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Molecular Identification of Hydrocarbonoclastic Bacteria from Sombreiro River in Niger Delta, Nigeria

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ABSTRACT

Hydrocarbon contamination poses a significant threat to aquatic ecosystems, necessitating the identification of indigenous microorganisms capable of degrading petroleum hydrocarbons. This study investigated the diversity and phylogenetic relationships of hydrocarbonoclastic bacteria isolated from Sombreiro River that is contaminated by hydrocarbons. Bacterial isolates were harvested and cultured following standard microbiological procedures. Isolates demonstrating hydrocarbon-utilizing potential were subsequently analyzed using the polymerase chain reaction (PCR) technique. Their 16S rRNA genes were sequenced and compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database to establish their taxonomic identities and assess their phylogenetic relationships. Phylogenetic analysis revealed the presence of diverse hydrocarbon-degrading bacterial species belonging to the genera *Bacillus*, *Enterobacter*, *Providencia*, *Shewanella*, and *Pseudomonas*. The isolates were identified as *Bacillus flexus* (AE1) (recent name *Priestia flexa*), *Bacillus subtilis* (DE2), *Enterobacter cloacae* (KE2), *Providencia startii* (IE2), *Shewanella algae* (OV2 and DE3), *Pseudomonas xiamenensis* (SE3), and *Pseudomonas plecoglossicida* (DV6) and their Accession numbers are MT645459.1, OQ726278.1, OR426319.1, MK641351.1, MT549169.1, MN611113.1 and MT498800.1 respectively. The occurrence of multiple hydrocarbonoclastic genera suggests the existence of a microbial consortium capable of synergistic degradation of complex hydrocarbon mixtures. Such microbial diversity enhances the potential for natural attenuation and bioremediation of polluted environments. The study highlights the importance of indigenous hydrocarbon-degrading bacteria from Sombreiro River and provides valuable molecular evidence supporting their application in bioremediation programs. The identification of genetically diverse and environmentally adapted bacterial strains offers promising prospects for the development of efficient, eco-friendly strategies for the remediation of hydrocarbon-contaminated ecosystems and the sustainable management of petroleum pollution.

Keywords: Sombreiro River, Hydrocarbonoclastic Bacteria, PCR, Bioremediation, Eco-Friendly Strategies.

Introduction

Petroleum hydrocarbons are complex mixtures of aliphatic hydrocarbons, aromatic hydrocarbons, cycloalkanes, resins, and asphaltenes, many of which are persistent, toxic, mutagenic, and carcinogenic to living organisms (Ehis-Eriakha et al., 2020; Ruth et al 2020; Orikpete & Ewim, 2024). The accumulation of petroleum hydrocarbon in aquatic environments poses serious ecological and public health concerns by reducing water quality, impairing aquatic productivity, and threatening biodiversity (Onwukwe et al., 2025). Despite these adverse environmental effects, certain indigenous microorganisms have developed adaptive mechanisms that enable them to survive and metabolize petroleum hydrocarbons as their primary source of carbon and energy. These specialized microorganisms, commonly referred to as hydrocarbonoclastic bacteria, are regarded as key biological agents responsible for the natural attenuation and biodegradation of petroleum pollutants (Ehis-Eriakha et al., 2020; Orikpete & Ewim, 2024).

The Sombreiro River, located in the eastern Niger Delta of Nigeria, is one of the region's major freshwater ecosystems, providing water for domestic use, fishing, transportation, irrigation, and other socio-economic activities for the surrounding communities.

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However, decades of crude oil exploration, production, transportation, pipeline vandalism, illegal refining, and accidental oil spillages have resulted in chronic petroleum hydrocarbon contamination of the river. These anthropogenic activities have significantly altered the physicochemical properties of the water and sediments, reduced aquatic biodiversity, and disrupted the ecological integrity of the river ecosystem (Okoye et al., 2024; Onwukwe et al., 2025).

Hydrocarbonoclastic bacteria possess diverse catabolic genes encoding enzymes such as alkane monooxygenases, dioxygenases, dehydrogenases, and cytochrome P450 oxygenases, which catalyze the degradation of aliphatic and aromatic hydrocarbons into less toxic intermediates that are eventually mineralized into carbon dioxide, water, and microbial biomass (Ehis-Eriakha et al., 2020; Okoye et al., 2024).

Consequently, these microorganisms constitute one of the most important biological resources for the remediation of petroleum-contaminated aquatic ecosystems through environmentally sustainable bioremediation processes (Orikpete & Ewim, 2024).

Accurate identification of hydrocarbonoclastic bacteria is fundamental to understanding their diversity, ecological roles, and biodegradation capabilities. Conventional identification techniques based on colony morphology, Gram staining, and biochemical characterization are often inadequate because many bacterial species exhibit similar phenotypic characteristics, while numerous environmentally significant microorganisms remain unculturable under laboratory conditions (Bodor et al., 2020).

These limitations have necessitated the adoption of molecular identification techniques, which provide greater accuracy, sensitivity, and reproducibility in bacterial taxonomy (Bodor et al., 2020; Eziuzor et al., 2021). Among molecular approaches, sequencing of the 16S ribosomal RNA (16S rRNA) gene remains the most widely accepted method for bacterial identification and phylogenetic analysis because the gene contains highly conserved regions interspersed with hypervariable regions that enable accurate differentiation among bacterial taxa (Eziuzor et al., 2021; Okoye et al., 2024).

