Molecular Diversity of Microbes with Probable Degradative Genes in Agricultural Soil Contaminated with Bonny Light Crude Oil

Ogbulie TE* and Nwaokorie FO

1Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO), Imo State, Nigeria
2Molecular biology and Biotechnology Division, Nigerian Institute for Medical Research (NIMR), Yaba Lagos State, Nigeria

Abstract

This study looked at the diversity of microorganisms persistent in agricultural soil sample polluted with 100 ml of 100% Nigerian Bonny light crude oil left for four years. DNA from crude oil polluted agricultural soil sample was extraction using ZYMO soil DNA extraction Kit. DNA sequencing was performed by Next Generation Sequencing Technique [NGST] using automated PCR cycle- Genome Sequencer™ FLX System from 454 Life Sciences™ and Roche Applied. Sequence analysis and alignment was performed using Vector NTI suite 9 (InforMax, Inc.). The resulting nucleotide sequences were compared to sequences obtained from GenBank by BLASTx analysis using CLO Bio software as well as BLASTn using NCBI. Molecular Identities of microbial community was obtained by creating different dendrograms. Gene sequencing carried out read 513 different nucleotide sequences. Seven phyla with 47 corresponding culture-dependent species and 169 culture-independent bacteria clone were obtained. The resultant tree showed cladogram of proteobacteria (b and g - proteobacteria), bacteria/enterobacteria, firmicutes, plantomycetes, acidobacteria group/ fibrobacteres, Bacteriodetes/chlorobi Actinobacteria/high G + C and chloroflexi phyla. Further taxonomical classification was carried out with reads of sufficient Q scores (> q30) and lengths and a total of 420 read count of top kingdom classification of 100% bacteria kingdom was obtained. Proteobacteria phyla of class betaproteobacteria, order Burkholderiales and family Comamonadaceae had the highest read count with percentage diversity of 57.14%, 53.81%, 53.81 and 53.57% respectively. The nucleotide sequences with no hit (208) was sent to Genbank for assigning of ascension number. The detection of these diverse organisms from crude oil polluted agricultural soil left for four years, depict that the organism probably, have degradative genes which aided their survival.

Keywords: Microbes; Diversity; Degradative genes; Crude oil; Agricultural soil; Sequence analysis

Introduction

Incidence of environmental pollution due to high rate of petroleum related activities in Nigeria and other oil producing areas of the world has been associated with frequent oil spills, especially through oil wells blow out, tanker accidents and bunkering. Disasters arising from such incidence results in the discharge of crude oil into the environment affecting both soil, air and water bodies. This threatens human health and that of organisms that are dependent on soil. Soil contains a variety of microorganisms including bacteria that can be found in any natural ecosystem. Microbial survival in polluted soil depends on intrinsic biochemical and structural properties, physiological and genetic adaptation including morphological changes of cells as well as environmental modifications [1]. Over the years, isolation and identification of hydrocarbon-degrading microorganisms have been carried out using isolation techniques. Previous studies on population dynamics showed that bacteria genera such as Pseudomonas, Bacillus, Brevibacterium, Corynebacterium, Acinetobacter and Mycobacterium are potential organisms for hydrocarbon degradation [2-4]. Shi et al. [5] compared culture-based diversity of agricultural soil communities with diversity obtained by molecular means and found that molecular methods revealed a much higher bacterial diversity than classical isolation techniques.

A variety of molecular methods have been developed to assay the presence of micro-organisms in soil. Most recently, the method of choice to determine what micro-organisms are present in environmental sample is to amplify the conserved small subunit RNA gene; where DNA is isolated from the soil using bead beating and Polymerase Chain Reaction (PCR) with universal or gene-specific primers used to amplify the specific gene from the sample. This study looked at the diversity of microorganisms persistent in agricultural soil sample polluted with 100 ml of 100% Nigerian Bonny light crude oil and left for four years with a view to ascertain the presence of microbes with probable degradative gene for crude oil degradation which can be harnessed for the creation of superbugs for faster clean up operations and to confirm similarities in microbial identities.

Material and Method

Procurement of samples

The crude oil used was bonny light Crude and was collected with sterile containers from Akiri in Oguta, Imo State, Nigeria. The agricultural soil subjected to pollution was obtained from Federal University of Technology Owerri (FUTO) farm land using surface sterilized soil auger at the depth of 15-30 cm.

