



Biochemical and PCR-based identification of Hydrocarbonoclastic Bacteria isolated from spent engine oil polluted soil

*^{1,2}Adeleye, A. O., ²Yerima, M. B. ³Nkereuwem, M. E. ⁴Onasanya, G. O., ³Onokebhagbe, V.O., ¹Bate, G.B. and ²Raji, M.

¹Department of Environmental Sciences, Federal University Dutse, Nigeria.

²Department of Microbiology and Biotechnology, Federal University Dutse, Nigeria.

³Department of Soil Science, Federal University Dutse, Dutse, Nigeria.

⁴Department of Animal Science, Federal University Dutse, Dutse, Nigeria.

*Corresponding author: adeniyi.adeleye@fud.edu.ng ; 08162784457

Abstract

This study was conducted to isolate and identify spent engine oil (SEO) utilizing bacteria from mechanic village, Dutse. Test tubes containing one (1) mL of the supernatant derived from the mixture of one (1) gram of SEO polluted soil and nine (9) mL sterile distilled water supplemented with mineral salt medium and overlaid with 0.5 mL of SEO were incubated at 37.3 °C for four (4) days. Bacterial growth in form of turbidity was sub-cultured into test tubes containing Trypticase Soy broth and Trypticase Soy agar, incubated at 37.3 °C for 48 hours. Developed colonies were observed and recorded. Six (6) distinct colonies were subsequently picked and transferred to glass slides to form smears for Gram staining. Biochemical tests based on morphological attributes of the bacterial isolates were conducted for its identification and further confirmed using molecular technique. Gram positive cocci in clusters, gram positive rods with squared ends, gram negative rods, gram negative rods in chains, gram positive cocci in chains and gram positive cocci bacteria were identified under the microscope. Results of the biochemical tests indicated Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Alcaligenes faecalis, Streptococcus pyogenes and Enterococcus faecalis as the isolated bacteria. Nucleotide similarity index (NSI) results generated showed 70.71% - 96.55% between the nucleotide sequences of all the bacteria isolated in this study when compared against the National Center for Biotechnology Information (NCBI) database. Owing to the results obtained in this study, the method employed for the isolation of hydrocarbonoclastic bacteria can therefore be recommended for subsequent isolation of such bacteria that can be employed in achieving bioremediation of hydrocarbon impacted environments.

Keywords: Spent engine oil, soil, bacterial isolates, biochemical tests and nucleotide similarity index

1. Introduction

Engine oil can be referred to as lard and petroleum product that has base oil emanating from fractions of crude oil and other related additives (Nwagwu *et al.*, 2020). These authors reiterated further that additives principally function as antioxidants, corrosion inhibitors and anti-foam agents in automobiles. According to Adeleye *et al.* (2018), engine oils are mostly mixed by using base oils that have got organic compounds comprising carbon and hydrogen completely, for example mineral oil. Spent engine oil (SEO) is the surviving brown-to-black oily liquid dispensed from motor vehicles that change their oil into a new one with a view to facilitating smooth running of automobile engines (Abdulsalam *et al.*, 2013; Adeleye *et al.*, 2018).

The utilization of the components of hydrocarbon compounds found in environmental pollution has been extensively associated with microorganisms that are hydrocarbonoclastic. Bacteria, most especially have been implicated to possess efficient systems that aid the synthesis of enzymes that can readily attack hydrocarbon



pollutants in their immediate environment. In a study conducted by (Ismail *et al.*, 2014), five bacterial isolates grew abundantly in SEO polluted soil samples coupled with showing high optical density in oil broth after incubation. These authors reiterated further that the SEO polluted soil degradation rate exhibited by various bacteria isolated in their study was significant.

Several indigenous microorganisms have been reported by Dhaker and Jain (2011); Jain *et al.* (2010) as having the capability of degrading petroleum hydrocarbon pollutants in water and soil. According to Prince (2005), there are more than seventy five (75) dependably described genera of bacteria that can degrade oil and other hydrocarbons that may be present in different environmental media. Specifically, Kaplan *et al.* (2004); Ayotamuno *et al.* (2006); Wolicka *et al.* (2009), have reported the isolation of many hydrocarbon degrading microorganisms from soils polluted by hydrocarbon.

