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Metagenomic Profiling Of Bacterial Communities in Crude-Oil Contaminated B-Dere River

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ABSTRACT
This study was aim at profiling the microbial diversity of petroleum-contaminated water and to determine the microbial adaptation to hydrocarbon degradation using a metagenomics approach in B-Dere, Niger delta region of Nigeria. The sequenced for 16S rRNA amplification, results in a total of 531,927 sequence reads. The sequencing of the PCR product using universal bacterial primers yielded 529,320 bacterial and 20 archaeal communities. The taxonomic profiles revealed 2,587 microorganisms (0.486%) that are unclassified. Proteobacteria, Tenericutes, Bacteroidetes, Planctomycetes, Firmicutes, Chlamydiae, Nitrospirae, Chloroflexi, Cyanobacteria, Actinobacteria, Chlorobi, Spirochaetes, Verrucomicrobia, Acidobacteria, Thermi, Caldithrix, Thermodesulfobacteria, Deferribacteres, Synergistetes, Thermotogae, Fibrobacteres, Caldiserica, Fusobacteria, Armatimonadetes and Chrysiogenetes were the bacterial phyla identified in this study. The top bacterial phyla with relative abundance greater 1 % were Proteobacteria (79.22 %), Bacteroidetes (3.56 %), Tenericutes (3.05 %), Planctomycetes (3.23 %) Firmicutes (2.07 %) and Chlamydiae (1.11). Abundance of the phyla Fusobacteria (0.0006 %), Armatimonadetes (0.0002 %) and Chrysiogenetes (0.0002 %) were less than 0.001%. This study thus confirms the presence of phyla like Fusobacteria, Armatimonadetes, and Chrysiogenetes, which were never known to inhabit petroleum-contaminated ecosystems (river).
Keywords: petroleum-contaminated water, microbial diversity, metagenomics

INTRODUCTION

With an increased crude oil exploration, production, transportation and marketing, oil spillage due to sabotage, illegal bunkering, pipeline vandalism and corrosion of pipeline and storage facilities accidents causes serious petroleum contamination on oilfields and surrounding soils, posing a serious threat to the ecosystems and human health. Fortunately, indigenous microorganisms such as *Alcanivorax* sp. (Guo and Iop, 2016), *Pseudomonas* sp. (Xu *et al*., 2018), *Marinobacter* sp. in bacteria (Li *et al*., 2018), and *Fusarium* sp. in fungi (Li *et al*., 2020a) harboring petroleum hydrocarbons (PH)-degrading genes can utilize petroleum as a carbon and energy source through heterotrophic metabolism or co-metabolic pathways and have been shown to efficiently degrade petroleum hydrocarbons (PHs) in oil-contaminated

areas. Bioremediation is a carbon-neutral and cost-effective method for the removal of petroleum contaminants (Naeem and Qazi, 2020).

Metagenomic analyses of the microbial community structure and functional gene abundance under different PH contamination stress conditions can provide helpful guidelines for the bioremediation of such environments (Liu *et al*., 2022). Next generation sequencing (NGS) methods such as whole-genome shotgun sequencing, RNA sequencing, and amplicon sequencing have been adopted to depict the profiles of microbial communities and functionally degrading genes in petroleum contaminated sites (Mukherjee *et al*., 2017).

According to Cury *et al*. (2015), who studied Differences in the microbial communities in high- and low-petroleum- contaminated soils detected the abundance of functional genes coding for aromatic ring-cleaving dioxygenases and alkane monooxygenase *alkB*. Metagenomic sequencing showed that *Gammaproteobacterium* and *Colwellia* species were enriched in the most heavily oil-impacted sediment samples from the Gulf of Mexico. (Mason *et al*., 2014). As mentioned above, metagenomic analysis of microbial communities and petroleum-degrading genes can illustrate the metabolic mechanisms possible (Datta *et al*., 2020). Due to the so-called uncultivability of many environmental microorganisms (Steen *et al*. 2019), several studies have concentrated on remediation by indigenous microorganisms (Kumar and Gopal 2015; Sarkar *et al*. 2016). More recent studies have shown that the inoculation of carefully cultivated hydrocarbon-degrading bacterial consortia or isolates enhances the effectiveness of various remediation techniques (Atashgahi *et al*. 2018a; Garrido-Sanz *et al*. 2019). Therefore, it is important to discover novel microbes that can be used for bioaugmentation (the introduction of additional microbiota), which is as an effective strategy for the remediation of organic contaminants (Atashgahi *et al*. 2018b; Ławniczak *et al*. 2020). The aim of this study was to investigate, through shotgun metagenomics, the diversity and genomic potential of bacterial consortia derived from a hydrocarbon contaminated water in B-Dere, Niger delta region of Nigeria.

