PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (11) 3601-3610 November, 2024

Physicochemical Properties and Bacterial Population in the Rhizosphere of Mangrove Plant Species at the Upper Reaches of Santa Barbara River, Central Niger Delta, Nigeria

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ABSTRACT: The objective of this paper is to evaluate the physicochemical properties and bacterial population of the rhizosphere of five mangrove plant species (*Rhizophora mangle, Rh. racemosa*, *Laguncularia racemosa, Avicennia africana,* and *Nypa fruticans*) at the upper reaches of Santa Barbara River, central Niger Delta, Nigeria using standard methods. The population of bacteria was highest in the rhizosphere of *Rh. racemosa*, being 8.38 x 10^6 cfu/g, which was followed by *N. fruticans* with a density of 5.04 x 10^6 cfu/g, while others were significantly lower, with the least density occurring under *A. africana* with a population density of 1.41 x 10^6 cfu/g. Nineteen species of bacteria were presumably identified in the study area. Bacillus sp dominated the rhizosphere of all the mangrove plants with a frequency of 15.52%, 20%, 19.67%, 20.51 and 13.43% for *Rh. racemosa, Rh. mangle, A. africana, L. racemosa* and *N. fruticans* respectively. The 16S rRNA sequencing of isolates confirmed the presence of diverse species of *Bacillus* including *B. mycoides, B. paramycoides, B. pumilus, B. siamensis, B. velezensis,* the closely related *Priestia megaterium,* among other species such as *Klebsiella quasipneumoniae,* and *Photobacteriuum ganghwense* in the rhizosphere of the mangrove trees. The study therefore revealed the culturable bacteria guides in the mangrove rhizospheres which could potentially be developed for restoration and rejuvenation of impacted mangrove ecosystem.

DOI: https://dx.doi.org/10.4314/jasem.v28i11.13

Cite this Article as: OHIMAIN, EI; ADA, YN; KENDABIE, P (2024). Physicochemical Properties and Bacterial Population in the Rhizosphere of Mangrove Plant Species at the Upper Reaches of Santa Barbara River, Central Niger Delta, Nigeria. *J. Appl. Sci. Environ. Manage*. 28 (11) 3601-3610

Dates: Received: 21 August 2024; Revised: 29 September 2024; Accepted: 08 November 2024; Published: 15 November 2024

Keywords: *Avicennia africana;* mangrove microbiome; molecular ecology; physicochemical properties; wetlands

Mangroves are important ecosystems located at the intertidal zones in the tropics and subtropics. These ecosystems perform several environmental, social and economic benefits; hence, they are key to sustainable development in tropical coastlines. The ecosystem services of mangroves cover provisioning, regulatory, supporting and cultural. Mangrove supply biomass for energy and timber, and provide medicinal plants. Mangrove provide habitat to diverse organisms that are adapted to this unique ecosystem including plants, animals and microbes (Ohimain 2016, 2022). Mangroves are important in climate change mitigation and resilience. For instance, they act as physical barriers to sea surges and protect the hinterlands from coastal erosion and generally stabilizes the coastline (Allard *et al*., 2020). They store carbon in biomass and

