Comparative Biochemical Characterization of Hydrocarbon-Degrading Bacteria on Petroleum-Contaminated Soils from Baranyonwa-Dere in Gokana and Non-Contaminated Soils from Rukpokwu in Obio/Akpor L.G.A, Rivers State, Nigeria

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Abstract:
This study presents a comparative biochemical evaluation of hydrocarbon-degrading bacteria (HDB) isolated from petroleum-contaminated soil in Baranyonwa-Dere (B-Dere), Gokana LGA, and non-contaminated soil from Rukpokwu, Obio/Akpor LGA, Rivers State, Nigeria. Using culture-dependent methods and standard biochemical assays, five bacterial genera were identified across both sites: *Pseudomonas sp., Salmonella sp*., *Citrobacter sp., Bacillus megaterium*, and *Bacillus subtilis*. However, isolates from the contaminated site demonstrated significantly higher enzymatic activities, including catalase, urease, succinate dehydrogenase, peroxidase, and protease. Among the isolates, *Bacillus subtilis* from the polluted soil exhibited the highest catalase (730.73 ± 79.92 µmol/mL/min) and urease (0.25 ± 0.01 mg/g/min) activities, while *Pseudomonas sp.* recorded peak succinate dehydrogenase activity (5.67 ± 0.01 µmol/mL/min). *Bacillus megaterium* excelled in peroxidase (76.04 ± 0.01 µmol/mL/min) and protease (91.08 ± 0.68 units/mg) activities. In contrast, isolates from the non-contaminated site exhibited comparatively lower enzymatic expression, indicating reduced metabolic demand and hydrocarbon stress. Catalase activity was significantly dominant ($p < 0.001$) in the polluted environment, suggesting its pivotal role in microbial oxidative stress management and survival in petroleum-rich soils. These findings confirm that oil contamination selectively enhances the diversity and enzymatic adaptation of indigenous bacteria, equipping them for effective hydrocarbon degradation. The elevated metabolic activities in contaminated soils underscore the potential of these native strains as viable bioremediation agents. Harnessing their enzymatic profiles offers a sustainable and eco-friendly strategy for restoring oil-polluted environments in the Niger Delta and similar regions globally.

Keywords: **Bioremediation, Enzymatic activity, Hydrocarbon degradation, Hydrocarbon degrading bacteria, Niger Delta, Oil pollution.**

1. Introduction

OIL exploration and related industrial activities remain heavily concentrated in the Niger Delta region, particularly in South-South Nigeria [18, 26]. This has resulted in chronic environmental pollution with significant ecological and health implications [28, 1, 20]. Recent surveys show that over 60% of Niger Delta residents report health issues due to oil pollution, and approximately 75% indicate biodiversity loss in their areas [21, 20]. Populations in affected regions are exposed to petroleum sludge and residues known for cytotoxicity, mutagenicity, teratogenicity, immunotoxicity, and carcinogenic potential [1]. The continuous influx of petroleum hydrocarbons from extraction, refining, and transportation degrades soils and water bodies [39], and spills often create rancid odors that exacerbate environmental degradation [45].

Pollutants such as total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), and volatile aromatics (BTEX) remain persistent, presenting major remediation challenges due to their toxicity and environmental persistence [13]. In response, regulatory bodies continue to recommend remediation techniques, with bioremediation emerging as the most sustainable, economically viable, and eco-friendly approach [12]. A study demonstrated the physiochemical properties of hydrocarbon degrading bacteria isolated from oil contaminated soils in B-Dere, Gokana Local Government Area of River State [15]. Similarly, a recent 2024 study in Khana LGA, Rivers State, demonstrated effective TPH degradation-achieving rate constants between 0.0013-0.0021 hr^{-1}using a microbial consortium of *Bacillus subtilis* and *Aspergillus niger* [9]. Similarly, mixed cultures and bioaugmentation strategies have been confirmed as superior to single strains [13]. Indigenous plant-microbe systems, such as *Vernonia* spp., have also been validated for their bioremediation potential in clayed soils of Ogoni land [11].

Bioremediation relies on microorganisms to metabolize hydrocarbons into harmless products. Enzymes like dehydrogenases, oxygenases, and lipases are central to this process, acting as bioindicators and have been proposed to enhance biodegradation efficiency [38], catalysts [31] and enzyme cocktails and biocatalysts [51]. Enzymes have a high catalytic activity under mild reaction circumstances, need little energy, react quickly at low contaminant concentrations, and produce fewer harmful byproducts [48, 29]. On the other hand, microorganisms can use carbon and nitrogen as energy sources as organic matter breaks down [5]. Bioremediation is safer, more environmentally friendly, and uses less energy because to these benefits. Consequently, microorganisms and enzymes

have emerged as crucial instruments for real-world use and are quickly attracting attention for environmental use [16]. Furthermore, the application of enzymes and microbial-based bioremediation is driven primarily by the growing usage of biological components, the requirement for mild reaction conditions, and cheap reaction costs [48].