Treatment of test soil sample

Surface sterilized plastic pot with no drainage holes was filled with 450 g of soil. Thereafter, 100 ml of crude oil was used to pollute the soil and 300 ml of sterile water added biweekly following modified method

*Corresponding author: Ogbulie TE, Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO) Imo State, Nigeria, Tel: +2348035472379; E-mail:ogbulie_toochi@yahoo.com

Received August 15, 2015; Accepted February 09, 2016; Published February 17, 2016

Citation: Ogbulie TE, Nwaokorie FO (2016) Molecular Diversity of Microbes with Probable Degradative Genes in Agricultural Soil Contaminated with Bonny Light Crude Oil. J Ecosys Ecogr 5: 002. doi: 10.4172/2157-7625.S5-002

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of Yee et al. [6]. The setup was kept in a chamber for a period of four years with a light cycle of 11 h darkness and 13 h light.

Molecular analysis

Molecular analysis was performed at the GS FLX Titanium Sequencing Service company- Inqaba, South Africa. Methodology was based on PCR and metagenomics analysis.

DNA extraction from soil sample

DNA extraction form soil sample was performed using ZYMO soil DNA extraction kit according to the manufactures. According to the method, genomic DNA was extracted by weighing out 0.25 grams of soil sample using an analytical Balance. The sample was then homogenized by mixing in a vortex mixer at maximum speed for 5 minutes. The ZR Bashing Bead™ Lysis Tube was centrifuged in a micro centrifuge at ≤10,000 x g for 1 minutes. 400 µl of the filtrate was added to a Zymo-Spin™ IV Spin Filter in a Collection Tube and centrifuged at 7,000 rpm (7,000 x g) for 1 minute. This was followed by the addition of 1,200 µl of soil DNA Binding Buffer to the filtrate in the Collection Tube. 800 µl of the mixture from above was added to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute. Flow through from the collection tube was discarded and this particular step was repeated with the remaining filtrate. 200 µl of DNA Pre-Wash Buffer was thereafter added to the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10,000 x g for 1 minute. Thereafter, 500 µl of Soil DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IIC Column was transferred into a clean 1.5 ml micro centrifuge tube and 100 µl of DNA Elution Buffer added directly to the column matrix. This was centrifuged at 10,000 x g for 30 seconds to elute the DNA. The eluted DNA was transferred into a filter unit of Zymo-Spin™ IV-HRC Spin Filter in a clean 1.5 ml micro centrifuge tube and centrifuged at exactly 8,000 x g for 1 minute. The filtered DNA was then used for PCR and DNA sequencing.

Polymerase Chain Reaction [PCR]

The PCR was carried out in a 20 µl reaction mixture containing a 5X HOT FIRE Pol blend master mix (ready to use) composed of FIREPol DNA polymerase, Proof-reading enzyme, 5X reaction buffer, 7.5 mM MgCl₂, 1 mM dNTPs of each have 200 µM of dATP, dCTP, dGTP, dTTP. A combination of 4 µl of master mix, 0.2 µl each of forward and reverse 16S rRNA primer and 2 µl of template gDNA constituted 6.4 µl. Hence 13.6 µl of sterile distilled water was added to make it up to the recommended PCR reaction mix of 20 µl. The entire mixture was then vortexed and loaded together with positive and negative control (dH₂O) into the thermal cycler (eppendorf vapor protect). The PCR reaction was carried out with an initial denaturation at 95°C for 5 min, followed by 30 consecutive cycles at 95°C for 30 sec, and annealing temperature of 55°C for 1 minute and then 72°C holding for 1 minute. This was then followed by a final extension step at 72°C for 10 minute.

DNA Sequencing

DNA sequencing was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of all microorganisms present in the soil sample using automated PCR cycle- Genome Sequencer™ FLX System from 454 Life Sciences™ and Roche Applied. Sequence analysis and alignment was performed using Vector NTI suite 9 (InforMax, Inc.) and the resulting nucleotide sequences were compared to sequences obtained from GenBank¹ by BLASTx. Analysis using CLO Bio software as well as BLASTn² using NCBI. For every sample set, every read was BLASTED and the result file saved. The top 5 hits for every BLAST result (i.e., species name) was counted and a record was kept of how many times each species appeared as a hit. The number in the last column basically is the number of times a read hit/ matched to that species. The frequency (i.e., count/total number of reads) and absolute count of each species were reported and used to name the specific organism (Table 1).

Results and Discussion

The study of identification of bacteria for the biodegrading capabilities is important in microbial ecology, especially with molecular techniques. In particular, analysis of the microbial communities that take part during in-situ hydrocarbon biodegradation activities has been a challenge to microbiologist. Interest in this area has been catalyzed by the rapid advancement of molecular ecological methodologies. Thus, the ability of an organism to degrade a specific substrate and persist within such environment is clear evidence that its genome harbored the relevant degrading gene [7]. The previous studies by Bindu and Satish [8] and Jyothi et al. [9] on hydrocarbon degradation by bacteria reveal that catechol 2, 3 dioxygenase is one of the enzyme involved in hydrocarbon degradation.

