom of the beaker is poured out, spread and allowed to dry for 24 hours.

Sterilization of the soil sample

One thousand grams each of the soil samples from the study sites were covered with foil in

a baking pan and sterilized in an oven at 190^0C for 30 minutes.

Pot experiment for the *Pseudomonas* isolates

Pot experiment with bacterized seeds was conducted as described by **[18]**. Exactly 465.5 grams of the sterile soil were introduced into a sterile container. The viable rice seeds were dipped in 5% alcohol for 15 seconds to remove wax and sterilized in 15% hydrogen peroxide for 30 seconds. The rice grain was bacterized by inoculating a loopful of the *Pseudomonas* species into the grain. The bacterised grains were allowed to dry for 30 minutes. After 30 minutes, the grains were planted in a sterile container. It was then transferred to green house and watered with sterile water once daily for three weeks. The shoot and root length was measured at interval of 7 days for 21days using a meter rule in cm.

Pot Experiment for the Positive Controls

Soil samples were collected from various sampling sites, exactly 465.5 grams was measured and poured into a pot and labelled accordingly. An unsterilized rice grain (4 grains each) was planted in each pot, watered every morning with a watering can. The length of shoot and leaf was taken and recorded every 7 days for 21 days using metre rule in cm.

Pot experiment for the negative controls:

Soil samples were collected from various sampling sites. The soil sample was sterilised using hot air-air oven at 190^0C for 30 minutes. The rice grain was sterilised with 5% alcohol to remove wax and 15% hydrogen peroxide for 30 seconds. Exactly 465.5 grams of the sterile soil was measured and poured into a pot and labelled accordingly. A sterile rice grain (4 grains each) was planted in each pot, watered every morning with sterile water the length of shoot and leaf was taken and recorded every 7 days for 3 weeks using metre rule in centimetres.

Statistical Analysis

The results obtained from the study were presented using tables and relevant data were statistically analyzed with the use of mean and standard deviation.

RESULTS

Morphological, Cultural and Biochemical Characterization of *Pseudomonas* species

The result of the morphological, cultural and biochemical characterisation, showed that sixty six (66) *Pseudomonas* species were isolated and identified. The isolates were gram negative, short rods and in singles. Cultural characteristics showed a cream yellow to greenish, round colonies to margin, single cell organism. The cells reacted positively to sucrose, dextrose, starch, lactose, oxidase, citrate utilization, Mannitol and nitrate reduction test and negative to hydrogen sulphide production (Table not shown).

Distribution of *Pseudomonas* species within the study area

The distribution of the sixty six (66) *Pseudomonas* species obtained from the six (6) local government areas is presented in **(Table 1)**. It shows that seven (7) species which represent 10.6% were isolated from Abakaliki, twenty two (22) species representing 33.3 % from Afikpo North, Eleven (11) species representing 16.7% from Ezza North, seven (7) species representing 10.6% from Ezza South, sixteen (16) species representing 24.4% from Ikwo and three (3) species representing 4.6% from Ohaukwu **(Table 1)**.

Siderophore production by *Pseudomonas* species

The result of the production of siderosphore by *Pseudomonas* species showed that, out of the seven (7) species of Pseudomonas isolated in Abakaliki Local Government. 3 species did not produce siderophore, one (1) specie showed low production of siderophore, one (1) specie showed moderate production of siderophore and two (2) showed high production of siderophore. Out of the twenty two (22) species from Afikpo North, 4 species showed low production of siderophore, 6 species showed a moderate production of siderophore, while twelve (12) species showed a high production of siderophore. Ezza North had eleven (11) species out of which, one (1) specie showed low production of siderophore, seven (7) showed moderate production of siderophore, six (6) species showed a high production of siderophore. A total of eleven (7) species were isolated from Ezza South, five (5) species showed moderate production of siderophore and two (2) species showed high production of siderophore. In Ikwo, a total number of sixteen (16) isolates were isolated, one (1) showed low production of siderophore, five (5) showed moderate production of siderophore and ten (10) showed high production of siderophore. In Ohaukwu a total of three (3) species were isolated, all the three (3) species showed low production of siderosphore **(Table 2)**.

