ISSN No:-2456-2165

Characterisation of Rhizosphere Soil Bacteria from Rice Varieties Grown under Greenhouse Environment

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Abstract:- The bacterial community in the rhizosphere region is critical for the growth of plants and the formation organic matter of the soil. This study focuses on the identification of rhizospheric soil bacteria from rice varieties grown under greenhouse environment. Samples of *Oryza sativa* and *Oryza glabberima* were collected from the National Cereals Research Institute (NCRI) Umuahia and Rice mill at Kpirikpiri, Ebonyi State. *Oryza sativa* grew more rapidly than *Oryza glabberima* because of the variations in rice species and nutritional requirements. The rice seeds were planted in nursery and transplanted in buckets under greenhouse conditions (75% humidity and at room temperature 25°C). The adhering soil samples from the rhizosphere region were collected from the roots and 1g of soil was weighed aseptically and dissolved in sterile distilled water. An aliquot was picked for serial dilution. The serially diluted aliquot was inoculated on nutrient agar and incubated at 25 degree centigrade for 24 hours. Individual colonies on nutrient agar, mannitol salt agar, Salmonella-Shigella agar, eosin methylene blue agar were subcultured. Microbiological, biochemical and sugar fermentation tests were used to classify the isolates from *Oryza sativa* into *Azotobacte*r spp., and *Bacillus* spp., and *Oryza glabberima* into *Bacillus* spp. and *Pseudomonas* spp. respectively. This study showed that there are a lot of soil bacteria in the rhizospheric region of *Oryza sativa* than *Oryza glabberima* because it is an improved variety which enhanced the utilization of available nutrients and minerals by microbial interaction which likely enabled rapid growth of the plant. From the observation in the study, it is suggested that, *Oryza sativa* should be cultivated more for high yield and large-scale agricultural production for its economic benefits.

Keywords:- *Oryza Sativa, Oryza Glabberima, Rhizosphere, Bacteria, Greenhouse, Nerica5, M306.*

I. INTRODUCTION

The immediate soil area that is in close proximity to plant roots is known as the rhizosphere. In Nigeria, rice is a staple diet, and the rhizosphere soil is vital to plant health and growth. After a light shake, the dirt that sticks to the roots may still contain the bacteria from this zone. Rhizobacteria are the bacteria that live in the rhizosphere. They erect a barrier against the actinomycetes, nematodes, bacteria, fungi, and other parasites that infect roots by living on the surface of the roots in the soil. Due to their toxic effects, rhizobacteria shield plant roots against plant parasites by excreting antibiotic compounds [1]. These bacyeria receive nourishment from the root exudates in return. Consequently, a symbiotic relationship develops between the rhizosphere bacteria and the roots of the host plant. *Pseudomonas* sp., *Enterobacter sakazakii*, and *Klebsiella oxytoca*, rhizosphere bacteria that produce ethylene, regulate root parasite striga infection in maize and sorghum [2]. The majority of toxins produced by rhizobacteria are really antibiotics. It was realized that plants and the rhizosphere population may directly compete for scarce mineral resources. Apart from the root, the rhizosphere has a denser microbial population than the soil. The number of organisms in rhizosphere soil as compared to the number in the same soil (non-rhizosphere) outside the impact of the roots is the commonly used measure of its effect on the soil population. The fact that the microflora in rhizosphere soil is more than in non-rhizosphere soil suggests that living roots have an impact on the soil. Numerous nitrogen-fixing and non-nitrogen-fixing rhizosphere soil bacteria are present in the roots, culms, and seeds of different wild, traditional, and farmed rice varieties [3]. The aim of the study was to isolate and identify bacterial species in rhizosphere soil using phenotypic characteristics and determine the growth rate of each rice plant varieties and the number of rhizosphere soil bacteria present in each rice plant.

II. MATERIALS AND METHODS

- *Experimental Design*

The soil was sampled from rice fields at Okpoto in Ebonyi state, air- dried and stored at room temperature prior to the beginning of the experiment. Soil samples collected from five spots in the rice field were sieved through a stainless steel screen (0.2 mm mesh) and 3.5 kg was added to opaque plastic pots (22.5 cm height, 18.5 cm diameter). The pots were flooded with deionized water one week before planting. Rice seeds (*Oryza sativa and Oryza glabberima*) were also obtained from the National Cereals Research Institute (NCRI), Umuahia and Rice mill at kpiriri Ebonyi state. The rice seeds were germinated at 25°C and

ISSN No:-2456-2165

75% humidity in a green house. Three germinated rice seedlings were planted each in a total of 5 bucket. One of the buckets was left unplanted which served as control. The buckets were incubated in a green house at 25°C and 75% humidity with a 12-hour light/dark cycle. The buckets were watered daily to maintain approximately 3cm water overlying the soil. Plant heights were recorded weekly. Plants were carefully removed from the buckets and shaken to remove large soil aggregates and adhering soil. The soil remaining attached on the roots was considered to be rhizosphere soil and was sampled using a sterile spatula. The sampling techniques and nursery practices carried out was employed following the methods of [4].

