BACTERIAL CARRIAGE AND CONSORTIUM DEVELOPMENT FROM ARTISANAL REFINERY CONTAMINATED SOIL FOR EFFECTIVE DEGRADATION OF PETROLEUM HYDROCARBONS

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ABSTRACT

The bacterial carriage and consortium development from indigenous bacterial flora in crude oil-contaminated soil from artisanal refineries was studied. Surface soil samples were collected from four different communities in Emohua LGA, Rivers State, Niger Delta, Nigeria. Total culturable heterotrophic bacterial counts (TCHBC) and total culturable hydrocarbon-utilizing bacterial counts (TCHUBC) were monitored using the spread plate technique on nutrient medium and Bushnell Haas medium (supplemented with 1% v/v Bonny light crude oil) respectively. Nutrient enrichment process, hydrocarbon degradation screening and biosurfactant production analysis were used to detect bacterial isolates with hydrocarbon-degrading abilities to develop bacterial consortium. The 16S Illumina amplicon metagenomic analysis was used to identify the bacterial community diversity present in the consortium. TCHBC ranged from 2.3 x 10^5 ± 0.28 CFU/g - 4.7 x 10^7 ± 0.32 CFU/g, while TCHUBC ranged from 1.92 x 10^4 ± 1.01 CFU/g - 3.7 x 10^6 ± 0.04 CFU/g. Members of the phylum Proteobacteria (96.12%) were dominant in the consortium, while members of the phylum Verrucomicrobia (0.02%) had the least dominance. The genera Acinetobacter (19.6%) and Morganella (10.97%) were dominant in the bacterial consortium, while Akkermansia (0.02%) showed the least dominance. This study has demonstrated the successful development of bacterial consortiums using indigenous bacterial communities, which can serve as a bioresource for the recovery of hydrocarbon-contaminated soils.

Keywords: Bacteria, Artisanal refinery, Crude oil, Hydrocarbons, Bacterial communities, Sequencing

INTRODUCTION

The discovery of crude oil in the Niger Delta, Nigeria, which is economically beneficial has also greatly impacted the environment as a result of the exploration and exploitation of petroleum (Onwuna *et al.*, 2022). This has led to widespread environmental contamination with hydrocarbons. The presence of petroleum-derived hydrocarbons in soil and water plays a significant role in determining the bacterial diversity and structure of the ecosystem (UNEP, 2011; Tudararo-Aherobo and Maya, 2023). Over the years, the major sources of crude oil pollution in the Niger Delta have been the operations of multinational oil companies and pipeline oil theft but with the sudden surging rate of numerous artisanal oil refineries, the rate of environmental pollution has greatly increased. This has posed serious environmental and public health challenges (Gijo *et al.*, 2016).

Artisanal crude oil refineries are small-scale makeshift operation plants that utilize the principles of fractional distillation to separate the hydrocarbon components of crude oil into individual petroleum fractions. This yields different petroleum products like dual-purpose kerosene (DPK), automotive gas oil (AGO) and bitumen among others (Onwuna *et al.*, 2022). Most of these refineries, also commonly called

kpo-fire, lack proper regulations because the majority of them are illegally operated.

The soil is adversely affected by this process as a result of the indiscriminate discharge of petroleum into the environment at various levels of refining and waste product release. This affects the soil biodiversity posing eco-toxicological effects on the environment (Fenibo *et al.,* 2024). Some of its impacts include; a reduction in biodiversity, changes in soil fertility, changes in soil physicochemical characteristics, groundwater contamination, adverse effects on microflora, bioaccumulation in aquatic fauna and flora, and carcinogenic effects on humans (Obire and Anyanwu, 2009; Kalantary *et al*., 2014).

Bioremediation, specifically the use of bacterial consortia, has emerged as an efficient approach for the recovery of hydrocarbon-contaminated sites because it is cost-effective and environmentally friendly. The concept of bacterial consortia involves the synergistic interaction of multiple bacterial species with complementary degradation capabilities, enhancing the degradation efficiency and broadening the range of hydrocarbons that can be targeted for remediation. Petroleum hydrocarbon-degrading bacteria are usually abundant in contaminated environments (Ali *et al.,* 2022). This relatively comprises autochthonous bacteria that play a special and effective role in the natural attenuation and degradation process (Fenibo *et al*., 2024). Several studies have demonstrated the effectiveness of bacterial consortia in degrading petroleum hydrocarbons in impacted systems (Haritash and Kaushik, 2009; Karigar and Rao, 2011; Fenibo *et al*., 2024). The selection and optimization of microbial strains with the ability to degrade different classes of hydrocarbons is imperative for the successful application of bacterial consortia in bioremediation processes (Varjani, 2017; Cao *et al.,* 2022). The development of a customized bacterial consortium from artisanal refinery polluted sites requires a thorough understanding of the microbial community structure, metabolic capabilities and environmental factors influencing microbial activity in hydrocarbon-contaminated soils.