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are therefore important in the mitigation of climate change. Mangrove ecosystems stores and purifies water (Mai *et al*., 2021), thus, preventing pollution from getting to the sea. They also fix heavy metals and radionuclides in their sediments (Perterson *et al*., 1997), thus making them unavailable to cause pollution. Different types of wetlands occur in the 853km long Nigerian coastline, with the largest expanse occurring in the Niger Delta region. The Niger Delta, which comprises of wetlands of about 76,000 sq km, hosts mangrove stands of over 11,000 sq km, which is the largest in Africa and the third largest in the world (Spalding *et al*., 1997; Spalding, 2010). The fisheries of the entire Atlantic Coastline come to breed in the Niger Delta mangrove ecosystem. Exploitation of mangrove resources especially its fisheries is the major occupation and source of livelihood for the people of the Niger Delta region. Since petroleum was discovered in the Niger Delta and commercial oil exploration began in the 1950s, the Niger Delta including its mangroves have come under immense pressure because of the influx of people and their activities in the area. Urban encroachment, agriculture, hunting and overfishing, solid waste disposal including plastics are among the major threats to the Niger Delta mangrove ecosystem (Ohimain 2016, 2022). Besides, oil exploration activities and incidents impact the environment such as oil spills, gas flaring and right of ways including roads, dredged canals and pipelines (Ohimain 2022). Oil exploration right of ways have modified the hydrology of the Niger Delta in certain areas and inadvertently creating access for further natural resource exploitation, all of which threatens the survival of the ecosystem. Turner and Ohimain (2024) assessed the impacts of petroleum industry canals on wetland loss. As a result of these and other anthropogenic factors, the Niger Delta wetlands including mangrove is under severe threat and appears to be changing (Ohimain and Eteh, 2021). With the current scale of destruction, it is predicted that the Niger Delta mangroves could be lost in less than 50 years (Corcoran *et al*., 2007), which will spell doom for the Niger Delta people, especially at a time when global energy focus is moving away from petroleum into renewables. It has been found that most of the ecological importance of mangroves is linked to the presence of healthy and unique microbiota that comprises the mangrove microbiome, including bacteria, archaea, fungi, and protists (Lin *et al*., 2019; Allard *et al*., 2020). Microbes, which occupies diverse microniches in mangrove ecosystems especially the rhizosphere, appear to be important drivers in the ecosystem functions of mangroves (Allard *et al*., 2020). These species can also play significant roles in the restoration and rejuvenation of impacted mangroves. For instance, rhizosphere microbes are involved in biogeochemical cycles of elements especially of sulphur, iron, carbon and nitrogen (Lin *et al*., 2019; Baker *et al*., 2021), which characterizes the mangrove ecosystems (Andreote *et al*., 2012), facilitates their survival in intertidal areas (Mai *et al*., 2021) and contributes to their high productivity (Haldar and Nazareth 2018). For instance, the microbial cycling of carbon and nitrogen are important in climate change mitigation (Mai *et al*., 2021). Rhizosphere microbes are also key to degradation of pollutants and xenobiotics including spilled crude oil, pesticides, solid wastes and fixing of heavy metals in sediment (Nealson, 1997). Microbes in marine and coastal sediments represent a major section of the biosphere, therefore understanding their ecology is crucial for understanding global ocean processes (Baker *et al*., 2021).

The rhizosphere of mangrove vegetation and the surrounding soils and sediments have been regarded as hotspots for various microbial activities which support nutrient cycling (Haldar and Nazareth 2018), and play major roles in the functioning and preservation of the ecosystem (Andreote *et al*., 2012), which open vista of opportunities for microbial-mediated methods for the protection and rehabilitation of mangrove ecosystems (Allard *et al*., 2020). For instance, microbial activities result in the fixing of heavy metal in mangrove sediments (Ohimain *et al*., 2008, 2009), could lead to disastrous consequences when the sediment are disturbed by dredging (Ohimain 2004, 2016). Literature on rhizosphere microbial diversity and microbiome-mangrove ecosystems function are still emerging. Besides, molecular biology techniques have opened a vista of opportunity for the exploration of microbial diversity of mangrove ecosystems. For instance, molecular studies have led to the recent discovery of the largest known bacteria species in the world, which was reported among Brazilian mangroves (Volland *et al*., 2022). Unfortunately, the potential roles of rhizosphere microbes and their specific activities in mangrove ecosystems are still poorly underappreciated globally and worst in Nigeria. Hence, the global Mangrove Microbiome Initiative was recently established with the first priority of characterizing mangrove microbiomes across the world, which could provide understanding on mangrove-microbial interactions and functions that could be useful in the sustenance of ecosystem services and remediation of impacted mangroves (Allard *et al*., 2020). Despite their global relevance, studies on the bacteriome of the Niger Delta mangrove ecosystem is not common. The objective of this study is to evaluate the physicochemical properties and bacterial population of sediment in the rhizosphere of five mangrove plant species (*Rhizophora mangle, Rh.*

racemosa, Laguncularia racemosa, Avicennia africana, and *Nypa fruticans*) at the upper reaches of Santa Barbara River, central Niger Delta, Nigeria

MATERIALS AND METHODS

Field sampling: Field sampling was undertaken in February 2023 to study the upper reaches of Santa Barbara River (Fig. 1, Plate 1). Soil auger and sediment grab were used to collect surface soil/sediment samples (at 0-10cm) in triplicates from 12 locations each at the root of the major mangrove vegetation species in the area, viz, *Rhizophora mangle, Rh. racemosa, Laguncularia racemosa, Avicennia africana,* and *Nypa fruticans*. The samples were collected into sterile ziplock bags and preserved in ice chest in transit to the laboratory. Electrical conductivity and pH were determined on-site electrometrically using Hach HQ 1140 conductivity/TDS meter and pH-211 meter (Hanna instruments) respectively.

