STUDY ON DIVERSITY AND PLASMID PROFILE OF BACTERIAL ISOLATES FROM CRUDE OIL ABANDONED WELL HEADS

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Abstract. Many indigenous bacteria have been reported to be capable of degrading petroleum hydrocarbon contaminants in soil because of their diverse metabolic capabilities. This study focused on isolation, identification and plasmid analysis of the bacterial isolates from crude oil polluted soil using culture- based methods and molecular methods. It was observed generally that polluted soil showed more viable count of bacterial colonies than unpolluted soil. The total viable count was highest in H3D1 sample (> 6.0 × 10^{10} CFU/g) and lowest in the control sample (< 2.0 × 10^{10} CFU/g). Samples collected at depth 30 cm showed an overall low level of total viable count compared to the other depths. The bacterial species isolated are from the genera; *Bacillus, Brochothrix*, *Lactobacillus*, *Microbacterium*, *Corynebacterium, Arcanobacterium*, *Actinomyces*, *Arthrobacter*, *Paenibacillus, Acinetobacter, Enterobacter*, *Pectobacterium*. All the isolates identified showed the presence of bands measuring from 12000 bp to 6000 bp except *Bacillus lichenformis* which showed no band. This study showed that many indigenous bacteria are capable of degrading petroleum hydrocarbon contaminants and the ability to degrade a pollutant is dependent on presence of plasmid bearing genes coding for pollutant degradation.

Key words; bacteria; contaminant; petroleum hydrocarbon; pollution.

INTRODUCTION

The discharge of crude oil is a major cause of pollution in the urban, semi-urban and industrialized world [15, 17]. Such substances that pose risk in the environment need to be degraded or removed to reclaim the polluted site for use. Crude oil pollution of agricultural soil has critical adverse effects on human health and the surrounding ecosystem. The toxicity of crude oil pollution is dependent on the concentration and components that make of the petroleum products [6, 14]. Environmental factors at time of pollution can cause increase or decrease in growth of microorganisms [12]. Microorganisms are ubiquitous and soil is an important natural resource that contains a vast diversity of these microbes which are important for maintaining soil quality and health. The use of microorganisms to detoxify environmental pollutants has been a promising technology because of its low cost, effectiveness, eco-friendly and economical in process [17]. Bacterial form a major group of microorganisms found in crude oil polluted soils and they degrade and may incorporate the break-down products into their metabolism. Thus, the ability to degrade a pollutant is dependent on presence of plasmid bearing genes coding for pollutant degradation or enzymes produced by the organism [22]. Indeed, many studies have revealed that, there are many hydrocarbon-degrading microorganisms in oil-rich environments, and their abundance are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors. However, no microorganism can degrade the entire petroleum hydrocarbon fraction, some microorganisms can metabolize certain alkanes, while others breakdown aromatic or resin fractions of hydrocarbons [27]. This indicates that different microbial strains have different catalytic enzymes, thus, their roles vary. A variety of molecular methods have been developed to identify the presence of micro-organisms in samples and to profile for the possible presence of plasmid and genes that code for degrading enzyme [22]. This study will be focused on isolation and characterization of the bacterial isolates, extraction of plasmid DNA and analysis of the bacterial isolates from crude oil polluted soil using culture-based methods and molecular methods. These methods are faced with problems of insufficient research to identify the diversity of these hydrocarbon utilizing microorganisms as well identify isolates with genes coding for catabolic enzymes mediating the degradation process. The information from this research would help to eradicate crude oil on polluted sites, thereby reducing the toxic effects on humans and plants.

MATERIALS

Study area

The study was conducted at Federal University of Technology Owerri in Imo State, South-Eastern Nigeria. The state is among the Niger Delta states, as one of the oil-rich states. It is located between latitude 4°45'N - 7°15'N and longitude 6°50'E - 7°25'E, with an area of about 5100 km^2.

Sample collection

The crude oil polluted soil samples were aseptically collected from three different locations of abandoned oil well heads, at three different distances of three meters (3 m) apart and different depths of the soil (soil surface, 10 cm, and 30 cm). All the samples measuring 250 g were from Assa and Ikwerede in Ohaji-Egbema, Imo State, using surface sterilized soil auger and were placed into sterile polyethylene bags. The samples were transported to laboratory within 2 hours of collection for further analysis.

Media preparation

The media used for the culture were nutrient agar and Bushnell-Hass agar media. The Media were prepared according to manufacturer's specifications as described by Asikong *et al.* [3] and sterilized by autoclaving under steam pressure of 15 psi at temperature of 121°C for 15 min. The composition of Mineral salt broth medium has K_2HPO_4 (0.36g) $MgSO_4 \cdot 7H_2O$ (0.04g), NaCl (0.02g), NH_4Cl (0.8g), $Na_2SO_4 \cdot 7H_2O$ (0.02g).