This research aims to isolate, characterize and optimize a bacterial consortium capable of effectively degrading petroleum-derived hydrocarbons in polluted sites. Developing a robust bacterial consortium can enhance the efficiency and sustainability of remediation efforts in petroleum-derived hydrocarbon-impacted soil, especially due to the activities of artisanal refineries. This represents an effective approach towards sustainable environmental remediation. By harnessing the synergistic interactions of specialised microbial strains, this study will contribute to the advancement of bioremediation technologies geared towards solving the unique challenges of hydrocarbon contamination in the Niger Delta region of Nigeria.

MATERIALS AND METHODS

Collection and Preparation of Samples: Hydrocarbon-polluted soils were collected from artisanal refineries located at Ndele (samples A1 and A2), Rumuji (samples B1 and B2), Ibaa (samples C1 and C2) and Ogbakiri (samples D1 and D2) communities in Emohua LGA, Rivers State, Niger Delta, Nigeria. The soil around these artisanal refineries has experienced high hydrocarbon contamination due to the indiscriminate spillage of petroleum products. The global positioning system (GPS) was used to compute each sample site's coordinates. The coordinates are Ndele: $04^0 58' 06'' N$ $06^0 45' 05'' E$, Ogbakiri: $04^0 50' 52'' N$ $06^0 53' 22'' E$, Ibaa: $04^0 56' 33'' N$ $06^0 52' 41'' E$, Rumuji: $04^0 56' 32'' N$ $06^0 46' 57'' E$. Soil samples were taken at 0 – 50 cm depths using soil auger from different points of each site, made into composite samples then wrapped in sterile black polyethene bags and moved to the laboratory in an ice chest ($05^0 C$).

Isolation and Enumeration of Culturable Bacterial Population: 1 g of the soil sample each and 9 mL sterile normal saline as a diluent (0.85% NaCl w/v in distilled water) was used to perform a ten-fold serial dilution in glass test tubes (Pyrex, USA). The total culturable heterotrophic bacterial counts (TCHBC) were carried out using nutrient agar (Accumedia, Sweden). In contrast, the total culturable

hydrocarbon-utilizing bacteria counts (TCHUBC) were done using Bushnell Haas Agar (with 1% v/v Bonny light crude oil) amended with 0.01% w/v nystatin. The media were prepared following the manufacturer's instructions. A volume of 100 µL each of 10^{-3} – 10^{-6} dilutions of the individual samples was inoculated using the spread plate technique. The inoculated plates were then incubated at 30^0C for 24 hours for TCHB and 7 days for TCHUB (APHA, 2005)

Enrichment for the Isolation of Hydrocarbonoclastic Bacteria: This was carried out using the method of Mittal and Singh (2009) and Mnif *et al.* (2009) with slight adjustments as explained by Maduwuba (2024). 10 g each of the soil samples in 100 mL of normal saline was used. The mixture was votexed and then allowed to settle. 5 mL of the supernatant from each sample was inoculated into 100 mL Bushnell Haas Broth (BHB) (containing in g/L: 0.42$MgSO_4.7H_20$, 0.02$CaCl_2.2H_20$; 1KH_2PO_4; 1K2HPO_4; 1 NH_4NO_3; 0.05$FeCl_3$; 15 g agar powder, 0.1 g nystatin) supplemented with 1% v/v Bonny light crude oil. Tween 80 (0.05% v/v) was used to enhance hydrocarbon degradation. The process was replicated for each sample in triplicate and then incubated in a shaker for 7 days at 150 rpm. The resulting colonies were sub-cultured into a fresh BHB medium supplemented with 1% v/v crude oil. The sub-culturing maintained the successful isolation of only hydrocarbonoclastic bacteria (Wedulo *et al.,* 2014).

Preparation of McFarland Standard and Inoculum Standardization: 0.5 McFarland standards which approximates to 1.5 x 10^8 CFU/mL, was used. The 0.5 McFarland standards were carried out by dissolving 0.05 mL of 1.175% of $BaCl_2.2H_2O$ in 9.95 mL of 1% H_2SO_4. The transmittance was 74.3, while the absorbance was 0.09 at the wavelength (λ) of 600 nm.

Inoculum standardization was performed by preparing a test suspension obtained by adding a 24 h pure culture of the bacterial isolates in sterile normal saline and votexed to mix completely. The turbidity of the bacterial suspensions was compared to the McFarland standards by checking the clarity of the lines on a Wickerham card. Turbidity that corresponds to that of the McFarland standards was achieved by the addition of either the test bacterial culture or sterile saline solution (Maduwuba, 2024).