Physicochemical analysis: The soil/sediment samples were air-dried under ambient temperature and analyzed using standard methods as described in Page *et al*. (1982) for pH, electrical conductivity, salinity, sulphur, phosphate, organic carbon and organic matter. Total organic matter (TOM) and carbon (TOC) were determined by the dichromate oxidation method. Sulphur was oxidized to sulphate and determine using barium chloride spectrophotometric method, while phosphorus was determined using ammonium molybdate-vanadate after perchloric acid digestion method. Spectrophotometric measurements were done using Jenway 6300 spectrophotometer.

Microbiological Analysis: The bacterial population of the soil/sediment samples were counted using nutrient agar with pour plate method reported by Pepper and Gerba (2005). Identification of bacterial colonies was done using cultural methods, while some of the dominant bacteria species were further characterized using molecular method based on 16S rDNA gene sequencing (Weisburg *et al*., 1991; Muyzer *et al*., 1993). Culture-based identification was carried out using cultural, morphological and biochemical characterization using procedures described by Cheesbrough (2004), Dubey and Maheshwari (1999) and Benson (2002), while the presumptive species were identified using the identification scheme in Bergey's manual (Bergey, 1994).

Molecular Identification: Molecular procedure involved DNA extraction, quantification, amplification, sequencing, and phylogenetic analysis. Bacterial DNA was extracted at the Niger Delta University, Wilberforce Island, Nigeria while sequencing was carried out at Inqaba Biotec, Pretoria, South Africa.

Bacterial genomic DNA extraction: The genomic DNA of bacterial isolates were extracted using a ZR bacterial DNA mini-preparation extraction kit. The pure cultures of the dominant isolates were suspended in 200 microliters (ul) of isotonic buffer in ZR Bashing Bead Lysis tubes, and 750 ul of lysis solution was dispensed into the tube. The tubes were fixed in a bead beater with a 2ml tube holder assembly and processed for 5 minutes at peak speed. The ZR bashing beads lysis tube was centrifuged for 60 seconds at 10,000g using Eppendorf® centrifuge microcentrifuge model 5424 (Eppendorf AG). Exactly 400ul of the supernatant were removed to the Zymo-Spin IV spin filter (top orange) in a collection tube and centrifuged for 60 seconds at 7,000g. Exactly 1,200 ul of bacterial DNA binding buffer were dispensed to the filtrate in the collection tube, which brought the final volume to 1,600 ul, of which 800 ul were removed to the Zymo-Spin IIC column in the collection tube, and centrifuged for 60 seconds at 10,000g. The flow-through was removed from the collection tube, while the remaining volume was moved to the same Zymo-spin IIC and spun. Exactly 200 ul of DNA prewash buffer were added to the Zymo-spin IIC in a fresh collection tube and operated for 60 seconds at 10,000g, which was followed by the addition of 500 ul of bacterial DNA wash buffer and then centrifuged for 60 seconds at 10,000g. The Zymo-spin IIC column was moved to a clean 1.5 ul centrifuge tube, exactly 100 ul of DNA elution buffer was added to the matrix of the column, and operated for 30 seconds at 10,000g to elute the DNA. The resultant pure DNA was preserved at -20^{o}C until further analysis.

DNA quantification: The concentration of extracted genomic DNA of the isolates were measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific). The spectrophotometer was operated with 2 ul of sterile distilled water and reagent blank using normal saline. Exactly 2 ul of the extracted DNA were put on the lower pedestal, while the upper pedestal was brought down to contact the extracted DNA on the lower pedestal, before the concentration of DNA in the sample was measured.