Enumeration of hydrocarbon-utilizing bacteria

The soil samples were diluted by using Ten-fold serial dilution method as reported by Obafemi *et al.* [20], using sterile water as diluent. In each of the sterile test tubes (10 test tubes), 9 mL of sterile water was pre-dispensed, thereafter, 1 mL of the stock culture (each of the samples) was serially diluted. An aliquot of 0.1 mL of dilution factor 10^{-5} was plated in duplicates on Bushnell-Haas agar medium using spread plate method. The inoculated plates were incubated for 7 days at room temperature. After the period of incubation, the cultured plates were examined for the presence of discrete colonies and the colonies were counted using colony counter and expressed as colony forming unit per gram (CFU/g) of the soil samples. Acceptable plates were those within 30-300 CFU/g and the CFU was calculated using the formula:

CFU/g= (no. of colonies × total dilution factor) / vol. of culture plated

Identification of hydrocarbon-utilizing bacterial isolates

Morphological and biochemical identification of the bacterial test strains were done according to Cowan and Steel's manual for identification of medical bacteria and Bergey's manual of systematic bacteriology as described by Erler *et al*. [8]. About 35 bacterial isolates were examined on Bushnell-Hass agar plates based on their morphological characteristics (Shape, color, size, elevation, and marginal characteristics) after the periods of incubation. Pure culture of the strains were obtained by streaking the inocula with wire loop on a nutrient agar medium and incubated for 18 hr at 37°C. The staining and biochemical tests carried out included Gram's stain, Indole test, methyl red (MR)test, motility test, ornithine test, citrate utilization test, cytochrome oxidase test, catalase test, urease test and sugar fermentation test.

Plasmid DNA extraction

TENS-MINI PREP method was carried out as described by Ogbulie *et al.* [22] and Akeredolu *et al*. [2]. The composition of TENS was Tris 25 mM, EDTA 10 mM, NaOH 0.1 N and SDS 0.5%. The bacterial isolates were cultivated on Luria Bertani medium, composing of 5 g of peptone, 10 g of NaCl and 5 g of yeast extract per liter. The bacterial cells were vortexed for homogenization and 1.5 mL of the broth was transferred to a pre-labeled Eppendorf tubes using micropipette, then centrifuged at 6500 rpm (revolutions per minute) for 4 min in a micro-centrifuge to pellet cells and gently decanted supernatant leaving about 50-100 µL together with cell pellet. It was vortexed to homogenize the solution. About 350 µL of TENS solution was added, then mixed by inverting tubes until the mixture became sticky (50 counts). About 150 µL of 3.0 M sodium acetate pH 5.2 was added to the tubes and vortexed to mix completely. The mixture was centrifuged for 8 min at 650 rpm in micro-centrifuge to pellet cell debris and chromosomal DNA. The supernatant was transferred into a fresh tube and mixed well with 900 µL of ice-cold absolute ethanol. The supernatant was centrifuged for 10 min at 650 rpm to pellet plasmid DNA and was discarded. The pellet was rinsed twice with 1 mL of 70% ethanol and air-dried. For further use, the pellet was re-suspended in 50 µL of TE buffer.

Gel Electrophoresis of extracted plasmid DNA

The separation of the DNA fragment was carried out using Agarose gel electrophoresis. This was achieved by preparing 0.8 g of agarose powder in mixture with 100 mL of 1×TBE buffer. The mixture was dissolved by heating for 3-5 min in a microwave oven. The dissolved mixture was allowed to cool at 50°C, 10 µL of ethidium bromide was added to it and gently mixed by swirling. The mixture was casted into electrophoresis tray with comb in place to obtain a gel thickness of about 4.5 mM avoiding bubbles and allowed to stand for 20 min to solidify. The combs were gently removed, and the tray was placed in the electrophoresis tank. The buffer (1×TBE buffer) was poured into the tank ensuring that it covers the surface of the gel casted. Mixture of 20 µL of the plasmid DNA sample and 2 µL of the loading dye was carefully loaded into the wells created by the combs with standard marker or ladder in a different lane to enable tracing or marking out the DNA molecular weight. The electrodes were connected to the power pack ensuring that the negative terminal was at the well side where the samples were loaded. The gel ran at 60-100 V until the loading dye has migrated to the end or about three-quarter of the gel field. The power was turned off and the electrodes were disconnected. The gel was placed on a UV-transilluminator and observed (a picture was taken) thereafter, the molecular weight of the target molecule was calculated [21].

Statistical analysis

All sample values were presented in mean ± standard error. Statistical Package for Social Sciences (SPSS) version 20 was used to represent the data in graphs.