It has been reported by Zucchi *et al.* (2003); Alquati *et al.* (2005); Ayotamuno *et al.* (2006); that there are numerous species of bacteria having the capability of utilizing hydrocarbons as their source of energy. The rapid disappearance of crude oil spills from the ground in oil fields, oceans, seas and beaches has been pin-pointed as the evidence in support of hydrocarbon utilization by these hydrocarbonoclastic microorganisms. We live in a modern world that requires unravelling more autochthonous bacterial candidates that can be adopted in combating and reclaiming petroleum hydrocarbon pollution that have been implicated in sterilizing agricultural soil (Adeleye *et al.*, 2021) and rendering water sources unfit for human consumption (Srivastava *et al.*, 2019). It is based on these background information that this study was conducted using the combination of biochemical and PCR-based techniques with a view to isolating and identifying SEO utilizing bacteria that can be readily employed in remediating and reclaiming possible hydrocarbon pollution in our study area.

2. Materials and Methods

2.1 Collection of spent engine oil polluted soil

Through the aid of sterilized soil auger and subsequently employing grid method of soil sampling from four spots, ten grams (10 g) of SEO polluted soil at the depth of five (5) centimeters was collected from the Dutse mechanic village, Jigawa State, Nigeria. The ambient temperature recorded with the aid of a thermometer during the sampling of the SEO polluted soil was 37.3 °C. SEO dumping at the site where the SEO soil was sampled has been going on for four (4) years (Personal communication with Head Mechanic at the workshop).

2.2 Preparation of mineral salt medium

The preparation mineral salt medium was done inside the Microbiology laboratory, Federal University Dutse. A medium having the essential nutrients (potassium, magnesium, sodium, phosphorus and ammonium salts) required by the targeted bacteria for their optimum growth was prepared by dissolving 0.1 g NaCl, 1.8 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O and 4.0 g NH₄Cl in one (1) Liter of distilled water as established by Mukred *et al.* (2008). The mineral salt medium (MSM) was subsequently sterilized in the autoclave at 121 °C for fifteen (15) minutes. It was later dispensed in test tubes.

2.3 Isolation procedure for spent engine oil utilizing bacteria

The isolation of SEO utilizing bacteria was also conducted inside the Microbiology laboratory, Federal University Dutse. SEO utilizing bacteria were isolated according to the procedure established by Adeleye and Yerima (2009). SEO polluted soil weighing one (1) gram each was suspended into three (3) test tubes containing 9 mL sterile distilled water. The mixture was vigorously shaken to give room for the formation of supernatant. Subsequently, 1mL of the supernatant was introduced into test tubes containing MSM overlaid with 0.5 mL of SEO each. The SEO overlaid as the carbon and energy source for the hydrocarbonoclastic bacteria present in the SEO polluted soil was obtained from one of the service pits in Dutse mechanic village. The mixture in the test tubes was then shaken vigorously and allowed to settle. The test tubes were subsequently incubated at 37.3 °C for four (4) days and bacterial growth in form of turbidity was observed. Bacterial growth observed after incubation was transferred into three (3) test tubes containing Trypticase Soy broth (TSB) and incubated at 37.3 °C for 48 hours. The TSB was



prepared according to the standard procedure described by the manufacturer (Himedia Laboratory Pvt Limited, India). After incubation of the test tubes, further transfer was carried out using three (3) Trypticase Soy Agar (TSA) plates. The TSA was prepared according to the standard procedure described by the manufacturer (Himedia Laboratory Pvt Limited, India). The TSA plates were incubated at 37.3 °C for 48 hours. The colonies that developed were observed and recorded. Distinct colonies were subsequently picked and transferred to glass slides to form smears.