Phosphate solubilisation by *Pseudomonas* species

The result in **Table 3** shows phosphate solubilisation test carried out on the sixty six (66) species, Out of the seven (7) species obtained from Abakaliki Local Government Area. Three (3) species did not solubilise phosphate and four (4) had a low phosphate solubilisation ability. In Afikpo North Local Government Area, a total of twenty two (22) species were isolated. Two (2) species showed low level of phosphate solubilisation ability, one (1) specie showed moderate level of phosphate solubilisation ability and nineteen (19) species showed high phosphate solubilisation ability. Out of the eleven (11) *Pseudomonas* species obtained from Ezza North, one(1) did not solubilise phosphate, three (3) species were moderate phosphate solubilizers and seven (7) species showed a

high phosphate solubilisation ability. Out of seven (7) species from Ezza South, three (3) showed moderate phosphate solubilisation ability and eight (8) showed high phosphate solubilisation ability. A total of sixteen (16) species were isolated from Ikwo Local Government Area. Five (5} species showed moderate phosphate solubilisation and eleven (11) species showed high phosphate solubilisation. In Ohaukwu Local Government Area, a total of three (3) species were isolated. One (1) specie did not solubilise phosphate while two (2) species showed low phosphate solubilisation **(Table 3)**.

Ammonia production by *Pseudomonas species*

Table 4 shows the plant growth promoting character of *Pseudomonas* species for Ammonia production. Among the six LGAs that was assayed for ammonia production, Afikpo North had the highest number of ammonia producers twenty one (21) followed by Ikwo LGA sixteen (16), while Ezza south recorded the least number of ammonia producers one (01).

Indole acetic acid (IAA) production by *Pseudomonas* species

Table 5 shows the plant growth promoting character of *Pseudomonas* species for indole acetic acid production. From the seven (7) species that were isolated from Abakaliki Local Government Area, 3 species were a non-producer of indole acetic acid, three (3) were low producers and one (1) specie was a moderate producer . From Afikpo North, 3 showed low production of indole acetic acid, 2 species showed a moderate production while, 17 species were high producers. Ezza North Local Government Area has a total of 11 species. One (1) specie are non producers of indole acetic acid, 2 were low producers, 1 specie showed moderate production and 7 species showed high production of indole acetic acid. Ezza south had 7 species, Out of which, 3 species showed non production of indole acetic acid, 1 specie showed moderate production and 3 species showed high production. Ikwo Local Government had a total of 16 species, the 16 species showed high production of indole acetic acid. Ohaukwu Local Government Area had a total of 3 species were isolated the 3 species showed low production of indole acetic acid **(Table 5)**.

The shoot and leaf length of rice inoculated with *Pseudomonas* species

Table 6 shows the result of the mean and standard deviation of the shoot and leaf length of rice bacterized with *Pseudomonas* species isolated from the six local government areas. It revealed that the maximum leaf length was from Ikwo which recorded 11.20±2.99, 22.52±6.00, 33.75±9.00 for first, second and third week respectively, while the lowest leaf length was obtained from Ohaukwu which recorded 4.00±0.00,

8.10±0.00, 15.00±0.00 respectively. Also the maximum shoot length was obtained from Ezza south which recorded 2.97 ± 0.07, 6.00±0.05, 11.47±0.69 for first, second and third week respectively. While the lowest leaf length was obtained from Ohaukwu which recorded 1.00 ± 0.00, 1.50 ± 0.00 and 02.50 ± 0.00 for the first, second and third weeks **(Table 6)**.