RESULTS

Total heterotrophic count of isolates

The total viable count of microbes isolated from the soil samples at three depth levels (ss, 10 cm, and 30 cm) is represented in Figure 1. It was observed that the total viable count was highest in H3D1 sample ($> 6.0 \times 10^{10}$ CFU/g) and lowest in the control sample ($< 2.0 \times 10^{10}$ CFU/g). The total viable count for samples at ss depth were below 2.0×10^{10} CFU/g except for samples

H1D1 (2.12 × 10^{10} CFU/g), H1D2 (3.0 × 10^{10} CFU/g), and H3D1 (3.0 × 10^{10} CFU/g). For samples collected at 10 cm depth, it was observed that H1D2, H1D3, H2D2, H2D3, H3D2, and H3D3 samples were below 2.0 × 10^{10} CFU/g. H1D1, H2D1 and H3D1 samples were observed to be 3.0 × 10^{10} CFU/g, 3.0 × 10^{10} CFU/g and 3.0 × 10^{10} CFU/g respectively. Samples collected at depth 30 cm showed and overall low level of total viable count compared to the other depths. All the samples collected at 30 cm depth were observed to be below 2.0 × 10^{10} CFU/g except sample H2D3 which showed total viable count of 3.0 × 10^{10} CFU/g .

Comparison of total viable count observed in the oil well head at different distances.

The total viable count observed in the study was compared based on the distances the samples were collected from the oil well head. As illustrated in Figure 2 (representing samples collected from soil surfaces of the oil well), on the surface of the soil, the total viable count of organisms was highest at distances H1D2 and H3D1 (3.0 × 10^{10} CFU/g for both locations). The lowest viable count observed for the soil surface sample was from H2D2 oil well head (1.2 × 10^{9} CFU/g). The total viable count for all the samples collected from soil surface of the oil well head were above the total viable count observed in the control sample (5 × 10^{8} CFU/g) (Fig. 2). For samples collected from 10 cm depth of the oil well heads (as shown in Fig. 3), the high total viable count was observed in samples from H1D1, H2D1, and H3D1 (3.0 × 10^{10} CFU/g for all three distances). The oil well head H2D2 showed the lowest total viable count, followed by oil well head H3D3 (1.0 × 10^{10} CFU/g)). The total viable counts of all the samples collected from 10 cm were above the total viable count observed in the control (7.0 × 10^{8} CFU/g) (Fig. 3). Samples collected from 30 cm depth in all the oil well distances were illustrated in Figure 4. According to the line graph (Fig. 4), the highest total viable count was observed in samples collected from oil well head H2D3 (3.0 × 10^{10} CFU/g). The total viable counts observed for samples collected from oil well head H3D2 (3.6 × 10^{9} CFU/g) was slightly higher than H3D3 (3.5 × 10^{9} CFU/g). The lowest total viable count was observed in samples collected from oil well heads H2D1 and H3D1 (4.0 × 10^{8} CFU/g and 4.9 × 10^{9} CFU/g respectively). These counts observed in H2D1 (4.0 × 10^{8} CFU/g) was below the counts observed in the control sample (5 × 10^{8} CFU/g). The other samples collected at 30 cm depth showed total viable count above the count observed in the control (Fig. 4).

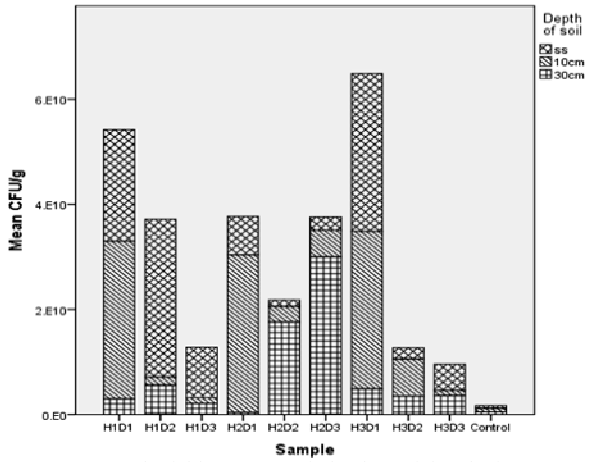

Figure 1. Total viable counts of the bacterial colonies. H1D1-headwell one and distance one, H1D2-headwell one and distance two H1D3- headwell one and distance three, H2D1- headwell two and distance one, H2D2- headwell two and distance two, H2D3- headwell two and distance three,H3D1- headwell three and distance one, H3D2-headwell three and distance two, H3D3- headwell three and distance three