Despite progress, there is a notable gap: few studies have compared the biochemical enzyme profiles of hydrocarbon-degrading bacteria (HDB) from both contaminated and uncontaminated soils within the same Niger Delta region. Addressing this gap is essential, as enzyme activity patterns may reflect adaptive microbial responses and inform targeted remediation techniques.

This study undertakes a comparative biochemical characterization of HDB isolated from contaminated soil in Baranyonwa-Dere (Gokana) and non-contaminated soil in Rukpokwu (Obio/Akpor)**,** Rivers State, Nigeria. By measuring key enzyme activities and metabolic functions, we aim to elucidate microbial adaptations to petroleum exposure and identify metabolic capabilities that support tailored, site-specific bioremediation strategies. The direct dual-site enzymatic comparison used in this study is innovative because it goes beyond straightforward presence-absence or degradation efficacy analyses and offers a deeper comprehension of functional microbial resilience in environments enriched with oil. Frameworks for ecological remediation that are optimized for Niger Delta ecosystems will be informed by these discoveries.

II. MATERIALS AND METHODS

I. Study Site

The study site is located at the oil polluted site in Baranyonwa-Dere (B-Dere) community in Ogoni land, Gokana Local Government Area, Rivers State, Nigeria. Gokana is located on longitude 04°66.92'N and latitude 07°28.69'E [33].

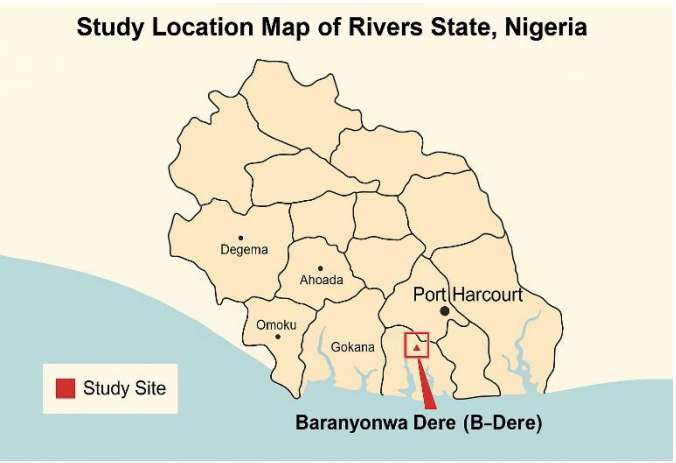

Figure 1: Map of River State showing B-Dere Community in Gokana L.G.A. [33]

II. Soil Collection and Pretreatment of Polluted Soils Samples

The oil soil samples were collected from three locations in B-Dere community, Gokana Local Government Area of Rivers State. A hand-held auger [37] was then used for collecting 50 g of the oil-polluted soil sample from sampling locations at the depths of 0 cm–15 cm (first layer) and 15 cm–30 cm (second layer), as well as core samples utilizing core cylinders. Following proper labeling and storage in sterile nylon bags, the samples were promptly taken to the lab to evaluate enzymatic activities using culture-dependent methods and standard biochemical assays. A garden in Rukpokwu, Obiakpor L.G.A, Rivers State, provided the uncontaminated soil samples which served as control. They were promptly taken to the lab for morphological and enzymatic studies.

III. Isolation of Crude Oil Degrading Bacteria

Contaminated soil samples were serially diluted to factor four (4) using ten-fold dilution (10^4) [24]. Pour plating was used to plate the fourth dilution on mineral salt medium, and sterile filter paper moistened in pure crude oil was placed on the Petri dish cover (vapour phase technique). Incubation of the plates lasted 21 days. Colonies that emerged after incubation were sub-cultured into fresh Nutrient agar plates and incubated for 18-24 hours at room temperature. Pure isolates were subjected to few biochemical tests, and the result obtained were compared with standard manual for identification of bacteria.

IV. Biochemical Characterization of Microbial Isolates

An array of biochemical analyses was conducted, encompassing catalase activity determination, oxidase activity testing, urease activity testing, spore staining and motility assessment. The data obtained from the examination of the different bacterial isolates were gathered and the identification process was conducted by means of a comparative analysis with the methodology outlined by Stancu [32].