Mean values of shoot and leaf length (cm) of the negative controls

The result of the mean and standard deviation of the shoot and leaf length of rice bacterized with *Pseudomonas* species isolated from the six local government areas for the negative controls was presented in **Table 7**. The result shows that samples collected from Ikwo recorded maximum leaf length 7.00±2.00, 17.75±3.25, 25.10±2.10 for first, second and third weeks respectively, while the lowest leaf length was from Ohaukwu with 4.00±0.00, 6.00±1.00, 7.55±2.35 (cm) values respectively. The maximum shoot length was equally from the same local government (Ikwo) with values ranging from 4.00±0.00, 6.00±1.00, 7.55±2.35, while the least values from Ohaukwu LGA were 1.00 ±0.00 , 1.45 ±0.45, 1.80 ±0.20 respectively **(Table 7)**.

Mean values of the shoot and leaf length of the positive controls

The result of the mean and standard deviation of the shoot and leaf length of rice bacterized with *Pseudomonas* species isolated from the six local government areas for the positive controls was presented in **Table 8**. The result shows that samples collected from Ikwo recorded maximum leaf length 17.65±0.07, 35.00±0.14, 52.60±0.14 for first, second and third weeks respectively, while the lowest leaf length was from Ohaukwu with 10.25±0.07, 20.30±0.28, 38.05±0.07 (cm) values respectively. The maximum shoot length was equally from the same local government (Ikwo) with values ranging from 6.05±0.07, 12.00±0.00, 19.00±0.00, while the least values from Ohaukwu LGA were 2.25 ±0.07 , 5.00 ±0.00, 8.20 ±0.28 respectively **(Table 8)**.

Table 1: Distribution of *Pseudomonas* species according to local government areas

S/N	Location	Number of species Isolated	Percentage distribution (%)
1	Abakaliki	07	10.6
2	Afikpo North	22	33.3
3	Ezza North	11	16.7
4	Ezza South	07	10.6
5	Ikwo	16	24.4
6	Ohaukwu	03	4.6
Total		66	100

Table 2: Siderophore production by the *Pseudomonas* species

S/N	L.G.A	Total no of species tested	No of species that did not produce siderosphore	No of species with Low production	Number of species with Moderate production	No of species with High production
1	Abakaliki	07	3	1	1	02
2	Afikpo North	22	0	4	6	12
3	Ezza North	11	1	1	3	06
4	Ezza South	07	0	0	5	02
5	Ikwo	16	0	1	5	10
6	Ohaukwu	03	0	3	0	00

Key: LGA = local government area

Table 3: Phosphate solubilisation by the *Pseudomonas* species

S/N	L.G.A	Total no of species tested	No of species that did not solubilise phosphate	No of species with Low solubilisation of phosphate	Number of spe cies with mode ratesolubilisati on of phosphate	No of species with High solubilisation of phosphate
1	Abakaliki	07	3	4	0	00
2	Afikpo North	22	0	2	1	19
3	Ezza North	11	1	0	3	07
4	Ezza South	07	0	0	0	07
5	Ikwo	16	0	0	5	11
6	Ohaukwu	03	1	2	0	00

Table 4: Ammonia production by the *Pseudomonas* species

	L.G.A	Total no of species tested	No of species that with no production of ammonia	Low production	Number of species with Moderate production of ammonia	No of species with High production of ammonia
1	Abakaliki	07	3	3	1	00
2	Afikpo North	22	0	0	1	21
3	Ezza North	11	1	0	1	09
4	Ezza South	07	0	5	1	01
5	Ikwo	16	0	0	0	16
6	Ohaukwu	03	0	3	0	00

Key: LGA = local government area

Table 5: Indole acetic acid production by the *Pseudomonas* species

S/N	L.G.A	Total no of species tested	No of species with no production of IAA	Low production	Number of species with Moderate production of IAA	No of species with High production of IAA
1	Abakaliki	07	3	3	1	00
2	Afikpo North	22	0	3	2	17
3	Ezza North	11	1	2	1	07
4	Ezza South	07	3	0	1	03
5	Ikwo	16	0	0	0	16
6	Ohaukwu	03	0	3	0	00