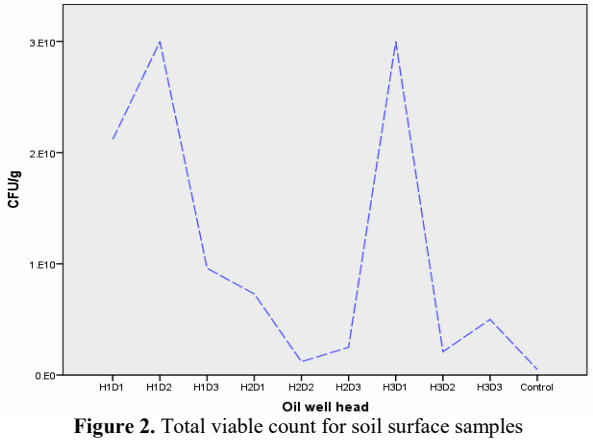

Figure 2. Total viable count for soil surface samples

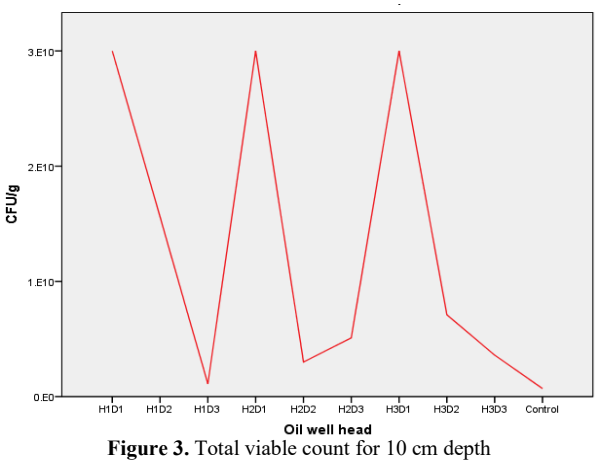

Figure 3. Total viable count for 10 cm depth

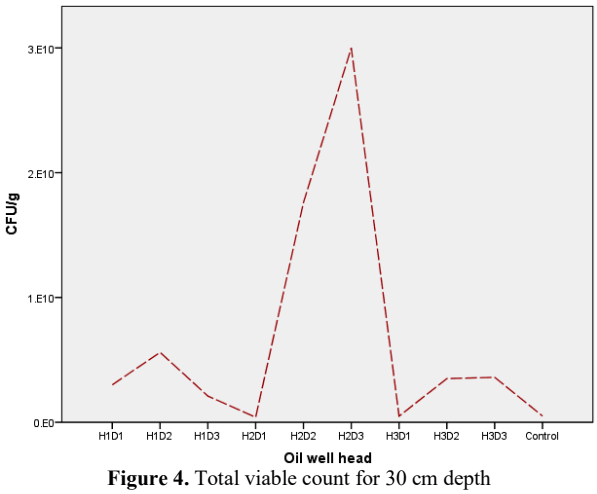

Figure 4. Total viable count for 30 cm depth

Comparison of total viable count for soil samples collected at different soil depths.

The comparison of total viable counts for one oil well head samples against the depth of collection was summarized using Figures 5, 6, and 7. For samples collected at oil well head one (H1D1, H1D2, H1D3) as illustrated in Figure 5, it can be seen that the total viable count observed in samples collected from the soil surface was higher than the total viable count observed in samples collected at 10 cm and 30 cm depth, except for samples at oil well head H1D1 which showed higher total viable count (3.0×10^{10} CFU/g) for samples collected at 10 cm depth than samples collected at the soil surface. The total viable count observed in samples collected at depth 10 cm was higher than the counts observed in samples from 30 cm depth. The total viable counts for samples collected at 30 cm were lowest for all the oil well head one (H1D1= 3×10^9, H1D2 = 5.6×10^9 CFU/g, and H1D3 = 1.1×10^9 CFU/g) (Fig. 5).

At oil well head two as illustrated in Figure 6, it can be observed that the lowest total viable count was observed in samples collected from the soil surface (H2D2 = 1.2×10^9 CFU/g and H2D3 = 2.5×10^9 CFU/g). For oil well heads H2D2 and H2D3, the highest total viable count was observed in samples collected at 30 cm depth (1.7×10^9 and 3.0×10^{10} CFU/g respectively), but samples at this depth from H2D1 distance showed the lowest total viable count (4×10^8 CFU/g). At oil well head H2D1, the highest total viable count was observed in samples collected at 10 cm depth (3.0×10^{10} CFU/g) (Fig. 6).