MATERIALS AND METHODS

3.1. Sample Collection

Near-surface water sample (40L) was collected from a flowing brackish water of crude-oil polluted environment in B-Dere, Gokana, Rivers State, South-South Nigeria. The sample was collected and prefiltered through a 105-µm pore-size spectra/mesh polypropylene filter (SpectrumLabs, Rancho Dominguez, CA, USA) to remove larger particles, then transported in ice chest park to the Department of Microbiology laboratory for further processing within 24 h.

3.2 Sample preparation

Water was filtered sequentially through 1 µm pore-size filter (Envirochek HV, Pall Corporation, Ann Harbor, MI, USA) then a 0.2 µm pore-size filter (142 mm Supor-200 membrane disk filter, Pall Corporation, Ann Harbor, MI, USA) to collect bacterial and archaeal sized cells. This pre-filtration did also remove larger and particle- associated bacterial cells, and of course bacteria adhering to large items like leaves. Cells were collected off the 0.2 µm pore-size filters by vortexing with tungsten beads (i.e., bead beating) and centrifugation. DNA was extracted using the PowerLyzer Powersoil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA). Cells were mainly bacterial and not archaeal (data not shown), so for simplicity, the microbial community is here referred to as bacterial. A positive control (mock community) was prepared by spiking de-ionized water with DNA extracted (NucleoSpin Tissue, Macherey-Nagel, Düren, Germany) from 12 cultured bacterial strains [Bacillus amyloliquefaciens FZB42, Bacillus cereus ATCC 14579, Burkholderia cenocepacia J2315, Escherichia coli K-12, Frankia sp. CcI3, Micrococcus luteus NCTC 2665, Pseudomonas aeruginosa PAO1, Pseudomonas aeruginosa UCBPP-PA14, Pseudomonas fluorescens, Pf-5,Pseudomonas putida KT2440, Rhodobacter capsulatus SB 1003, Streptomyces coelicolor A3(2)]. Ultrapure (Type 1) water (Milli-Q, Millipore Corporation, Billerica, MA, USA) was used as a negative control. Shotgun sequencing libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina, Inc., San Diego, CA, USA). Gel-size selection was automated with Ranger Technology (Coastal Genomics Inc., Burnaby, BC, USA) to ensure consistent and specific fragment lengths, targeting 500–800 bp (Uyaguari- Diaz *et al.*,, 2015).

3.3 DNA Sequencing

Sequencing was performed using a MiSeq platform (Illumina, Inc., San Diego, CA, USA) using the MiSeq Reagent Kit V2 (2× 250 bp paired end reads, 500 cycles) at the British Columbia Public Health Microbiology and Reference Laboratory. Samples were sequenced over seven runs and a positive and negative control sample was included in each run. All raw sequences are deposited in the NCBI Sequence Read Archive under BioProject ID: 287840.

3.4 Metagenome Compositional Analysis

Shotgun sequenced reads were trimmed to remove low quality bases using Trimmomatic (Bolger *et al.,* 2014). A sliding window of length 5 and a minimum Phred score of 20 was used at the 3end. At the 5end, sequences of one or more nucleotides with scores less than 20 were trimmed. Sequencing adapters were removed using Cutadapt (Martin, 2011), overlapping paired- end reads were merged using PEAR (Zhang *et al.,* 2014), and reads shorter than 100 bp were discarded. After this processing, samples had 418538 to 2165162 high quality reads and samples were subsampled to the number of reads in the smallest sample: 418500 reads.