V. Investigation of Some Biochemical Studies of Hydrocarbon Degrading Bacteria in the Polluted Soil Samples

Catalase Test

In order to ascertain whether the isolates could break down H_2O_2 and produce O_2 and H_2O, the test was conducted. The test was conducted by exposing the test isolates to 3 % H_2O_2. Three milliliters of H_2O_2 solution were placed in separate test tubes, and some colonies of the separated organisms were introduced into the H_2O_2-containing test tube. The test's contents were examined for gas bubbling. Effervescence indicated the presence of bacteria that tested positive for catalase, while absence of effervescence indicated bacteria not positive for catalase [2].

Indole Test

According to standard methodologies [54, 22], the test was conducted. Each test tube contained precisely 3 mL of sterile typtone water, and the experimental organisms were injected within the tubes. At 30 °C, it was incubated for 48 hours. A red ring was formed at the top layer of the test tubes after 10 minutes, which signified a successful outcome. After 48 hours, 0.5 mL of Kovac's reagent was added to each test tube and thoroughly mixed. If there fails to be a red ring, the outcome is negative.

Oxidase Test

On a piece of filter paper, 2-3 drops of a recently prepared oxidase reagent were applied [54]. Aseptic technique was employed to select a colony of the experimental microorganism using a sterile glass rod. The selected colony was then transferred onto a filter paper and subsequently monitored for any alterations in coloration within a time frame of 10 seconds. The manifestation of a blue hue signified an unfavourable outcome.

Urease Test

In this experiment, the test organisms were inoculated onto slants containing urea agar in test tubes, which were subsequently incubated at 30 °C for 24 hours. The solid medium developed by Christensen was formulated by dissolving 1 gram of peptone, 5 grams of NaCl, 2 g of K_2HPO_4, and 10 g of agar in 100 mL of distilled water. In addition, a volume of 60 mL of phenol red was introduced into the solution, at a neutral pH. The sample was sterilized at 121ºC for 20 minutes under the pressure of 15 psi. The temperature was then adjusted to 5ºC. Aseptically, a mixture of 10 mL of a 10 % glucose solution and 100 mL of a 20 % urea solution was combined with agar medium. This mixture was then dispensed in 5 mL quantities into bijou bottles and left to solidify in a sloped position. The cultural specimens were examined for chromatic transition, specifically from a yellow hue to a pink hue, which served as an indication of a favourable outcome. Conversely, bacteria that tested negative for urease activity did not exhibit any alteration in coloration [53].

Citrate Utilization Test

The agar medium was sterilized through autoclaving at 121ºC for 15 minutes. The agar was cooled and subsequently poured into plates, where it was left undisturbed to solidify. After the test organisms were injected onto the plate, they were cultured for 48 hours at 37 °C. The growth development was evaluated by examining the test tubes, specifically searching for a shift in color from the medium's initial green hue to a blue hue. This change in pigment signifies the use of citrate, which suggests a successful outcome [53].

Methyl Red - Voges Proskauer broth (MR - VP) Test

This differentiation pertains to organisms that exhibit the ability to generate and sustain a significant level of acidity, as opposed to those that initially possess a lower level of acidity that gradually returns to a state of neutrality. The Voges Proskauer test is utilized in order to ascertain the production of acetyl methylcarbinol (acetone) resulting from the metabolism of dextrose. The reagent oxidizes this compound to form diacetyl, resulting in the generation of a red colour when interacting with guanidine residues present in the medium. The test organism was inoculated into Methyl Red – Voges Proskauer broth, a dextrose phosphate medium, and cultured for five days at 30°C for the purpose of conducting the methyl red test. A small amount of 0.1 % methyl red solution was subsequently added. A red hue signifies a positive outcome, whereas a yellow hue signifies a negative response. In the Voges Proskauer test, a 3 mL aliquot of a 40 % potassium hydroxide (KOH) solution was added to the broth culture of the test organism, along with 6 % α-naphthol. A positive result was indicated by the emergence of a bright pink coloration within a time frame of 5 minutes, whereas a negative reaction was denoted by a yellow colour [53].

Motility Test

Growing the isolates in semi-solid nutrient agar allowed for the detection of their motility [22, 34]. The inoculating needle was used to stain the semi-solid nutritional agar in the test tube with test organisms. After being picked with a needle, the organisms were stabbed into the agar, incubated for 24 hours at 30ºC, and then their growth was monitored. The organism's motility was demonstrated by the growth that occurred along the stabbing line.