At oil well head 3, as illustrated in Figure 7 samples collected from 10 cm depth showed the highest total viable count (H3D1 = 3×10^{10} CFU/g and H3D2 = 7.1×10^9 CFU/g) except for samples from distance H3D3 (1.0×10^{10} CFU/g) which had the lowest total viable count. Equal number of counts was observed in samples collected at distance H3D1 for both depth 10 cm and surface soil (3.0×10^{10} CFU/g). Samples collected at depth 30 cm showed the lowest total viable count for distance H3D1 (4.9×10^9 CFU/g) and the highest viable count for distance H3D3 (3.6×10^9 CFU/g). The samples collected from soil surface of the oil head three showed the lowest viable count at distance H3D2 (2.1×10^9 CFU/g), while samples collected from 10 cm depth of the oil well head showed the lowest viable count at distance H3D3 (1.0×10^9 CFU/g) (Fig. 7).

Plasmid profiling analysis

Detection of bands was seen under UV-Transilluminator and denotes the presence of plasmid DNA. Figure 8 showed the plasmid DNA profile of strains from lanes A-L, Letter M, representing 1 kb marker. All the strains have plasmids measuring up to 12000 bp except isolate from lane I, which shows no visible band. Hence, absence of plasmid DNA that confers degradative ability to it, instead the gene could be borne in the chromosomal DNA which enabled its persistence in the polluted soil. Figure 9 showed the plasmid DNA of strains from lanes 1-14, lane M, representing 1kb marker. All the strains in Figure 9 have plasmids measuring up to 6000 bp. Figure 10 showed the plasmid DNA of strains from lanes A-E and lanes F-I, and lane M representing 1kb marker. All the strains in Figure 4 have visible plasmid DNA measuring 11000 bp in molecular weight.

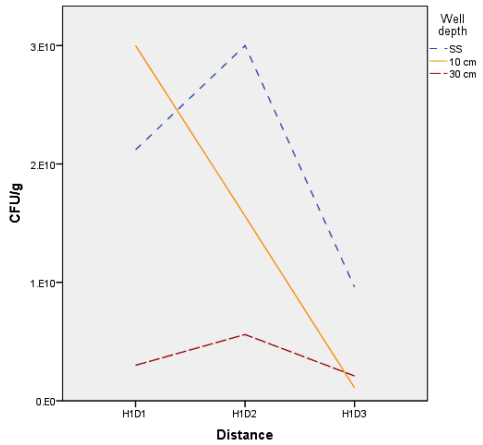

Figure 5. Comparative line gragh from different depths and distances of oil well head 1

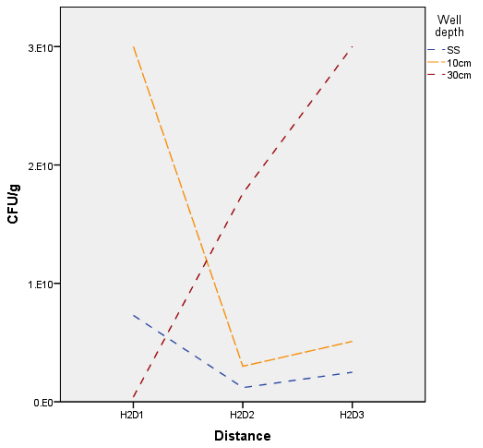

Figure 6. Comparative line gragh from different depths and distances of oil well head 2

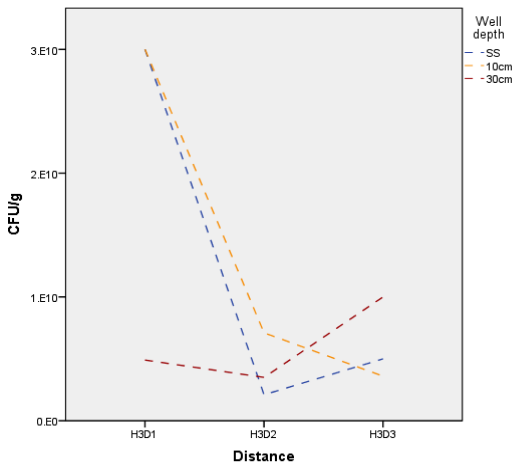

Figure 7. Comparative line gragh from different depths and distances of oil well head 3