2.4 Determination of morphological characteristics

Gram staining procedure was carried out according to the procedure outlined by Olutiola *et al.* (2000). The smear on the slide was subsequently flooded with crystal violet solution for one (1) minute, and rinsed with water. All the cells turned purple and the smear was again flooded with Iodine for one (1) minute and the slide was rinsed with 75% alcohol until the crystal violet was completely washed off. It was subsequently counter stained with safranin for one (1) minute and finally rinsed with water. The slides were then viewed under an oil immersion objective and resultant colours, sizes and shapes of bacterial isolates were recorded accordingly

2.5 Biochemical tests for the identification of bacterial isolates

All the reagents used for the biochemical tests conducted on the bacterial isolates were obtained from the Microbiology laboratory, Federal University Dutse wherein the tests were equally done. The choices made on the types of biochemical tests conducted were based on the morphological characteristics described by Barrow and Feltham (1993) and thirty nine (39) biochemical tests were done according to the procedures outlined by Olutiola *et al.* (2000); Choopun *et al.* (2002); Cheesebrough (2006); Ochei and Kolhatkar (2008); Wilson (2012); Hemraj *et al.* (2013); Himedia (2015); [Pokhrel](#) (2015); Microbeonline (2019).

2.6 Genomic DNA Extraction from cultured bacterial cells

Bulk of the molecular work was conducted in DNA Laboratory Kaduna, Kaduna State. DNA extraction protocol used in this study was in accordance with the procedures of ExiPrep™ Genomic DNA extraction kits (Bioneer ExiPrep Plus Bacteria Genomic DNA Kits). About 20 µL of Proteinase K was added to 1 mL eppendorf tube after which 104-108 cultured cells were added, this was made to a total volume 200 µL using phosphate buffer saline (PBS). A 200 µL of binding buffer was added to the mixture and was immediately vortexed for thorough mixing. Thereafter, the vortexed mixture was re-suspended to achieve maximum lysis. After the above, the sample was incubated at 60 °C for 10 minutes using ACCUBLOCK™ digital dry bath until total digestion was achieved. 100 µL of Isopropanol was added to the digested mixture and was mixed thoroughly by up-down pipetting. After the above, mixture was briefly spun using micro-centrifuge (ThermoFisher Scientific, Waltham, MA U.S.A) to obtain clean lysate. The lysate was poured into the upper reservoir of the binding column tube without touching the rim and was centrifuged at 8,000-10,000 rpm for 1 minute.

Consequently, the binding column tube was transferred into a new 2 mL eppendorf tube for filtration. Subsequently, 500 µL of Washing buffer 1 (W₁) was added to the binding column without touching the rim, the tube was closed, and centrifuged at 8,000 rpm for 1 minute. Solution obtained after centrifugation was poured into a disposable tube. A 500 µL of Washing buffer 2 (W₂) was poured into the binding column without touching the rim and again centrifuged at 8,000 rpm for 1 minute. Further centrifugation was also carried out at 12,000 rpm for 1 minute to completely remove remains of ethanol droplets clinging to the bottom of binding column tube. The binding column tube was transferred to a new 1.5 mL tube for elution of DNA.

Finally, 200 µL of elution buffer was added onto binding column tube and this was allowed to wait for at least 5 minutes at temperature of 15-25 °C until elution buffer was completely absorbed into the glass fiber of binding column tube, this was done to increase the yield of the genomic DNA. The eluted genomic DNA was stored in the refrigerator.



2.7 PCR-based detection of *16S rRNA* gene in the bacterial isolates

The primer set used for this study was obtained from previously published work quoted by Ebu *et al.* (2018). The primer set was optimized for primer specificity and primer dimers were eliminated. The fragment size of *16S rRNA* gene was 789 bp covering the coding region (Table 1). From the literature, *16S rRNA* is generally known as the conserved region in wide varieties of bacteria and is acceptably used in the detection of bacteria for genetic diversity studies.

2.8 Amplification of *16S rRNA* gene

The PCR reaction was carried out in a total volume of 20 uL reaction containing template DNA of 2 uL, 1 uL each of forward and reverse primer set, 9.5 uL of master mix (Bioneer AccuPower^R PCR PreMix) and 6.5 uL of nuclease free water. The amplification of *16S rRNA* gene was performed in MJ Research PTC-100 thermal cycler (1000 Alfred Nobel Drive Hercules, CA USA).

The amplification condition involved initial denaturation at 95 °C for 5 minutes followed by 25 cycles of denaturation at 94 °C for one minute, annealing temperature of 52 °C for 1 minute, extension temperature of 72 °C for 1 minute followed by final extension at 72 °C for 5 minutes.

Table 1. *16S rRNA* Primer Sequences and Target region

Primer Sequence	Number of bases	Fragment size of PCR product (bp)
F (5 ¹ -3 ¹) GGACTACAGGGTATCTAAT	19	789
R (3 ¹ -5 ¹) AGAGTTTGATCCTGG	15	

Source: Quoted by Ebu *et al.* (2018).