Hydrogen Sulphide Production

The TSI agar test is commonly employed for the purpose of identifying enteric bacteria in laboratory settings. Additionally, this method is employed to differentiate enterobacteriaceae from other gram-negative bacteria found in the intestinal tract based on their capacity to metabolise glucose, lactose, or sucrose, as well as their ability to release sulphides from ferrous ammonium sulphate or sodium thiosulphate. A quantity of the test organism was introduced onto the surface of the slant and inoculated at 37ºC for 48 hours. The presence of hydrogen sulphide production is indicated by the blackening of the medium, which signifies a positive outcome. Conversely, the absence of any alteration in the coloration of the media indicates a negative result [22].

Sugar Fermentation Test

The test was conducted in order to assess the tendency of isolates to metabolize various carbohydrates through either fermentation or oxidation, which leads to the simultaneous generation of acid and gas. A sterile basal medium was supplemented with precisely 1 g of each test carbohydrate, including glucose, sucrose, fructose, xylose, lactose, maltose, mannitol, and galactose. Phenol red with peptone was added to each medium, which was freshly prepared. A volume of 10 mL of each medium was carefully transferred into separate test tubes, with a Durham's tube placed in an inverted position within each tube. The test tubes were sterilized at a 121ºC for 15 minutes. Subsequently, the mixture was allowed to undergo the cooling process prior to the introduction of distinct isolates via a sterile inoculating loop. The resulting mixture was then inoculated at 30ºC for 24 - 48 hours. The test tubes were analyzed to determine the existence of gas and the production of acid which was identified through the observation of air space in Durham's tubes [3].

Lipase Test

The basis of this test lies in the observation that lipolytic microorganisms exhibit a distinct area of lipolysis, characterized by a clear zone surrounding their bacterial growth. This phenomenon is observed when these microorganisms are inoculated on a nutrient agar medium that has been supplemented with triglyceride tributyrin. A volume of 15 mL of tributyrin agar medium was aseptically transferred into sterile petri dishes. The test organism was then introduced

onto the agar surface using a single line streak technique. The inoculated plates were incubated at 30°C for 48 hours. A clear zone surrounding the organism's development indicates a positive lipid hydrolysis test result, whereas the absence of a clear zone indicates a negative reaction [3].

Starch Hydrolysis Test

The test organisms were introduced onto a medium containing starch and subsequently placed in an incubator set at of 37°C for 24 hours. Lugol's iodine was applied to the surface, resulting in flooding. The isolates possessing the amylase enzyme exhibited hydrolysis of starch, resulting in a positive outcome. However, the absence of blue-black coloration was indicative of a negative outcome [50].

Spore Staining Test

To determine whether spores were present in the test isolates, this test was conducted. Gram staining was performed using the same bacterial smears that were produced. The slides were passed over the flame multiple times to heat-fix them. Following that, the slides were submerged in boiling water, inundated with malachite green stain, and allowed to sit for ten minutes. Following a 20 second safranin deluge, the slides were cleaned with slow-running water, rinsed under tap water, and then blot dried. The purpose used to view the slides was oil immersion. The bacterium's spore dyed green, while its vegetative component stained pink [22].

VI. Determination of Enzymatic Activity in Hydrocarbon Degrading Bacteria

Assay of Catalase

An enzyme extract volume of 0.5 mL was added to a reaction mixture that contained 0.4 mL of H2O, 0.5 mL of 0.2 M H_2O_2, and 1 mL of 0.01 M phosphate buffer at pH 7.0. The combination was then incubated for different amounts of time. In order to finish the reaction, 2 mL of an acid reagent made of a combination of acetic acid and dichromate were added. Glacial acetic acid was mixed with petroleum dichromate in a 1:3 volume ratio to create this reagent. Control was established in the experimental setup by introducing the enzyme after the acid reagent. At a wavelength of 610 nm, the absorbance of the tubes was measured following a 10-minute heating operation. In order to determine the enzyme activity, an extinction coefficient of 0.036/µmol/mL was used. Following that, the results were converted to micromoles of hydrogen peroxide consumed per minute per milligram of protein (umol H_2O_2/min/mg protein) [46].

Assay of Peroxidase

The experimental procedure involved incubating a reaction mixture containing specific volumes of various components at different time intervals. The reaction mixture consisted of 0.4 mL of a 0.4 M sodium phosphate buffer with a pH of 7.0, 0.1 mL of a 10 mM sodium azide solution, 0.2 mL of a 4 mM reduced glutathione solution, 0.1 mL of a 2.5 mM H_2O_2 solution, 0.2 mL of water, and 0.5 mL of a plant extract. The incubation was carried out for 0, 30, 60, and 90 seconds, respectively. The reaction was concluded by adding 0.5 mL of Tricarboxylic acid (TCA) solution with a concentration of 10 %. After centrifugation, 2 mL of the resulting liquid above the sediment (supernatant) was combined with 3 mL of phosphate buffer and 1 mL of DTNB reagent, also known as Ellman's reagent. The DTNB reagent consisted of 5,5'-dithiobis-(2-nitrobenzoic acid) (0.04 %) dissolved in a solution of 1 % sodium citrate. The developed colour was measured at a wavelength of 412 nm, and the activity of the enzyme was quantified in units per milligramme (micrograms of glutathione utilized per minute per milligramme of protein) [38].

