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Molecular Characterization of Root-Nodule Bacteria from Soybean (*Glycine Max L. Merrill*) Rhizosphere in Three Agro-Ecological Systems in Nigeria

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Abstract

The worldwide increase in population continues to threaten the sustainability of agricultural systems since agricultural output must be optimized to meet the global rise in food demand. This study examined the symbiotic compatibility between indigenous rhizobia population and soybean plant. Soil samples 5kg each were randomly collected from agricultural fields in a total of nine different local governments from Southwest and North central zones of Nigeria at a depth of 0-30cm using sterile soil auger (5cm diameter). The control soil samples were obtained from Badagry beach (*a location with no previous history of soybean cultivation*). The indigenous microbial population were isolated using serial dilution technique and they were cultured using nutrient agar (NA), yeast-extract mannitol salt agar (YEMA) and sabouraud dextrose agar (SDA) incubated at 26±2^{o}C for 48 hours and 3-5 days respectively. Soybean seeds were sown in each of twenty pots aseptically. The mean pH of soil samples from this study was 6.0 (slightly acidic) while NO_3 - N and NH_4 - N was relatively low. Molecular characterization of these rhizobia species as well as their genetic diversity in soil were investigated. A total of nine different rhizobia species were isolated from all the soil samples and three of the nine isolates were randomly selected for molecular characterization using 16S rRNA gene sequencing method. The accession number for the selected organisms were confirmed as follows; 0J7 (Accession number: MN0677451.1), *Stenotrophomonas maltophilia* OJ8 (Accession number: MF767518.1) *Agrobacterium tumefaciens*, OJ9 (Accession number: NR_181268.1) *Rhizobium rhizolycopersici*. The other isolates were characterized as *Bradyrhizobium japonicum*.

Sustainability Statement

The observations and inferences from this study would further strengthen food security (UN SDG2: Zero hunger) and healthy land (UN SDG15: Life on land) when fully integrated into sustainable agricultural practice.

Keywords: Bradyrhizobium; DNA extraction; N_2 – fixation; Nodulation; Soil fertility; 16S rRNA

1. Introduction

The worldwide increase in population and decreasing productivity due to excessive cultivation of farmlands continues to threaten the sustainability of agricultural systems since agricultural output must be optimized to meet the global rise in food demand [1]. Soybean (*Glycine max L. Merrill*) *is* among the world's important crops due to its high-quality plant-based protein and oil content [2]. Soybean roduction in Africa is constrained by several factors, including the lack of compatible rhizobia in many African soils, promiscuous soybean varieties which nodulate with indigenous soil rhizobia have been bred to overcome the nodulation problem [3, 4]. However, there is currently insufficient information on the diversity of indigenous rhizobial populations in the soils of southwest and north central agro-ecological zones of Nigeria. Once a field has been inoculated with rhizobia and cropped to soybean, a large population of soil rhizobia (*Bradyrhizobium* sp.) resides in that field in subsequent years [5]. The promiscuous nodulating group of soybean cultivars form nodules and fix atmospheric N_2 with native soil rhizobia, hence farmers seldom inoculate soybean with *B. japonicum* in many agroecosystems in Africa. The residual effect of introduction of *Bradyrhizobium* inoculants into farmlands resulted in enhanced nodulation, grain yield and selected soil health indicators after inoculation [6-8]. In order to feed the expected 2.5 billion people by 2050, Africa will need to double its agricultural production [1]. The persistence of inoculants from year to year in soybean cultivation and the residual benefits in soybean production made its adoption in sub-Saharan Africa as a means of soil fertility maintenance necessary [5, 6]. *Bradyrhizobium* is the major symbiont leading to an effective symbiosis with soybean [9]. The legume/ *Rhizobium* symbiosis has several advantages including improved agricultural productivity, maintenance and restoration of soil fertility, economy of expensive fertilizers and limitation of groundwater pollution by nitrates playing therefore a significant ecological and economical function [8, 10]. Soybean (*Glycine max L. Merrill*) is one of the prolific nitrogen - fixing leguminous crop with different groups of root-nodule bacteria as its microsymbiont [4, 5]. However, since soybean can be nodulated by cross - nodulating rhizobia, this necessitated the search for effective and compatible indigenous rhizobia isolates as well as effective cross-nodulating rhizobia species. The study of rhizobia diversity is a valuable biological resource and attempts to find bacterial strains with interesting features to maximize agricultural productivity, the discovery of new strains of rhizobia and consequently select efficient combinations of *Rhizobium*-legume association [11]. Soybeans are a rich source of proteins, vitamins, minerals, low saturated fats and fibres, the presence of these many biologically active compounds made the use of soybean in the pharmaceutical industry and other dietary products an added nutritional benefit. Soybean is cultivated as the major oil seed crop which have lots of importance and health benefits [12]. Despite the growing cultivation of soybean in Africa at large, there is very limited information in the literature on the type of rhizobia nodulating this legume crop, about 109 980 000 ha of land are under soybean production worldwide [13]. Despite the economic importance of soybean as a major source of protein and oil, the low yield of soybean in Nigeria is partly due to the inadequate knowledge of the indigenous microsymbionts that associate with soybean plants to fix atmospheric nitrogen. Therefore, the lack of molecular characterization of these microsymbionts from different ecological zones in Nigeria makes it difficult to understand their diversity and potential benefits to soybean production, thereby, hindering the development of effective strategies for improving soybean yield in Nigeria.