Molecular confirmation of similarities in microbial Identities was obtained by creating different dendrograms/distance trees. Gene sequencing carried out read 513 different nucleotide sequences. Every read was BLASTED and the result file saved. The report on the frequency of reads of each species is as shown in Table 1. Seven phylum with 47 corresponding culture-dependent species and 169 culture-independent bacteria clone was obtained. The resultant haplotype/cladogram however, showed clades of proteobacteria (b-and g-proteobacteria), bacteria/enterobacteria, firmicutes, plantomycetes, acidobacteria group/ fibrobacteres, Bacteriodetes/chlorobi, Actinobacteria/high G+C and chloriflexi phyla (Figure 1). The nucleotide sequences with no hit was sent to Genbank for assigning of ascension number. The isolation of the aforementioned organisms from crude oil polluted agricultural soil left for four years, depict that the organism probably, have degradative genes coding for enzymes for hydrocarbon catalysis which aided their survival. These have been confirmed by the presence of plasmid DNA in culture - dependent isolates obtained and published elsewhere [10].

Taxonomical classification and percentage diversity

Further taxonomical analysis was carried out with reads of sufficient Q scores (>q30) and lengths and a total of 420 read count of top kingdom classification of 100% bacteria kingdom was obtained. Top phyla classification depict that that Proteobacteria had the highest diversity of 57.14% followed by Acidobacteria (20.24%), Unknown (16.67%), Firmicutes (3.33%), Bacteroidetes (2.38%), and Planctomycetes (0.24%) in that order (Figure 2). Top class and order classification of phylum proteobacteria, class beta proteobacteria and order Burkholderiales also had similar highest values of 53.81% (Figure 3 and Figure 4) whereas in top family classification, Burkholderiaceae recorded the lowest diversity of 0.24% (Figure 4). Furthermore, the family of unknown increased by 2.38% while diversity of Acidobacteria phyla, class, order and family remained constant (20.24%). Generally, the dendrogram of the BLAST hit showing resultant clades with their leaves and height of the branch points indicating the similarity and differences of isolates from each other (the greater the height, the