Table 6: Shoot and leaf length of rice inoculated with *Pseudomonas* species

S/N	Locations	leaf length (cm)			shoot length (cm)		
		1st week	2nd week	3rd week	1st week	2nd week	3rd week
1.	Abakaliki	04.97 ± 4.66	10.30 ± 9.63	15.89 ±14.87	2.16 ± 2.05	4.14 ±3.88	06.59 ± 6.20
2.	Afikpo North	09.09 ± 0.21	18.13 ± 0.24	27.56 ±0.87	2.93 ± 0.16	5.01 ± 0.08	10.19 ± 0.58
3.	Ezza North	10.04 ±0.06	20.00 ± 0.03	29.99 ± 0.03	2.84 ±0.20	5.01 ±0.04	09.94 ± 0.32
4.	Ezza South	09.91 ± 0.15	19.38 ±0.89	26.00 ±10.10	2.97 ± 0.07	6.00 ± 0.05	11.47 ± 0.69
5.	Ikwo	11.20 ± 2.99	22.52 ±6.00	33.75 ± 9.00	2.80 ±0.74	4.69 ± 1.25	09.41 ± 2.51
6.	Ohaukwu	04.00 ± 0.00	08.10 ± 0.00	15.00 ± 0.00	1.00 ±0.00	1.50 ±0.00	02.50 ± 0.00

Table 7: Shoot and leaf length of the negative controls

S/N	Locations	Length of leaf (cm)			Length of shoot (cm)		
		1st week	2nd week	3rd week	1st week	2nd week	3rd week
1	Abakaliki	4.65± 1.65	11.45±3.05	19.00±1.00	1.15±0.35	1.65±0.35	2.9±0.90
2	Afikpo North	7.10±1.70	17.00±2.00	24.20±1.00	3.61±0.59	5.65±0.65	7.15±1.85
3	Ezza North	4.85±1.05	13.05±2.15	21.75±0.25	2.01±0.49	3.45±0.45	4.00±1.00
4	Ezza South	5.25±1.25	12.4±2.40	22.50±0.50	1.85±0.15	2.90±0.30	4.75±1.25
5	Ikwo	7.00±2.00	17.75±3.25	25.10±2.10	4.00±0.00	6.00±1.00	7.55±2.35
6	Ohaukwu	2.62±0.415	7.70±1.20	15.05±0.95	1.00±0.00	1.45±0.45	1.80±0.20

Table 8: Shoot and leaf length of the positive controls from the six local Governments Areas

		Length of leaf (cm)			Length of shoot (cm)		
		1st week	2nd week	3rd week	1st week	2nd week	3rd week
S/N	Locations						
1	Abakaliki	10.95±0.20	21.75±0.35	32.15±0.35	3.11± 0.01	7.65±0.21	10.6±0.14
2	Afikpo North	13.4±0.28	27±0.00	40.3±0.42	5.25±0.35	9.9±0.14	15.1±0.14
3	Ezza North	14.7±0.28	29±1.41	42.2±0.42	5.00±0.00	10.15±0.21	15.3±0.14
4	Ezza South	15.05±0.07	30.60±0.56	45.2±0.00	5.25±0.07	10.15±0.07	15.75±0.07
5	Ikwo	17.65±0.07	35.00±0.14	52.6±0.14	6.05±0.07	12±0.00	19±0.00
6	Ohaukwu	10.25±0.07	20.3±0.28	38.05±0.07	2.25±0.07	5±0.00	8.2±0.28

DISCUSSIONS

The morphological, cultural and biochemical identification tests carried out revealed that *Pseudomonas* species were present in the soil samples analyzed. A total of 66 *Pseudomonas* species were successfully identified in this study **(Table 1)**. The presence of this organism is well understood because the organisms are frequently isolated from soil samples. This observation agrees with the report of **[19]** who isolated *Pseudomonas* strains from roots and rhizospheric soil samples of rice. **[20]** reported that *Pseudomonas* bacteria can be found in many different environments such as soil, water, marshes coastal marine habitats, and plant and animal tissue. Generally, these bacteria can tolerate a variety of physical conditions. The soil environment provides a maximum of ecological opportunity and competition for microorganisms, it seems plausible that diversification among soil microorganisms including *Pseudomonas* species bacteria is indeed related to the heterogeneity of this environment.