The objectives of the current study were to evaluate; (i)for the presence of specific microsymbionts for the promiscuous soybean cultivar and its growth in tropical soils from two different agroecological regions in Nigeria (ii) to determine the symbiotic compatibility between indigenous rhizobia population and legume host.

2. Materials and Methods

2.1. Collection of Soil Samples

Soil samples were randomly collected at a depth of 0 - 30cm from agricultural fields in Alimosho local government Area (LGA), Ikorodu LGA (Lagos State), Ejigbo LGA (Osun state), Akinyele LGA (Oyo State), Ado-Odo/Ota LGA (Ogun State), Offa LGA (Kwara State), Batsari LGA (Katsina State) and Soba LGA (Kaduna state) using sterile auger (5cm diameter). Thereafter, composite soil samples were made from each of the nine different local government areas and transported to the laboratory in sterile polythene bags. The soils were later air-dried at room temperature. Soil samples with no previous history of being cultivated with legumes were obtained from Badagry beach tightly wrapped with Aluminium foil paper and sterilized at 121℃ for 15mins to serve as the control. The composite soil samples from each of the eight different locations were then used for the pot experiment in the greenhouse.

2.1.1. SEEDS

Legumes seeds were obtained from the Genetic Resources Unit, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2.1.2. Media and Plant Nutrients

Yeast-extract mannitol agar (YEMA), Nutrient agar (Himedia Laboratories Pvt. Ltd. India), Sabouraud dextrose agar (Biomark Laboratory, India), Jensen's Nutrient solution and potassium nitrate (KNO_3) solution at 0.05% N were used [14].

2.2. Chemicals

The different chemicals of analytical grade used for this study include: KNO_3, $NaNO_3$, $KHSO_4$ molecular biology reagents (Zymo research corp., U.S.A.).

2.3. Scarification of Seed

Forty-four viable seeds of *Glycine max* obtained from the genetic resources unit, IITA Ibadan were treated with 95% ethanol in a beaker. The beaker was shaken for 5 minutes before being drained away. The seeds were then soaked with 3% hydrogen peroxide for four minutes before the seeds were drained of the hydrogen peroxide. The seeds were rinsed in several changes of sterile water inside the beaker, they were left to imbibe in the water for 24 hours in a refrigerator. Then, the seeds were sown aseptically in pots filled with the composite soil samples [14].

2.4. Soil Analysis Procedures

2.4.1. Preparation of Soil Samples

The composite soil samples from each location was passed through a 6-mm mesh sieve and mix thoroughly [15].

Determination of physic-chemical properties of soil samples

The composite soil samples obtained were taken to the laboratory for proximate analysis to determine pH, Nitrogen content, NO_3^-, PO_4^{-3}, SO_4^{2-}, Cl^-, and NH_4^+ Organic C, NO_3-N, NH_4^+- N, dissolved in the K_2SO_4 extracts were determined colorimetrically using automated equipment as described by Voroney, *et al.* [16]; Bareither, *et al.* [17].

2.5. Determination of Heterotrophic Microbial Population in Composite Soil Samples

Serial dilution technique was used to determine the heterotrophic microbial population as well as the indigenous rhizobia population in each of the composite soil samples using Nutrient agar, sabouraud dextrose agar and YEMA [18, 19].

2.6. Nodulation Experiment

The composite soil samples were tested with scarified soybean seeds to evaluate their capacity to nodulate with promiscuous native rhizobia population in the soils. Twenty plastic pots of 20mm diameter were used to cultivate the legume species. The control pots were two pairs with one pair watered with 0.05% KNO_3 and the other pair watered with Jensen nutrient solution while the tests pots (sixteen pots) received sterile water (Vincent,1970). A total of four control soil samples were used, this was arranged in a randomized complete block fashion in the greenhouse. Three seeds of soybeans were sown aseptically in each pot and later thinned to two plants per pot with sterile forceps 2 weeks after planting (WAP). The experiment was monitored for 12 weeks, thereafter the plant shoot and root materials were harvested with sterile surgical blades and then oven-dried at 65℃ for 48h. Roots were carefully removed and examined for nodulation. Fresh nodules were counted, cleaned of soil particles and then used for strain identification [19].

2.7. Nodule-typing on YEMA

Harvested nodules were typed on YEMA supplemented with 0.0005% bromothymol blue (BTB) and incubated at 26±2℃ following aseptic procedures as described by Somasegaran and Hoben [20], Ojo-Omoniyi, *et al.* [21]. Thereafter, pure cultures of bradyrhizobia strains were kept on YEMA agar slants for molecular analysis.

2.8. Morphological and Biochemical Characterization of Soybean Microsymbiont

The morphological and biochemical characterization of rhizobia from harvested nodules of *G. max L. merrill* were done following the methods described by Gerhardt, *et al.* [18]; Somasegaran and Hoben [20].