- *Measurement of Plant Height*

After planting the seeds, the heights and tiller numbers was taken note of and observed. The heights were measured weekly using a metre rule. Measurement was taken on each plant from top soil to the shoots/straw.

- *Isolation and Culturing of Rhizosphere Soil Bacterial Isolates*

After extracting samples of adhering soil (rhizosphere) from the roots, 1 g of the soil was weighed aseptically and dissolved in sterile distilled water. Then, for serial dilution, an aliquot (1 ml) was selected and added to the same buffer (9 ml). In order to create a ten-fold serial dilution, this process was repeated. After preparation of the nutrient agar, mannitol salt agar and Cetrimide agar. They were dispensed into sterile Petri dishes and inoculated using pour plate method. On the agar plates, an aliquot (1 ml) serially diluted from the 10^{-4} and 10^{-5} was thereafter inoculated. After that, the cultures were kept in an incubator set at 25°C for 24 hours to promote bacterial growth. To create pure cultures, individual colonies were selected and streaked on new media for purification. Following that, morphological and biochemical characterisation was carried out on the pure cultures.

- *Colony Counts of Bacteria*

Colony counter was used to count distinct colonies on the agar plates. The number of bacterial colonies on the agar plates were expressed in colony forming unit (CFU/ ml).

- *Morphological and Biochemical Characterization of Rhizosphere Soil Bacterial Isolates*

The traditional Gram staining technique was used in the morphological characterisation process to ascertain the bacterial cells' cell shape [5]. After making and heat-fixing smears of the bacterial isolates, they were doused with crystal violet (Sigma Aldrich, Steinheim, Germany) and allowed to stand for a minute. After giving the smears a mild rinse with tap water, Grams of iodine (Sigma Aldrich, Steinheim, Germany) was added. After a minute, it was washed with tap water. Subsequently, 95% ethanol (Scharlab S.L., Spain) was used for decolorisation, and safranin was used as a counter-stain for 45 seconds. After lightly washing the smears with tap water, it was blot dried and examined under a light microscope using x10 objective lens, oil immersion. Biochemical tests were carried out such as catalase, indole acetic acid production test and sugar fermentation tests (D-glucose, sucrose, mannitol and lactose). The bacterial isolates were identified according to Bergy's manual [6].

III. RESULTS

It was observed that *Oryza sativa* grew more rapidly than *Oryza glabberima* (Figure 1).

In the 10^{-4} dilution, N51 had a higher number of bacterial colonies than M3061, while in the 10^{-5} dilution, M3062 had a higher number of colonies (Table 1).

A total of five (5) isolates were recovered from the rice's rhizosphere. Morphological characterisation of the five isolates based on Gram test revealed that all isolates were rod shaped (Table 2). Two of the rhizosphere bacterial isolates were Gram positive rods while three were Gram negative rods (Table 2). Both Gram's positive and Gram's negative outcomes were observed for the Catalase test, Sugar tests (D-glucose, sucrose, mannitol and lactose) and indole test. All the bacterial isolates were positive for catalase. Only N52 was positive for indole. Three (3) of the isolates were negative for sugar tests while two (2) were positive (Table 2).

Table 1 Colony Counts of Bacteria Showing the Number of Colonies in each Sample

Dilution factor	Isolates	Number of colonies (CFU/ml)
10^{-4}	N51	6.0×10^{5}
	M3061	2.4×10^{5}
	S1	3.5×10^{5}
10^{-5}	N52	4.0×10^{6}
	M3062	6.0×10^{6}