Hydrocarbon Degradation Screening: Hydrocarbon utilization screening was done using the turbidometric method. A UV-5200 spectrophotometer (Shanghai Metash, China) was used to measure turbidity. A volume of 50 mL Bushnell Hass broth supplemented with crude oil 1% (v/v) and 0.05% Tween 80 was prepared and dispensed into Erlenmeyer's flasks and sterilized in an autoclave at 121 ^{0}C (15 psi) for 15 minutes. After sterilization, the broth was inoculated with 5% (v/v) of the standardized inoculum of the test bacterial isolate. The inoculated flask containing the test bacterial isolates was incubated for 15 days in a shaker incubator at 150 rpm. The turbidity of each broth was measured using a UV-5200 spectrophotometer (Shanghai Metash, China) every five days throughout the incubation period. Biodegradation was scored every three days based on the turbidity of the broth and oil emulsification in the medium.

The straight chain and polycyclic aromatic hydrocarbon (PAH) utilization screening was done by culturing each of the test isolates on Bushnell Haas agar plates supplemented with 1% hexadecane (C_{16}) for straight chain hydrocarbon and 1% naphthalene ($C_{10}H_8$) for polycyclic aromatic hydrocarbon, incubated for 168 hours and scored based on the ability of the test isolates to grow on either or both of them.

Biosurfactant Production Analysis: The individual isolates were examined for the production of biosurfactants using the oil spreading assay. The isolates were further subjected to the oil spreading test using the procedure of Morikawa *et al.* (2000). 20 mL of distilled water was introduced into a Petri dish followed by the addition of 20 µL of crude oil and then 10 µL of cell-free culture broth was added to the oil surface. The presence of biosurfactants is indicated by oil displacement with an oil-free clearing zone. The diameter of the clearing zone indicates surfactant or oil displacement activity. Distilled water served as the negative control

(without surfactant) in which no oil displacement or clear zone appeared.

Emulsification Index Assay: The pure cultures of the individual isolates were inoculated into 2 mL of Bushnell Haas medium in test tubes. After 2 days of incubation, 2 mL hexadecane was introduced into each test tube then vortexed for 1 minute and allowed to settle for 24 hours. The emulsion index (E_{24}) was calculated using the method stated by Maduwuba (2024).

Bacterial Consortium Identification Using 16S Amplicon Sequencing: The metagenomic sample preparation was done using the method of Minich *et al.* (2018). The bacterial metagenomic DNA extraction of each sample was carried out using Laragen's validated proprietary bacterial DNA extraction protocol (Caporaso *et al.*, 2012).

The 16S V4 metagenomic libraries were generated using methods adapted from Minich *et al.* (2018) from the Earth Microbiome Project. The protocol comprises a miniaturised (5-µL volume), high-throughput (384-sample) amplicon library preparation and the Echo 550 acoustic liquid handler. The samples were concentrated by adding 5 – 10% PhiX (Caporaso *et al.*, 2012).

The primer sets used for amplification were adapted based on the original 515F–806R primer pair modification (Caporaso *et al.*, 2012). Amplifications were performed in triplicate in 25 µL PCR reactions. The thermocycling conditions were also considered. The PCR reactions for the samples were pooled together into a single volume (75 µL). Amplicons were run on an agarose gel with a band size of ~300– 350 bp. Amplicons were quantified using a Quant-iTPicoGreends DNA assay kit (ThermoFisher /Invitrogen Cat. No. P11496) following the manufacturer's instructions. Equal quantities of amplicons (240 ng) were combined in a single sterile tube. Larger quantities were also used as long as the final pool could be isolated using the gel. The Amplicon pool was cleaned using a MoBioUltraClean PCR clean-up kit. Concentrations of the amplicon were measured to determine the A260: A280 ratio of the cleaned final pool. For best results, the A260: A280 ratio usually falls between 1.8 and 2.0. The following sequencing primers were used to sequence the aliquot; Sequencing Primer (Read I): TATGGTAATTGTG TGYCAGCMGCCGCGGTAA, Sequencing Primer (Read II): AGTCAGCCAGCCGGACTACNVGGGTW TCTAAT, Sequencing primer (index): AATGATAC GGCGACCACCGAGATCTACACGCT.

The Ribosomal Database Project (RDP) classifier was used to assign sequences derived from bacterial 16S genes to the corresponding taxonomic model (McMurdie and Holmes, 2012). Phyloseq package objects containing OTU tables were created from the classifier output.