Assay of Succinate Dehydrogenase

The experimental procedure involved preparing a reaction mixture consisting of 1 mL of a 0.3 M phosphate buffer, 0.1 mL of a 0.03 M solution of Ethylenediaminetetracetic acid (EDTA), 0.1 mL of a 3 % Bovine Serum Albumin (BSA) solution, 0.3 mL of a 0.4 M sodium succinate solution, and 0.2 mL of a 0.075 M potassium ferricyanide solution. The total volume of the reaction mixture was adjusted to 2.8 mL by adding water. The initiation of the reaction was facilitated by the introduction of 0.2 mL of mitochondrial suspension. The alteration in optical density is measured at intervals of 15 seconds over a duration of 5 minutes, specifically at a wavelength of 420 nm. The activity of succinate dehydrogenase was quantified by measuring the amount of succinate oxidised per minute per milligramme of protein [32].

Assay of Protease

A standard biochemical method was employed for assessing protease activity involved the utilization of casein as a substrate [40]. A volume of 100 microliters of enzyme solution was introduced into a solution containing 900 µL of substrate solution, specifically casein at a concentration of 2 mg/mL (w/v), in a Tris-HCL buffer with a pH of 8.0, at a concentration of 10 mM. The solution was incubated at 45°C for 30 minutes. The reaction was brought to an end by introducing an equivalent amount of 10 % (w/v) trichloroacetic acid, which had been cooled. The reaction mixture was allowed to remain at a low temperature on ice for 15 minutes in order to induce the precipitation of proteins that are insoluble. The supernatant was subjected to centrifugation at 4°C for 10 minutes.

Assay of Urease (Amidohydrolase)

Urease (0.5 mL) was added to 1 mL of 0.1 M phosphate buffer (pH 7.6), 0.023 M adenosine – 5' – diphosphate (ADP), in 0.01 M NADH, 0.023 M α- ketoglutarate, 1.8 M urea and 500 units/mL of glutamate dehydrogenase [42].

Data Analysis

The result was expressed as Mean ± SEM. Test of statistical significance was carried out using one-way analysis of various (ANOVA) followed by Tukey pairwise comparison. A p-value of $p < 0.001$ was considered as significant.

III. RESULTS AND DISCUSSION

Characterization and identification of bacterial isolate

The results have indicated that most of the bacteria are gram negative, rod shaped, motile and less spore forming. The enzymes produced are catalase, urease and oxidase. Catalase is highly present while urease and oxidase is present in low

amount as seen in contaminated soil in Tables 1.0a and 1.0b.

TABLE 1.0A: CHARACTERIZATION AND IDENTIFICATION OF BACTERIA ISOLATED FROM CONTAMINATED SOIL USING CULTURE-DEPENDENT METHODS

Compounds	Probable organisms				
	Bacillus subtilis	*Citrobacter* sp	*Pseudomonas* sp. *putida*	*Salmonella* sp.	*Bacillus Megaterium*
Xylose	A	AG	A	A	AG
Dextrose	AG	-	A	-	-
Galactose	A	A	-	-	-
Mannose	-	-	-	AG	-
Sucrose	-	-	-	-	AG
Fructose	A	AG	A	-	A
Lactose	-	AG	-	-	-
Glucose	AG	AG	AG	A	AG
Indole	-	-	-	-	-
Oxidase	-	-	-	-	-
Hydrogen sulphide	-	-	-	+	-
Spore	+	-	-	-	+
Voges Proskauer	+	-	+	-	-
Methyl red	-	+	-	+	+
Urease	-	-	-	-	-
Citrate	+	+	+	+	+
Starch hydrolysis	+	-	-	-	+
Motility	+	-	+	+	+
Coagulase	-	-	-	-	-
Catalase	+	-	+	+	+
Shape	Rod (Tiny)	Rod	Rod	Rod	Rod
Gram Staining	+	-	-	-	+

A: Acid; Ag: Acid and gas; +: positive; - negative

TABLE 1.0B: CHARACTERIZATION AND IDENTIFICATION OF BACTERIA ISOLATED FROM NON-CONTAMINATED SITE (CONTROL) USING CULTURE-DEPENDENT TECHNIQUES