PCR products were evaluated on 1.5% agarose gel electrophoresis after staining with 2 uL of ethidium bromide and the DNA fragments were visualized under Bio-RAD DocTM XR + Imaging System version 5.1 (Gel documentation Molecular imager, Bio-Rad laboratories, Inc., U.S.A).

2.9 Sequencing, Evolutionary and Nucleotide Sequence Analyses of *16S rRNA* gene of the Bacterial Isolates.

The PCR products of the DNA extracted from the bacterial isolates were sequenced in Inqaba Biotechnical Industries (Pty) Limited in South Africa using automated ABI 3100 Series sequencer/genetic analyzer (ThermoFisher Scientific, Waltham, MA, U.S.A). Nucleotide sequence map/chromatogram map was edited using BioEdit Sequence Alignment Editor (ABI, ThermoFisher Scientific, Waltham, MA, U.S.A). Rooted neighbor-joining tree diagram was constructed from nucleotide sequence of *16S rRNA* gene of the bacterial isolates compared with sequences obtained from National Center for Biotechnology Information (NCBI) database and were analyzed using MEGA 5.2 software with a view to evaluating the degree of relatedness, ancestral evolution and common ancestry of *16S rRNA* gene of bacteria (Tamura *et al.*, 2011).

3. Results

3.1 Morphological and biochemical identification of SEO utilizing bacteria

The following colonial characteristics observed on the TSA plates after incubation are presented in Table 2. The six distinct colonies that were seen on the plates ranged between C₁ (creamy yellow) and C₆ (light green) (Table 2). Having gram stained the isolated bacteria, the observed morphological characteristics are presented in Table 3. Four (4) gram positive bacteria and two (2) gram negative bacteria with different shapes ranging from cocci in clusters, rods, cocci in chains, cocci and rods in chain were seen under the microscope and recorded (Table 3).

Table 2. Colonial characteristics of hydrocarbonoclastic bacterial isolates.

Number ascribed to colony	Colonial characteristics
C ₁	Creamy yellow colonies
C ₂	Glistening white colonies
C ₃	Creamy white colonies
C ₄	Creamy colonies with scanty growth
C ₅	Creamy colonies with moderate growth
C ₆	Light green colonies

Table 3. Morphological characteristics of hydrocarbonoclastic bacterial isolates

Colonial characteristic	Gram staining
Creamy yellow colonies	Gram positive cocci in clusters
Glistening white colonies	Gram positive rods with squared ends
Creamy white colonies	Gram negative rods
Creamy colonies with scanty growth	Gram negative rods in chains
Creamy colonies with moderate growth	Gram positive cocci in chains
Light green colonies	Gram positive cocci

Having conducted suitably relevant biochemical tests on the bacterial isolates, results indicated *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Streptococcus pyogenes* and *Enterococcus faecalis* as the hydrocarbonoclastic bacteria isolated from the SEO polluted soil in Dutse mechanic village, Nigeria (Tables 4 and 5). Fifteen (15) suitably relevant biochemical tests ranging from oxidase (negative) to protease (positive) were done to identify gram positive cocci in clusters, seventeen (17) biochemical tests ranging from oxidase (positive) to growth in 10% NaCl (positive) were employed to identify gram positive rods with squared ends, thirteen (13) tests were conducted to identify gram positive cocci in chains while fifteen (15) biochemical tests ranging between oxidase (negative) and starch fermentation (positive) were done to identify gram positive cocci (Table 4). In the same vein, sixteen (16) biochemical tests ranging from oxidase (positive) to 10% lactose (positive) were done to identify gram negative rods while fourteen (14) biochemical tests ranging between oxidase (positive) and growth on centrimide agar (positive) were done to identify gram negative rods in chain (Table 5).