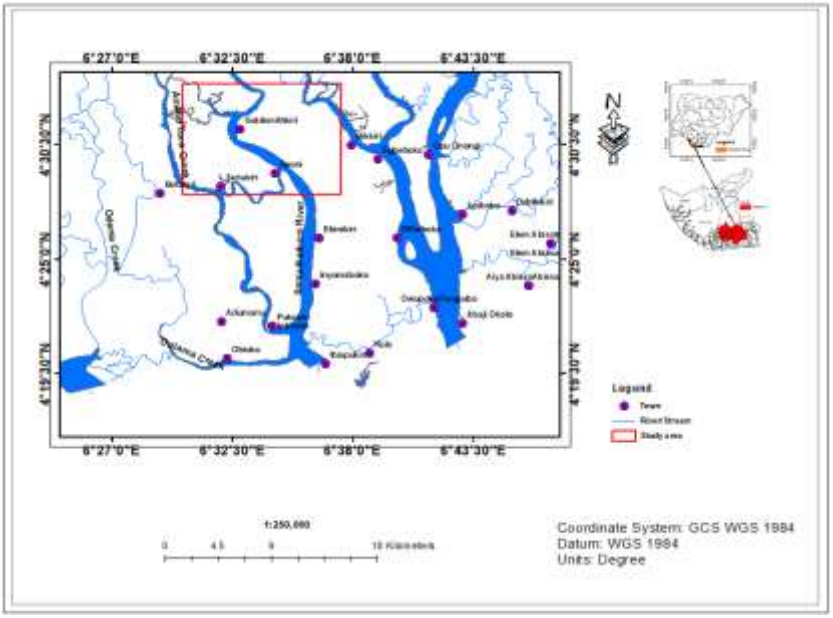

Fig 1: Study area at Santa Barbara River

Plate 1: Mangrove forest at Santa Barbra River

16S rRNA Amplification: The 16S rRNA region of the rRNA genes of bacterial isolates was amplified using the following primers; 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTTTCGACTT-3' on ABI 9700 thermal cycler (Applied Biosystems) at a final volume of 50 ul for 35 cycles. The PCR reaction mixture comprised of the X2 Dream Taq Master mix (DNTPs, Taq polymerase, MgCl), the primer at a concentration of 0.4M with the DNA extracted from the isolates were used as a template. The operating conditions of the PCR reaction were as follows: initial denaturation at 95ºC for 5 minutes; denaturation at 95ºC for 30 seconds; annealing at 52ºC for 30 seconds; extension at 72ºC for 30 seconds for 35 cycles; final extension at 72ºC for 5 minutes. The product was resolved for 15 minutes on a 1% agarose gel at 120V and visualized on an UV transilluminator (PI-1002 PrepOne manufactured by Embi Tec).

DNA sequencing: Sequencing was performed with a 3510 ABI sequencing kit (Applied Biosystems), using a BigDye Terminator kit (Thermo Fisher Scientific). The sequence was made at a final volume of 10ul, with the following components; 0.25ul BigDye® terminator v1.1/v3.1, 2.5ul BigDye sequencing buffer, 10uM PCR primer and 2-10ng PCR template per 100bp. The sequencer was operated under the

following conditions; 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds, and 60°C for 4 min.

Phylogenetic analysis: The sequences obtained were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded using BLASTN from the database of the National Center for Biotechnology Information (NCBI). These sequences were aligned using ClustalX. The evolutionary history was deduced using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei 1987). The bootstrap consensus tree, which was derived from 500 replicates (Felsenstein, 1985), was used to represent the evolutionary history of the taxa studied. The evolutionary distance was calculated using the Jukes-Cantor method (Jukes and Cantor 1969).

Statistical analysis: The data were compiled using Microsoft Excel and analyzed using SPSS version 26 (IBM SPSS Inc). Descriptive and inferential statistics was done using one-way analysis of variance, while multiple comparison was carried out using Duncan statistics. Results were presented as mean ± standard deviation with alpha set at 0.05.

RESULTS AND DISCUSSION

The physicochemical properties and bacterial population of the soil/sediment in the rhizosphere of the mangrove vegetation is presented in Table 1. The pH, which was slightly acidic, varied significantly ($P<0.05$) among the plant species. Soils under *A. africana* was the most acidic with a pH 5.67, which was not significantly different from 5.97 recorded for *Rh. racemosa* ($P>0.05$). The highest pH of 6.99 tending towards neutrality was obtained under *Rh. mangle*, while the pH recorded for the soil under *L. racemosa* and *N. fruticans* was in-between both extremes. Mangrove soils and sediments are known to exhibit acidic character principally due to the presence of sulphur and its minerals including pyrites (Ohimain et al 2004). But sulphur content during this study is low, being 0.08 mg/kg in the rhizosphere of *Rh. racemosa and L. racemosa*, which were significantly different from the 1.0 mg/kg recorded in the rhizosphere of *A. africana*, *Rh. mangle* and *N. fruticans* ($P<0.05$). The relatively low sulphur content might be because the study area is located at the upper reaches of Santa Barbara River, which is relatively far from the coastline.