¹http://www.laihey.org/studies/webt.html
²http://blast.ncbi.nlm.nih.gov/Blast.cgi
| Accession | Organism Description | Identity Score | Minimum ID Value | Count |
|-----------|----------------------|----------------|-----------------|-------|
| gb|GU599159.1| Uncultured soil bacterium clone HB_Ca_M_285 | 1A | 0.001592357 | 1 |
| gb|HQ120802.1| Uncultured bacterium isolate 1112865261764b 16S | 2A | 0.003184713 | 2 |
| gb|GQ18974.1| Uncultured soil bacterium clone 21_77KE06 | 3A | 0.001592357 | 1 |
| gb|JX186556.1| Uncultured bacterium clone YB61 16S | 4A | 0.011146497 | 7 |
| gb|JN168313.1| Uncultured bacterium clone WLBL550 16S | 5A | 0.001592357 | 1 |
| gb|H322283.1| Uncultured bacterium clone W4-84 16S | 6A | 0.001592357 | 1 |
| emb|FR716374.1| Uncultured bacterium partial 16S rRNA | 7A | 0.001592357 | 1 |
| gb|JN865443.1| Uncultured bacterium isolate DS-3 16S | 8A | 0.001592357 | 1 |
| gb|HM019522.1| Burkholderia phytophym strain GR06 16S | 9A | 0.001592357 | 1 |
| gb|JQ119629.1| Uncultured bacterium isolate 1112842460007a 16S | 10A | 0.001592357 | 1 |
| gb|EF667534.1| Uncultured bacterium clone LaC15L18 16S | 11A | 0.001592357 | 1 |
| gb|JN168399.1| Uncultured bacterium clone WLCLC424 16S | 13A | 0.001592357 | 1 |
| gb|JN168229.1| Uncultured bacterium clone WLBL429 16S | 14A | 0.001592357 | 1 |
| gb|JQ476801.1| Uncultured bacterium clone 071071_067 16S | 15A | 0.007961783 | 5 |
| gb|JQ710440.1| Nevskia sp. F2-63 16S ribosomal | 16A | 0.003184713 | 2 |
| gb|JX041839.1| Uncultured proteobacterium clone APC_4_G1 16S | 18A | 0.001592357 | 1 |
| emb|FR687596.1| Uncultured bacterium partial 16S rRNA | 19A | 0.003184713 | 2 |
| gb|GU598830.1| Uncultured soil bacterium clone HB_R_M_212 | 20A | 0.001592357 | 1 |
| gb|EF911130.1| Uncultured bacterium clone Bbf10-02C12 16S | 21A | 0.003184713 | 2 |
| gb|EU382007.1| Uncultured rumen bacterium clone P5_B07 | 22A | 0.001592357 | 1 |
| gb|JQ861367.1| Uncultured Acidobacteria bacterium clone XH15 | 23A | 0.001592357 | 1 |
| gb|HQ674808.1| Uncultured Acidisphaera sp. clone LWM1-70 | 24A | 0.001592357 | 1 |
| gb|JN168198.1| Uncultured bacterium clone WLBL342 16S | 26A | 0.001592357 | 1 |
| gb|EF020266.1| Uncultured Acidobacteriaceae bacterium clone Elev... | 36A | 0.00477707 | 3 |
| gb|HQ445474.1| Uncultured bacterium clone Luq_GS470_003 16S | 37A | 0.001592357 | 1 |
| gb|JX172839.1| Uncultured bacterium clone PB17026-1A_G11 16S | 38A | 0.015923567 | 10 |
| emb|HE660678.1| Uncultured bacterium partial 16S rRNA | 39A | 0.00477707 | 3 |
| gb|JX171869.1| Uncultured bacterium clone PB17007-2_E01 16S | 40A | 0.001592357 | 1 |
| gb|HQ264667.1| Uncultured bacterium clone SCP117 16S | 41A | 0.003184713 | 2 |
| gb|JF440522.1| Uncultured bacterium clone CG364 16S | 42A | 0.001592357 | 1 |
| gb|HQ023258.1| Burkholderia unamae strain CACua-11 16S | 43A | 0.003184713 | 2 |
| gb|JQ968935.1| Uncultured bacterium clone Gra-Bac073 16S | 44A | 0.001592357 | 1 |
| gb|JN911353.1| Uncultured microorganism clone GF13U7304.JZZZD 16S... | 45A | 0.001592357 | 1 |
| gb|JF648701.2| Burkholderia sp. SWF66247 16S ribosomal | 46A | 0.003184713 | 2 |
| gb|GQ140333.1| Comamonas testosteroni strain SJ9 16S | 47A | 0.001592357 | 1 |
| gb|JQ665348.1| Pseudomonas aeruginosa isolate H23 16S | 48A | 0.001592357 | 1 |
| emb|HE856926.1| Uncultured bacterium partial 16S rRNA | 49A | 0.001592357 | 1 |
| gb|JQ730653.1| Uncultured Acidobacterium sp. clone JL123_4 | 50A | 0.001592357 | 1 |
| gb|JF625119.1| Uncultured bacterium clone H_C122 16S | 51A | 0.001592357 | 1 |
| gb|HQ210555.1| Uncultured Acidobacterium bacterium clone An465_C4 | 52A | 0.001592357 | 1 |
| gb|JF451723.1| Uncultured bacterium clone ORFRC-FW102-670d-2.8 1... | 53A | 0.001592357 | 1 |
| gb|JN172802.1| Uncultured soil bacterium clone em_emp435 | 54A | 0.001592357 | 1 |
| Accession | Organism Type | Species | Similarity (%) | E-value |
|-----------|--------------|---------|---------------|---------|
| gb| JF797204.1 | Pseudomonas aeruginosa strain ITCC B0030 | 55A | 0.001592357 |
| gb| EU881261.1 | Uncultured bacterium clone KGB200711-007 16S | 56A | 0.001592357 |
| gb| EF600579.1 | Uncultured bacterium clone E5-47 16S | 57A | 0.007961783 |