Table 4. Biochemical characterization tests for gram positive bacterial isolates

Biochemical Tests	Clusters	Rods with squared ends	Cocci in chains	Cocci
Oxidase	–	+	ND	–
Voges Proskauer	+	+	–	+
Coagulase	+	ND	ND	ND
Lactose	+	–	+	+
Maltose	+	ND	ND	ND
Mannitol	+	ND	ND	+
Fructose	+	+	ND	ND
Sucrose	+	ND	+	+
Xylose	–	–	–	ND
Cellobiose	–	ND	ND	ND
Mannose	+	–	ND	ND
Phosphatase	+	ND	+	–
Nitrate	+	+	ND	+
Arginine	+	ND	ND	ND



Biochemical Tests	Clusters	Rods with squared ends	Cocci in chains	Cocci
Protease	+	ND	ND	ND
Methyl Red	ND	-	ND	ND
Indole	ND	-	ND	-
Citrate	ND	-	ND	ND
Urease	ND	-	ND	ND
Simmon's Citrate	ND	+	ND	ND
Glucose	ND	+	+	+
Sorbitol	ND	-	-	ND
Galactose	ND	-	ND	ND
Tyrosine hydrolysis	ND	+	ND	ND
Growth in 10% NaCl	ND	+	ND	ND
Motility	ND	ND	-	+
Starch hydrolysis	ND	ND	-	-
Production of H ₂ O ₂	ND	ND	-	-
Growth on 40% bile agar	ND	ND	-	+
Growth on 6.5% NaCl	ND	ND	-	ND
Catalase	ND	ND	ND	-
Starch fermentation	ND	ND	+	+
Identity	<i>S aureus</i>	<i>B cereus</i>	<i>S pyogenes</i>	<i>E faecalis</i>

Legend: + = Positive, - = Negative, ND= Not done

Table 5. Biochemical characterization tests for gram negative bacterial isolates

Biochemical Tests	Rods	Rods in chains
Oxidase	+	+
Lactose	-	ND
Maltose	-	-
Mannitol	ND	-
Fructose	+	-
Sucrose	-	-
Xylose	+	-
Nitrate	+	-
Urease	-	-
Simmon's Citrate	+	+
Glucose	+	-
Tyrosine hydrolysis	ND	+
Motility	+	ND
Growth on MacConkey agar	+	ND
Christensen's Citrate	+	+
Gluconate	+	ND
10% Glucose	+	ND
10% Lactose	+	ND

Biochemical Tests	Rods	Rods in chains
Casein Hydrolysis	ND	-
Growth on Centrimide agar	ND	+
Identity	<i>P aeruginosa</i>	<i>A faecalis</i>

Legend: + = Positive, - = Negative, ND= Not done

3.2 PCR-Based Amplification of 16S rRNA Gene of Bacterial Isolates

Plate 1 shows the fragment size of the 16S rRNA gene of the bacterial isolates isolated in this study.

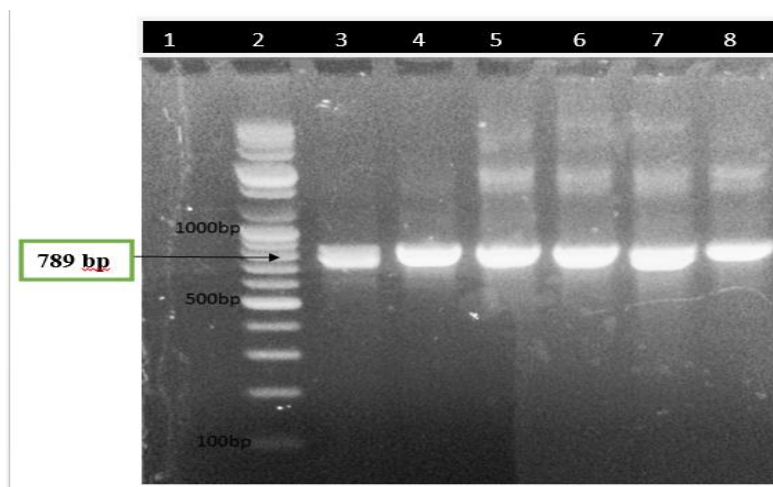


Plate 1. Amplified fragments of 16S rRNA gene (789 bp). Lane 1: negative control, Lane 2: Molecular ladder, Lanes 3-8 are representatives of amplified DNA fragments

3.3 Nucleotide Sequence Analysis of the Bacterial Isolates

The nucleotide sequence analysis was carried out to evaluate nucleotide similarity index (NSI) between the bacterial isolates and related sequences obtained from NCBI. Basic Local Alignment Search Tool (BLAST) was used for the analysis. NSI results generated showed 70.71% - 96.55% between the nucleotide sequences of all the bacteria isolated in this study when compared with those obtained from NCBI database (Table 6). *Enterococcus faecalis* EnGen0336 that recorded the highest NSI with *Enterococcus faecalis* and other bacterial isolates with corresponding high percentages of NSI identified biochemically in our study can be explored for hydrocarbon utilizing potential due the recorded similarity index (Table 6).