Hierarchical clustering was performed and evaluated using the pvclust R package (Suzuki and Shimodaira, 2006). Bootstrap p-values were based on multiscale bootstrap resampling with 10000 repetitions. The k-mer abundance profile of each sample was calculated by counting the frequencies all nucleotide sequences (k-mers) of length 12 in each dataset using Jellyfish (Marçais and Kingsford, 2011). These k-mer profiles were compared using Manhattan distances, as appropriate for high dimensional datasets (Aggarwal *et al.,* 2001), clustered using Ward's method (R: ward.D2) and visualized using hierarchical clustering and NMDS with two axes. Major sample clustering patterns were consistent with k-mer lengths 4, 9, and 10, but were most distinct with higher k-mer lengths.

3.5 Calculation and Normalization of Functional Gene Group Abundances

Reads were compared against NCBI's nr database using RAPSearch2 (Zhao *et al.,* 2012). Resulting protein alignments longer than 30 amino acids were analyzed using MEGAN version 5.10 (Huson *et al.,* 2011) with default parameters,including aminimumbitscore of35andamaximum e-value of 0.01, to determine the gene families present, using the SEED (Overbeek *et al.,* 2005) and KEGG gene functional group databases (Kanehisa, 2000). Gene group abundance profiles were analyzed for differential abundance using the Wilcox test after removing low abundance features (mean abundance <0.01% in all samples). Subsamples of 100,000 reads were also analyzed using MG-RAST (Meyer *et al.,* 2008) with default parameters for comparative purposes. The quality of the MEGAN assignments of reads to gene groups was assessed using the mock community samples. These samples of known taxonomic composition were annotated using MEGAN and the KEGG database of ortholog groups. These mock community KEGG profiles were compared against reference profiles, compiled from annotated genomes. The KEGG database was used for this analysis due to the availability of annotated genomes, however, the version of the KEGG database used for this reference annotation was not the same as the version used in the MEGAN analysis. Ortholog groups missing from one of the two profiles under comparison, possibly due to differences in database version, were omitted from this analysis, leaving 1725 KEGG ortholog groups to compare. Annotation profiles were fairly well correlated between the MEGAN and reference datasets when looking at KEGG ortholog groups ($r = 0.74$, $p < 2.2e{-}16$, Pearson correlation used due to interest in linear relationship). This correlation improved when looking at KEGG pathways ($r = 0.96$, $p < 2.2e{-}16$). Of the 208 pathways, two were predicted as less abundant in the MEGAN profiles relative to the reference profiles: "Ribosome" and "ABC transporter". When these pathways were removed, the correlation rose to $r = 0.98$.

Adjusting abundance profiles by the average KEGG ortholog group gene length improved the correlations between ortholog group profiles ($r = 0.86$, $p < 2.2e{-}16$) but the improvement was minimal for pathway profiles ($r=0.99$, $p < 2.2e{-}16$). Normalization of gene functional group abundance profiles by AGS was performed on subsampled reads with two approaches. The first used MicrobeCensus to estimate AGS values (Nayfach and Pollard, 2015), which were then divided by the mean AGS across samples (to avoid inconveniently large numbers) and then multiplied by group abundances; the second used MUSiCC, which adjusts group abundances directly (Manor and Borenstein, 2015). Both tools are based on the same

goal: to calculate normalization factors such that normalized universal, single copy gene abundances will be constant across samples. These tools assume that all reads are bacterial and so can be affected by the presence of eukaryotic DNA sequences. Due to the filtration strategy used during sample processing, very little eukaryotic DNA was present in the samples (median 2 ± 0.7% of domain-assigned reads). Both tools gave very similar results, with an overall Pearson correlation of 0.998 ($p < 2.2e–16$) between KEGG ortholog group abundance profiles across all samples, and a correlation score of 0.997 (p-values < 2.2e–16) within each sample. Currently, MUSiCC only accommodates KEGG and COG profiles and normalizes assigned reads, whereas MicrobeCensus works directly on reads to estimate AGS and therefore allows the flexibility of using any downstream functional assignment tool. In the analyses that follow, MicrobeCensus normalization is used.