| gb| JX083379.1 | Burkholderia kuriburiensis strain PR1 16S | 58A | 0.003184713 |
| gb| UE880360.1 | Uncultured bacterium clone S7-68 16S | 59A | 0.003184713 |
| gb| JF166800.1 | Uncultured bacterium clone PLQ3_C01 16S | 60A | 0.001592357 |
| gb| JN817761.1 | Firmicutes bacterium enrichment culture clone | 61A | 0.001592357 |
| gb| DQ264622.1 | Uncultured bacterium clone BANW64 16S | 62A | 0.001592357 |
| gb| GQ376582.1 | Uncultured bacterium clone D1G_F10 16S | 63A | 0.001592357 |
| gb| EFS516204.1 | Uncultured bacterium clone FCPP711 16S | 64A | 0.001592357 |
| gb| JF440427.1 | Uncultured bacterium clone CG208 16S | 65A | 0.001592357 |
| gb| JX047141.1 | Uncultured bacterium clone KWB121 16S | 66A | 0.006369427 |
| gb| JX083379.1 | Burkholderia kuriburiensis strain PR1 16S | 67A | 0.001592357 |
| gb| EU265982.1 | Uncultured bacterium clone Nit2A0626_56 16S | 68A | 0.00477707 |
| gb| JN082688.1 | Uncultured Schlegelella sp. clone 262 | 69A | 0.003184713 |
| gb| JF833857.1 | Uncultured bacterium clone E30 16S | 70A | 0.001592357 |
| gb| JF833857.1 | Uncultured bacterium clone E30 16S | 71A | 0.001592357 |
| emb| AM159259.1 | Uncultured Chloroflexi bacterium 16S rRNA | 72A | 0.001592357 |
| gb| EF471223.1 | Dyella sp. CHNCT13 16S ribosomal | 73A | 0.00477707 |
| gb| JN873119.1 | Uncultured bacterium isolate DGGE gel | 74A | 0.017515924 |
| gb| JF361451.1 | Uncultured soil bacterium clone G00VNXF07H1XC7 | 75A | 0.003184713 |
| gb| HM544542.1 | Uncultured bacterium clone ZM9-198 16S | 76A | 0.003184713 |
| gb| GQ376832.1 | Uncultured bacterium clone D10H_G08 16S | 77A | 0.001592357 |
| gb| HM488701.1 | Uncultured Myxococcales bacterium clone BOM_f02 | 78A | 0.006369427 |
| gb| DQ50730.1 | Uncultured Chloroflexi bacterium clone B12_WMSP1 | 79A | 0.001592357 |
| emb| AJ233524.1 | Uncultured eubacterium 16S ribosomal RNA, | 80A | 0.001592357 |
| gb| EF018933.1 | Uncultured bacterium clone G00VNXF07H1XC7 | 81A | 0.003184713 |
| gb| HM544542.1 | Uncultured bacterium clone ZM9-198 16S | 82A | 0.003184713 |
| gb| JQ433554.1 | Uncultured bacterium clone GOP_C 16S | 83A | 0.001592357 |
| gb| HM63734.1 | Uncultured bacterium clone GB7N7003GAA6JB small | 84A | 0.02388535 |
| gb| HM488701.1 | Uncultured Myxococcales bacterium clone BOM_f02 | 85A | 0.001592357 |
| gb| DQ450730.1 | Uncultured Chloroflexi bacterium clone B12_WMSP1 | 86A | 0.001592357 |
| emb| JX083379.1 | Uncultured bacterium clone ITCC B0030 | 87A | 0.001592357 |
| gb| HM582700.1 | Uncultured bacterium clone LCH_B101 16S | 88A | 0.00955414 |
| gb| JQ796741.1 | Burkholderia sp. 10-18 16S ribosomal | 89A | 0.001592357 |
| gb| GQ376832.1 | Uncultured bacterium clone D10H_G08 16S | 90A | 0.001592357 |
| gb| JQ926999.1 | Uncultured soil bacterium clone M1R20 16S | 91A | 0.001592357 |
| gb| GU366823.1 | Uncultured bacterium clone C2_A25 | 92A | 0.001592357 |
| emb| JQ726640.1 | Uncultured bacterium clone F1G32TO06G2XSI 16S | 93A | 0.001592357 |
| gb| EU465058.1 | Uncultured bacterium clone AFYEL_aaj76d08 16S | 94A | 0.001592357 |
| gb| JF004759.1 | Uncultured bacterium clone MR20 16S | 95A | 0.001592357 |
| gb| GQ366823.1 | Uncultured bacterium clone C2_A25 | 96A | 0.001592357 |
| gb| CP003782.1 | Burkholderia pseudomallei chromosome II, | 97A | 0.02292994 |
| gb| JQ726640.1 | Uncultured bacterium clone F1G32TO06G2XSI 16S | 98A | 0.001592357 |
| gb| JF019209.1 | Uncultured soil bacterium clone MR20 16S | 99A | 0.001592357 |
| gb| JQ726640.1 | Uncultured bacterium clone F1G32TO06G2XSI 16S | 100A | 0.001592357 |
| Accession | Description | Band | Similarity | Number |
|-----------|-------------|------|------------|--------|
| emb|FR687637.1| Uncultured bacterium partial 16S rRNA | 110A | 0.00477707 | 3 |
| gb|HQ684418.1| Uncultured bacterium clone OI2132 16S | 111A | 0.00159235 | 1 |
| emb|CU234118.1| Bradyrhizobium sp. ORS278, complete sequence | 112A | 0.00159235 | 1 |
| gb|HQ264456.1| Uncultured bacterium clone SC3D30 16S | 113A | 0.003184713 | 2 |
| gb|EU662545.1| Uncultured bacterium clone MC1B_16S_181p 16S | 114A | 0.00159235 | 1 |
| gb|HQ706109.1| Burkholderia silvatlantica strain AB284 16S | 115A | 0.00159235 | 1 |