2.9. DNA Extraction and Amplification of the I6S rRNA Gene

2.9.1. DNA Extraction

A measurement of 80mg (wet weight) of bacterial cell that had been re-suspended in up to 200µl of deionized water to a ZR BashingBead™ Lysis Tube was obtained. It was secure in a bead beater fitted with a 2.0ml tube holder assembly (Scientific Industries' Disruptor Genie™, Cat. No. S6001-2 from Zymo Research Corp.) and processed at maximum speed for 5 minutes. The ZR BashingBead™ Lysis Tube was centrifuged in a micro-centrifuge at ≥10,000 x g for 1minute. Up to 400µl supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 rpm (7,000 x g) for 1 minute. The base of the Zymo-Spin™ IV Spin Filter was snapped off prior to use. 1,200µl of Bacterial DNA Binding Buffer was added to the filtrate in the collection tube of step four. Thereafter, 800µl of the mixture from step five was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x g for 1minute. The flow through was discarded from the Collection Tube and step six was repeated. Then, 200µl DNA Per-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 x g for 1minute. Thereafter, 500µl Fungal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1minute. The Zymo-Spin™ IIC Column was transferred to a clean 1.5ml micro-centrifuge tube and 100µl DNA Elution Buffer

was added directly to the column matrix. It was then centrifuged at 10,000 x g for 30seconds to elute the DNA. The bacterial DNA were extracted following Zymo research protocols [22-24].

2.10. PCR Amplification of the ITS/16S rRNA Gene and Sequencing

Polymerase chain reaction (PCR) was carried out to amplify the ITS gene of the fungal isolates using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction was carried out using the Solis Biodyne 5× HOT FIREPol Blend Master mix. The bacterial isolates 16S rRNA gene was amplified following the protocols of Qiagen [24].

PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5× concentration to 1× concentration containing 1× Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 25pMol of each primer (BIOMERS, Germany) and 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne). Additional Taq DNA polymerase was incorporated into the reaction mixture to make a final concentration of 2.5 units of Taq DNA polymerase, Proof reading Enzyme, 2µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series U.S.A.) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 58°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. [22, 23, 25].

2.11. 16S rRNA / ITS Analysis

PCR amplification of the 16S rRNA gene was performed using the following primers:
Forward Primer (with T7 tail):
TAATACGACTCACTATAGGGAGAGTTTGATCCTGGCTCAG
Reverse Primer: GYTACCTTGTTACGACTT
PCR amplification of the ITS gene was performed using the following primers:
Forward primer (ITS1): TCCGTAGGTGAACCTGCGG
Reverse primer (ITS4): TCCTCCGCTTATTGATATGC
PCR is performed using the amplification program:
96℃ (5 min), followed by 30 cycles of 96℃ (15 sec.), 52℃ (15 sec.), 72℃ (90 sec.).
PCR completed with 5 min incubation at 72℃.
Approximately, 5µl aliquot from each PCR reaction was run on 1% TAE Agarose gel to confirm positive PCR outcome.
PCR products are treated with ExoSAP and are sequenced (Sanger Sequencing)
[22, 24].
The sequence information was analyzed using NCBI Blast.
(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch)

2.12. Sequencing

The extracted DNA was subjected to amplification with a thermal cycler (Helena Biosciences, Sunderland, United Kingdom) and the primers listed in Table 3 were used. All the primers, synthesized by Sigma Aldrich House, Suffolk, United Kingdom, were used in four sets of PCR procedures [23, 24].

2.13. Analysis

In order to identify organisms, DNA sequence data generated from this study was blasted against
ITS/16S rRNA type strain database on NCBI. Threshold for identification was set according to Wang, *et al.* [26] with modification to accommodate species with high congeneric sequence divergence range. Phylogeny was constructed to support the identification by ITS sequence using Fast Minimum Evolution algorithm as deployed on NCBI and visualized on MEGA11 [27].

3. Results

The soils from Kaduna and Katsina (pH 5.4 and 5.2 respectively) were the most acidic and they had the highest PO_4^{-3} concentrations. However, soybean plant was not established in the soil from the two locations. Although, organic C was highest in Ikorodu soil followed by Katsina soil. Meanwhile, the organic matter content of the soils from these two locations (28% and 19% respectively) suggested that they were the most fertile in spite of being from two different agro-ecological zones. Contrary to expectations, soybean failed to be established in Katsina soil despite having low NO_3-N and NH_4-N (Table 1). Low NO_3-N and NH_4-N has been reported as a trigger for inducing N_2 - fixation in legume/Rhizobium symbiosis. It thus suggested that the presence of favourable edaphic factors in an agro-ecological zone alone does not influence the symbiotic relationship between legume and its microsymbiont.

