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Isolation and Identification of Nitrogen fixing Bacteria (Rhizobium) from Root Nodule of a Legume Plant in the Bori Garden soil, Rivers State, Nigeria

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ABSTRACT
The roots of common beans *(Phaseolus vulgaris*) were collected fresh by uprooting carefully from a garden soil in Bori, in Khana Local Government Area, Rivers State, Nigeria. The root nodules were prepared using the Gwyn and Handelsman,(1989) technique, and analyzed by the spread plate method upon Yeast Mannitol agar, for the Isolation and Identification of nitrogen fixing bacteria (Rhizobium), obtained from the leguminous plant. *Rhizobium* japonicum, *R.* leguminosarum and *R.aggregatum* were isolated and identified. *Rhizobium japonicum* and *Rhizobium leguminosarum* were the most regular isolates, whereas, *Rhizobium aggregatum* were scarcely isolated. The frequency of occurrence of the microbial colony forming units were in order of 6.7, 20, 26.7, 33.3 and 13 for the sample plates,T1, T2, T3, T4 and T5 as assay of root nodules formicrobial concentration of isolates were found, in the legume plant.

Introduction

Common beans plant *[Phaseolus vulgaris*] is an all important economic and ecological leguminous plant with good adaptability in hot and humid area, such as the Bori metropolis, located within the Niger Delta Basin of Nigeria. Farmers within the area are often apprehended with poor crop yield, following environmental pollution by industrial activities within the area, resulting to soil infertility (Uneka,et al., 2015). Alternating planting of legumes over the time had been the natural source of plant nutrient by farmers. Rhizospheric bacteria are those bacteria that prefer to live in closeness to the root, or upon the surface of roots of the legume plants. The Rhizobia species are entirely free living nitrogen fixers and stand for or represent a wide range of other relative micro-organisms, including Saprophytes living on host plant residues and Endophytes which live completely within host plants [Gothwal, et. al, 2007]. Fixed nitrogen is limited in most soil environments. Being that the main reserve of nitrogen in biosphere is the molecular or elemental nitrogen, which cannot be directly taken in by plants; it is therefore made available through the biological nitrogen fixation. A process that can only be developed by prokaryotes, known as Rhizobium [Claudine, et. al, 2009].

Particulate matters such as ash, hydrocarbons, photochemical, Oxidants and hydrogen sulphide gas (H2S), eventually constitute acidification of soil, depletion of soil nutrient with varying degree of negative effect on crop leading to High accumulation of heavy metals, stunted growth, defoliation of leaves, wrinkling and withering of leaves and premature ripening of fruits (Echem and Lipnee, 2013, Odjugo, 2002).

Nitrogen-fixing organisms are always active in the plant root zone soil. Plants that are therefore capable of releasing exudates usually exhibit high nitrogen fixation within the soil. Nitrogen is an all important nutrient required for plant growth. It is however true that certain tropical soil environment may be deficient in available nitrogen, and therefore short of nutrient. In ecosystems with such low nutrient inputs, and without any fertilizer or soil amendment by human, the nitrogen available to plants come from atmosphere or from biological nitrogen fixation by the Rhizobacteria, converting elemental nitrogen to plant usable form [Gothwal, et.al,2007; Bagali, 2012]. The property of symbiotic nitrogen fixing within root nodules of vascular plants is found in two major groups of bacteria which are not phyto-genetically related, namely Rhizobia and Frankia. Whereas Rhizobia associates essentially with leguminous plants, Frankia is associated with a wide range of plants from eight families [Claudine, et. al, 2009].

Legumes such as pear, beans, soya beans, alfalfa and clover help to feed the meat producing animals and humans. Legumes can grow well in poor soil where there is not enough fixed nitrogen to support other types of plants. After harvest, legume roots left in the soil decay, releasing organic nitrogen compound for uptake by plants in the next generation. Farmers take advantage of this natural fertilization to rotating leguminous crops with non leguminous. Nitrogen fixation needed for plant growth is of great importance to humans. This is because sustainable living is powered basically by plant. The better the crop yield, the better the human food source, and Rhizospheric bacteria are the farmer friendly bacteria which form baseline support to soil fertility through Nitrogen fixation.