Therefore, the molecular identification of hydrocarbonoclastic bacteria from the Sombreiro River is essential for understanding the composition, diversity, phylogenetic relationships, and functional potential of indigenous microbial communities inhabiting this petroleum-impacted ecosystem (Okoye et al., 2024). Such knowledge contributes to environmental monitoring, ecological risk assessment, and the development of effective and sustainable bioremediation technologies for restoring the ecological integrity of the Sombreiro River and other oil-contaminated aquatic ecosystems in the Niger Delta (Onwukwe et al., 2025; Orikpete & Ewim, 2024). Hence the study aimed at molecularly identifying hydrocarbonoclastic bacteria from Sombreiro River in Niger Delta, Nigeria.

Materials and Methods

Description of Study Area

The lower Sombreiro River is one of the major distributaries of the eastern Niger Delta in southern Nigeria. It is located primarily in Rivers State**,** flowing through several riverine communities before discharging into the New Calabar River estuary and ultimately the Atlantic Ocean through interconnected creeks and estuarine channels. The Lower Sombreiro River originates from the middle reaches of the Sombreiro River around the Ahoada axis and extends southward through Degema**,** Abonnema**,** Buguma**,** Tombia**,** Krakrama, and adjoining coastal settlements within the Niger Delta. Numerous tributaries, tidal creeks, and floodplain channels connect the river with neighboring water bodies, resulting in a complex hydrological network characterized by seasonal flooding and tidal influences from the Atlantic Ocean (Eke & Nwankwoala, 2021). GPS (Geographic Positioning System) coordinates of various sampling locations along Sombreiro River were: station 1 Degema 4º 44’33”(N) 6º 46’32”(E), station 2 Abonnema 4º 43’66”(N) 6º 40’63”(E), station 3 Krakrama 4º 44’36”(N) 6º 46’28”(E), station 4 Obonoma 4º 43’53”(N) 6º 46’76”(E), station 5 Idama 4º 37’42”(N) 6º 48’51”(E) and station 6 Soku 4º 40’42”(N) 6º 41’02”(E). Map generated from these coordinates using Google map 2024 and NDDC map 2007 is shown in Figure 1.

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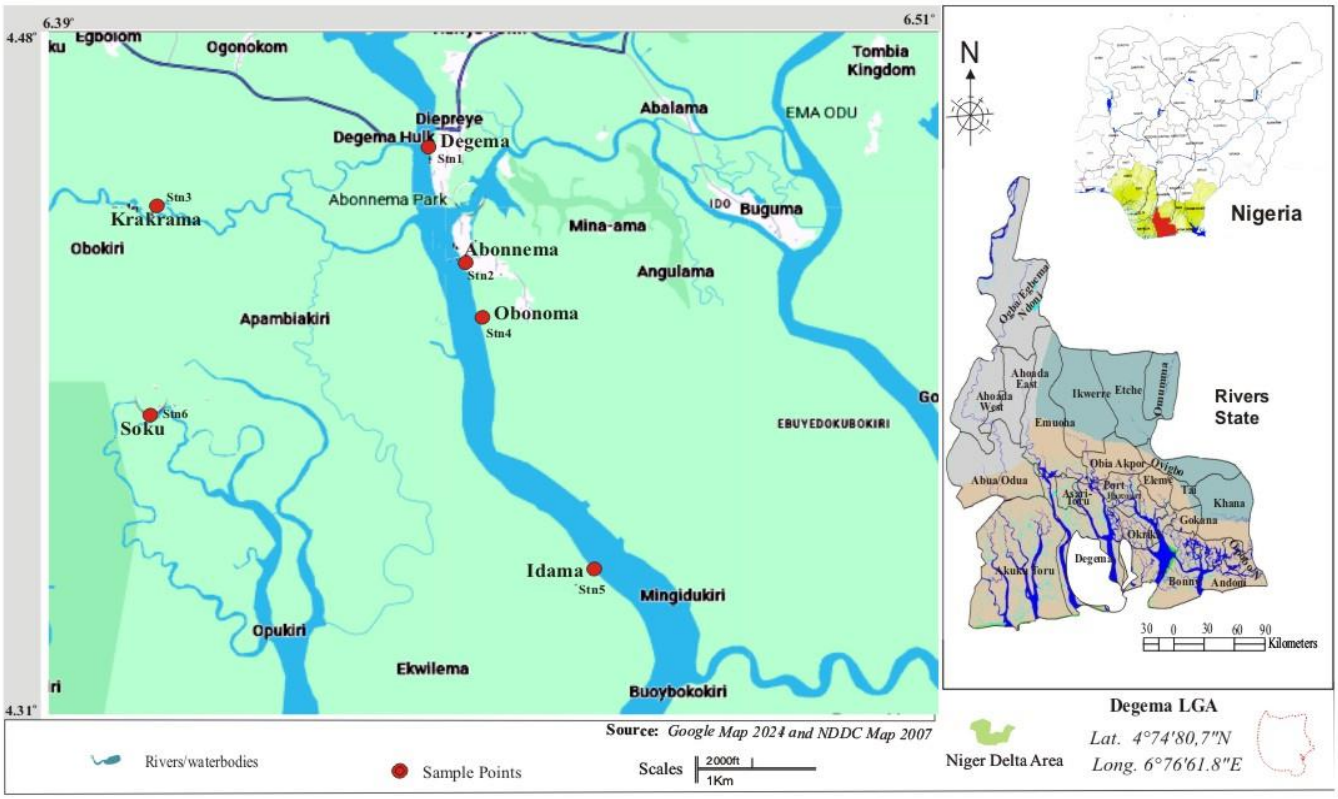

Fig. 1: Map Showing the Sampling Locations along the Lower Sombeiro River (Google Map, 2024)

Collection of Surface Water and Sediment Samples

Sample Collection

Water samples were collected from various stations along Sombreiro River using sterile amber bottles from a depth of approximately 10–15 cm below the water surface following standard sampling procedures (ISO, 2022). To minimize contamination, each bottle was opened beneath the water surface with its mouth facing the direction of water flow, filled while still submerged, and capped before being brought to the surface. Sediment samples were obtained using a Van Veen grab sampler (manufactured by Royal Eijkelkamp). The collected sediment was transferred into a sterile stainless-steel bowl, after which a sterilized spatula was used to place representative portions into sterile amber bottles. The bottles were then tightly sealed and appropriately labeled. All collected samples were preserved in ice packed cool box and transported immediately to the laboratory for subsequent analyses.