Table-1. Selected Physic-Chemical Characteristics of the Composite Soil Samples

Parameters	Control soil	Ikorodu	Osun	Kaduna	Alimosho	Katsina	Kwara	Oyo	Ogun
pH H_2O	6.4	5.8	6.2	5.4	6.1	5.2	5.7	6.5	6.8
pH Ca_2Cl_2	5.1	3.9	5.0	4.1	5.1	3.8	4.3	6.0	5.2
PO_4 (mg/kg)	1.43	1.96	1.66	2.50	1.99	1.73	1.69	1.70	1.81
TOC (%)	5.10	23.80	10.20	10.20	11.90	16.15	9.75	13.6	11.90
TOM (%)	6.0	28.0	12.0	12.0	14.0	19.0	13.5	16.0	14.0
NO_3 (mg/kg)	0.27	0.38	0.22	0.55	0.24	0.24	0.35	0.45	5.33
NH_4 (mg/kg)	0.02	0.01	0.03	0.01	0.04	0.03	0.02	0.03	0.04

Key:
TOC: Total Organic Carbon
TOM: Total Organic Matter

Table-2. Analysis of Variance (ANOVA) for Selected Physic-Chemical Characteristics of the Composite Soil Samples

Source		DF	Mean Square	F	Sig.
Soil	Hypothesis	8	17.153	2.072	0.047
	Error	48	8.278		
Parameters	Hypothesis	6	304.562	36.790	0.001
	Error	48	8.278		

The result presented in Table 2 showed that there existed a significant difference in both the soil and the parameter under investigation since the p-values for both the soil and parameters (0.047 and 0.001 respectively) were less than 0.05 level of significance. This suggested that the soil samples across the eight locations are significantly different from that of the control soil which attest to the fact that they are not the same. Also, the concentration of each parameter across the soil sample locations are not the same as well.

3.1. Colonial Morphology

Pure cultures of the isolated rhizobia were observed on yeast extract mannitol agar (YEMA) after 48 hours of incubation at 26±2^{o}C which has the morphological characteristics shown in Table 3.

Table-3. Colonial morphology of isolates from the sampled locations.

Isolate	Colour	Surface	Colony size	Cell shape	Optical characteristics
Badagry	C	D	SL	R	O
Kwara	T	S	M	R	TR
Osun	LC	D	SL	R	O
Ogun	Y	S	M	R	TR
Oyo	T	S	M	R	TR
Ikorodu	C	D	SL	R	O
Alimosho	LC	D	SL	CC	O
Katsina	T	S	M	R	TR
Kaduna	Y	D	SL	CC	O

KEYS:
CC*= Cocci,* ***C****= cream,* ***D****= Dull,* ***LC****= Light cream,* ***M****= Moderate,* ***O****= Opaque,* ***R****=Rod,* ***SL****= Small,* ***S****= Smooth,* ***T****= Transparent,* ***TR****= Translucent.* ***Y****= yellow*

3.2. Molecular Identification Test

3.2.1. 16S rRNA Gene Analysis

The extracted DNA sequence data generated from this research was blasted against 16S rRNA type strain database on NCBI GenBank and the following organisms were identified;

Table-4. Molecular identity of test isolates using Basic Local Alignment Search Tool (BLAST).

Isolates code	Predicted organism	Percentage ID	GenBank Accession
OJ7	*Stenotrophomonas maltophilia*	98.93%	MN067745.1
OJ8	*Agrobacterium tumefaciens*	98.25%	MF767518.1
OJ9	*Rhizobium rhizolycopersici*	98.41%	NR_181268.1

KEY : *OJ7(Lagos State), OJ8(Oyo State), OJ9(Osun State).*

The agronomic features evaluated 12 Weeks after planting (WAP) were the mean shoot and root biomass, nodules number and percentage moisture content. The agronomic features of the legume host were greatest with Oyo soil samples, similarly nodulation with specific soybean microsymbiont occurred more with soils from Oyo. This is evidenced by the nodule number and shoot dry weight (Table 5).

Table-5. Mean shoot biomass, nodule number and percentage moisture content of *Glycine max* per pot at 12 (WAP).

Soil	shoot Fresh weight	Dry weight	Nodules number	Moisture content
OSUN	25.0	5.8	50	76.8%
OGUN	10.6	2.2	20	79.3%
LAGOS IKD	17.1	3.0	52	82.5%
OYO 1	21.1	8.7	75	58.7%
LAGOS ALM	31.5	6.0	100	80.9%
OYO 2	32.8	6.7	110	79.6%

Table-6. Analysis of Variance (ANOVA) for Mean shoot biomass, nodule number and percentage moisture content of *Glycine max* per pot at 12 (WAP).

Source		Df	Mean Square	F	Sig.
Soil	Hypothesis	5	479.097	1.749	0.184
	Error	15	273.895[a]		
Parameter	Hypothesis	3	7077.205	25.839	0.001
	Error	15	273.895[a]		

Testing for the significance of the mean biomass, nodule number and percentage moisture at 12 WAP, there existed a significant difference among parameters of fresh weight, dry weight, nodule number and moisture content that were under investigation (Table 6) since the p-values of 0.001 is less than 0.05 level of significance while for soil locations, the result of samples across the different locations were not significantly different from one another at p-values of 0.184.

The biomass development in the root was greatest with Alimosho soil in Lagos followed by Oyo soil. The establishment of soybean was relatively more successful in the southwest agroecological zone than in the northcentral zone. This is evidenced by both the nodulation and biomass production (Table 7).