According to the result presented in **Table 1**, the highest number of *Pseudomonas* species were isolated from soil samples obtained from Afikpo North (22) and Ikwo local governments (16). The isolation of such high number of *Pseudomonas* species could be attributed to the fact that these two localities have a sandy-loamy soil which is highly favourable for the proliferation of soil microorganisms including *Pseudomonas* species **[21]**. This could also be credited to the number of swamps, lakes and other water sources found in those areas. It was observed that soil samples from Ohaukwu local government area had the least number of *Pseudomonas* species (3) which could be as a result of poor farming activities like deforestation, bush burning, use of toxic chemicals and overgrazing **[22]**. Other possible factors which could be responsible for the low number of *Pseudomonas* species could be as a result of the sandy-clay soil type found at the study site. The result of this study is consistent with several previous studies that demonstrated isolation of a number of novel bacterial species in rice and other plants **[23, 24]**. The observed differences in the number of *Pseudomonas* species isolated in the different local governments studied may be as a result of demographical differences and variations in native agricultural practices. The environmental conditions therefore could be a factor affecting bacterial populations.

Result shown in **Table 2** revealed that *Pseudomonas* species isolated from soil

samples gotten from Afikpo north and Ikwo had the highest level of siderophore production with 12 and 10 respectively. Other local governments except Abakaliki (1 specie) had *Pseudomonas* species that showed zero (0) siderosphore production. The isolation of *Pseudomonas* species with a high level of siderophore production could be linked to the natural availability of mineral deposits found in those locations. The observation made in this study is similar to the report of **[25]** who reported the isolation of *Pseudomonas putida, Pseudomonas fluorescens* and *Azospirillum lipoferum* form the rice rhizospheric soil. **[26]** opined that organisms with high level of siderosphore production are beneficial to the soil community as they boost the iron-content of the soil. Iron is required by aerobic bacteria and other living organisms for a variety of biochemical reactions in the cell. Iron is a common intermediate in many biochemical oxidation reactions. It is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals **[27]**. Chemical compounds produced by microorganisms in the rhizosphere can also increase the availability and uptake of iron **[28]**. Organisms that produce siderophore like *Pseudomonas* have an advantage due to the extreme acid stability of these molecules. Siderosphore therefore becomes extremely important in an ecological niche defined by low iron availability, iron being one of the critical growth limiting factors for virtually all aerobic microorganisms **[29]**.

From the result presented in **Table 3**, it was observed that a majority of the *Pseudomonas* species isolated in this study were high phosphate solubilizers. The local government that had the highest number of *Pseudomonas* species with high phosphate solubilization ability was Afikpo north (19) followed by Ikwo (11). The local government that had *Pseudomonas* species with lowest phosphate solubilization ability were Abakaliki and Ezza south. The isolation of *Pseudomonas* species with high phosphate solubilization ability from soil samples from Afikpo north and Ikwo is not surprising being that these two local governments are currently the leading producers of rice in Ebonyi state. This was consistent with previous studies by **[30, 31]**. In a similar study **[32]** isolated nifH-containing endophytic bacteria from Korean rice cultivars and demonstrated their ability to solubilize phosphates in rice plants. This is in agreement with previous works in which these phosphate solubilizers have been described as dominant groups in various soils **[33]**. The ability of bacteria to solubilize

mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth **[34]**. Bacterial genera like Pseudomonas, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Rhizobium *and* Serratia have been reported as the most significant phosphate solubilizing bacteria **[35]**, hence lending support to the observations of this study. In ecological terms, because of its important role in biological systems, phosphate is a highly sought after resource. Once used, it is often a limiting nutrient in environments, and its availability may govern the rate of growth of organisms.