RESULTS

In the study, water sample was sequenced for 16S rRNA amplification, resulting in a total of 531,927 sequence reads. The sequencing of the PCR product using universal bacterial primers yielded 529,320 bacterial and 20 archaeal communities. The taxonomic profiles revealed 2,587 microorganisms (0.486%) that have yet to be classified. Relative abundance bacterial phyla, genus and species in water samples are presented in Tables 4.2. Proteobacteria, Tenericutes, Bacteroidetes, Planctomycetes, Firmicutes, Chlamydiae, Nitrospirae, Chloroflexi, Cyanobacteria, Actinobacteria, Chlorobi, Spirochaetes, Verrucomicrobia, Acidobacteria, Thermi, Caldithrix, Thermodesulfobacteria, Deferribacteres, Synergistetes, Thermotogae, Fibrobacteres, Caldiserica, Fusobacteria, Armatimonadetes and Chrysiogenetes were the bacterial phyla identified in this study. The top bacterial phyla with relative abundance greater 1 % were Proteobacteria (79.22 %), Bacteroidetes (3.56 %), Tenericutes (3.05 %), Planctomycetes (3.23 %) Firmicutes (2.07 %) and Chlamydiae (1.11). Abundance of the phyla Fusobacteria (0.0006 %), Armatimonadetes (0.0002 %) and Chrysiogenetes (0.0002 %) were less than 0.001% (Table 4.7).

Precisely 575 bacterial genera were detected in this study, however the different genus varied in number of their relative abundance (Table 4.8). About seven (7) genera (*Shewanella*, *Acinetobacter*, *Enterobacter*, *Mycoplasma*, *Wautersiella*, *Desulfonatronum* and *Planctomyces*)were the most the dominant in sample. Overall, *Shewanella*was the most prominent with 90216 sequences read while 38.90 % of the bacterial genera detected are yet to be classified (Table 4.8). Bacterial genera with relative abundance ≥ 1 % were *Shewanella* (17.04 %), *Acinetobacter* (9.37 %), *Enterobacter* (5.42 %), Mycoplasma (3.03 %), *Wautersiella* (2.19 %), *Desulfonatronum* (1.82 %) and *Planctomyces* (1.79 %).

Table 4.1: Distribution of sequence reads in water sample

Microbial group	No. of hits	Relative abundance
Bacteria	529320	99.510
Archaea	20	0.004
Unclassified	2587	0.486

Table 4.2: Diversity and Distribution of Bacterial Phylum in water sample

S/n	Phylum	Number of hits	Relative abundance
1	Proteobacteria	419344	79.2232
2	Tenericutes	16119	3.0452
3	Bacteroidetes	18828	3.5570
4	Planctomycetes	17091	3.2289
5	Chlamydiae	5869	1.1088
6	Nitrospirae	2466	0.4659
7	Firmicutes	10934	2.0657
8	Chloroflexi	5043	0.9527
9	Cyanobacteria	3972	0.7504
10	Actinobacteria	3116	0.5887
11	Chlorobi	1960	0.3703
12	Spirochaetes	1330	0.2513
13	Verrucomicrobia	1408	0.2660
14	Acidobacteria	439	0.0829
15	Thermi	664	0.1254
16	Caldithrix	302	0.0571
17	Thermodesulfobacteria	119	0.0225
18	Deferribacteres	221	0.0418
19	Synergistetes	48	0.0091
20	Thermotogae	73	0.0138
21	Fibrobacteres	7	0.0013
22	Caldiserica	6	0.0011
23	Fusobacteria	3	0.0006
24	Armatimonadetes	1	0.0002
25	Chrysiogenetes	1	0.0002
	Unclassified	19956	3.7701

Precisely, 2 archaeal (Fig. 4.1) phyla were identified in the water samples. Euryarchaeota was the most prominent archaea with 90 % abundance. The abundance of Crenarchaeota phylum detected in the sample was 10 % indicating a 9 times lower abundance than Euryarchaeota (Fig. 4.1).