| gb|JN412269.1| Uncultured Oxalobacteraceae bacterium clone CM67 | 116A | 0.00159235 | 1 |
| gb|UN172799.1| Uncultured soil bacterium clone em_emp427 | 117A | 0.00159235 | 1 |
| gb|HM580555.1| Uncultured bacterium clone cs1H11 16S | 118A | 0.00159235 | 1 |
| emb|AJ292885.1| uncultured eubacterium WR828 partial 16S | 119A | 0.00159235 | 1 |
| gb|JX091743.1| Uncultured bacterium clone BAC27A5 16S | 120A | 0.003184713 | 2 |
| gb|GQ376973.1| Uncultured bacterium clone PI_C12 16S | 121A | 0.00159235 | 1 |
| ref|XM_001977801.1| Drosophila erecta GG19261 (DereGG19261), mRNA | 122A | 0.00159235 | 1 |
| gb|GU599104.1| Uncultured soil bacterium clone HB_Ca_M_182 | 123A | 0.00159235 | 1 |
| gb|JN172666.1| Uncultured soil bacterium clone DSM-R34 16S | 124A | 0.00159235 | 1 |
| gb|JX391481.1| Uncultured bacterium clone N0045 16S | 125A | 0.00159235 | 1 |
| gb|JF402916.1| Uncultured soil bacterium clone GOOVNXF07IGU40 | 126A | 0.00159235 | 1 |
| gb|HQ904139.1| Uncultured bacterium clone sa0.62 16S | 127A | 0.00159235 | 1 |
| gb|JF809205.1| Uncultured bacterium clone CP2-B2 16S | 128A | 0.00159235 | 1 |
| gb|EU800550.1| Uncultured bacterium clone 2C228685 16S | 129A | 0.00159235 | 1 |
| emb|AJ292885.1| Uncultured bacterium WR828 partial 16S | 130A | 0.00159235 | 1 |
| gb|JF178166.1| Uncultured bacterium clone TY-F-II-OTU3 16S | 131A | 0.003184713 | 2 |
| gb|GU172206.1| Uncultured bacterium clone DSM-R34 16S | 132A | 0.00159235 | 1 |
| gb|JQ178187.1| Uncultured Thermoanaerobacterales bacterium clone... | 133A | 0.00159235 | 1 |
| gb|JN168234.1| Uncultured bacterium clone WLBL437 16S | 134A | 0.00159235 | 1 |
| gb|EU464591.1| Klebsiella pneumoniae strain ECU-21 genomic | 135A | 0.00159235 | 1 |
| gb|HQ397045.1| Uncultured Bacillus sp. clone HAHS13.81 | 136A | 0.00159235 | 1 |
| gb|JQ655798.1| Uncultured bacterium clone N24 16S | 137A | 0.00477707 | 3 |
| gb|JX391481.1| Uncultured bacterium clone N0045 16S | 138A | 0.00159235 | 1 |
| gb|JQ514083.1| Bradyrhizobium sp. R34_Vidisha 16S ribosomal | 139A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 140A | 0.00159235 | 1 |
| gb|JQ178187.1| Uncultured Acidobacteria bacterium clone GASP-WB2... | 141A | 0.00159235 | 1 |
| gb|JN178272.1| Uncultured bacterium clone TX2_4M08 16S | 142A | 0.00159235 | 1 |
| gb|HM688413.1| Uncultured bacterium clone GB7N87001BH8QO small | 143A | 0.003184713 | 2 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 144A | 0.00159235 | 1 |
| gb|JF402916.1| Uncultured soil bacterium clone GOOVNXF07IGU40 | 145A | 0.003184713 | 20 |
| gb|JY571493.1| Uncultured bacterium clone FAahw485 16S | 146A | 0.00159235 | 1 |
| gb|JN178272.1| Uncultured bacterium clone TX2_4M08 16S | 147A | 0.00159235 | 1 |
| gb|JX286412.1| Uncultured bacterium clone abscm03.0.46 16S | 148A | 0.00159235 | 1 |
| gb|JQ178187.1| Uncultured Acidobacteria bacterium clone GASP-WB2... | 149A | 0.00159235 | 1 |
| gb|JQ655798.1| Uncultured bacterium clone N24 16S | 150A | 0.00477707 | 3 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 151A | 0.003184713 | 2 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 152A | 0.00159235 | 1 |
| gb|JQ598830.1| Uncultured Acidobacteria bacterium clone SEW_08_1... | 153A | 0.003184713 | 2 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 154A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 155A | 0.00159235 | 1 |
| gb|U6335380.1| Uncultured bacterium clone BacC-u_034 16S | 156A | 0.00159235 | 1 |
| gb|JQ514083.1| Bradyrhizobium sp. R34_Vidisha 16S ribosomal | 157A | 0.007961783 | 5 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 158A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 159A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 160A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 161A | 0.007961783 | 5 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 162A | 0.00159235 | 1 |
| gb|JQ684238.1| Uncultured bacterium clone DSM-R34 16S | 163A | 0.00159235 | 1 |
| gb|JX133661.1| Uncultured bacterium clone WB123 16S | 164A | 0.003184713 | 2 |
| gb|FJ178119.1| Uncultured bacterium clone TY-D-I-OTUS 16S | 165A | 0.001592357 | 1 |