Table 1: physicochemical properties and culturable bacteria population density in the rhizosphere of different mangrove vegetation

	Rh. Racemosa	*Rh. mangle*	*A. africana*	*L. racemosa*	*N. fruticans*
pH	5.97±1.15a	6.99±0.23c	5.68±0.58a	6.73±0.59bc	6.16±0.75ab
EC, µS/cm	720.42±163.58b	737.00±30.03b	666.33±78.39b	742.75±103.73b	572.08±144.97a
Salinity, mg/kg	358.33±77.41b	362.17±36.14b	335.00±34.87b	371.92±55.85b	280.83±72.97a
TOC, %	7.40±1.56a	8.01±0.70a	8.35±1.16a	7.79±0.59a	8.25±2.36a
TOM, %	14.10±0.92ab	14.59±0.51ab	14.37±2.00ab	13.40±1.02a	15.78±4.12b
Sulphur, mg/kg	0.08±0.01a	0.98±0.08b	1.03±0.18b	0.08±0.01a	1.05±0.17b
Phosphorus, mg/kg	5.52±1.61a	5.39±1.13a	8.69±2.15c	5.80±1.18ab	7.42±3.27bc
Bacteria, x 10^6 cfu/g	8.38±5.89c	1.68±0.52a	1.41±0.55a	3.50±0.62ab	5.04±0.11b

Results presented as mean ± standard deviation (mean ± SD, N =12). Across the rows, mean with the same alphabets are not significantly different (P>0.05)

Table 2: Percentage frequency (%) of presumptive culturable bacteria isolated from the rhizosphere of different mangrove plants in Santa Barbara River

BACTERIA	*Rh. racemosa*	*Rh. mangle*	*A. africana*	*L. racemosa*	*N. fruticans*
Citrobacter spp		8		7.69	4.48
Pseudomonas spp					10.45
Proteus sp					8.96
Serratia sp		10	11.48	15.38	7.46
Actinomycete spp	12.07	12	9.82	12.83	10.45
Vibrio spp	12.07				
Corynebacteria spp	6.9				5.97
Bacillus spp	15.52	20	19.67	20.51	13.43
Micrococcus spp	5.17		6.56		
Staphylococcus spp	10.35		6.56		5.97
Enterobacter spp			6.56	15.38	
Enterococcus spp		6	8.2		
Alcalligenes spp		4	4.92	10.26	
Clostridium spp		8	8.2	10.26	4.48
Acinetobacter spp	8.62				7.46
Flavobacter spp	3.44	6	4.92		5.97
Klebsiella spp	12.07	14	13.11		7.46
Aeromonas sp	5.17				
Photobacterium sp	8.62	12		7.69	7.46

Salinity and electrical conductivity exhibited a similar pattern, with significantly higher values in the true mangrove species (*Rh. racemosa, Rh. mangle, A. africana* and *L. racemosa*) compared to the exotic *N. fruticans* ($P<0.05$). The highest salinity and electrical conductivity values of 371.92 mg/kg and 742.75 µS/cm were obtained at the rhizosphere of *L. racemosa*. Mangrove soil are known to be brackish in nature.

TOC was apparently highest in the rhizosphere of *N. fruticans*, which was not significantly different among the other species ($P>0.05$). The highest TOM value of 15.78±4.12% was also obtained from *N. fruticans*, which was significantly different from the lowest value of 13.40±1.02% that was obtained from *L. racemosa* ($P<0.05$), whereas the TOM of the other three species was not significantly different from both extremes ($P>0.05$). Phosphorus concentration varied significantly among the various species. The highest phosphorus value of 8.69±2.15 mg/kg was recorded in the rhizosphere of *A. africana*, followed by 7.42±3.27 mg/kg under *N. fruticans*, while the least values of 5.39±1.13 mg/kg and 5.52±1.61 mg/kg were recorded under *Rh. mangle* and *Rh. racemosa* respectively ($P<0.05$).