Compounds	Probable organisms				
	Bacillus cereus	*Pseudomonas* sp.	*Salmonella* sp.	*Citrobacter* sp	*Bacillus subtilis*
Sorbitol	-	A	A	AG	A
Sucrose	A	AG	-	-	-
Lactose	-	AG	-	AG	-
Fructose	A	AG	-	-	A
Galactose	A	A	-	AG	-
Xylose	-	-	A	AG	-
Dextrose	A	AG	AG	AG	-
Mannose	-	-	-	A	-
Maltose	A	A	A	AG	A
Glucose	AG	A	A	AG	AG
Indole	-	-	-	-	-
Oxidase	-	-	-	-	-
Hydrogen sulphide	-	-	-	-	-
Spore form	+	-	-	-	+
Vogues Proskauer	+	+	+	-	+
Methyl red	-	-	-	+	-
Urease	-	-	+	-	-
Citrate	-	+	+	+	+
Starch Hydrolysis	+	-	-	-	+
Motility	+	+	+	+	+
Coagulase	-	+	-	-	-
Catalase	+	-	+	+	+
Shape	Rod	Rod	Rod	Rod (in pairs)	Rod
Gram Rxn	+	-	-	-	+

A: Acid; Ag: Acid and gas; +: positive; - negative

Enzymes activity from Bacterial isolate (hydrocarbon degrading bacteria) involved in Bioremediation

The HDB isolate indicated the presence of *Pseudomonas* sp., *Salmonella* sp., *Citrobacter* sp., *Bacillus megaterium* and *Bacillus subtilis* as shown on Table 1.0a. The enzymes isolated from the hydrocarbon degrading bacteria (HDB) indicated the presence of catalase, urease, succinate dehydrogenase, peroxidase and protease as presented in Table 1.0a. The results revealed that catalase activity in the five HDB include *Pseudomonas* sp- 207.82 ɥmol/mL/min ± 1.10, *Salmonella* sp- 520.14 ± 13.73, *Citrobacter* sp- 605.60 ± 1.17, and *Bacillus subtilis*-668.16±79.92. The urease activity was 0.19±0.01 (*Pseudomonas* sp), 0.11±0.01 (*Salmonella* **sp)**, 0.05±0.01 (*Citrobacter* sp), 0.21±0.04 (*Bacillus megaterium*), and 0.25±0.01 (*Bacillus subtilis*). The succinate dehydrogenase activity was 5.67±0.01 (*Pseudomonas* sp), 0.68±0.01 (*Salmonella* sp), 2.91±0.04 (*Citrobacter* sp) 1.14±0.35 (*Bacillus megaterium*), and 2.73±0.13 (*Bacillus subtilis*). The Peroxidase activity was 53.15±0.09 (*Pseudomonas* sp), 60.86±0.09 (*Salmonella* sp), 27.92±0.22 (*Citrobacter* sp), 76.04±0.01 (*Bacillus megaterium*), and 70.91±4.54 (*Bacillus subtilis*). The Protease activity was 36.2±0.01 (*Pseudomonas* sp), 37.84±0.14 (*Salmonella* sp), 41.23±1.90 (*Citrobacter* sp), 91.08±0.68 (*Bacillus megaterium*), and 62.27±3.85 (*Bacillus subtilis*). The catalase activity is the highest among all the enzymes (Table 2.0 and Table 3.0), the concentration of catalase activity was prominent in *Bacillus subtilis*. Urease activity was the least among all the enzymes as presented in Table 2.0 and Table 3.0. The high presence of catalase activity indicated that catalase had the highest antioxidant activity as presented in Table 2.0.

TABLE 2.0:
ENZYMES ACTIVITY FROM BACTERIAL ISOLATE (HYDROCARBON DEGRADING BACTERIA) INVOLVED IN BIOREMEDIATION

Names of Microorganism	Catalase (ɥmol/mol/min)	Urease (pH) unit	Succinatede-hydrogenase (ɥmol/mL/min)	Peroxidase (ɥmol/mL/min)	Protease (ɥnits/mg)
Pseudomonas sp.	207	0.190	5.68	53.2	36.2
	205	0.180	5.67	53.3	36.2
	209	0.190	5.67	52.9	36.2
Salmonella sp.	520	0.130	0.68	60.8	37.7
	496	0.100	0.68	60.7	38.1
	543	0.100	0.68	61.0	37.7
Citrobacter sp.	605	0.050	2.95	27.9	44.3
	603	0.060	2.83	27.6	41.7
	607	0.040	2.96	28.3	37.7
Bacillus megateriu m	654	0.250	1.82	76.0	91.8
	710	0.140	0.68	76.0	89.7
	616	0.250	0.91	76.0	91.7
Bacillus subtilis	730	0.250	2.73	68.0	66.1
	509	0.240	2.95	68.7	54.6
	764	0.250	2.50	76.0	66.1