Table-7. Mean Root biomass, nodule number and percentage moisture content of *Glycine max* per pot at 12 weeks after planting (WAP).

Soil	Fresh weight	Dry weight	Nodules number	Moisture content
OSUN	4.9	1.7	50	65.3%
OGUN	1.6	0.4	20	75%
LAGOS IKD	5.5	1.6	25	70.9%
OYO 1	7.2	2.3	75	68.1%
OYO 2	8.2	2.2	110	73.2%
LAGOS ALM	7.8	2.5	100	68%

Table-8. Analysis of Variance (ANOVA) Mean Root biomass, nodule number and percentage moisture content of *Glycine max* per pot at 12 weeks after planting (WAP).

Source		df	Mean Square	F	Sig.
Soil	Hypothesis	5	401.870	1.143	0.381
	Error	15	351.522[a]		
Parameter	Hypothesis	3	7970.863	22.675	0.001
	Error	15	351.522[a]		

The mean root biomass, nodule number and percentage moisture at 12 WAP, showed that there existed a significant difference among parameters of fresh weight, dry weight, nodules number and moisture content that were under investigation since the p-values of 0.001 was less than 0.05 level of significance while the result of soil samples across the different locations established that there was no significant different among the soil samples across the six (6) locations with p-values of 0.381 (Table 8). Soybean plant was not established in Kaduna and Katsina soils in spite of the presence of indigenous rhizobia populations and the level of fertility in the soil from the two locations.

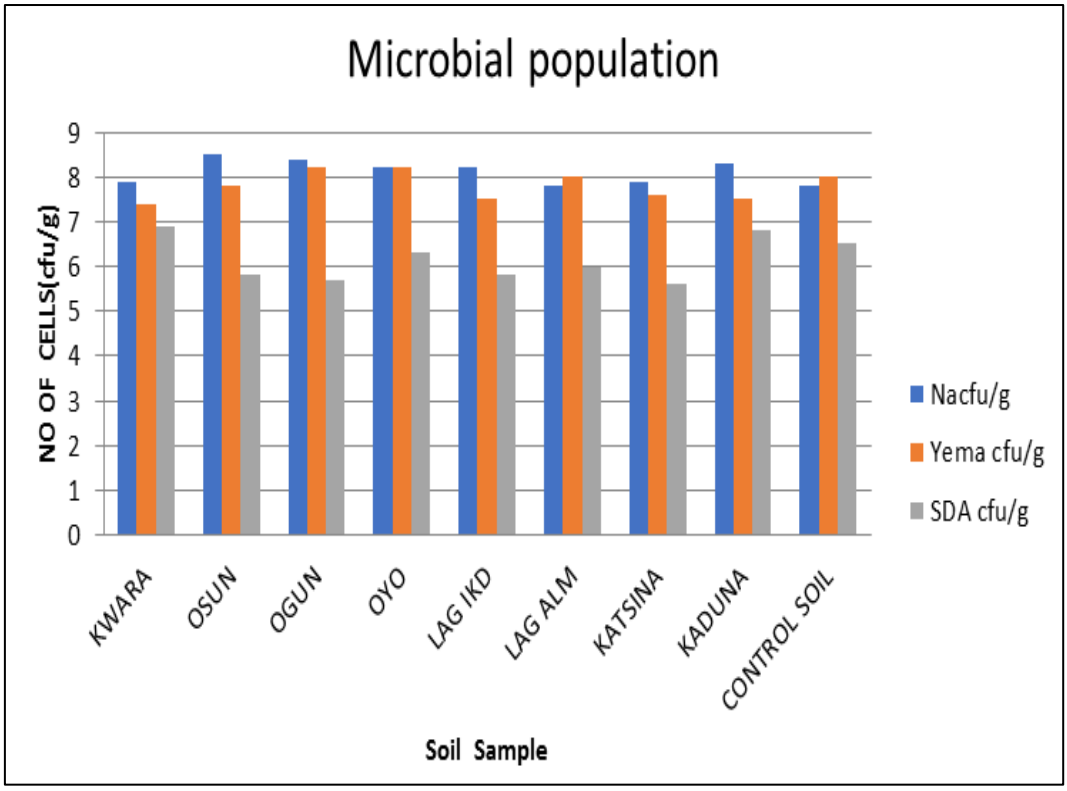

Fig-1. Enumeration of microbial population in the soil samples.

Enumeration of microbial population in the soil samples using the dilution factor of $10^{-5.}$ as represented in the chart. Osun, Ogun, and Kaduna soil had the highest heterotrophic bacterial population while Oyo and Ogun state soil samples relatively contained the highest population of rhizobia cells as detected with yeast extract mannitol salt agar (YEMA).