ISSN No:-2456-2165

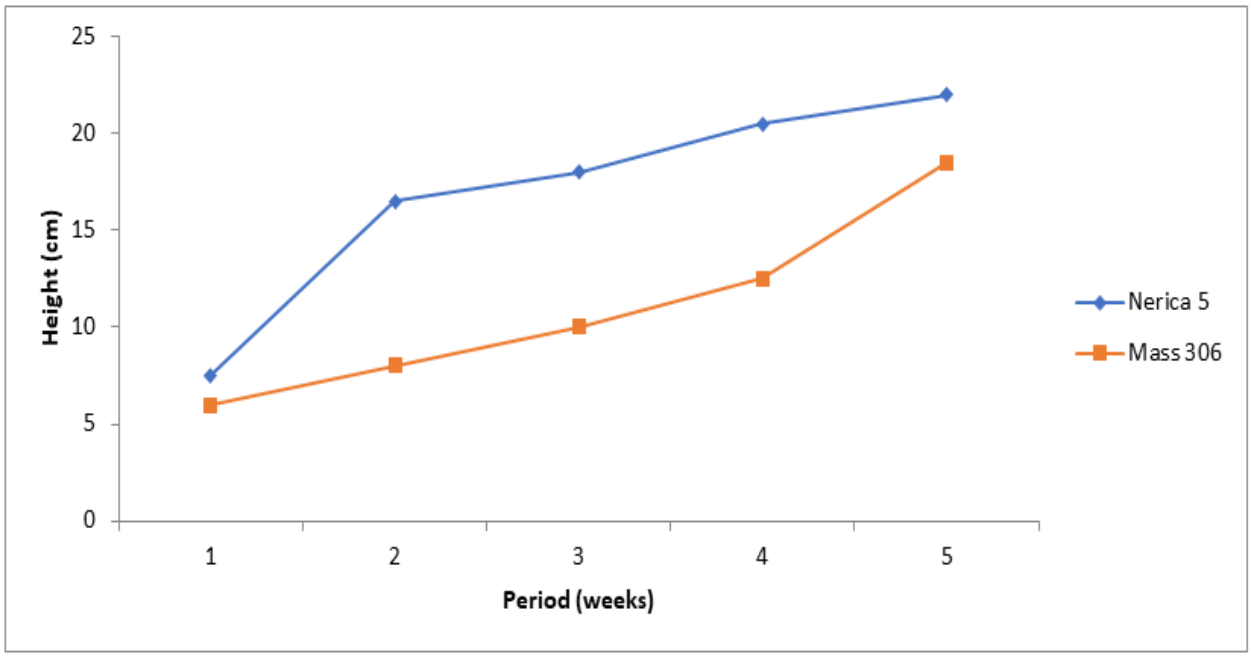

Fig 1 The Plant Height of Oryza *sativa* (Nerica5) and *Oryza glabberima* (Mass306) Showing five (5) Weeks of Growth

Table 2 Summary of Result on Biochemical Characterization of the Five (5) Rhizosphere Soil Bacterial Isolates Showing Gram Test and Morphology. + (Positive), - (Negative)

Isolate code	Gram test	Morphology	Colour on plate	Catalase test	D-glucose	Sucrose	Lactose	Indole	Probable isolate
N51	+	Rods	Creamy	+	+	+	+	-	*Bacillus* spp.
M3061	-	Rods	Blue-green	+	+	-	-	-	*Pseudomonas* spp.
N52	-	Rods	White	+	+	-	-	-	*Azotobacter* spp.
M3062	-	Rods	Creamy	+	+	+	+	-	*Bacillus* spp.
S1	-	Rods	Green	+	+	-	-	-	*Pseudomonas* spp.

IV. DISCUSSION

It was observed that *Oryza sativa* (Nerica5) grew more rapidly than *Oryza glabberima* (Mass306) as shown in Figure 1, because it is an improved species which can grow on most paddy soil environments and due to the utilization of available nutrients and minerals in the soil with the aid of the microorganisms present [7][8].

Table 2 reports that every isolated bacteria tested positive for Catalase. This is a crucial component that the bacteria need in order to proliferate without endangering cells. This is in concordance with [9] who emphasised that certain bacteria have flavoproteins that reduce oxygen and produce superoxide and hydrogen peroxide, which are highly toxic to cells because they are strong oxidisers and can quickly destroy cellular components. The isolated rhizosphere soil bacteria were found to be Catalase positive, indicating that they have the ability to defend against this harmful influence. All isolates fermented the D-glucose, while three isolates tested negative for lactose and sucrose. All isolates tested negative for indole. This is due to the lack of the enzyme tryptophanase, to produce indole from the aminoacid tryptophan.