Preparation of Sediment Samples

Sediment samples were first homogenized using a ceramic mortar and pestle that had been cleaned with 100% ethanol and allowed to air-dry before use. The homogenized sediment was then passed through a 2 mm mesh sieve to remove coarse debris, following the procedure described by ASTM International. (2022).The sieved samples were subsequently air-dried in a dark environment to minimize photodegradation before further analysis.

Media Used

Mineral salt medium (MSM) was prepared by dissolving 0.20 g/L $MgSO_4 \cdot 7H_2O$, 0.02 g/L $CaCl_2$, 1.0 g/L KH_2PO_4, 1.0 g/L K_2HPO_4, 1.0 g/L NH_4NO_3, 0.05 g/L $FeCl_3$, and 15 g/L agar in 1 L of distilled water. Nutrient agar was similarly prepared by dissolving 5 g/L peptone, 3 g/L beef extract, 5 g/L NaCl, and 15 g/L agar in 1 L of distilled water.

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Cultivation and Enumeration of Total Heterotrophic and Hydrocarbonoclastic Bacterial Counts

Total heterotrophic bacteria (THB) and hydrocarbonoclastic bacteria (HCB) were enumerated using the tenfold serial dilution technique as described by Harrigan and McCance (1990) and in accordance with the procedures outlined by APHA (2017), with sterile normal saline serving as the diluent. Before serial dilution, 1 mL of each water sample and 1 g of each sediment sample were aseptically transferred into separate test tubes containing 9 mL of sterile normal saline to obtain the initial dilution. Subsequent serial dilutions were prepared, and dilution factors of 10^{-5} and 10^{-6} were used for the enumeration of heterotrophic bacteria, while 10^{-2} and 10^{-3} were used for hydrocarbonoclastic bacteria.

Thereafter, 0.1 mL aliquots from the appropriate dilutions were inoculated, in duplicate, onto nutrient agar and mineral salt medium plates using the spread plate technique (APHA, 2017). For the isolation of hydrocarbonoclastic bacteria, hydrocarbons were supplied to the mineral salt medium through the vapour-phase transfer method. The inoculated plates were incubated at 35°C for 24 hours for heterotrophic bacteria and for 7 days for hydrocarbonoclastic bacteria. At the end of the incubation period, the developed colonies were counted, and the bacterial populations were expressed as colony-forming units (CFU) per millilitre of water or per gram of sediment.

Molecular identification of Hydrocarbonoclastic Bacterial

DNA Extraction

Genomic DNA was extracted from selected crude oil-utilizing bacterial isolates using the ZR Fungal/Bacterial DNA Prep Kit **(**Zymo Research, supplied by Inqaba Biotec, South Africa), following the manufacturers protocol. As described by Sokolo et al. (2025). A pure culture of each isolate was suspended in 200 µL of isotonic buffer in a ZR BashingBead™ lysis tube. Subsequently, 750 µL of lysis solution was added, the tube was tightly capped, and the sample was homogenized in a cell disruptor fitted with a 2 mL holder assembly at maximum speed for 5 minutes to ensure complete cell disruption.

The lysate was then centrifuged at 10,000 × *g* for 1 minute to separate cellular debris from the released nucleic acids. After centrifugation, 400 µL of the supernatant was transferred into a Zymo-Spin™ IV Spin Filter placed in a collection tube and centrifuged at 7,000 × *g* for 1 minute. The filtrate was mixed with 1,200 µL of DNA Binding Buffer, giving a final volume of 1,600 µL. An aliquot of 800 µL of this mixture was loaded onto a Zymo-Spin™ IIC Column and centrifuged at 10,000 × *g* for 1 minute. The flow-through was discarded, and the remaining sample was applied to the same column and centrifuged again under the same conditions to allow complete DNA binding.

The bound DNA was purified by washing the column first with 200 µL of DNA Pre-Wash Buffer, followed by centrifugation at 10,000 × *g* for 1 minute. This was followed by the addition of 500 µL of Fungal/Bacterial DNA Wash Buffer and a further centrifugation at 10,000 × *g* for 1 minute to remove residual contaminants. The spin column was then transferred to a sterile 1.5 mL microcentrifuge tube, and 100 µL of DNA Elution Buffer was added directly onto the column matrix. After centrifugation at 10,000 × *g* for 30 seconds, purified genomic DNA was recovered in the collection tube. During the purification process, DNA molecules bind to the positively charged matrix of the spin column while impurities are removed through successive washing steps. The purified genomic DNA was carefully labelled and stored at −20 °C until further molecular analyses (Sokolo et al., 2025).

Polymerase Chain Reaction (PCR) Amplification

The bacterial 16S rRNA gene was amplified using the universal primer pair 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′). For fungal isolates, the Internal Transcribed Spacer (ITS) region was amplified using the universal fungal primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). All primers were synthesized by Inqaba Biotec, South Africa. PCR amplification was carried out using a 2× PCR master mix containing Taq DNA polymerase, $MgCl_2$, deoxynucleotide triphosphates (dNTPs), reaction buffer, nuclease-free water, and the appropriate forward and reverse primers.