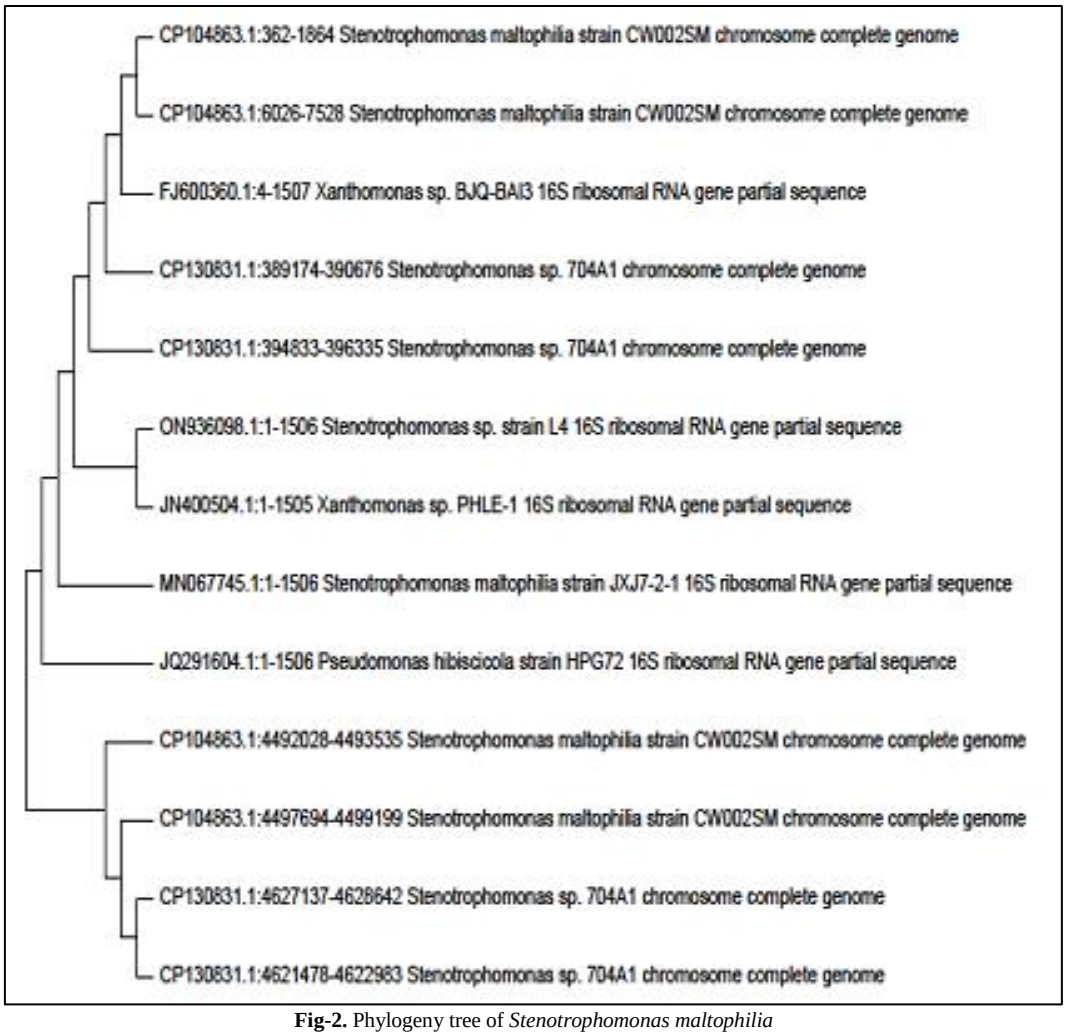

Fig-2. Phylogeny tree of *Stenotrophomonas maltophilia*

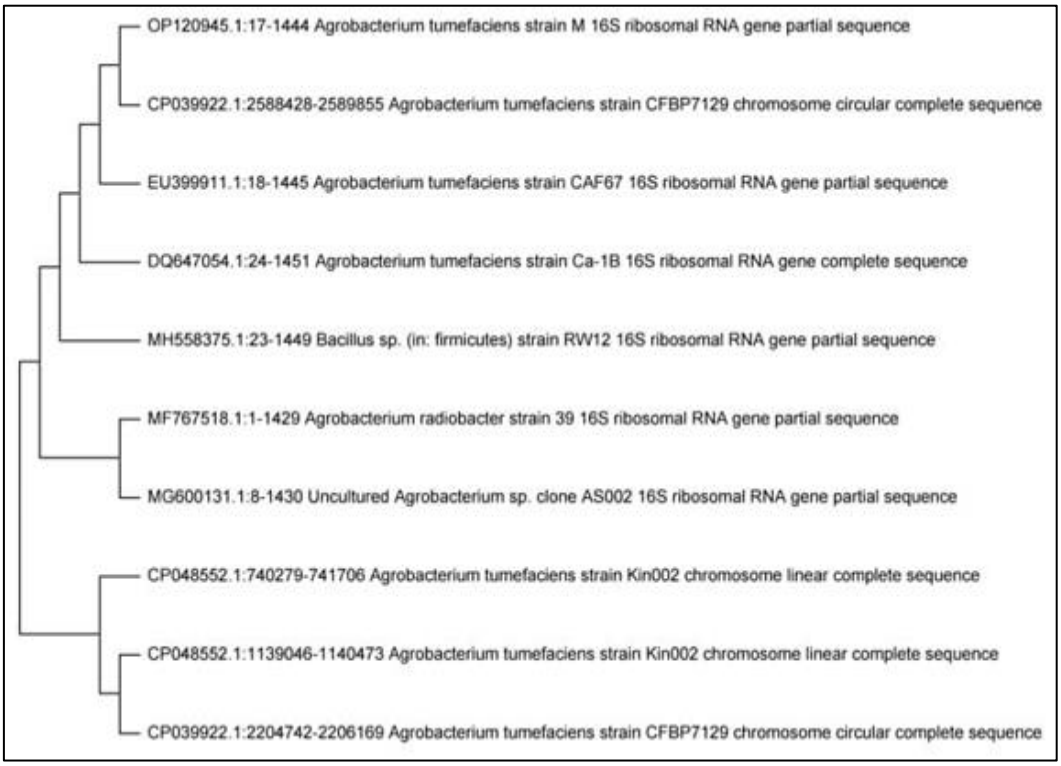

Fig-3. Phylogeny tree of *Agrobacterium tumefaciens*

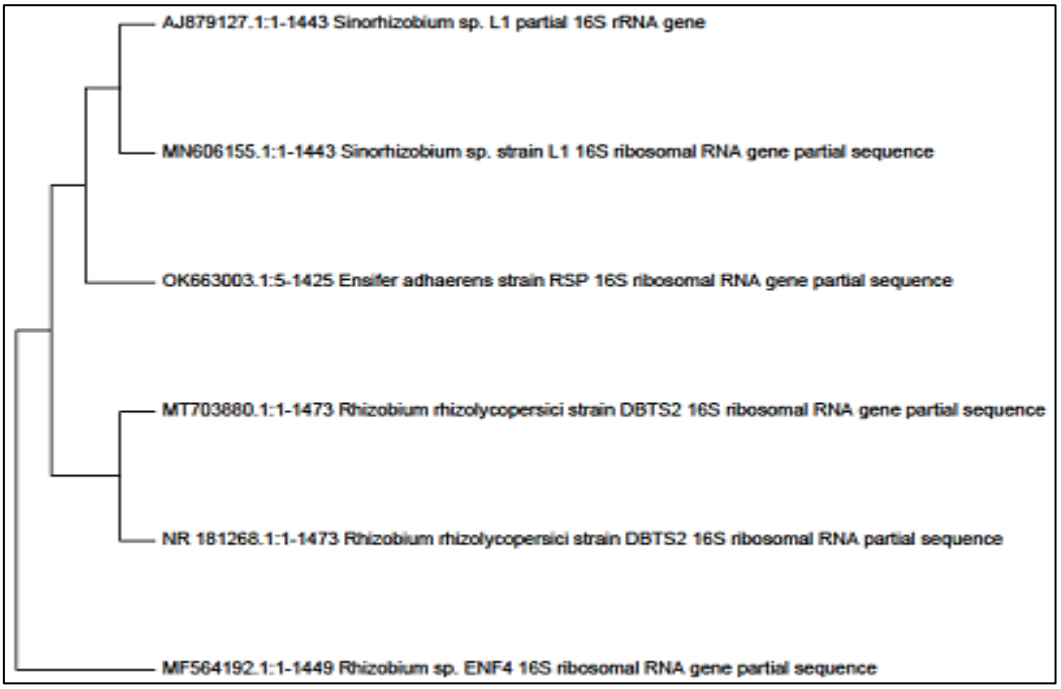

Fig-4. Phylogeny tree of Rhizobium rhizolycopersici strain DBTS2

Discussion

Nitrogen is an essential component of all amino acids and nucleic acids, thus making it an important plant nutrient. Although, the atmosphere consists of 78.1% nitrogen, plants cannot use it unless it is converted into NH_3 which is the form utilizable by plants and this makes the use of chemical nitrogen fertilizer unnecessary in sustainable agriculture [28]. One of the strategies through which N_2 is incorporated into plant is the formation of root nodules in symbiotic legumes which involves a complex molecular signalling between the legume host and the microsymbiont [28, 29]. The physiochemical properties of the soil samples analysed prior to Soybean cultivation in the Greenhouse (Table 1), provided evidence of the presence of phosphate, nitrate, ammonium and total organic matter (TOM) in the soil which subsequently revealed the presence of minimal fertility in the investigated soils, however, they have strong influence on the diversity of rhizobial strains within diverse agro-ecological regions [30]. The ability of a native rhizobia or an introduced rhizobia strain to survive or persist in the soil depends on cropping history and the health of the soil, especially soil moisture, texture, pH, and nutrients (available N, P, and K; soil organic C [SOC]; and total N) [31]. The detection of root-nodule bacteria in the cultivated soybean roots

corroborated the fact that promiscuous root-nodule bacteria capable of nodulating soybean were present in tropical soils investigated. These findings corroborated earlier report by Ojo-Omoniyi, *et al.* [21].