TABLE 3.0
AVERAGE ENZYMES ACTIVITY FROM BACTERIA ISOLATE (HYDROCARBON DEGRADING BACTERIA THAT IS INVOLVED IN BIOREMEDIATION)

Names of Microorganism	Catalase (ɥmol/mL/min)	Urease (pH) unit	Succinate de-hydrogenase	Peroxidase (ɥmol/mL/min)	Protease (ɥnits/mg)
Pseudomonas sp.	207.82± 1.10	0.19± 0.01	5.67±0. 01	53.15 ±0.09	36.2± 0.01
Salmonella sp.	520.14± 13.73	0.11± 0.01	0.68±0. 01	60.86 ±0.09	37.84 ±0.14
Citrobacter sp.	605.60± 1.17	0.05± 0.01	2.91±0. 04	27.92 ±0.22	41.23 ±1.90
Bacillus megaterium	660.71± 27.26	0.21± 0.04	1.14±0. 35	76.04 ±0.01	91.08 ±0.68
Bacillus subtilis	668.16± 79.92	0.25± 0.01	2.73±0. 13	70.91 ±4.54	62.27 ±3.85
P Value	<0.001*	<0.00 1*	<0.001*	<0.00 1*	<0.00 1*

IV. DISCUSSION

The present study revealed significant hydrocarbon-degrading capabilities among bacterial isolates from petroleum-contaminated soils in Baranyonwa Dere (B-Dere), Gokana LGA, Rivers State, Nigeria. The identified bacterial strains demonstrated substantial enzymatic activity, notably catalase, peroxidase, urease, succinate dehydrogenase, and protease, which collectively contribute to hydrocarbon degradation. These findings align with recent global studies, reinforcing the critical role of indigenous microbial populations in bioremediation [8, 47, 49, 10].

As shown in Table 1.0a, the biochemical studies of the bacteria isolate revealed that gram negative bacteria occur more than gram positive bacteria during gram staining in the contaminated site. This is in consonance with the findings of other researchers who in their respective studies reported a similar trend [15, 43, 6, 38]. Gram-negative bacteria are more prevalent in oil-contaminated sites because of their lipopolysaccharide (LPS), which functions as a biosurfactant to speed up the biodegradable process [44]. The LPS molecules make gram negative bacteria initially resistant to pollutants like hydrocarbons by converting the outer membrane (OM) into an efficient permeability barrier against tiny hydrophobic compounds that could otherwise pass through phospholipid bilayers (52). A similar study confirmed that gram negative bacteria predominate in test samples due to the complexity of their cell wall which hinders the penetration of antimicrobials and their entry into the cytoplasm [36]. They possess porins which help in selective uptake of substances by the cell [14].

As shown in Tables 2 and 3, enzymes derived from hydrocarbon degrading bacteria (HDB) are catalase, urease, succinate dehydrogenase, peroxidase and protease. These results agree with the findings of many others [15, 38, 41]. Their reports revealed the prevalence of these enzymes in oil contaminated soils. The findings of the present study further reveal that catalase is readily present in aerobic and anaerobic microorganisms indicating that catalase is dominant among all the enzymes in oil contaminated sites. The high catalase activity observed in *Bacillus subtilis* and *Bacillus megaterium* corresponds with some recent findings [30]. The result of the study showed enhanced oil degradation through biosurfactants and enzymatic activities, particularly from *Bacillus subtilis*, isolated from oil-polluted environments in Iraq [30]. Their work highlighted catalase and protease as key contributors to hydrocarbon breakdown, consistent with the findings of the present study. Similarly, studies also revealed the prevalence of catalase activity in fish contaminated with oil in River State and oil contaminated soils in Ugheli Delta State Nigeria respectively [4, 17]. Catalase plays an important role of decomposing the substrate hydrogen peroxide to oxygen and water thereby giving the required oxygen supply needed in the process of bioremediation [48, 35].

Similarly, the present study also reveals the prevalence of protease and peroxidase activity recorded in *Bacillus megaterium.* This is in consonance with a similar study that demonstrated fungal-bacterial consortia, including *Bacillus* species, to achieved over 90% degradation of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) [25]. Their study emphasized the synergistic role of

enzymatic mechanisms in complex pollutant breakdown, supporting the observed biochemical efficiency in the isolates.