Table 1. Biochemical tests results

Isolate	Morphological Description	Staining reaction	C U	C	O	MR	U	M	OR	I	Sugar fermentation						
											L	M	S	MN	G	GA	I
CU1	Small transparent colony	+ve rods	-	+	-	-	-	+	+	-	-	AG	AG	A	A	A	AG
CU2	Small white colony	-ve short rods	+	-	-	-	-	+	+	-	-	AG	AG	AG	A	A	-
CU3	White pinpoint colony	-ve rods	-	+	-	-	-	-	+	-	AG	AG	AG	AG	A	AG	AG
CU4	Small transparent flat colony	+ve short rods	-	+	-	-	-	-	+	-	-	AG	AG	AG	AG	-	AG
CU5	Pinpoint white colony	-ve rods	-	+	-	-	-	+	+	-	-	AG	AG	AG	AG	AG	AG
CU6	Pinpoint transparent colony	-ve rods	-	+	-	-	-	-	+	-	-	AG	-	A	A	A	-
CU7	Light yellow flat large transparent colony	-ve rods	-	+	-	-	-	+	+	-	A	AG	AG	AG	A	A	-
CU8	Pinpoint transparent colony	+ve rods	+	-	-	-	-	-	+	-	A	AG	AG	A	A	-	A
CU9	Medium sized milky with white dot at center colony	+ve short rods	+	+	-	-	-	+	+	-	AG	AG	AG	AG	AG	-	AG
CU10	Large transparent flat colony	+ve short rods	-	+	-	-	-	-	+	-	AG	AG	A	AG	AG	AG	AG
CU11	Pinpoint light yellow colony	+ve rods	-	-	-	-	-	-	+	-	A	AG	AG	-	A	A	A
CU13	Pinpoint orange colony	-ve rods	-	+	-	-	-	-	+	-	-	AG	AG	-	AG	-	AG
CU15	Small milky colony	+ve rods	-	+	-	-	-	-	+	-	AG	AG	AG	AG	AG	AG	A
CU16	White transparent spreading colony	-ve rods	-	+	-	-	-	+	+	-	AG	AG	AG	AG	AG	AG	AG
CU17	Small white colony	+ve large rods	+	+	-	-	-	-	+	-	A	AG	AG	A	AG	-	AG
CU18	Small white colony	+ve rods	-	-	-	+	-	-	+	-	-	AG	AG	AG	A	AG	-
CU19	Small white colony	+ve rods	+	+	+	-	-	-	+	-	AG	AG	AG	A	A	A	-
CU20	Transparent flat colony	-ve rods	-	+	-	-	-	-	+	-	-	AG	AG	A	AG	A	AG
CU21	Transparent small colony	+ve rods	-	-	+	-	-	-	+	-	-	AG	AG	AG	A	AG	AG
CU22	Small white colony	+ve rods	-	+	-	-	-	+	+	-	AG	AG	A	AG	AG	AG	-
CU23	Small white colony	+ve rods	-	+	-	-	-	-	+	-	AG	AG	AG	AG	AG	AG	AG
CU25	Spreading milky colony	-ve rods	-	+	-	-	-	+	+	-	A	AG	AG	AG	A	AG	A
CU26	Transparent small colony	-ve rods	-	+	-	-	-	+	+	-	AG	AG	AG	AG	AG	AG	A
CU27	Transparent small colony	+ve rods	-	+	+	-	-	-	+	-	A	AG	AG	AG	A	A	A
CU28	Transparent small colony	+ve rods	-	+	+	-	-	-	+	-	A	AG	AG	A	A	AG	AG
CU29	Transparent small colony	+ve rod / cocci	-	+	+	-	-	-	+	-	-	AG	AG	AG	A	A	AG
CU30	Transparent small colony	+ve rods	-	+	+	+	-	-	-	-	A	AG	A	-	A	-	AG
CU31	White pinpoint colony	+ve rods	-	+	+	-	-	+	+	-	AG	AG	AG	AG	AG	AG	-
CU32	Small white colony	-ve rods	-	+	-	-	-	+	+	-	AG	AG	AG	AG	AG	A	AG
CU34	Small transparent white colony	-ve rods	-	+	-	+	-	+	+	-	AG	-	AG	-	AG	AG	AG
CU36	White fluffy colony	+ve fat / large rods	-	+	+	+	-	-	+	-	AG	AG	A	-	AG	-	AG
CU37	Pinpoint white colony	-ve rods	+	+	-	-	-	-	+	-	A	-	A	A	A	A	-
CU38	Uniform transparent colony	+ve rods	-	+	+	-	-	+	+	-	AG	AG	AG	AG	AG	AG	A
CU39	Uniform transparent colony	+ve rods	-	+	+	-	-	-	+	-	-	AG	AG	AG	AG	A	AG
CU40	Uniform transparent colony	+ve rods	-	+	+	+	-	+	+	-	AG	AG	AG	AG	AG	AG	AG

Legend: CU= citrate utilization, C= catalase, O=oxidase, MR=methyl red, U=urease, M=motility, O=ornithine, I=Indole tests. Sugar fermentation: L= lactose, M=maltose, S=sucrose, MN=mannitol,G=glucose, GA=galactose and I=innositol. AG= acid gas production. A= acid production.