Figure 4.2 shows six (6) archaeal genera which were *Methanobacterium*, *Methanosaeta*, *Methanocorpusculum, Methanobrevibacter, Methanosphaera* and Acidianus. Overall, the predominant archaeal genera were *Methanobacterium*(25 %),*Methanosaeta* (20 %), *Methanocorpusculum* (10 %) and Acidianus(10 %). *Methanobrevibacter*and *Methanosphaera* were the minor phyla with 5 % abundance each. The abundance of unclassified genus was 25%

Diversity and distribution of archaeal species in water sample is presented inFigure 4.3. A total of 6 archaeal (Table 4.3) were detected in this study. The abundance of the bacterial species was in the order;Unclassified species >*Methanosaeta harundinacea* (10 %), *Methanocorpusculum parvum* (10 %), *Methanosaeta concilii* (10 %) >*Methanobrevibacter gottschalkii* (5 %), *Methanobacterium beijingense* (5 %),*Methanosphaera cuniculi* (5 %).

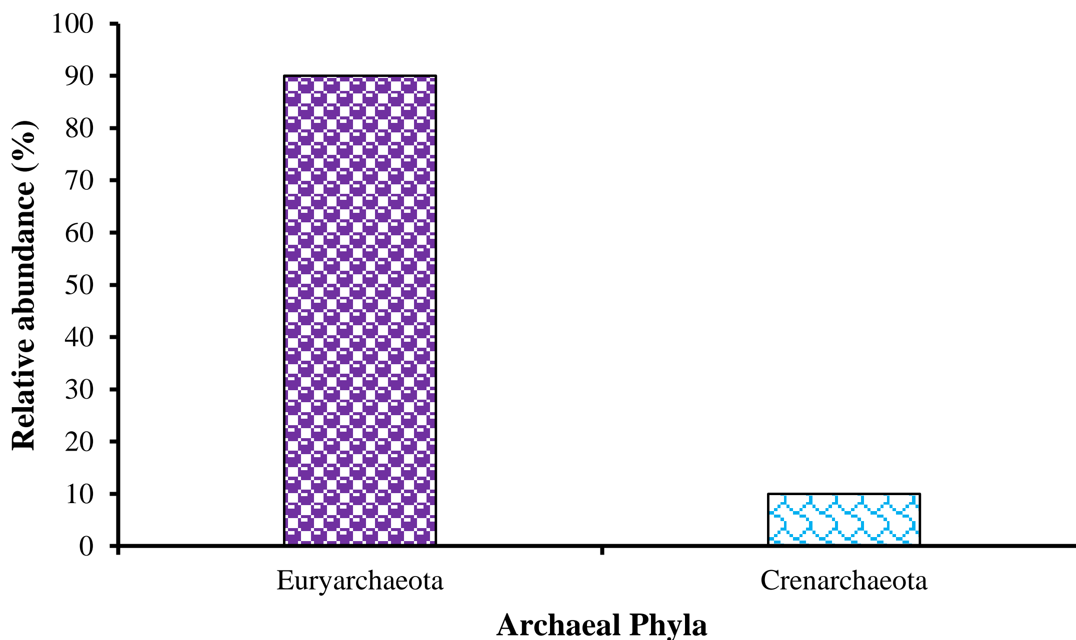

Figure 4.1: Relative abundance of archaeal phyla in water sample

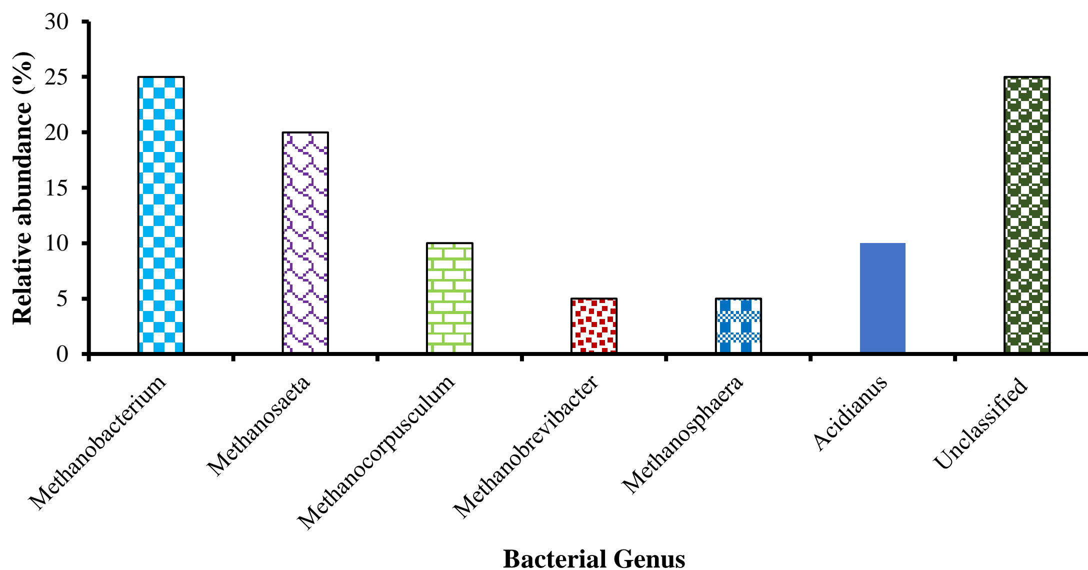

Figure 4.2: Relative abundance of archaeal genus in water sample

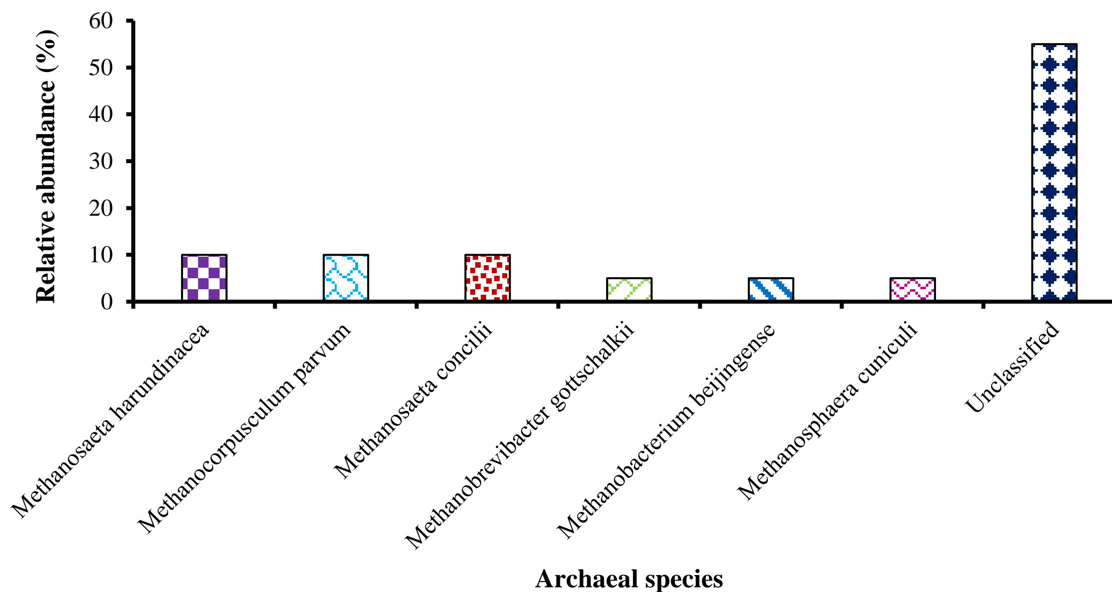

Figure 4.3: Relative abundance of archaeal species in water sample

DISCUSSION

Bacterial and archaeal Community of B-Dere polluted water sample using 16 S Metagenomics