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Taq DNA polymerase, a thermostable enzyme isolated from *Thermus aquaticus*, catalyzes DNA synthesis during repeated thermal cycling. Magnesium chloride acts as an essential cofactor that enhances the activity of the DNA polymerase, while dNTPs provide the nucleotide building blocks required for DNA strand synthesis. The reaction buffer maintains optimal pH and ionic strength, and nuclease-free water serves as the reaction diluent. The primers specifically anneal to the target DNA sequences, enabling selective amplification of the genes of interest. Each PCR reaction was prepared to a final volume of 25µL. The reaction tubes were briefly mixed, centrifuged, and placed in a thermal cycler for amplification. The cycling conditions consisted of an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 secs, annealing at 52°C for 30 secs, and extension at 72°C for 1 min. A final extension was performed at 72°C for 7 min, after which the reactions were held at 4°C until further analysis (Sokolo et al., 2025).

Agarose Gel Electrophoresis

The PCR amplicons were analyzed by 1% agarose gel electrophoresis to verify the successful amplification of the bacterial 16S rRNA gene. The amplified DNA fragments were compared with a DNA molecular weight marker to confirm the expected amplicon size of approximately 1,500 base pair (bp). Electrophoresis was carried out at 120 V for 15 minutes, after which the gel was placed on a UV transilluminator to visualize and photograph the DNA bands (Ce Bro et al., 2008).

Sequencing

The amplified 16S rRNA gene products were sequenced using the BigDye Terminator Cycle Sequencing Kit following the manufacturer's protocol.

Phylogenic Tree

The obtained 16S rRNA sequences were edited and quality-checked using standard bioinformatics tools before being submitted to the National Center for Biotechnology Information (NCBI) database. The edited sequences were compared with reference sequences in the NCBI database using the Basic Local Alignment Search Tool for Nucleotide (BLASTN) (Johnson et al., 2008) to determine their closest genetic relatives. Multiple sequence alignment was carried out using Clustal Omega (Sievers & Higgins, 2018), after which phylogenetic relationships among the isolates and related reference sequences were reconstructed using the Neighbour-Joining method implemented in MEGA version 6.0 (Kumar et al., 2018; Saitou & Nei, 1987). The reliability of the phylogenetic tree was assessed by bootstrap analysis with 500 replicates, and the resulting bootstrap consensus tree was used to infer the evolutionary relationships among the taxa analysed (Felsenstein, 1985).

Results

Total heterotrophic bacteria counts in surface water from the six stations ranged from $4.98 \pm 2.42 \times 10^6$ CFU/ml at Abonnema to $5.84 \pm 1.72 \times 10^6$ CFU/ml at Obonoma in December and January *(Dry Season)*, while the hydrocarbon utilizing bacteria counts ranged from $2.80 \pm 1.71 \times 10^3$ CFU/ml at Obonoma to $5.20 \pm 0.75 \times 10^3$ CFU/ml at Abonnema.

In the sediment samples, THBC varied between $3.98 \pm 1.48 \times 10^6$ CFU/g at Krakrama and $7.24 \pm 2.04 \times 10^6$ CFU/g at Idama. Whereas the hydrocarbon utilizing bacteria in sediment ranged from $3.32 \pm 1.84 \times 10^3$ CFU/g at Abonnema to $6.54 \pm 1.95 \times 10^3$ CFU/g at Soku as shown in Table 1.

Results presented in Table 2 revealed that, during March/April (beginning of Wet season), total heterotrophic bacteria counts in surface water from the six stations ranged from $4.98 \pm 2.42 \times 10^6$ CFU/ml at Abonnema (ABM) to $5.84 \pm 1.72 \times 10^6$ CFU/ml at Obonoma (OBO) and hydrocarbon utilizing bacteria counts ranged from $2.80 \pm 1.71 \times 10^3$ CFU/ml at Obonoma to $5.20 \pm 0.75 \times 10^3$ CFU/ml at Abonnema.

In the sediment, total heterotrophic bacteria counts ranged from $3.98 \pm 1.48 \times 10^6$ CFU/g at Krakrama to $7.24 \pm 2.04 \times 10^6$ CFU/g at Idama and hydrocarbon utilizing bacteria counts ranged from $3.32 \pm 1.84 \times 10^3$ CFU/g at Abonnema to $6.16 \pm 1.80 \times 10^3$ CFU/g at Idama (Table 2).

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Table 1: Total Heterotrophic and Hydrocarbon Utilizing Bacteria Counts of Surface Water and Sediment Samples from Sombreiro Rivers During December/January (Dry Season)

Location/Station	Surface Water		Sediment	
	THBC ($\times10^6$CFU/ml)	HUBC ($\times10^3$CFU/ml)	THBC ($\times10^6$CFU/g)	HUBC ($\times10^3$CFU/g)
Degema	5.24±1.78[a]	4.68±1.24[a]	6.64±1.94[a]	5.04±1.60[a]
Abonnema	4.98±2.42[a]	5.20±0.75[a]	4.32±0.81[a]	3.32±1.84[a]
Krakrama	5.48±3.03[a]	4.46±1.23[a]	3.98±1.48[b]	4.46±1.94[a]
Obonoma	5.84±1.72[a]	2.80±1.71[b]	4.90±2.04[a]	3.70±1.91[a]
Idama	5.16±2.69[a]	3.16±1.45[a]	7.24±2.04[a]	6.16±1.80[a]
Soku	5.38±2.03[a]	5.14±1.12[a]	5.14±1.46[a]	6.54±1.95[a]

Keys: THBC= Total Heterotrophic Bacteria Counts; HUBC= Hydrocarbon utilizing bacteria counts.
Post Hoc (Tukey's Test): Values in the same column with different superscripts (a, b) differ significantly at $p<0.05$ while values in same column with same superscripts (a) shows no significant difference.