Table 6. Nucleotide Sequence Analysis of Bacterial Isolates

Experimental Bacteria	Variants detected from NCBI	NSI (%)
<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> ATCC12600	77.89
<i>Bacillus cereus</i>	<i>Bacillus cereus</i> B4264	78.25
<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i> PA01	79.27
<i>Alcaligenes faecalis</i>	<i>Alcaligenes faecalis</i> C16	76.02
<i>Streptococcus pyogenes</i>	<i>Streptococcus pyogenes</i> NGAS638	85.71
<i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i> EnGen0336	96.55

3.4 Evolutionary Study of the Bacteria Isolates

The result obtained from the evolutionary analysis of the bacterial isolates isolated in this study showed that the six bacteria fall into same clade (Figure 1).

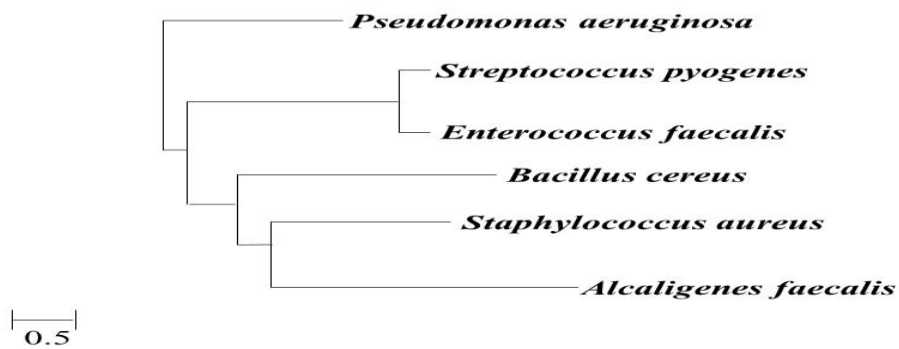


Figure 1. Rooted Neighbor Joining Dendrogram of *16S rRNA* gene within the DNAs of the bacterial isolates

4. Discussion

The capability of the enrichment of TSB and TSA with MSM overlaid with SEO described by Ebakota *et al.* (2017) and adopted with a view to isolating SEO utilizing bacteria in this study has proved to be effective as six (6) distinct bacterial colonies were isolated. In this study, topsoil containing SEO was considered for sampling for the possible presence of hydrocarbon utilizing bacteria due to the submission of Ebakota *et al.* (2017) about the vast availability of decayed organic matter and presence of oxygen needed for microbial metabolism therein. Interestingly, three (3) of the SEO utilizing bacteria; *Pseudomonas* sp., *Streptococcus* sp., and *Bacillus* sp., isolated in this current study had been isolated and reported by Makut and Ishaya (2010) in Keffi town, Nigeria. Again, in a similar environment with the current study area, Kawo and Faggo (2017) isolated similar bacteria; *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Kano city, Nigeria. The results of the isolation of the bacteria responsible for SEO utilization in this study are in concord with the report of Yahaya and Bappa (2019) on the degradative potential of bacteria on different soil particles in Kano, Nigeria. These authors reported the isolation of *Bacillus cereus* and *Staphylococcus aureus* as part of those bacteria associated with such hydrocarbon degradation.

The presence and isolation of *Bacillus cereus* and *Pseudomonas aeruginosa* in SEO polluted soil of Dutse mechanic village are in line with the submission of Ogunbayo *et al.* (2012), that *Bacillus* species and *Pseudomonas* species were predominantly found and isolated in SEO polluted soil across numerous automobile workshops in the city of Lagos, Nigeria. Two (2) of the SEO utilizing bacteria; *Staphylococcus aureus* and *Bacillus cereus* isolated and identified in this study have been previously implicated in the biodegradation of petroleum hydrocarbon related pollution by Ajayi *et al.* (2008); Allamin (2014); Romanus *et al.* (2015).