| gb|JX255255.1| Uncultured bacterium clone abscm03.0.575 16S | 166A | 0.001592357 | 1 |
| gb|JF405890.1| Planctomycetacia bacterium WF3-27 16S ribosomal | 167A | 0.001592357 | 1 |
| gb|JN911190.1| Uncultured microorganism clone GF13U730415R3Y 16S... | 168A | 0.001592357 | 1 |
| gb|HO322962.1| Uncultured bacterium clone W9-11 16S | 169A | 0.001592357 | 1 |
| gb|JQ684492.1| Uncultured Rhodobacter sp. clone Deep95 | 170A | 0.001592357 | 1 |
| gb|EF588371.1| Uncultured Acidobacteria bacterium clone WSD-045 | 171A | 0.00477707 | 3 |
| gb|FJ193705.1| Uncultured bacterium clone WB19 16S | 172A | 0.078025478 | 49 |
| gb|JX174263.1| Burkholderia sp. 2386 16S ribosomal | 174A | 0.003184713 | 2 |
| gb|HM108406.1| Uncultured Clostridia bacterium clone SHA049 | 175A | 0.001592357 | 1 |
| gb|JX133573.1| Uncultured bacterium clone FB31 16S | 176A | 0.001592357 | 1 |
| dbj|AB188624.1| Uncultured bacterium gene for 16S | 178A | 0.001592357 | 1 |
| gb|EU381918.1| Uncultured rumen bacterium clone P5_C17 | 179A | 0.001592357 | 1 |
| gb|HM138688.1| Uncultured bacterium clone GQ25 genomic | 180A | 0.001592357 | 1 |
| gb|JF712828.1| Uncultured bacterium clone CV16 16S | 181A | 0.003184713 | 2 |
| gb|JX172662.1| Uncultured bacterium clone PB17024-1_H03 16S | 182A | 0.001592357 | 1 |
| gb|HQ121355.1| Uncultured bacterium clone G40 16S | 183A | 0.001592357 | 1 |
| gb|JQ690672.1| Uncultured bacterium clone WB123 16S | 184A | 0.05247771 | 33 |
| gb|JN172776.1| Uncultured soil bacterium clone em_em4s11 | 185A | 0.001592357 | 1 |
| gb|JN688802.1| Uncultured bacterium clone MW47 16S | 186A | 0.001592357 | 1 |
| gb|GU931381.1| Uncultured bacterium clone GQ25 genomic | 187A | 0.001592357 | 1 |
| gb|JX133661.1| Uncultured bacterium clone WB123 16S | 188A | 0.001592357 | 1 |
| gb|JN168386.1| Uncultured bacterium clone WLCLC404 16S | 189A | 0.001592357 | 1 |
| gb|JQ433573.1| Uncultured bacterium clone GOP_H 16S | 190A | 0.2070064 | 17 |
| gb|JN168386.1| Uncultured bacterium clone WLCLC404 16S | 191A | 0.001592357 | 1 |
| gb|CP000494.1| Bradyrhizobium sp. BTA1, complete genome | 192A | 0.001592357 | 1 |
| gb|JQ770095.1| Uncultured bacterium clone YT-47 16S | 193A | 0.001592357 | 1 |
| gb|CP002299.1| Frankia sp. Eul1c, complete genome | 194A | 0.00477707 | 3 |
| gb|JF829579.1| Uncultured bacterium clone M2_247 16S | 195A | 0.08598726 | 54 |
| gb|JQ389743.1| Streptomyces tanashiensis strain BAB1573 16S | 196A | 0.001592357 | 1 |
| gb|HQ674949.1| Uncultured Acidobacteria bacterium clone MWM2-7S | 197A | 0.001592357 | 1 |
| gb|JQ433573.1| Uncultured bacterium clone GOP_H 16S | 198A | 0.2070064 | 17 |
| gb|JN168386.1| Uncultured bacterium clone WLCLC404 16S | 199A | 0.001592357 | 1 |
| gb|JN168386.1| Uncultured bacterium clone WLCLC404 16S | 200A | 0.001592357 | 1 |
| gb|JQ349505.1| Bradyrhizobium sp. DW6.4 16S ribosomal | 201A | 0.001592357 | 1 |
| gb|GU598779.1| Uncultured soil bacterium clone HB_R_M_105 | 202A | 0.001592357 | 1 |
| gb|JF800677.1| Uncultured bacterium clone BT41 16S | 203A | 0.00477707 | 3 |
| gb|HQ852966.1| Uncultured bacterium clone C8 16S | 204A | 0.003184713 | 2 |
| gb|JF412274.1| Lysobacter sp. ATCC 53042 lysobactin | 205A | 0.001592357 | 1 |
| gb|EF588337.1| Uncultured Acidobacteria bacterium clone WSD-011 | 206A | 0.001592357 | 1 |
| gb|JX240938.1| Uncultured bacterium clone ONGS77 | 207A | 0.001592357 | 1 |
| gb|EU471806.1| Uncultured bacterium clone AE2_aaa02a11 16S | 208A | 0.003184713 | 2 |
| gb|GU482873.1| Uncultured bacterium clone F1Q32TO5GFAJR 16S | 209A | 0.001592357 | 1 |
| gb|JQ183105.1| Uncultured bacterium clone 10 16S | 210A | 0.001592357 | 1 |
| gb|JQ183105.1| Uncultured bacterium clone 10 16S | 211A | 0.001592357 | 1 |
| gb|JN428405.1| Uncultured organism clone SBXV_2668 16S | 212A | 0.003184713 | 2 |
| gb|HM581210.1| Uncultured bacterium clone mgH04 16S | 213A | 0.001592357 | 1 |
| gb|CP002521.1| Acidovorax avenae subsp. avenae ATCC | 214A | 0.001592357 | 1 |
| gb|JX684408.1| Uncultured bacterium clone OI2144 16S | 215A | 0.001592357 | 1 |
| gb|JG684400.1| Uncultured bacterium clone HWGB-142 16S | 216A | 0.003184713 | 2 |
| gb|JQ397486.1| Uncultured bacterium clone BSS101 16S | 217A | 0.001592357 | 1 |