The efficiency of biological nitrogen fixation (BNF) may vary in different legumes as it depends on various biotic and abiotic components such as legume species, rhizobia efficiency, and soil physic-chemical properties, this affirmed the different observations in terms of evaluated agronomic features produced as a consequence of the legume/ *Rhizobium* symbiosis in soils from the two agro-ecological zones [7, 30]. This discovery agrees with the findings of Naamala, *et al.* [32]. The high cost of chemical fertilizers, inaccessibility and its potentials to cause environmental pollution among many other factors have limited the use of chemical N -fertilizers in agriculture, hence, the need for a more environment-friendly in-put for sustainable agricultural practice [33]. Rhizobia are particularly important to plants in nitrogen-deficient soils since low soil N triggers N_2 - fixation in *Rhizobium*/ legume symbiosis [34].

Sugiyama, *et al.* [35], reported changes in the rhizospheric bacteria and especially *Bradyrhizobium* during soybean growth, suggesting that the symbiosis of host plant with rhizobia may be selective but it thus improved soil health and legume yield [8]. Comparing the soil samples and their performance for a sustainable agricultural practice, Lagos, Oyo and Osun soil proved to be more fertile from the observed agronomic features of the cultivated soybean plant. The potted experiment showed that soybean did not grow in the soils from Katsina and Kaduna, this suggested that apart from edaphic factors and biotic factors, adaptability of legume cultivars to the soil dictates its establishment. Although, it had been reported that there is a great genetic diversity among bradyrhizobia in African soil, the detection of root-nodule bacteria in the cultivated soybean that showed more alignment with other root-nodule bacteria other than *Bradyrhizobium japonicum* corroborated the fact that there existed site-dependent genomic diversity, lateral gene transfer or recombination events (Fig. 2, 3, and 4) [36]. This underscores the high genetic variability associated with soybean *Bradyrhizobium* as well as the presence of important reservoir of novel soybean-nodulating bradyrhizobia strains in Nigeria. Although, nine root-nodule bacteria were isolated from the two agro-ecological zones under investigation, three of the isolates identified as *Bradyrhizobium* sp. using biochemical methods were randomly selected for 16S rDNA and gene sequence analysis and were characterized as *Stenotrophomonas maltophili*a (MN067745.1) and *Agrobacterium tumefaciens* (MF767515.1) *Rhizobium rhizolycopersici* (NR_181268.1). The relationship between *Stenotrophomonas maltophila* and soybean is complex and not fully understood because apart from being able to cause disease, some studies have reported that it can promote growth and improve nitrogen fixation in soybean plant by facilitating nutrient uptake [37]. The ability of *S. maltophilia* to interact with soybean plants in either a beneficial or detrimental way depends on various factors, as observed with this strain of *S. maltophilia* that established an efficient association with soy bean as well as being competitive enough to form root nodule [8, 36].

The transformation ability of *Agrobacterium tumefaciens* has been widely exploited for the genetic engineering of soybean plants. The bacterium was used as a vector to introduce desired traits such as resistance to herbicides and pests into soybean plants. [32, 38]. Further to the discovery of the gene transformation capacity, the occurrence of site-dependent genetic diversity and lateral transfer of gene among root nodule-bacteria was corroborated by this study since the genetic sequence of these three roots - nodule bacteria were different from those of the type strain *Bradyrhizobium japonicum* in the genebank [32, 38].

Soil with the *Rhizobium rhizolycopersic*i has been reported by Khan, *et al.* [39], to improve agronomic features in legumes, nutrient uptake and soil fertility. There were other cross-nodulating root-nodule bacteria associated with the rhizosphere of legumes whose complete genome (*Achromobacter xylosoxidans*) have been studied and found to be N_2-fixer, that both induce systemic resistance to pathogens and improves soil fertility [40].

Conclusion

Rhizobium is very relevant to plant growth and maintenance of soil fertility due to its ability to convert atmospheric nitrogen to ammonia for its utilization by plants. The introduction of effective and persistent rhizobia species into soybean smallholder farms would enhance soybean production as well as encourage sustainable agricultural practice. However, increasing the performance of the soybean crop is a major challenge in the country and the efficacy of symbiotic nitrogen fixation may be an important factor for enhancing productivity through the successful management of the soybean and indigenous-rhizobia symbiosis. The use of biological fertilizer would serve as a low-input and cheap bio-fertilizer input into agriculture, thus preventing the use of scarce resources in procurement of chemical fertilizers as well as impacting positively on adjacent farmlands and further research is needed to explore its potential for sustainable agriculture practice in Africa.

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Author Contributions

Olusola Ojo-Omoniyi: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Supervision, Validation; Writing– original draft; Writing– review & editing. Opeyemi Oseni: Soil sample collection, Preparation of media & isolation of soil bacteria

Michael Animashaun: Soil sample collection, Preparation of media & isolation of soil bacteria

Abdullateef Mohammed: Soil sample collection, Preparation of media& isolation of soil bacteria

Dataset

All Dataset are available in GenBank and NCBI repository, the Accession numbers for isolates are displayed and trackable. (*www.ncbi.nlm.nih.gov/genome*).

Ethical Consent

Ethical approval is not required for this type of work in Nigeria, since we did not test any material on human and animal subjects.

Statements and Declarations / Funding

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Competing Interests

The authors have no competing interest to disclose because this study was self-sponsored it was as a collaborative study. The authors did not receive support from any organization for the conduct of the study and the submitted manuscript.

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