RESULTS

The total culturable heterotrophic bacterial counts (TCHBC) revealed the presence of a high bacterial load (Figure 1).

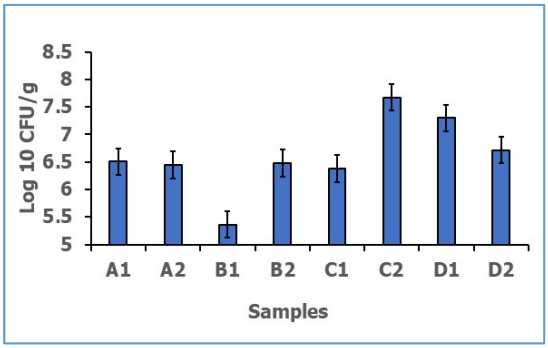

Figure 1: Total culturable heterotrophic bacterial counts in all the samples

Sample A1 had TCHBC of 3.2 x 10^6 ± 0.41 CFU/g, while sample A2 had 2.8 x 10^6 ± 0.25 CFU/g. Sample B1 had 2.3 x 10^5 ± 0.28 CFU/g TCHBC, while sample B2 had 3.0 x 10^6 ± 0.21 CFU/g. The TCHBC recorded for sample C1 and C2 were 2.4 x 10^6 ± 0.44 CFU/g and 4.7 x 10^7 ± 0.32 CFU/g respectively. A 2.0 x 107 ± 0.37 CFU/g bacterial load and 5.2 x 106 ± 0.31 CFU/g were also recorded for samples D1 and D2, respectively. Sample C2 recorded the highest TCHBC of 4.7 x 10^7 ± 0.32 CFU/g followed by sample D1 with 2.0 x 10^7 ± 0.37 CFU/g, while sample B1 recorded the lowest TCHBC of 2.3 x 10^5 ± 0.28 CFU/g.

The total culturable hydrocarbon-utilizing bacterial counts (TCHUBC) (Figure 2) revealed that sample A1 recorded a TCHUBC of 3.0 x 10^5 ± 0.33 CFU/g and A2 recorded a TCHUBC of 2.5 x 10^5 ± 0.29 CFU/g. Samples B1 and B2 revealed TCHUBC values of 1.92 x 10^4 ± 1.01 CFU/g and 3.85 x 10^5 ± 0.98 CFU/g respectively.

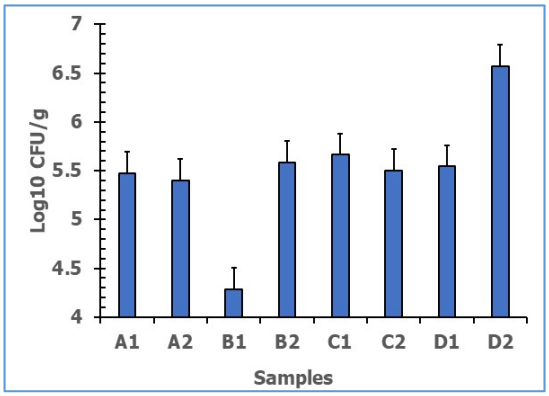

Figure 2: Total culturable hydrocarbon utilizing bacterial counts in all the samples

The TCHUBC of 4.6 x 10^5 ± 1.11 CFU/g and 3.2 x 10^5 ± 0.99 CFU/g were recorded in samples C1 and C2 respectively, while sample D1 and D2 had TCHUBC values of 3.5 x 10^5 ± 0.03 CFU/g and 3.7 x 10^6 ± 0.04 CFU/g. Sample D2 recorded the highest TCHUBC value of 3.7 x 10^6 ± 0.04 CFU/g followed by sample C1 with a TCHUBC value of 4.6 x 10^5 ± 1.11 CFU/g, while sample B1 had the lowest TCHUBC value of 1.92 x 10^4 ± 1.01 CFU/g.

The major portion of the sequence of bacterial diversity in the consortium obtained at the phylum level showed that the bacteria present were affiliated to the top 5 phyla namely; Proteobacteria (96.12%), Firmicutes (1.94%), Bacteroides (1.47%), Actinobacteria (0.45%) and Verrucomicrobia (0.02%) (Figure 3). The dominant phylum was Proteobacteria with the highest relative abundance of 96.12%, while the phylum with the lowest relative abundance was Verrucomicrobia (0.02%).