These bacterial isolates' morphological and biochemical traits (Table 2) indicated a close relationship to other members of the Bacillus genus. This supports the findings of [10], which indicated that the genus Bacillus comprises rod-shaped, Gram-positive bacteria. Primarily, *Bacillus* cultures are Gram-positive by nature. *Bacillus* species are generally catalase positive. Moreover, *Bacillus* tests negative for indole and positive for D-glucose, sucrose, and lactose (Table 2). The rice rhizosphere soil bacterial isolates' biochemical characterisation process yielded results that were in line with the previously reported findings among rhizosphere bacteria. *Bacillus* species are important rhizosphere soil bacteria that play a significant role in promoting rice plant growth by fixing atmospheric nitrogen and converting it into a form that can be utilised by rice plants, this enhancing plant health, and contributing to biofertilization [11]. *Bacillus* strains such as *Bacillus subtilis* and *Bacillus amyloliquefaciens*, have been shown to exhibit strong phosphate-solubilising activity on inorganic phosphates in the soil, thereby making phosphorus more available to rice plants [12]. It has also been found to enhance plant health as a bio-control agent and bio-pesticide by producing antimicrobial compounds such as antibiotics and lipopeptides which can suppress the growth of pathogenic microorganisms in the rhizosphere [13]. Certain

Bacillus strains, like *Bacillus pumilus*, have been used as bio-fertilizers in rice cultivation due to their ability to fix nitrogen and promote rice plant growth [12].

The morphological and biochemical characteristics of some of the isolates (Table 2) indicated that they are closely related to the genus Pseudomonades *which* are described as aerobic, rod shaped, Gram-negative bacteria with one or more flagella providing motility. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonis e a wide range of niches [14]. The plant growth promoting *Pseudomonas fluorescens* has been found to be responsible for phosphate solubilisation, production of plant growth-promoting hormones and nitrogen fixation [15]. According to [16], *Pseudomonas* strains produce compounds like 2,4-diacetylphloroglucinol (DAPG) that can stimulate plant growth and act as natural fertilizers. These compounds contribute to improved rice plant health and yield. This study identified isolates M3061 and S1 as *Pseudomonas* spp. These findings agree with the literature [14].

Azotobacter spp. is one of the isolates present in the rhizosphere of N52 (Table 2). It is a genus of free-living aerobic, Gram-negative bacteria that are known for their ability to fix atmospheric nitrogen and convert it into ammonia, which can be used by plants as a source of nitrogen. In a study conducted by [17], it was found that *Azotobacter* sp. was able to fix atmospheric nitrogen and produce plant growth-promoting compounds such as indole acetic acid, which can stimulate plant growth and development. The use of *Azotobacter* sp. as a biofertiliser can reduce the need for synthetic or chemical nitrogen fertilisers, which have environmental drawbacks such as eutrophication and algal bloom. This sustainable approach promotes eco-friendly rice cultivation practices [18].

From this study, the isolation of rhizosphere soil bacteria from the rice varieties revealed that *Oryza sativa* harboured more rhizospheric bacteria than *Oryza glabberima*. The phenotypic characteristics of the isolates clustered them into three different genera namely *Pseudomonas*, *Bacillus and Azotobacter*. This is in agreement with the biochemical characteristics reported by [17].

V. CONCLUSION

This study revealed that the presence and number of rhizosphere soil bacteria in the rhizosphere region of the rice plants account for the growth of the plant, thereby improving and increasing plant yield. This further emphasizes the significance of rhizhosphere bacteria to rice growth and productivity. Further studies using molecular characterisation of the isolates should be done to establish any importance other than their role in agriculture. This study showed that there are more rhizosphere bacteria in the rhizosphere region of *Oryza sativa* than *Oryza glabberima*, because it is an improved variety and enhances the utilization of available nutrients and minerals by microbial interaction which enable rapid growth of the plant. Therefore, *Oryza sativa* should be the rice variety of choice to be cultivated due to its high yield and large-scale agricultural production. It is evident from the study that these rhizobacteria could be used as biofertilisers in rice cultivation in Nigeria to promote plant to enhance the economic importance of rice, which forms a major part of the diets of most Nigerians.

ACKNOWLEDGEMENTS

We acknowledge the Department of Microbiology, Faculty of Science, Evangel University, Akaeze, Abakaliki, Nigeria, the laboratory technologists and the rice farmers Kpirikpiri, Ebonyi State, for their assistance in this research and specially, LASU-Humboldt Kolleg Conference for providing an International Conference as a platform for scientists to communicate their findings. We are eternally grateful to God for the grace upon all the researchers to accomplish this arduous task.

- *Funding*
  This research received no external funding.

- *Conflicts of Interest*
  The authors declare no conflict of interest.