The population of culturable bacteria was highest in the rhizosphere of *Rh. racemosa*, being 8.38 x 10^6 cfu/g, which was followed by *N. fruticans* with a density of 5.04 x 10^6 cfu/g, while others were significantly lower, with the least density occurring under *A. africana* with a population density of 1.41 x 10^6 cfu/g. Nineteen species of culturable bacteria were presumably identified in the study area; 11 each for *Rh. racemosa* and *A. africana*, 10 for *Rh. mangle*, 8 for *L. racemosa* and 13 for *N. fruticans.* Hence, *L. racemosa* is the least diverse. *Bacillus* sp. dominated the rhizosphere of all the mangrove plants with a frequency of 15.52%, 20%, 19.67%, 20.51 and 13.43%, whereas *Actinomycetes* spp had a frequency of 12.07%, 12%, 9.82%, 12.83%, and 10.45% for *Rh. racemosa, Rh. mangle, A. africana, L. racemosa* and *N. fruticans* respectively. *Photobacterium* sp was detected at all the stations except under *A. africana*. *Serratia* sp and *Clostridium* spp were detected in all the rhizosphere except *Rh. racemosa. Klebsiella* spp and *Flavobacter* spp were detected in all the rhizosphere except *L. racemosa*. Among the rhizosphere of the plants, *Vibrio* spp and *Aeromonas* sp were only detected in *Rh. racemosa*, while *Pseudomonas* spp and *Proteus* sp were only detected in *N. fruticans* rhizosphere. *Corynebacteria* spp and *Acinetobacter* spp were only detected under *Rh. racemosa* and *N. fruticans* rhizosphere, *Enterococcus* spp. was only detected in *Rh. mangle* and *A. africana* rhizosphere, *Micrococcus* spp was only detected in *Rh. racemosa* and *A. africana*, while *Enterobacter* spp. was only detected in *A. africana* and *L. racemosa* rhizosphere. The rest bacteria species were detected in the rhizosphere of three plants; *Citrobacter spp (Rh. mangle, L. racemosa, N. fruticans), Staphylococcus* spp *(Rh. racemosa, A. africana, N. fruticans)* and *Alcaligenes* spp *(Rh. mangle, A. africana, L. racemosa).*

The 16s rRNA sequences gotten from the selected rhizosphere isolates matched other species with 61-100% similarity observed during the megablast search for analogous sequences from the NCBI non-redundant nucleotide database (Fig. 2). The calculated evolutionary distances were consistent with the phylogenetic placement of the isolates within the following genera; *Klebsiella, Bacillus, Priestia* and *Photobacteriuum*, with closely relatedness to the following species; *Klebsiella quasipneumoniae, Bacillus mycoides, Bacillus cereus, Bacillus paramycoides, Bacillus pumilus, Bacillus siamensis, Bacillus velezensis, Priestia megaterium* and *Photobacteriuum ganghwense* (Fig. 3). To the best of our knowledge, some of the species detected have not been previously reported in the Niger Delta mangrove ecosystem especially *Klebsiella quasipneumoniae, Bacillus mycoides, Bacillus paramycoides, Bacillus pumilus, Bacillus siamensis, Bacillus velezensis, Priestia megaterium* and *Photobacteriuum ganghwense.* Of particular note, is the detection of *Priestia megaterium*, which was previously known as *Bacillus megaterium*, which is a known plant growth-promoting bacterium that is being explored for other potential applications such as biorestoration (Guzmán-Moreno et 2022) and plant diseases resistance (Li *et al*., 2022).

Few studies have been done in the Santa Barbra soils mostly relating to oil spills. Goodluck (2024) reported the physicochemical properties of the sediment around Santa Barbra oil well No. 1 with pH ranging from 2.08 - 6.80 and TOC from 0.18 - 2.00%. Oyibo and Obire (2022) used cultural methods to isolate the following bacteria genera in the wastewaters from an oilfield in Santa Barbara River; *Bacillus, Alcaligenes, Enterococcus, Pseudomonas, Salmonella, Aeromonas, Staphylococcus*, and *Escherichia.* Allen-Adebayo *et al*., (2024) used cultural and molecular methods to characterize culturable hydrocarbon utilizing bacteria in Santa-Barbara River and identified the following organisms; *Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa*, and *Micrococcus luteus*. Elsewhere in the Niger Delta, Chikere *et al*. (2019) used physicochemical and metagenomic techniques to assess crude oil-polluted