Table 2: Total Heterotrophic and Hydrocarbon Utilizing Bacteria Counts of Surface Water and Sediment Samples from Sombreiro Rivers During March/April (beginning of Wet season)

Location/Station	Surface Water		Sediment	
	THBC ($\times10^6$CFU/ml)	HUBC ($\times10^3$CFU/ml)	THBC ($\times10^6$CFU/g)	HUBC ($\times10^3$CFU/g)
Degema	5.24±1.78[a]	4.68±1.24[a]	6.64±1.94[a]	5.04±1.60[a]
Abonnema	4.98±2.42[a]	5.20±0.75[a]	4.32±0.81[a]	3.32±1.84[a]
Krakrama	5.48±3.03[a]	4.46±1.23[a]	3.98±1.48[b]	4.46±1.94[a]
Obonoma	5.84±1.72[a]	2.80±1.71[b]	4.90±2.04[a]	3.70±1.91[a]
Idama	5.16±2.69[a]	3.16±1.45[a]	7.24±2.04[a]	6.16±1.80[a]
Soku	5.38±2.03[a]	5.14±1.12[a]	5.14±1.46[a]	6.54±1.95[a]

Keys: THBC= Total Heterotrophic Bacteria Counts; HUBC= Hydrocarbon utilizing bacteria counts;
Post Hoc (Tukey's Test): Values in the same column with different superscripts (a, b) differ significantly at $p<0.05$ while values in same column with same superscripts (a) shows no significant difference

In July and August, the peak months of raining season, total heterotrophic bacteria counts in surface water ranged from $3.66 \pm 1.19 \times 10^6$ CFU/ml to $6.44 \pm 1.24 \times 10^6$ CFU/ml and hydrocarbon utilizing bacteria counts ranged from $3.74 \pm 1.60 \times 10^3$ CFU/ml to $5.68 \pm 1.41 \times 10^3$ CFU/ml. In the sediment, total heterotrophic bacteria counts in surface water ranged from $4.50 \pm 1.42 \times 10^6$ CFU/g to $5.90 \pm 1.67 \times 10^6$ CFU/g and hydrocarbon utilizing bacteria counts ranged from $4.50 \pm 1.42 \times 10^6$ CFU/g to $5.90 \pm 1.67 \times 10^6$ CFU/g (Table 3).

The electrophoretic profiles shown in Plates 1 verified the successful amplification of the target genes. Following amplification, the nucleotide sequences were analyzed against the National Center for Biotechnology Information (NCBI) database to identify closely related organisms.

A phylogenetic tree was subsequently constructed to depict the evolutionary relationships between the obtained sequences and their corresponding reference sequences, as presented in Figure 1.

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Table 3: Total Heterotrophic and Hydrocarbon Utilizing Bacteria Counts of Surface Water and Sediment Samples from Sombreiro Rivers during July/August (Wet Season)

Location/Station	Surface Water		Sediment	
	THBC ($\times10^6$CFU/ml)	HUBC ($\times10^3$CFU/ml)	THBC ($\times10^6$CFU/g)	HUBC ($\times10^3$CFU/g)
Degema	4.70±1.73[a]	5.06±1.73[a]	5.44±2.17[a]	5.54±2.23[a]
Abonnema	5.16±1.38[a]	5.40±0.59[a]	4.68±1.69[a]	4.77±1.48[a]
Krakrama	3.66±1.19[a]	5.68±1.41[a]	4.50±1.42[a]	5.26±1.39[a]
Obonoma	6.44±1.24[a]	4.60±2.17[a]	5.90±1.67[a]	5.44±2.03[a]
Idama	5.08±0.91[a]	3.74±1.60[a]	5.10±1.11[a]	5.36±2.58[a]
Soku	5.50±1.78[a]	5.06±1.85[a]	5.52±0.92[a]	5.38±1.74[a]

Keys: THBC= Total Heterotrophic Bacteria Counts; HUBC= Hydrocarbon utilizing bacteria counts;
Post Hoc (Tukey's Test): Values in the same column with different superscripts (a, b) differ significantly at $p<0.05$ while values in same column with same superscripts (a) shows no significant difference.

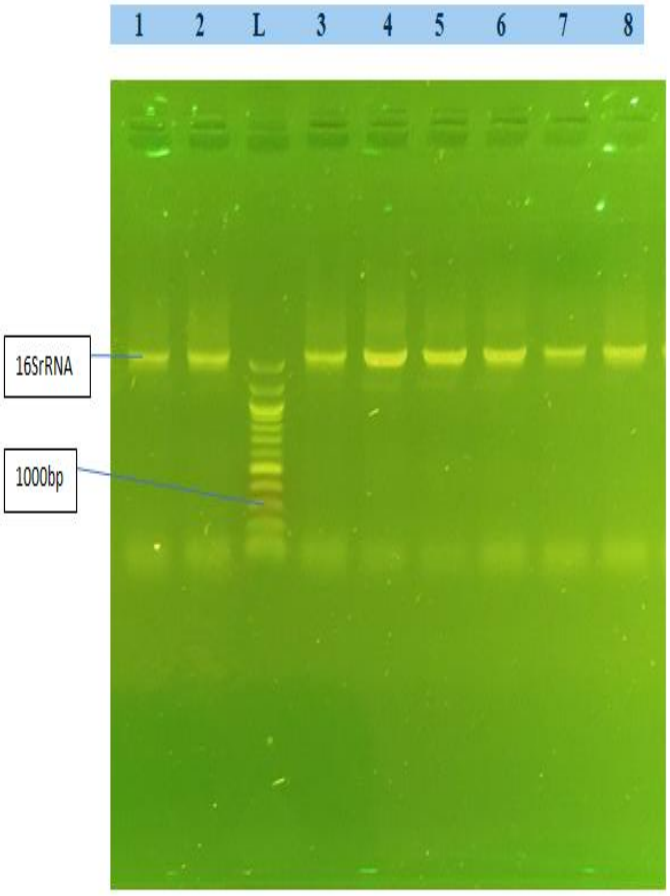

Plate 1: Agarose Gel Electrophoresis Showing the Amplified 16SrRNA of Hydrocarbonoclastic Bacteria Isolates. Lanes 1-8 shows the 16SrRNA bands at 1500bp while Lane L represents the 1000bp DNA molecular ladder.