Table 2. Identified hydrocarbon utilizing bacterial strains

ISOLATE CODE	ISOLATE IDENTITY	ISOLATE CODE	ISOLATE IDENTITY
CU1	*Bacillus Amyloliquefaciens*	CU21	*Actinomyces* spp
CU2	*Enterobacter* spp	CU22	*Bacillus siamensis*
CU3	*Acinetobacter* spp	CU23	*Arthrobacter* spp
CU4	*Bacillus siamensis*	CU25	*Pectobacterium betavasculorum*
CU5	*Enterobacter bugandensis*	CU26	*Pectobacterium betavasculorum*
CU6	*Acinetobacter* spp	CU27	*Bacillus amyloliquefaciens*
CU7	*Enterobacter turiensis*	CU28	*Bacillus amyloliquefaciens*
CU8	*Brochothrix* spp	CU29	*Bacillus amyloliquefaciens*
CU9	*Bacillus lichenformis*	CU30	*Paenibacillus* spp
CU10	*Bacillus siamensis*	CU31	*Paenibacillus macquariens*
CU11	*Lactobacillus ultuensis*	CU32	*Pectobacterium betavasculorum*
CU13	*Acinetobacter* spp	CU34	*Enterobacteria* spp
CU15	*Microbactrium* spp	CU36	*Paenibacillus koreensis*
CU16	*Enterobacter aerogens*	CU37	*Acinetobacter* spp
CU17	*Corynebacterium matrichotii*	CU38	*Paenibacillus koreensis*
CU18	*Arcanobacterium* spp	CU39	*Bacillus* spp
CU19	*Bacillus lichenformis*	CU40	*Paenibacillus koreensis*
CU20	*Enterobacter bugadensis*		

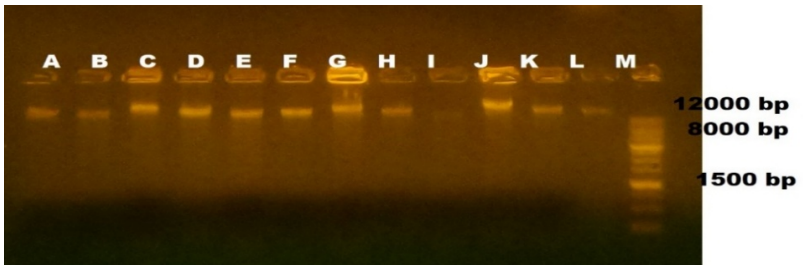

Figure 8. Lanes A-L: cu2, cu1, cu8, cu6, cu7, cu5. cu3, cu4, cu9, cu10, cu11, cu13, Lane M=1kb marker

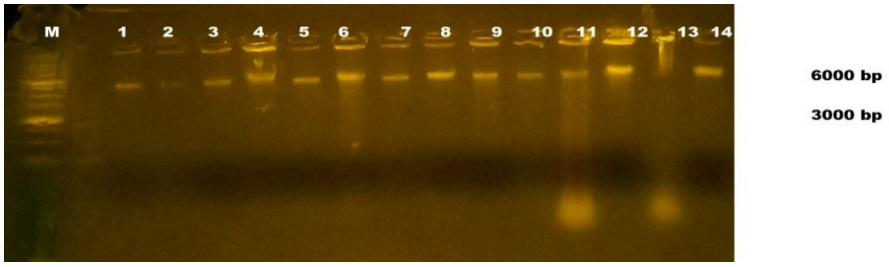

Figure 9. Lane M=1kb marker, Lanes 1 – 14: cu17, cu15, cu16, cu22, cu21, cu19, cu20, Cu18, cu25, cu23, cu34, cu38, cu36 and cu37

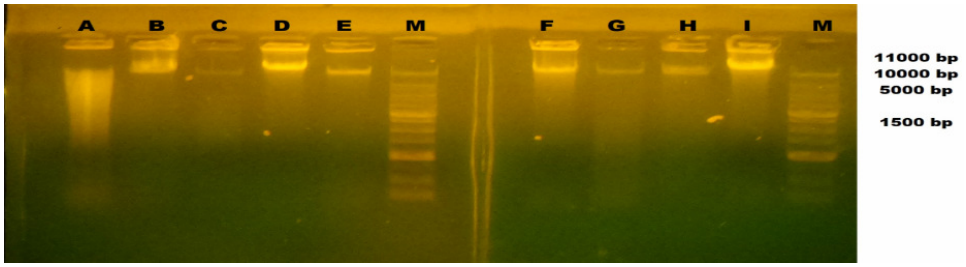

Figure 10. Lanes A – E: Cu26, cu27, cu28, cu29, cu30; Lane M =marker; Lanes F – I: cu31, cu32, cu39 and cu40