mangrove soils in Bodo West in Ogoni, and reported pH of 6.5, EC of 105 µS/cm, TOC of 0.60%, phosphate of 11.60 mg/kg, and the following bacterial families were detected; Alcanivoracaceae, Oceanospirillaceae, Acidithiobacillaceae and Desulfobacteraceae among others. Iturbe-Espinoza *et al*., (2022) used molecular methods to observe the changes in rhizosphere microbial communities associated with oil spill remediation in Bodo mangrove forests. They found that oil impacted soil contained highly diverse microbiota, but upon clean-up by water flushing resulted in reduced microbial diversity, which resulted in the dominance of the microbiota by members of the phyla Firmicutes and Proteobacteria.

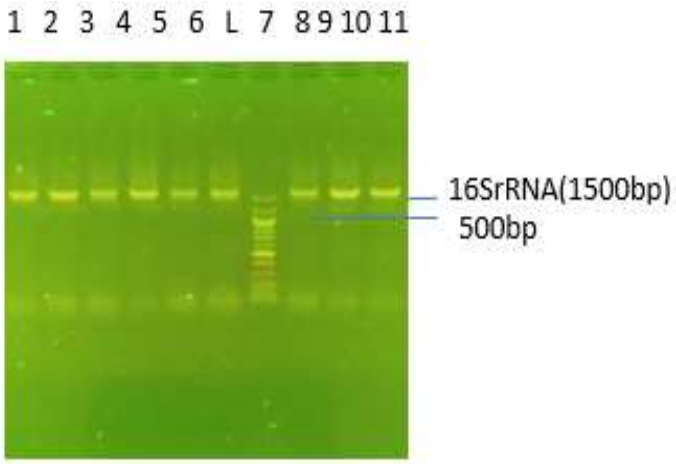

Fig 2: Agarose gel electrophoresis showing the amplified 16s rRNA. Lanes 1-9 represent the amplified 16srRNA at 1500bp while lane L represents the 100bp DNA ladder.

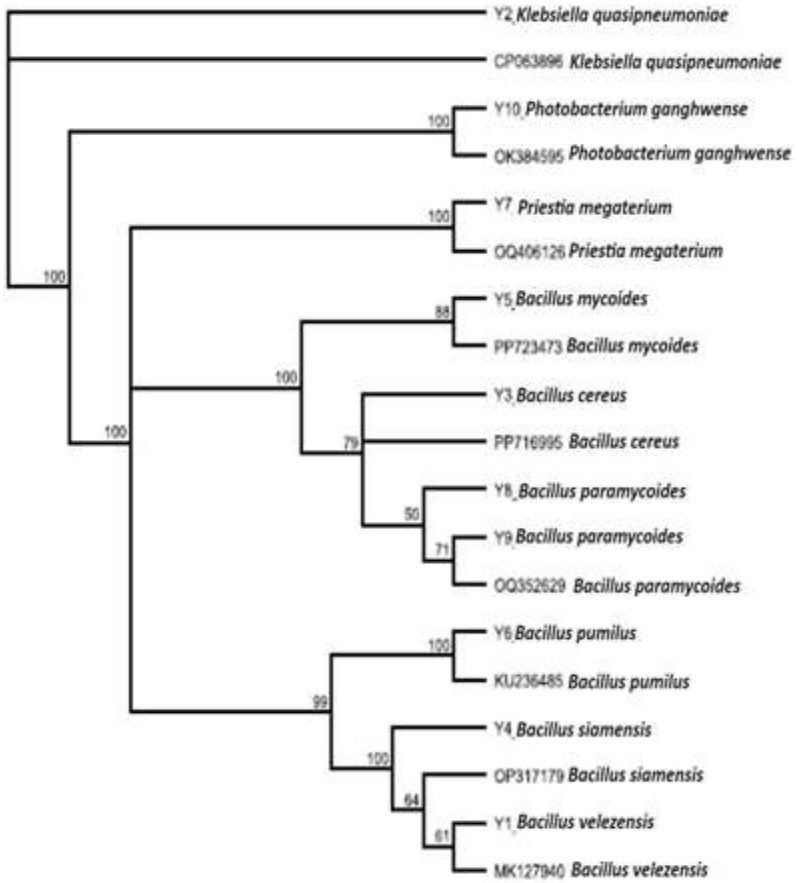

Fig 3: Phylogenetic tree showing the evolutionary relationship between the bacterial isolates