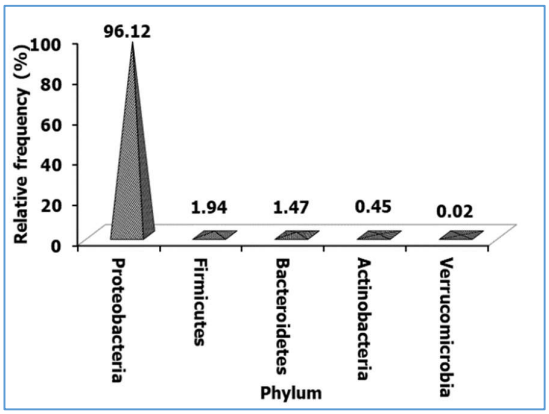

Figure 3: Relative abundance of the phylum present in the bacterial consortium

The distribution of the phyla into two different superclass further revealed that they were shared into the top 9 classes (Figure 4) namely; Betaproteobacteria (47.13%), Gammaproteobacteria (46.8%), Alpha-proteobacteria (2.2%), Bacilli (1.4%), Clostridia (0.54%), Actinobacteria (0.45%), Sphingobacteria (0.43%) and Verrucomicrobiae (0.02%) with the relative abundance of Betaproteobacteria being the highest followed by Gammaproteobacteria, while the class with the lowest relative abundance was Verrucomicrobia.

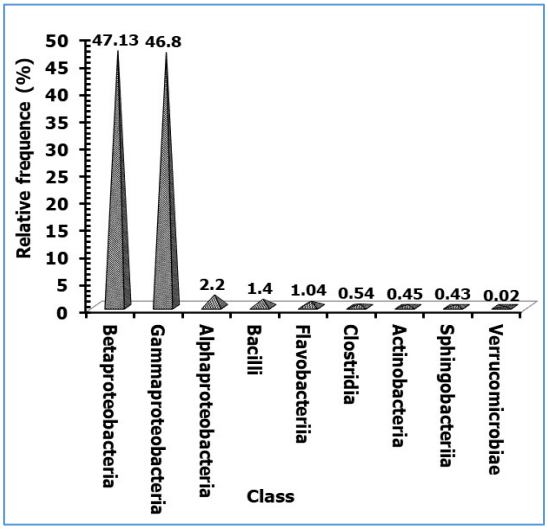

Figure 4: Relative abundance of the classes present in the bacterial consortium

The distribution of the class into different order further revealed that they were shared into 12 top order (Figure 5) namely: Burkholderiales (47.13%), Enterobacteriales (25.65%), Pseudomonadales (19.65%), Xanthomonadales (1.50%), Lactobacillales (1.4%), Rhizobiales (1.38%), Flavobacteriales (1.04%), Caulobacterales (0.81%), Clostridales (0.54%), Actinomycetales (0.45%), Sphingobacteriales (0.43%) and Verrucomicrobiales (0.02%) with the relative abundance of Burkholderiales being the highest and most dominant followed by Enterobacterales, while the lowest relative abundant was Verrucomicrobiales.

The distribution of order into different families further revealed that they were shared into 16 top families (Figure 6) as follows: Alcaligenaceae (43.67%), Enterobacteriaceae (25.65%), Moraxellaceae (19.65%), Comamonadaceae (3.46%), Xanthomonadaceae (1.5%),

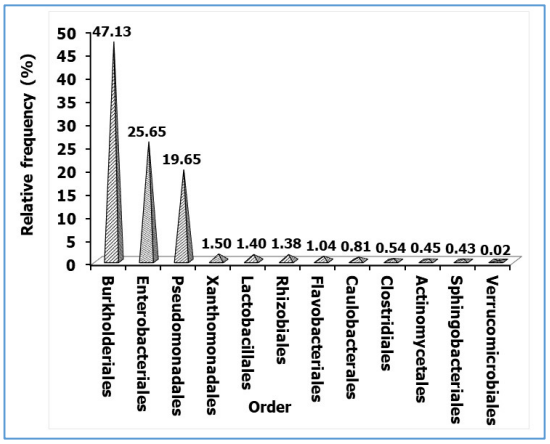

Figure 5: Relative abundance of order present in the bacterial consortium

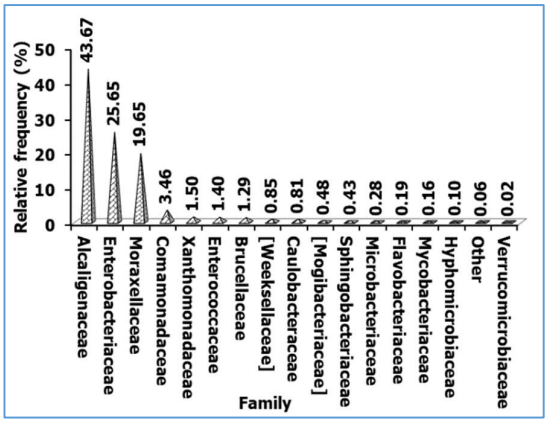

Figure 6: Relative abundance of family present in the bacterial consortium

Enterococcaceae (1.4%), Brucellaceae (1.29%), Weeksellaceae (0.85%), Caulobacteraceae (0.81%), Mogibacteriaceae (0.48%), Sphingobacteriaceae (0.43%), Microbacteriaceae (0.28%), Flavobacteria (0.19%), Mycobacteriaceae (0.26%), Hyphomicrobiaceae (0.1%) and Verrucomicrobiaceae (0.02%). The family with the highest relative abundance was Alcaligenaceae followed by Enterobacteriaceae, while the lowest was Verrucobacteriaceae.