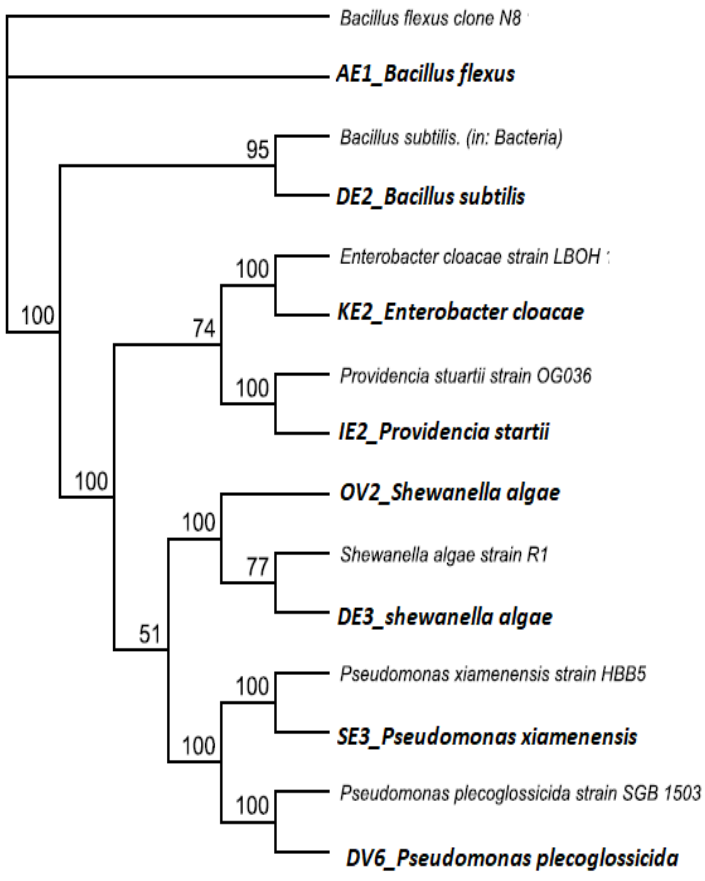

Figure 1: Phylogenetic Tree analyses of Hydrocarbon Utilizing Bacteria Isolated from Sombreiro River

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Table 4: The Molecular Identities of Hydrocarbonoclastic Bacteria Isolates and their Accession Numbers

Code	Percentage Similarity	Identity	Accession Numbers
AE1	100	*Bacillus flexus strain* S6c	MT645459.1
DE2	97.19	*Bacillus subtilis*	OQ726278.1
DE3	100	*Shewanella algae* strain R1	MT549169.1
DV6	100	*Pseudomonas plecoglossicida* strain SGB1503	MT498800.1
IE 2	100	*Providencia startii* strain OG036	MK641351.1
KE2	100	*Enterobacter cloacae* strain LBOH	OR426319.1
OV2	100	*Shewanella algae* strain R1	MT549169.1
SE3	100	*Pseudomonas xiamenensis strain HBB5*	MN611113.1

Discussion

The results presented in the Table 1 shows that although Obonoma recorded the highest THBC while Abonnema recorded the lowest, all stations shared the same superscript (a), indicating that there was no significant spatial variation ($p > 0.05$) in heterotrophic bacterial populations across the river during the dry season. This relatively uniform distribution suggests that the physicochemical characteristics of the river water and nutrient availability were fairly similar among the sampling stations during the study period. Heterotrophic bacteria constitute the dominant microbial community in aquatic ecosystems because they utilize a wide variety of organic substrates, and their abundance generally reflects the availability of biodegradable organic matter and environmental stability (APHA, 2023; Atlas & Bartha, 2002).

Unlike THBC, Obonoma was assigned a different superscript (b), indicating that its HUBC was significantly lower ($p < 0.05$) than those of the other stations. The comparatively high hydrocarbon-utilizing bacteria counts (HUBC) in surface water observed at Abonnema, Soku ($5.14 \pm 1.12 \times 10^3$ CFU/ml), and Degema ($4.68 \pm 1.24 \times 10^3$ CFU/ml) may indicate previous exposure to petroleum hydrocarbons or continuous low-level hydrocarbon contamination, which selectively enriches indigenous hydrocarbon-degrading microorganisms. Hydrocarbon-utilizing bacteria are known to increase in abundance in environments with chronic petroleum inputs because hydrocarbons serve as carbon and energy sources for these specialized microorganisms (Varjani, 2017; Das & Chandran, 2011).

Conversely, the significantly lower count recorded at Obonoma may suggest reduced hydrocarbon availability or environmental conditions less favorable for hydrocarbon-degrading bacterial proliferation.

In the sediment samples, Krakrama was the only station assigned a different superscript (b), indicating that its bacterial population was significantly lower ($p < 0.05$) than those of the other stations. The higher bacterial counts observed at Idama and Degema ($6.64 \pm 1.94 \times 10^6$ CFU/g) are expected because sediments provide greater nutrient availability, organic matter accumulation, moisture retention, and protection from environmental disturbances than the overlying water column. Sediments therefore serve as reservoirs for diverse microbial communities and support greater microbial biomass than surface water (Azubuike et al., 2016; Head et al., 2006).