REFERENCES

[1]. Ferris H., Venette R. C., Van der Meulen H. R., Lau S. S., and Sanchez-Moreno S. (2012). Molecular characterization of bacterial communities in rhizosphere soils from container-grown hardwood trees. Journal of Environmental Horticulture, 30(1), 12-21. https://doi.org/10.24266/0738-2898-30.1.12

[2]. Babalola O. O., Osir E. O. Sanni A. I., and Odunfa S. A. (2019). Rhizosphere microbial communities and their applications in plant growth enhancement: A review. *Journal of Plant Growth Regulation*, 8(4), 1113-1134. https://doi.org/10.1007/s00344-019-09910-

[3]. Barraquio W. L., Ladha J. K., Watanabe I., and Klingmuller W. (2019). Effect of rice cultivar on nitrogen-fixing bacterial populations in the rhizosphere. *Biology and Fertility of Soils*, 55(8), 829-835. https://doi.org/10.1007/s00374-019-01402-0.

[4]. Breidenbach, B., Pump, J., and Dumont, M. G. (2016). Microbial Community Structure in the Rhizosphere of Rice Plants. *Frontiers in Microbiology*, 6. https://doi.org/10.3389/fmicb.2015.01537.

[5]. Bartholomew, J.W. (1962). Variables influencing results and precise definition steps in gram staining as a means of standardizing the results obtained. *Stain Technology*, **37**:139-155.76.

[6]. Parte, A., Krieg, N. R., Ludwig, W., Whitman, W. B., Hedlund, B. P., Paster, B. J., Staley, J. T., Ward, N., & Brown, D. (Eds.). (2010). *Bergey's Manual of Systematic Bacteriology, Vol. 4* (2nd ed.). Springer. ISBN-13 : 978-0387950426.

[7]. Kobata T., Koguchi M., and Kanzaki M. (2019). Community Structure of root associated fungi in a primary subtropical evergreen forest in Japan. *Fungal Ecology*, 41, 239-246. https://doi.org/10.1016/j. funeco.2019.05.006.
[8]. Fujii K., and Horie R. (2021). Rhizosphere bacteria and plant growth promotion. *Microbes and Environments*, 36(1), ME20122. https://doi.org/10.1264/jsme2.ME20122.
[9]. Cappuccino, J.G. and Sherman, N. (2017). Microbiology: A Laboratory Manual (11th Edition) Pearson. ISBN-13: 978-0805325782. *Jaypee Brothers Medical Publishers* (P) Ltd, New Delhi.
[10]. Kamal S., Khan S. A., Maqbool Z., and Zaidi A. (2018). Plant growth promoting rhizobacteria and sustainable agriculture: A review. *Journal of Pure and Applied Microbiology*, 12(4), 1677-1688. https://doi.org/10.22207/JPAM.12.4.29.
[11]. Verma, S. and Ladha, J. K. (2019). Rhizobial Formulations for Enhanced N2 Fixation and Nitrogenase Activity in the Rice Rhizosphere. *Applied and Environmental Microbiology*, *85*(13), e00467-19.
[12]. Subramanian, P., Kim, K., Krishnamoorthy, R. and Mageswari, A. (2016). A New Fungus, *Trichoderma asperellum* T42 Reprograms Tobacco for Enhanced Nitrogen Utilization Efficiency. *Frontiers in Plant Science*, 7, 680.
[13]. Ongena, M. and Jacques, P. (2008). Bacillus Lipopeptides: Versatile Weapons for Plant Disease Biocontrol. *Trends in Microbiology, 16*(3), 115-125.
[14]. Madigan, M. and Martinko, J. (2018). Brock Biology of Microorganisms (15th ed.). *Prentice Hall. Benjamin-Cummings Publishing Company*. ISBN 9780321649638.
[15]. Spaepen, S. and Vanderleyden, J. (2011). Auxin and Plant-Microbe Interactions. *Cold Spring Harbor Perspectives in Biology, 3*(4), a001438.
[16]. Raaijmakers, J. M. and Mazzola, M. (2012). Diversity and Natural Functions of Antibiotics Produced by Beneficial and Plant Pathogenic Bacteria. *Annual Review of Phytopathology*, 50, 403-424.
[17]. Singh D., Yadav A., and Kumar V. (2019). Isolation and Chracterisation of plant growth promoting rhizobacteria from the rhizosphere of rice plants. *Journal of Soil Science and Plant Nutrition*, 19(4), 806-819.
[18]. Choudhary, D. K. and Johri, B. N. (2009). Interactions of Bacillus Spp. and Plants with Special Reference to Induced Systemic Resistance (ISR). *Microbiological Research, 164*(5), 493-513.