Further distribution of the family into genera revealed that they were affiliated with the top 20 bacterial genera (Figure 7). The top 20 genera OTUs in the consortium were revealed in the order which showed that *Acinetobacter* had the highest relative abundance of 19.6% followed by *Morganella* which had 10.97%, while the lowest was *Akkermansia* which had 0.02% relative abundance.

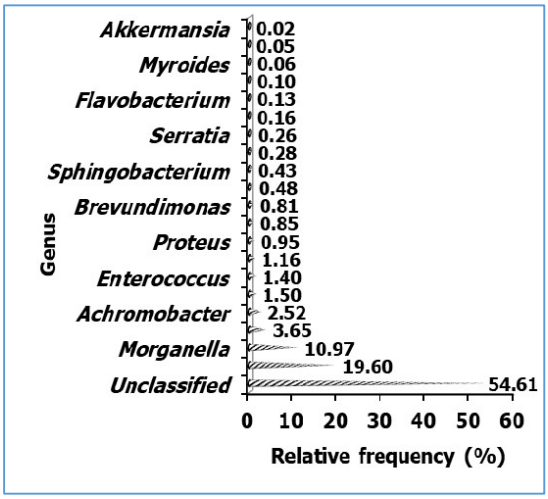

Figure 7: Relative abundance of genera present in the bacterial consortium

The results of the bacterial consortium screened for the presence of xenobiotic degradation genes and xenobiotic metabolic genes revealed that the relative abundance of xenobiotic degradation genes present in the bacterial consortium was 3.51%, while the relative abundance of xenobiotic degradation genes present was 5.82%. The xenobiotic metabolic genes were higher in abundance than the xenobiotic degradation genes. The benzoate degradation gene had the highest percentage abundance followed by the aminobenzoate degradation gene. Also, geraniol and naphthalene degradation genes had differential significant abundance. DDT degradation gene had the lowest relative abundance.

DISCUSSION

This study explored the bacterial carriage of crude oil artisanal refinery contaminated soil environment and the development of bacterial consortium from resident soil bacterial flora for the remediation of petroleum-derived hydrocarbon contaminated soils. The mean bacterial population revealed a range of 5.36 log CFU/g – 7.67 log CFU/g for heterotrophic bacteria and 4.28 log CFU/g – 6.57 log CFU/g for hydrocarbon-utilizing bacteria. This range is considered to be relatively high which is similar to the observation of Kalantary *et al.* (2014) and Onwuna *et al.* (2022)

who recorded changes and a relatively high bacterial population in crude oil-polluted soils. The development and identification of bacterial consortium using genome analysis was imperative in producing a rapid and effective bioresource needed for the restoration and conservation of crude oil-impacted soils.

This study revealed the development of a robust bacterial consortium which was made up of bacteria of the phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia with Proteobacteria having the highest relative abundance of 96.12%. This interaction is similar to those recorded in other hydrocarbon-impacted environments (Gałązka *et al*., 2018; Chikere *et al.,* 2019). The phylum Proteobacteria has been demonstrated as a distinct bacteria group which includes hydrocarbon-utilizers and plant growth-promoting bacterial (PGPB) species due to their possession of degradative genes and enzymes that help the organisms to breakdown complex substances present in petroleum compounds during metabolism (Bruto *et al.,* 2014; Gkorezis *et al.,* 2016).

At class distribution, Gammaproteobacteria dominated the bacterial communities followed by the Alphaproteobacteria in the consortium. similar findings have been reported by researchers about oil-contaminated soils (Huettel *et al*., 2018; Chikere *et al.,* 2019). Gammaproteobacteria have been known to be dominant in oil-impacted environments due to their hydrocarbon-degrading ability (Gontikaki *et al*., 2018). This class is also excellent at breaking down alkanes in natural environments (Chikere *et al.,* 2019). Also, the presence of these dominating classes of bacteria is significantly dependent on both the contaminated sites and the petroleum derivative found in these sites (Gałązka *et al.,* 2018).