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Material/Methodology

The roots of common beans (*Phaseolus vulgaris*) were collected fresh by uprooting carefully from garden soil in Bori town of Khana Local Government Area, Rivers State, Nigeria. Enough tap water was initially poured to moisten the base of the plant before it was pulled out. The roots were rinsed in tap water to remove loosely adhering soil particles before they were analyzed.

The method of Gwyn and Handlesman (1989), was adopted. By this method, 5 nodules were aseptically removed with sterile forcept and placed in a microfuge tube containing 1.3ml of glycerol. The microfuge tube was vigorously agitated for 1minute. Using a vortex mixer, the nodules were transferred to 15ml of sterile water for 1 minute. The nodules were finally placed individually in the wells of a sterile 96 well microtitre. Nodules were crushed individually with a flamed aluminum rod in wells containing 120ul of Yeast Mannitol broth. Sterile pipettes were used to collect samples from each well by which 0.1ml of bacterial suspensions were spread over surface of fresh plates of Yeast Mannitol agar, labeled T1 –T5 and incubated at 30oC within 48hours. Pure cultures were made from successive subcultures and used for both Gram's reaction and biochemical tests for purposes of bacterial identification.

Result/Discussion

Result of identification of isolates realized from root nodules of a Leguminous plant, the common beans *(Phaseolusvulgaris*), Heterotrophic count, Colony forming unit and frequency of occurrence is as illustrated in Tables 1 and 2 respectively.

Table 1. Biochemical, Motility and Gram'sReaction Tests for identification of Bacterial isolates.

Code Gram's Morph MotilIndole Methylred Citrate Urease Catalas Isolate

T1-Bacilli+ --+ + +*Rhizobium japonicum*

T2 -Bacilli+---++ *Rhizobium leguminosarum*

T3 -Bacilli+ -+ + + -*Rhizobium Aggregatum*

T4 -Bacilli + --+ + +*Rhizobium japonicum*

T5-Bacilli+ ---+ + *Rhizobium Leguminosarum*

KEY:

- = Negative or No reaction
+ = Positive or Reaction
Bacilli = Rod shaped
Morph = Morphology
Motil = Motility
Catalas = Catalase

Table 2.Heterotrophic count, Colony forming units and frequency of bacterial occurrence.

Plate code	Viable count	Colony forming unit(cfu/ml)	Frequency
T1		101.0 x 1026.7	
T2	30	3.0 x 10220	
T3	40	4.0 x 10226.7	
T4	50	5.0x 10233.3	
T5	20	2.0 x 10213	

Result revealed the presence of three species of Rhizobia, viz; *Rhizobium Japonicum, Rhizobium leguminosarum and Rhizobiumaggregatum* which also had then least frequency of occurrence. Research showed that nodule with plate code T4 gave growth to the highest bacterial population indicating good presence of Rhizospheric bacteria, whereas nodule with plate code T1 gave the least bacterial growth, showing less presence of Rhizospheric bacteria. It was also observed that all of the bacterial isolates were Indole negative, motile and Gram's negative bacilli.

Conclusion and Recommendation

For so many years ago, a limited number of bacterial species were believed to be nitrogen fixers, but in the last 30years, Nitrogen fixation has been shown to be a property with representatives in most of the Phyla of bacteria and also in methano-genic Archaea. The property of symbiotically fixing Nitrogen within nodules of vascular plants (Claudine, et. all. 2009). Leguminous plants are nodule forming plants and harbor Rhizobial bacteria. Result agreed with this fact because the common beans plant *(Phaseolus vulgaris*) had nodules, and from the nodules viable colonies of *Rhizobium japonicum, Rhizobium leguminosarum* and *Rhizobium aggregatum* were recovered. This became a proof that in the Bori area of Rivers State, Nigeria, such Rhizobia could be realized in legumes plant nodules in symbiotic relationship.

Since it is obvious that Rhizobial organisms living within the root nodules of leguminous plants play key role in soil fertility through Nitrogen fixation, it is my recommendation that leguminous plants be planted within or alternatively with the seasonal farm crops to support soil bio-fertility.

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