This work demonstrated that cultural approach of microbial enumeration in the ecosystem counting is inadequate because it represents less than 1% of the ecosystem's total microbiota. Notwithstanding the substantial amount of study that has been done thus far, little is known about the prokaryotic distribution and community structures in the B-Dere water. Human activity has an impact on the quantity and quality of rivers as they flow through various forms of settlement. The overall microbial composition and diversity of the specific water body may be impacted by these human activities. A total of 25 bacterial phyla were found to be present in B-Dere contaminated water samples according to metagenomic analyses. The top bacterial phyla associated with the water sample were Proteobacteria, Tenericutes, Bacteroidetes, Planctomycetes, Chlamydiae, Unclassified and Firmicutes while the remaining phyla made the remaining 4.0013%. The high read counts of bacteria found in this investigation are consistent with multiple reports suggesting that they are the predominant microbial domain in aquatic environments (Wang *et al.,* 2023; Udofia *et al.,*, 2022). Proteobacteria (69.8%) and Bacteroidetes (2.7%) were found to be among the most prevalent phyla in all of the samples, according to a study by Abia *et al.,* (2023). A significant percentage of the sequences were not classified at the phylum level, according to Meneghine *et al.,* (2017), who reported the existence of 22 bacterial phyla. Proteobacteria was the most common phylum among the 95 bacteria phyla found in the study conducted by Wang *et al.,* (2023).

Precisely 575 bacterial genera were detected with seven (7) genera (*Shewanella*, Acinetobacter, *Enterobacter*, *Mycoplasma*, *Wautersiella*, *Desulfonatronum* and *Planctomyces*) being the most the dominant in sample. The Shewanella bacterial genus primarily consists of aquatic γ-proteobacteria that have been isolated worldwide in a variety of settings, including the deepest marine trenches and surface freshwater (Lemaire *et al.*, 2020). The *Shewanella* bacterial genus is primarily an aquatic γ-proteobacteria that have been isolated worldwide in a variety of settings, including the deepest marine trenches and surface freshwater (Lemaire *et al.,* 2020). A number of these *Shewanella* bacteria are essential species for a wide range of biotechnological uses, most notably the bioremediation of metals and hydrocarbon contaminants (Joe *et al.,* 2019). Scholarly publications have documented the prevalence of Shewanella in hydrocarbon-contaminated environments and their function in the process of bioremediation (Rathour *et al.*, 2018; Ram *et al* 2019). For instance, Martín-Gil (2004) isolated Shewanella putrefaciens from the Prestige oil spill in Spain. Among the most common bacteria that produce biosurfactants, *Shewanella alga* and *Shewanella upenei* were identified from seawater samples and sediments polluted with crude oil that were collected from five stations in the Persian Gulf (Hassanshahian, 2014). The inherent capacity of this genera to proliferate and detoxify harmful substances accounts for their importance for the resilience of aquatic ecosystems, their use in wastewater treatment, and their employment in microbial fuel cells for energy generation (Lemaire *et al.,* 2020).

Acinetobacter is a genus of gram-negative, oxidase-negative, and strictly aerobic bacteria that belongs to the γ-Proteobacteria and Pseudomonadales order (Jung and Park, 2015). Acinetobacter holds a significant role in nature due to its widespread distribution over a variety of habitats, including soils, freshwater bodies of water, oceans, sediments, and polluted sites. This genus's versatile metabolic traits enable it to catabolize a broad variety of natural chemicals, suggesting that it actively participates in cycling of nutrient in the ecosystem (Jung and Park, 2015). It is well known that Acinetobacter breaks down hydrocarbons, particularly alkanes with varying chain lengths. The potential for Acinetobacter to biodegrade alkane is demonstrated by its common presence in a variety of hydrocarbon-contaminated habitats, such as soils, mangrove sediments, Antarctic marine sediments, and pristine settings (Kuhn *et al.,* 2009; Kang *et al.,* 2011; Rocha *et al.,* 2013). The majority of bacteria that break down hydrocarbons belong to the genus Acinetobacter (Li *et al.*, 2023). According to earlier studies (Atakpa *et al.,* 2022; Czarny *et al.,*, 2020), Acinetobacter is capable of degrading a variety of hydrocarbons, including n-alkanes and PAHs, and it has also been shown to be successful at remediating polluted soil caused by crude oil in an alkaline environment (Zhang *et al.,*, 2021).

The presence of *Enterobacter*, *Mycoplasma*, *Wautersiella*, *Desulfonatronum* and *Planctomyces* have reported previously detected in hydrocarbon polluted water. Members of the genus Enterobacter were

detected from the crude oil polluted Iko river estuary and freshwater ecosystem of the Niger Delta Region of Nigeria (Johnny *et al.,* 2018). The genus *Wautersiella* were most *abundant* in polluted River water samples in a study by Liu *et al.,*(2024).