Despite these numerical differences seen in hydrocarbon-utilizing bacteria counts in sediment, all stations shared the same superscript (a), indicating no significant difference ($p > 0.05$) in sediment HUBC among the sampling locations. The generally higher HUBC observed in sediment than in surface water supports previous reports that petroleum hydrocarbons tend to adsorb onto sediment particles where they persist for longer periods, creating favorable habitats for hydrocarbon-degrading microorganisms (Prince et al., 2018); Varjani, 2017). Sediment therefore functions as an important ecological niche for indigenous hydrocarbonoclastic bacteria that contribute to the natural attenuation of petroleum pollutants.

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The result demonstrates that bacterial populations were consistently abundant throughout the Sombreiro River during the dry season. Total heterotrophic bacteria showed no significant spatial variation in surface water but exhibited a significant reduction in sediment at Krakrama. In contrast, hydrocarbon-utilizing bacteria displayed significant variation only in surface water, where Obonoma recorded the lowest count, while sediment HUBC remained relatively uniform across all stations.

The generally higher bacterial populations observed in sediments compared with surface water highlight the ecological importance of sediments as reservoirs of both heterotrophic and hydrocarbon-degrading microorganisms. The results suggest the presence of indigenous microbial communities with the capacity for natural biodegradation of hydrocarbons within the Sombreiro River ecosystem, indicating an inherent potential for bioremediation following petroleum contamination (Das & Chandran, 2011; Varjani, 2017; Prince et al., 2018).

The total heterotrophic bacteria counts (THBC) and hydrocarbon-utilizing bacteria counts (HUBC) recorded during March/April, which marks the beginning of the wet season, demonstrated that the Sombreiro River supports abundant bacterial populations in both the water column and sediments. The onset of the rainy season is typically characterized by increased runoff from surrounding terrestrial environments, which introduces organic matter, nutrients, and petroleum-derived contaminants into aquatic ecosystems, thereby stimulating microbial growth and metabolic activities (Varjani et al., 2023; Ayangbenro et al., 2024).

In the surface water, although Obonoma recorded the highest heterotrophic bacterial density, followed by Krakrama ($5.48 \pm 3.03 \times 10^6$ CFU/ml), Soku ($5.38 \pm 2.03 \times 10^6$ CFU/ml), Degema ($5.24 \pm 1.78 \times 10^6$ CFU/ml), and Idama ($5.16 \pm 2.69 \times 10^6$ CFU/ml), all stations carried the same superscript ("a"), indicating that these differences were not statistically significant ($p > 0.05$). The relatively uniform distribution of heterotrophic bacteria suggests that environmental conditions across the river were generally similar during the early wet season, allowing bacterial communities to proliferate throughout the study area.

Similar observations have been reported in tropical estuarine ecosystems where rainfall enhances nutrient availability and promotes microbial productivity without causing marked spatial differences in heterotrophic bacterial abundance (Zhang et al., 2023; Ayangbenro et al., 2024). Hydrocarbon-utilizing bacteria in the surface water varies and Obonoma carried a different superscript ("b"), indicating that its HUBC was significantly lower ($p < 0.05$) than those of the other stations.

This lower abundance may reflect reduced hydrocarbon contamination, limited availability of biodegradable petroleum substrates, or local physicochemical conditions that were less favorable for hydrocarbon-degrading microorganisms. Hydrocarbon-utilizing bacteria generally proliferate where hydrocarbons are consistently available as carbon and energy sources; therefore, their abundance often reflects the extent of petroleum contamination in aquatic environments (Varjani et al., 2023; Saxena et al., 2024).

Sediment samples contained greater bacterial populations than the overlying water, confirming that sediments provide a more favorable habitat for microbial colonization due to their higher organic matter content, nutrient accumulation, and protection from environmental disturbances. The significantly lower count observed at Krakrama (superscript "b") suggests localized environmental conditions such as sediment texture, lower organic carbon, or hydrodynamic disturbances that may have limited bacterial proliferation. Previous studies have similarly shown that sediment microbial communities are strongly influenced by organic matter availability, oxygen gradients, and contaminant accumulation, resulting in higher bacterial densities than those found in surface waters (Zhang et al., 2023; Mishra et al., 2022).

Hydrocarbon-utilizing bacteria counts in the sediments vary and despite these numerical differences, all sediment HUBC values shared the same superscript ("a"), indicating that the variations were not statistically significant ($p > 0.05$). This suggests that hydrocarbon-degrading bacteria were widely distributed across the sediments of the Sombreiro River during the onset of the rainy season.

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The widespread occurrence of these microorganisms indicates chronic exposure of the sediments to petroleum hydrocarbons, allowing indigenous hydrocarbonoclastic bacteria to become established throughout the ecosystem. Similar findings have been reported in oil-producing regions of the Niger Delta, where sediment environments consistently harbor abundant hydrocarbon-degrading bacterial communities because of long-term hydrocarbon deposition (Varjani et al., 2023; Chikere et al., 2022).

The results demonstrate that the beginning of the wet season supports active microbial communities within the Sombreiro River ecosystem. The absence of significant spatial differences in most THBC and sediment HUBC values suggests relatively uniform environmental conditions across the sampling stations, whereas the significantly lower surface-water HUBC at Obonoma and sediment THBC at Krakrama indicate localized ecological variations. The consistently higher bacterial populations in sediments compared with surface waters further confirm the ecological importance of sediments as reservoirs of indigenous heterotrophic and hydrocarbon-degrading bacteria, which play a critical role in the natural attenuation and biodegradation of petroleum contaminants in aquatic environments (Ayangbenro et al., 2024; Saxena et al., 2024).