Relative to the hydrocarbon utilizing bacteria isolated in this current study, Ebakota *et al.* (2017) equally isolated *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp. in a study conducted in selected mechanic workshops in Benin city, Nigeria. In a similar environment to this study, Okerentugba *et al.* (2003), isolated and reported similar bacteria; *Pseudomonas*, *Bacillus*, *Streptococcus* and *Staphylococcus* sp. The isolation of *Alcaligenes* sp of different strains has been reported by Wokem *et al.* (2017) in a similar hydrocarbon polluted environment in Niger Delta, Nigeria. The isolation of these hydrocarbonoclastic bacteria in oil polluted environment suggests their ability to utilize these hydrocarbons as their carbon and energy source.



In this study, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Streptococcus pyogenes* and *Enterococcus faecalis* isolated from SEO polluted soil in Dutse mechanic village can be said to be autochthonous and indigenous in that environment. These bacteria have been implicated by Nwachukwu (2001); Mohammed *et al.* (2011); Akpomie (2013); Ebakota *et al.* (2017) as having the required enzymatic prowess to utilize hydrocarbons, most especially *Pseudomonas* sp. and *Bacillus* sp. Again, the isolation of *Pseudomonas aeruginosa* from a typical SEO polluted soil in this current study is a strong indication of its capability to utilize hydrocarbons extensively as Bhattacharya *et al.* (2002); Pokethitiyook *et al.* (2003); Van Hamme *et al.* (2003); Osarumwense and Igiebor (2018) have implicated *Pseudomonas* species as one of the most common hydrocarbonoclastic bacteria reported in the literature. The isolation of these hydrocarbonoclastic bacteria from a typical SEO polluted environment is a pointer to the development of approaches and strategies they employ in adjusting to such environment. The presence and subsequent isolation of *Bacillus cereus* in this study is in line with the report of Ghazali *et al.* (2004), that ascribed its capability of producing spores which ultimately confers enablement to survive in hydrocarbon polluted soils and harsh environmental conditions as obtained in the study area.

Results obtained from nucleotide sequence analysis of the bacterial isolates and related sequences as obtained from NCBI showed a very high NSI, this implies that the nucleotide sequences of the bacterial isolates obtained in this study are highly similar to those obtained from NCBI database and therefore this can form the basis for strain level identification (Nwagwu *et al.*, 2020). Also, this result raises the potentials of related bacteria for possible hydrocarbon cleaning capacity. Nucleotide sequence similarity index is a bioinformatics tool used to evaluate the extent of relatedness or similarity between sets of given nucleotide sequence base on the complexity of the nucleotide sequence and nucleotide sequences found to have high NSI are found to be genetically similar at molecular levels and as such they can perform similar functions (Onasanya *et al.*, 2021). The result obtained from the phylogenetic analysis of this study shows that all the bacterial isolates that the six bacteria originated from common ancestral lineage, this suggests that they have shared ancestry because they belong to common clade. The implication of common clade and shared ancestry is that they evolve from common ancestor and as such they are genetically the same (Onasanya *et al.*, 2020), this genetical similarity informs shared functions. Furthermore, the detection of shared ancestry between the experimental confirmed that *16S rRNA* gene is conserved in a wide range of bacteria (Onasanya *et al.*, 2019; Onasanya *et al.*, 2018). To the best of our knowledge, little or no works had been carried out on bioinformatics and genomics of hydrocarbonoclastic bacteria in Nigeria and as such there are no references to establish any similarities or differences with our works.

5. Conclusion

Results of the biochemical tests and molecular analysis conducted on the SEO utilizing bacteria isolated in this study indicated the presence of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Streptococcus pyogenes* and *Enterococcus faecalis* in the SEO polluted soil sampled. However, we found out that the hydrocarbonoclastic bacteria obtained in this study had a very high nucleotide similarity with those reported elsewhere as in the sequences obtained from the NCBI gene bank. Owing to their availability in a typical hydrocarbon polluted environment and their established appetite for utilizing hydrocarbons in the literature, there is no doubt that the hydrocarbonoclastic bacteria isolated and identified in this current have got massive prospect in achieving bioremediation of hydrocarbon impacted environments.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work

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