The dominant species with relative abundance ≥ 0.9 % in B-Dere polluted water were *Shewanella decolorationis* (16.27 %), *Pseudomonas plecoglossicida* (14.03 %), *Acinetobacter baumannii* (3.54 %), *Wautersiella falsenii* (2.19 %), *Enterobacter hormaechei* (1.99 %), *Desulfonatronum thiosulfatophilum* (1.82 %), *Pseudomonas entomophila* (1.76 %), *Enterobacter amnigenus* (1.58 %), *Acinetobacter baylyi* (0.97 %) and *Pseudomonas putida* (0.97 %). Shewanella decolorationis is very appealing because of its metabolism, which enables it to grow effectively at temperatures between 24 and 40°C while preserving its capacity to respire different substrates and break down harmful substances like chromate or poisonous dyes (Lemaire *et al.,* 2019). *Shewanella decolorationis* demonstrates the mechanisms underlying chromate resistance and the potential to be a strong agent for bioremediation (Lemaire *et al.,* 2019). *Acinetobacter baumannii*were detected from river water and wastewater samples in a study by Hubeny *et al.,* (2022). The unexpected capacity of A. baumannii to develop resistance to antibiotics makes it one of the most clinically significant isolates in modern medicine (Hubeny *et al.,*2022; Lin and Lan, 2014). The primary resistance mechanism in *A. baumannii* is the inactivation of beta-lactam antibiotics through the synthesis of beta-lactamases. Numerous accounts in the literature have detailed the diversity of genes in *A. baumannii* that confer resistance to beta-lactams, including carbapenems (Lee *et al.,* 2017; Qi *et al.,*, 2016). *Pseudomonas putida* is a recognized hydrocarbon user that has been connected to crude oil deterioration. *P. putida*had the highest percentage utilization of 80.27% and a rate of utilization of 0.46 mg/ml/day (Ezeji *et al.,* 2006).

Archaeal Community of B- Dere polluted water sample

The roles of archaea in global biogeochemical cycles are very important (Offre *et al.,* 2014). These microorganisms are widely distributed in different habitats such as hot springs (Cousins *et al.,*, 2018), marine (deep-sea) sediments,permafrost (Shcherbakova *et al.,* 2016), marine seawaters,and freshwater as well as hypersaline lakes (Wemheuer *et al.,*, 2019). The two phyla (Euryarchaeota and Crenarchaeota) found in this study were previously documented in freshwater habitat and estuaries by Wang *et al.,* (2020).

Euryarchaeota was the predominant archaeal phylum in the present study. According to Baker *et al.,* (2020) the most numerous and diverse group of cultured lineages is found in Euryarchaeota. These microorganisms appear to play a variety of functions in biogeochemical cycles, as evidenced by their involvement not only in the anaerobic oxidation of methane and its synthesis (Wang *et al.,* 2020), but also in the anaerobic oxidation of other short-chain hydrocarbons (Wang *et al.*, 2019). *Methanosaeta harundinacea* (10 %), *Methanocorpusculum parvum* (10 %), *Methanosaeta concilii* (10 %) *Methanobrevibacter gottschalkii* (5 %), *Methanobacterium beijingense* (5 %)and *Methanosphaera cuniculi* (5 %) are also species implicated in methane production (Bomberg *et al.,* 2008). The research indicates that B-dere has a very strong microbial diversity, suggesting a high potential for heterotrophic activity, even in the face of growing anthropogenic activities in and around the study area. This is critical because it maintains microbial capacity to adapt to unfavourable environmental circumstances. The metagenomic assay also validates the diversity of microorganisms in B-Dere water samples which is critical to the bio-geochemical cycling and maintenance of environmental health and quality.

CONCLUSION

Based on the metagenomic data analysis, the petroleum-contaminated sites were inhabited by a diverse array of bacterial species. These microorganisms play an important role in the bioremediation process as they are found to possess rich sources of different and diverse enzymes useful for the degradation of different hydrocarbon groups. This study thus confirms the presence of phyla, which were never known to be playing any role in hydrocarbon degradation or inhabiting these petroleum oil-contaminated areas.

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