During July and August**,** corresponding to the peak of the rainy season, both surface water and sediment supported high bacterial populations, reflecting increased microbial activity associated with enhanced nutrient availability and hydrocarbon inputs. The consistently higher THBC than HUBC indicates that the majority of bacteria utilized general organic substrates, whereas only a specialized fraction possessed the metabolic capability to degrade petroleum hydrocarbons. This observation agrees with recent studies showing that hydrocarbon-degrading bacteria constitute a subset of the indigenous heterotrophic microbial community and become enriched in oil-contaminated aquatic environments (Ławniczak et al., 2020; Laczi et al., 2020).

The elevated bacterial populations recorded during the rainy season may be attributed to increased surface runoff, which transports dissolved organic matter, nutrients, suspended sediments, and petroleum residues into the river.

These inputs stimulate microbial metabolism and promote the proliferation of both heterotrophic and hydrocarbon-utilizing bacteria. Rainfall also redistributes hydrocarbons within the aquatic environment, increasing their bioavailability to indigenous degraders and enhancing natural biodegradation processes. Similar seasonal increases in microbial abundance have been reported in polluted estuarine ecosystems, where wet-season conditions favors bacterial growth and hydrocarbon degradation (Okereke & Ike-Obasi, 2021).

In the sediment, high microbial abundance reflects their function as repositories for organic matter and petroleum hydrocarbons. Sediments provide stable physicochemical conditions, moisture, and nutrients that support diverse microbial communities and facilitate the persistence of hydrocarbon-degrading microorganisms. Consequently, sediments often serve as the primary sites for petroleum hydrocarbon biodegradation in aquatic ecosystems (Dell'Anno et al., 2021). The appreciable numbers of hydrocarbon-utilizing bacteria observed in both water and sediment demonstrate the adaptive response of indigenous microorganisms to chronic hydrocarbon contamination.

Continuous exposure to petroleum hydrocarbons selectively enriches bacteria capable of utilizing these compounds as carbon and energy sources, thereby enhancing the natural attenuation capacity of the ecosystem. Such microbial communities are fundamental to the biodegradation and eventual mineralization of petroleum pollutants and represent valuable biological resources for the remediation of oil-contaminated rivers (Wang et al., 2024).

The phylogenetic tree (Figure 1) revealed that the hydrocarbon-utilizing bacterial isolates from the Sombreiro River were genetically diverse and clustered with known hydrocarbon-degrading bacteria in the NCBI database. The close relationship between the isolates and reference strains confirms their taxonomic identity and suggests that they possess conserved genes involved in petroleum hydrocarbon degradation. The occurrence of multiple bacterial clades indicates the presence of a diverse indigenous microbial community capable of utilizing hydrocarbons as sources of carbon and energy.

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Such microbial diversity is advantageous for natural attenuation and enhances the bioremediation potential of the river ecosystem, as different bacterial species contribute complementary metabolic pathways for degrading a wide range of hydrocarbon compounds (Das & Chandran, 2011; Varjani, 2017; Prince et al., 2018).

The molecular characterization of the hydrocarbonoclastic bacterial isolates identified members of the genera *Bacillus*, *Shewanella*, *Pseudomonas*, *Providencia*, and *Enterobacter*, with sequence similarities ranging from 97.19% to 100%, confirming accurate species identification based on 16S rRNA gene sequencing (Janda & Abbott, 2007; Stackebrandt & Ebers, 2006). *Bacillus flexus* has undergone taxonomic reclassification and is currently designated as *Priestia flexa*. Furthermore, Sokolo et al. (2025) molecularly identified *Priestia flexa* having the potential to degrade hydrocarbons from Orashi River. The predominance of *Bacillus* and *Pseudomonas* species suggests that these bacteria are major contributors to petroleum hydrocarbon degradation because of their metabolic versatility and biosurfactant-producing capabilities (Das & Chandran, 2011; Varjani, 2017). The occurrence of *Shewanella algae*, *Providencia startii*, and *Enterobacter cloacae* further demonstrates the presence of a phylogenetically diverse microbial community capable of utilizing hydrocarbons under varying environmental conditions (Hau & Gralnick, 2007; Varjani & Upasani, 2017). Overall, the high sequence similarities and assigned GenBank accession numbers indicate that the Sombreiro River harbors indigenous hydrocarbon-degrading bacteria with significant potential for the natural attenuation and bioremediation of petroleum-contaminated aquatic ecosystems (Das & Chandran, 2011; Wang et al., 2021).

Conclusion

This study demonstrated that the Sombreiro River harbors a diverse population of indigenous hydrocarbonoclastic bacteria capable of utilizing petroleum hydrocarbons as carbon and energy sources. Molecular identification revealed the presence of *Bacillus flexus*, *Bacillus subtilis*, *Shewanella algae*, *Pseudomonas plecoglossicida*, *Pseudomonas xiamenensis*, *Providencia startii*, and *Enterobacter cloacae*, with sequence similarities ranging from 97.19% to 100%, confirming the reliability of the taxonomic identification. The occurrence of these well-known hydrocarbon-degrading bacteria indicates that the river possesses an inherent microbial capacity for the natural attenuation of hydrocarbon pollutants. Their distribution across the sampling stations suggests adaptation to hydrocarbon contamination and highlights their ecological importance in maintaining the self-purification potential of the aquatic ecosystem. Overall, the findings provide valuable baseline information on the hydrocarbon-degrading bacterial community of the Sombreiro River and support the potential application of these indigenous microorganisms in the bioremediation of petroleum-contaminated aquatic environments.

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