It was observed from the result of ammonia production test **(Table 4)** that the local government areas which had the highest number of *Pseudomonas* species with the highest ammonia production ability were Afikpo north and Ikwo with 21 and 16 respectively. It also showed that Abakaliki had species with lowest level of ammonia production. Soil samples from Afikpo north and Ikwo having *Pseudomonas* species with high level of ammonia production indicates that such soil is biologically satisfactory for the growth of cereals like rice and other closely related plants. Ammonia is an important source of nitrogen for living systems. Many plants like rice, wheat, oat, rely on ammonia and other nitrogenous waste incorporated into the soil by decaying matter **[36]**. Globally, approximately 88 % (as of 2014) of ammonia is used as fertilizers either as its salt, solutions or anhydrously when applied to soil, it helps provide increased yield of rice, maize, wheat and other crops **[37]**. This result is similar to that obtained by **[38]**, who found cultivar-specific differences for ammonia-oxidizing bacteria (AOB) in the rhizosphere of rice. In several plant developmental stages, *Pseudomonas* species could provide a great benefit for growth promotion and fitness of their host, as described by **[37]**.

Table 5 shows the plant growth promoting character of *Pseudomonas* species for Ammonia production. Afikpo North had the highest number of ammonia producers twenty one (21) , while Ezza south recorded the least number of ammonia producers one (01). Similar studies as obtained in this study was obtained previously where IAA production is very common among PGPR **[39]**.

From the result presented in **Table 6** it was observed that rice seedlings bacterized with *Pseudomonas* species from Ikwo and Ezza south recorded the highest length in leaf and

shoot, having a mean and standard deviation of 33.75 ± 9.00 cm and 11.47 ± 0.69 cm in their third week respectively. While the least length in leaf and shoot was obtained from Ohaukwu. Rice plant bacterized with *Pseudomonas* species from Ikwo and Ezza south having the highest leaf and shoot length could be as a result of traditional farming practices in those areas such as mulching, fallowing and shifting cultivation have rendered the soil conducive and favourable for the growth and multiplication of *Pseudomonas* species. This could also be attributed to the availability of different water sources like lake, streams, dams; and the loamy and swampy nature of the soil. Increased nutrient uptake by plants inoculated with plant growth PGP bacteria has been attributed to the production of plant growth regulators at the root interface, which stimulate root development and better absorption of water and nutrients from soil. Increase in shoot dry mass has been reported in response to bacterial inoculation in rice **[40]**. **[41]** demonstrated that seed treated with *Pseudomonas* species significantly enhanced early growth of winter wheat in low fertility acquit soil. This is in concurrence with the observation of **[42]** who stated that enhance uptake of nutrients in black pepper and sweet basil due to seed bacterialized with *Pseudomonas* spp. This shows that congenial conditions of the soil perhaps, facilitates the plant growth promoting (PGP) activities of these isolates as soil nutritional conditions are reported to be influencing the performance of PGPR. Conversely, rice plant bacterized with *Pseudomonas* species isolated from Ohaukwu had the lowest length of leaf and shoot. This could be as a result of the sandy nature of the soil and the scarcity of the natural water sources like lakes, streams, damps, wrong agricultural practices such as bush burning, continuous cropping (due to lack of large availability of land for agricultural practices) could also be some of the causes. This is probably due to the soil characteristics and biotic and abiotic stresses **[43]**.

From the result of the positive and negative controls in this study as presented in **Tables 7 and 8**, it was observed that the values obtained for the negative control was lower than that obtained for rice plants bacterized with *Pseudomonas* species. This observation agrees with the findings of other researchers who carried out related studies on plant growth promoting activities of rhizobacteria on cereals **[44, 45]**.

CONCLUSION

The results obtained in this study have revealed the presence of *Pseudomonas*

species in rice fields in Ebonyi State. The bacterial isolates could be potential candidates for future application as biofertilizers in rice farms in Ebonyi State. Therefore, judicious application of plant growth promoting rhizobacteria to rice holds immense potential that is still to be explored and it can also provide an answer to the global demand for the development of a sustainable strategy for enhanced crop production.

Data Availability

The datasets used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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