Molecular biology methods have also been used for the study of microbiome of mangrove ecosystems in other countries. In South America, Andreote *et al*. (2019) used metagenomic approach to study the microbiome of a Brazilian mangrove sediment and found the presence of bacterial families; Rhodobacteraceae, Burkholderiaceae, Planctomycetaceae, and Desulfobacteraceae, which are involved in the metabolism of nitrogen, methane, and sulphur. da Costa *et al*. (2022) used physicochemical and 16S rDNA methodology to assess the changes in mangrove rhizosphere due to highway construction in Brazilian Amazon, and reported pH ranging from 5.1 – 6.4, TOC 8.4 – 24.8 g/kg, TOM 14.5 – 42.8 g/kg and phosphate 16 – 91 cmol/dm^3. They also found that the phyla Proteobacteria, followed by Firmicutes and Bacteroidetes were most abundant in all the mangroves studied. They further observed that intact mangrove had the highest microbial diversity followed by the recovering mangrove, while impacted mangrove had the least. They also observed that the intact mangrove contained genera such as *Collinsella, Desulfuromonas, Dorea, Desulfatiglans*, and *Dialister*, which are not found in the impacted areas.

In Asia, Mai *et al*. (2021) used 16S metagenomic analyses to assess microbial community structure in the rhizosphere of three mangrove species (*Rhizophora apiculata, Sonneratia alba*, and *Bruguiera parviflora*) along the Merbok River in Malaysia. They found out that each of the mangrove species harboured their own unique microbiota, but generally observed the dominance of Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Actinobacteria, and Anaerolineae with occurrence frequencies that varied significantly among the three-mangrove species, with *Rh. apiculata* exhibiting the highest microbial diversity. In India, Haldar and Nazareth (2018) used 16S rDNA and culture-based techniques to assess the taxonomic diversity of bacterial species in mangrove sediments and found *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Acidobacteria* and *Gemmatimonadetes* as the dominant phyla. They also reported the presence of pathogenic *Vibrio* sp. and other ecologically important bacterial classes including *Bacilli, Alphaproteobacteria, Gammaproteobacteria,* and *Deltaproteobacteria*. In China, Lin *et al*. (2019) similarly used 16S rRNA metagenomic methodology to assess the sediment microbiome at the Yunxiao National Mangrove Reserve, and detected the presence of guilds of bacteria involved in carbon, nitrogen and sulphur cycles and observed the following microbes as drivers of these cycles; *Chloroflexi, Nitrospira, Sulfurovum, Syntrophobacter*, and *Anaerolinea*.

It should however be noted that cultural techniques are quite limiting in scope because it grossly underestimates the population and diversity of bacteria in the rhizosphere. Hence, does not present the complete picture of the rhizosphere bacteriome of the area. 16S rRNA-based metagenomic approaches such as was carried out in Kenya mangroves would have revealed greater population, diversity and ecological roles of the rhizosphere bacteria (Muwawa *et al*., 2021). But our focus was on the isolation and characterization of culturable bacteria that could be further developed for the maintenance of intact mangroves and restoration of impacted mangroves.

Conclusion: The study assessed the physicochemical and the culturable bacterial content of the rhizosphere of the five major mangrove plants at the upper reaches of the Santa Barbara River in the central Niger Delta, Nigeria. The results showed that the sediment is slightly acidic though tending toward neutrality, brackish in nature with relatively low levels of sulphur and phosphorus. The bacteria population and diversity in the rhizosphere of the plants differed significantly. *Bacillus* sp dominated the rhizosphere of the mangrove plants followed by *Serratia* sp and *Klebsiella* sp. The study show that the bacteria population and diversity differ in the rhizosphere of different mangrove species in Santa Barbra River. The isolated bacteria can be further developed to play critical role in the sustenance of the health and vitality of intact mangrove or restoration of impacted mangroves.

Acknowledgement: The authors extend their appreciation to Nigerian Tertiary Education Trust Fund (TETFund) for funding this research with a Grant Number NDU/TETFUND-IBR/03

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the corresponding author.

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