The result of the present study further reveals high succinate dehydrogenase activity by *Pseudomonas* sp. This enzyme plays a major role in energy metabolism during hydrocarbon degradation. A similar study revealed that *pseudomonas* species isolated from oil-polluted sites in Saudi Arabia demonstrated enhanced succinate dehydrogenase activity, contributing to their efficient breakdown of complex hydrocarbons [23]. Similarly, recent study in Nigeria reported enhanced succinate dehydrogenase activity by *pseudomonas* sp [13]. These studies indicate the metabolic competence of succinate dehydrogenase to generate energy from complex hydrocarbons degradation.

Enzyme activity is quantified in micromoles of substrate transformed per minute, which represents the rate of reaction that the enzyme catalyzes. The factories of enzyme manufacturing are bacteria, which are live cells with the capacity to produce enzymes [7]. Hence, they are the main source of enzymes since they can be genetically modified to increase the production of enzymes and are cultivated in great quantities in a short amount of time [27]. The large hydrocarbon molecules that bacteria can readily ingest are degraded by them. Their effects include lowering the activation energy of chemical processes within cells and promoting cell-specific reactions. Cell activity is largely dependent on enzymes, which ultimately dictate the type of chemical reaction a cell can perform and how quickly it can do it. Their capacity to alter or degrade petroleum hydrocarbons encapsulates the importance of enzymes in the bioremediation process [19].

V. Conclusion

The findings of the present study validate that oil contamination promotes diversity and enzymatic adaptation of indigenous bacteria, equipping them for effective hydrocarbon degradation. The elevated metabolic activities in contaminated soils underscore the potential of these native strains as viable bioremediation agents. Harnessing their enzymatic profiles offers a sustainable and eco-friendly strategy for restoring oil-polluted environments in the Niger Delta and similar regions globally.

Scientific Implications of the Findings

Inference from the study suggest that oil contaminated soil in B-dere Gokana Local Government Area, Rivers State, Nigeria support more bacteria population that are diverse. The results provide imperative scientific information on bioremediation of petroleum contaminated soil that maybe vital in justifying further studies that are related to bioremediation of oil polluted soil in B-Dere.

Limitation of the study and future plan

Despite the insightful findings, this study presents certain limitations that should be considered;

I. Limited Microbial Diversity Assessment**:** The study focused solely on culture-dependent methods, which may not capture the full spectrum of hydrocarbon-degrading bacteria present in the contaminated soils. Many microorganisms involved in hydrocarbon degradation are unculturable under standard laboratory conditions.
II. Absence of Molecular Characterization: The identification of bacterial isolates was primarily based on morphological and biochemical tests. Molecular techniques such as 16S rRNA gene sequencing were not employed, which could have provided more precise taxonomic resolution and insights into the genetic potential of the isolates.
III. Site-Specific Findings**:** The study was confined to a single oil-polluted site in Baranyonwa Dere (B-Dere), Gokana LGA, Rivers State. As such, the results may not be generalized to other oil-contaminated environments with different ecological or contamination profiles.
IV. Limited Enzyme Spectrum**:** While important enzymes like catalase, urease, succinate dehydrogenase, peroxidase, and protease were analyzed, other key enzymes involved in hydrocarbon metabolism (e.g., alkane hydroxylases, monooxygenases, dioxygenases) were not investigated.
V. No In-Situ Bioremediation Trials: The study evaluated the enzymatic potential of the bacterial isolates in controlled laboratory conditions. Actual bioremediation effectiveness in the field, considering complex environmental interactions, was not assessed.

Future plans

To build upon the current findings and address the identified gaps, the following future directions are recommended:

I. **Molecular Characterization**: Future studies will incorporate molecular tools such as 16S rRNA sequencing and metagenomics to achieve accurate identification of hydrocarbon-degrading bacteria and explore their genetic diversity and functional potential.
II. **Broader Enzyme Profiling**: Comprehensive enzyme profiling, including hydrocarbon-specific degrading enzymes (e.g., oxygenases, hydrolases), will be undertaken to better understand the metabolic pathways involved in petroleum hydrocarbon degradation.
III. **Community-Level Analysis**: Employing culture-independent approaches such as metagenomics and metatranscriptomics will reveal the structure and functional dynamics of the entire microbial community in oil-polluted soils.
IV. **Field-Based Bioremediation Trials**: The effectiveness of the identified bacterial strains will be tested under in-situ or simulated field conditions to validate their bioremediation potential and optimize application strategies.

Comparative Studies across Multiple Sites: Expanding the study to other oil-impacted sites within and outside Rivers State will help ascertain spatial variations in microbial populations and enzymatic activities, enhancing the applicability of bioremediation interventions.

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Disclosure of Conflict of Interest

The authors claim that there are no conflicting interests.

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