The presence of the order Pseudomonadales and Acidobacteriales in significantly differential abundance can be linked to the acidic pH present in most of the hydrocarbon-polluted sites. The United Nations Environmental Programme (UNEP) assessment of crude oil-contaminated sites in different habitats reported an acidic pH for most of the sites studied. The Pseudomoadales have been shown to exhibit more versatile catabolic potentials and are good PCB (Polychlorinated biphenyls) degraders especially in natural environments (Lindén and Pålsson, 2013).

The presence of the bacterial genera *Acinetobacter*, *Morganella, Providencia* and *Achromobacter* in significant abundance showed that they play pertinent roles in the breakdown of the different hydrocarbon components present in the polluted sites. These bacterial genera have been reported to consistently dominate hydrocarbon-polluted sites (Morrison *et al.,* 2021) and have excellent biodegradation ability because of the flexibility and plasticity of their metabolic pathways (Palleroni *et al.* 2010). This is manifested in their ability to degrade several aliphatic, aromantic, poly-aromatic hydrocarbons and various derivatives among a vast variety of organic compounds (Palleroni *et al.* 2010; Morrison *et al.,* 2021).

Conclusion: Overall, this study has revealed the successful development of bacterial consortium and its bacterial community profile and functions in crude oil-contaminated soils from artisanal refineries. This has also shown the need to harness indigenous bacterial flora residents in polluted sites in remediating impacted systems since this environment contains microorganisms that possess the necessary potential to give the desired output.

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REFERENCES

ALI, S. S., AL-TOHAMY, R., MAHMOUD, Y. A. G., KORNAROS, M., SUN, S. and SUN, J. (2022). Recent advances in the life cycle assessment of biodiesel production linked to azo dye degradation using yeast symbionts of termite guts: A critical review. *Energy Reports*, 8: 7557 – 7581.

APHA (2005). *Standard Methods for the Examination of Water and Wastewater.* 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington D.C., USA.

BRUTO, M., PRIGENT-COMBARET, C., MULLER, D. and MOËNNE-LOCCOZ, Y. (2014). Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. *Scientific Reports*, 4: 6261. https://doi.org/10.1038/srep06261

CAO, Z., YAN, W., DING, M. and YUAN, Y. (2022). Construction of microbial consortia for microbial degradation of complex compounds. *Frontiers in Bioengineering and Biotechnology*, 10: 1051233. https://doi.org/10.3389/fbioe.2022.1051233

CAPORASO, J.G., LAUBER, C.L., WALTERS, W.A., BERG-LYONS, D., HUNTLEY, J., FIERER, N., OWENS, S.M., BETLEY, J., FRASER, L., BAUER, M. and GORMLEY, N. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6(8): 1621 – 1624.

CHIKERE, C. B., MORDI, I. J., CHIKERE, B. O., SELVARAJAN, R., ASHAFA, T. O. and OBIEZE, C. C. (2019). Comparative metagenomics and functional profiling of crude oil-polluted soils in Bodo West Community, Ogoni, with other sites of varying pollution history. *Annals of Microbiology*, 69: 495 – 513.

FENIBO, E. O., NKUNA, R. and MATAMBO, T. (2024). Impact of artisanal refining activities on bacterial diversity in a Niger Delta fallow land. *Scientific Reports*, 14*:* 3866. https://doi.org/10.1038/s41598-024-53147-4

GAŁĄZKA, A., GRZĄDZIEL, J., GAŁĄZKA, R., UKALSKA-JARUGA, A., STRZELECKA, J. and SMRECZAK, B. (2018). Genetic and functional diversity of bacterial microbiome in soils with long term impacts of petroleum hydrocarbons. *Frontiers in Microbiology*, 9: 1923. https://doi.org/10.3389/fmicb.2018.01923

GIJO, A. H., HART, A. I. and SEIYABOH, E. I. (2016). The impact of makeshift oil refining activities on the physico-chemical parameters of the interstitial water of the Nun River Estuary, Niger Delta, Nigeria. *Biotechnological Research*, 2(4): 193 – 203.

GKOREZIS, P., DAGHIO, M., FRANZETTI, A., VAN HAMME, J. D., SILLEN, W. and VANGRONSVELD, J. (2016). The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: an environmental perspective. *Frontiers in Microbiology*, 7: 1836. https://doi.org/10.3389/fmicb.2016.01836

GONTIKAKI, E., POTTS, L. D., ANDERSON, J. A. and WITTE, U. (2018). Hydrocarbon-degrading bacteria in deep-water subarctic sediments (Faroe-Shetland Channel). *Journal of Applied Microbiology*, 125(4): 1040 – 1053.

HARITASH, A. K. and KAUSHIK, C. P. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *Journal of Hazardous Materials*, 169(1-3): 1 – 15.