Table 1: Report on the frequency (counts/total number of reads of each species).
greater the difference) as well as the percentage diversity of all the taxonomical classification of bacterial isolates from the BLAST output result based on hits and non-hit read counts are shown in Figure 6 and Figure 7 respectively. The result (Figure 5) depict that about 33.12% had no hit.

Indeed, microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment [10,11]. The recognition of biodegraded petroleum hydrocarbons in the environment as observed in previous studies [2,3,9,12,13], which was evident through detectable biodegradation of n-alkane profile of the crude oil by microorganisms supports the findings of this study. The microbial genera, namely, *Arthrobacter, Alcaligenes, Burkholderia, Mycobacterium, Micrococcus, Pseudomonas, Acinetobacter, Bacillus, Sphingomonas, Corynebacterium and Rhodococcus* have been incriminated to be involved in hydrocarbon degradation as observed in the percentage diversity of the taxonomical classification in this study; as these organisms fall within similar identified phyla, class, order and family of bacterial isolate during this metagenomic analysis. From the findings of previous studies and in...
line with this study, bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in the environment having in them enzymes for hydrocarbon degradation. This corroborates the report made by Rahman et al. [14] and Brooijmans et al. [15] who studied on hydrocarbon degrading bacterial isolates in petroleum sludge. The persistence of the identified bacterial isolates in this study could also be due to the ability of the isolates to produce bio surfactants which aids in the formation of micelles to enhance uptake of hydrocarbons. Studies have also shown that total bacteria population in polluted soil are more than that in unpolluted soil [16-18] which implies that those organisms are the active degraders of that oil.

Metagenomic analysis carried out in this study have actually helped in detection of Acidobacteria phyla which are under- represented in culture even though they are physiologically diverse and ubiquitous as well as so many uncultured genera. The low diversity of Planctomycetes is not surprising since they are aquatic bacteria phyla and are found in samples of brackish, marine and fresh water. Further molecular studies are therefore needed to detect specific catabolic genes resident in these hydrocarbon degrading isolates. This can help to produce superbugs required for faster remediation, cost effective and efficient bioremediation protocol for Nigerian oil polluted soil.

**Conclusion**

The isolation of the aforementioned organisms from crude oil polluted agricultural soil left for four years, depict that the organism probably, have degradative genes which aided their survival.

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