DISCUSSION

The process of bioremediation and biodegradation has been used to eradicate environmental pollutants. The process is very effective, and pose no harm to man and its environment. This study was conducted with the aim of investigating the bacterial diversity and plasmid profile of strains in crude oil polluted agricultural soil. In this study, a total of thirty five (35) bacterial strains were identified from thirty (30) samples; HIDI10CM, HIDISS, HIDI30CM, HID2SS, HID210CM, HID230CM, HID3SS, HID310CM, HID330CM, H2DI10CM, H2DISS, H2DI30CM, H2D2SS, H2D210CM, H2D230CM, H2D3SS, H2D310CM, H2D330CM, H3DI10CM, H3DISS, H3DI30CM, H3D2SS, H3D210CM, H3D230CM, H3D3SS, H3D310CM, H3D330CM, CTSS, CT10CM and CT30CM.The study showed that, the bacterial counts of the strains were observed to be more at the soil depths of 0-10 cm when compared to the depth of 30 cm. This agreed with the findings of Nwankwo and Okpokwasili [19], that reported bacterial counts were observed to be more at the top soil (0-15 cm) when compared to the sub soil (15-30 cm). Saadoun *et al.*

[25] also reported that the bacterial counts declined to 65-95% in the depth of 10-20 cm when compared to 0-10 cm. This could be because of the concentration of the crude oil at the lower soil. The bacterial increase could be because of the hydrocarbon nutrients available in the soil sample. The distances, D1 in some samples (HID110CM, H2D110CM, H3D110CM) showed higher number of microbial population compared to other distances. The variations in microbial population could be related to time of soil contamination on different sites. As expected in uncontaminated soil (negative control), showed least CFU values. As reported by Umar and Ummar [26], bacteria have been described as more efficient hydrocarbon degraders than other microorganisms. The result of the bacterial strains identified from this study belong to twelve (12) bacteria genera: *Bacillus, Acinetobacter, Paenibacillus, Enterobacter*, *Brochothrix, Corynebacterium, Arcanobacterium, Arthrobacter, Actinomyces, Pectobacterium, Lactobacillus, Microbacterium.* Many bacterial strains have been reported to be hydrocarbon utilizers. In this study, the population of hydrocarbon utilizing strains showed that majority of the bacteria were Gram positive belonging to the genera, *Bacillus*. Although some studies have shown that oil-polluted soils are dominated by Gram negative bacteria [5]. This study showed that the dominant species belonged to *Bacilli,* they use rapid growth as survival strategists when there is abundant nutrient availability. With this advantage, this could account for the presence of more *Bacillus* species isolated [10], followed by *Enterobacter* spp. The stains identified were like the findings of Nwankwo and Okpokwasili [19], who isolated *Bacillus, Paenibacillus, Enterobacter, Aspergillus,* and other isolates in their work. Also, Boboye *et al.* [4] and Olukunle [23], obtained *Bacillus, Acinetobacter*, *Lactobacillus, Micrococcus, Aerococcus, Clostridium, Staphylococcus, Streptococcus, Pseudomonas* and *Enterobacter* in their work on degradation activity of bacteria obtained from crude oil polluted sites. This work corresponds to the report of [7], reporting *Bacillus* spp., *Acinetobacter, Micrococcus and Corynebacterium* spp as hydrocarbon utilizers. Similar results were obtained by Phulpoto *et al.* [24], Adams *et al.* [1], Kawo and Bacha [16] and Mohammad *et al.* [18]. In this study, *Arcanobacterium, Pectobacterium* and *Brochothrix* species were isolated as hydrocarbon utilizers and confers degradative gene in plasmid DNA, but no previous studies have been recorded. The presence of fragments on the bacterial strains depicts presence of plasmids measuring different molecular weights. The isolated hydrocarbon degraders correspond with the findings of [13, 11] that several species of Gram-positive bacterial carry multiple plasmids which serves as adaptive mechanism. All the bacterial strains isolated showed the presence of plasmid except the isolate (cu9) *Bacillus lichenformis* that shows no visible band, this indicated no plasmid DNA that confers degradative ability to it. Hence, the degradative gene could be present in the chromosomal DNA of the bacterial cell for it to be persistence in the polluted soil as reported by Ogbulie *et al.* [22]. The plasmids that bear genes encoding enzymes capable of hydrocarbon degradation are considered important as these plasmids known as catabolic plasmids can give the organisms harboring them the ability to degrade certain compounds**.**

The presence of bacteria in contaminated soils indicated that microorganisms are everywhere, diverse in nature and can adapt in extreme environments. This indicates that they utilize crude oil as energy and carbon sources by breaking down part of the oil and use it to grow. The result of this study revealed that some bacterial strains were able to grow effectively on crude oil. The utilizing capabilities in most of these bacterial strains appear to be plasmid encoded. These bacterial strains could therefore be employed in large scale oil spill clean-up, as well as the genes could be incorporated into another organism to confer degradative ability to such organism for enhanced biodegradation and bioremediation of crude oil polluted sites.

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