HUETTEL, M., OVERHOLT, W. A., KOSTKA, J. E., HAGAN, C., KABA, J., WELLS, W. B. and DUDLEY, S. (2018). Degradation of Deepwater Horizon oil buried in a Florida beach influenced by tidal pumping. *Marine Pollution Bulletin*, 126: 488 – 500.

KALANTARY, R. R., MOHSENI-BANDPI, A., ESRAFILI, A., NASSERI, S., ASHMAGH, F. R., JORFI, S. and JA'FARI, M. (2014). Effectiveness of biostimulation through nutrient content on the bioremediation of phenanthrene contaminated soil. *Journal of Environmental Health Science and Engineering*, 12: 143. https://doi.org/10.1186/s40201-014-0143-1

KARIGAR, C. S. and RAO, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. *Enzyme Research*, 2011: 805187. https://doi.org/10.4061/2011/805187

LINDÉN, O. and PÅLSSON, J. (2013). Oil contamination in Ogoniland, Niger Delta. *Ambio*, 42(6): 685 – 701.

MADUWUBA, M. C. (2024). Bacteriological and physicochemical analysis of a crude oil-polluted soil undergoing laboratory scale bioremediation. *Animal Research International,* 21(2): 5443 – 5452.

MCMURDIE, P. J. and HOLMES, S. (2012). Phyloseq: A bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. *Pacific Symposium on Biocomputing*, 17: 235246. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0061217

MINICH, J. J., HUMPHREY, G., BENITEZ, R. A., SANDERS, J., SWAFFORD, A., ALLEN, E. E. and KNIGHT, R. (2018). High-throughput miniaturized 16S rRNA amplicon library preparation reduces costs while preserving microbiome integrity. *mSystems*, 3(6): 00166-18. https://doi.org/10.1128/msystems.00166-18

MITTAL, A. and SINGH, P. (2009). Studies on biodegradation of crude oil by *Aspergillus niger. The South Pacific Journal of Natural and Applied Sciences*, 27(1): 57 – 60.

MNIF, S., CHAMKHA, M. and SAYADI, S. (2009). Isolation and characterization of *Halomonas* sp. strain C2SS100, a hydrocarbon-degrading bacterium under hypersaline conditions. *Journal of Applied Microbiology*, 107(3): 785 – 794.

MORIKAWA, M., HIRATA, Y. and IMANAKA, T. (2000). A study on the structure-function relationship of lipopeptide biosurfactants. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1488(3): 211 – 218.

MORRISON, E. S., THOMAS, P., OGRAM, A., KAHVECI, T., TURNER, B. L. and CHANTON, J. P. (2021). Characterization of bacterial and fungal communities reveals novel consortia in tropical oligotrophic peatlands. *Microbial Ecology*, 82: 188 – 201.

OBIRE, O. and ANYANWU, E. C. (2009). Impact of various concentrations of crude oil on fungal populations of soil. *International Journal of Environmental Science and Technology*, 6: 211 – 218.

ONWUNA, D. B., STANLEY, H. O., ABU, G. O. and IMMANUEL, O. M. (2022). Impact of artisanal crude oil refinery on physicochemical and microbiological properties of soil and water in Igia-Ama, Tombia Kingdom, Rivers State, Nigeria. *Asian Journal of Environment & Ecology*, 19(3): 48 – 59.

PALLERONI, N. J., PIEPER, D. H. and MOORE, E. R. B. (2010). Microbiology of hydrocarbon-degrading *Pseudomonas.* Chapter 12, Pages 1787 – 1798. *In:* TIMMIS, K. N. (Eds.). *Handbook of Hydrocarbon and Lipid Microbiology*. Springer -Verlag, Heidelberg, Berlin.

TUDARARO-AHEROBO, L. and MAYA, B. (2023). Assessment of the impacts of artisanal refinery of crude oil on surface water, groundwater and sediment quality at Obi-Ayagha community in Delta State Nigeria. *International Journal of Scientific Research in Science and Technology*, 10(3): 1164 – 1181.

UNEP (2011). *Environmental Assessment of Ogoniland Report*. http://www.unep.org/disastersandconflicts/countryOperations/Nigeria/EnvironmentalAssessment/ Accessed June 23, 2024.

VARJANI, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology*, 223: 277 – 286.

WEDULO, A., ATUHAIRE, D. K., OCHWO, S., MUWANIKA, V., RWENDEIRE, A. J. J. and NAKAVUMA, J. L. (2014). Characterisation and evaluation of the efficiency of petroleum degrading bacteria isolated from soils around the oil exploration areas in western Uganda. *African Journal of Biotechnology*, 13(48): 4458 – 4470.