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Molecular Characterization and Determination of Bioremediation Potentials of Some Bacteria Isolated from Spent Oil Contaminated Soil Mechanic Workshops in Kaduna Metropolis

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Abstract: Spent oil contaminated Soil from ten selected mechanic workshops were investigated for their bacteria and bioremediation potentials. The bacterial isolates were morphologically and molecularly identified as *Enterobacter hormaechei*, *Escherichia coli, klebsiella pneumoniae, Shigella flexneri, Wesiella cibaria, Lactobacillus planetarium*. The singles and a consortium of these bacteria incubated in the minimal salt medium incorporated with 1% engine oil exhibited various biodegradation rates, with the mixed consortium exhibiting the highest for this oil. The gene for the hydrocarbon enzyme Catechol 2, 3 dioxygenase (C2,30) was detected and amplified in *Enterobacter hormaechei*, *Escherichia coli and Shigella flexneri* using PCR and Agarose gel electrophoresis. The detection of the (C2,30) enzyme gene in and the spent oil biodegradation activity exhibited by these bacteria suggest their possible possession of bioremediating potentials for the spent engine oil. It is therefore suggested that a pilot study on the field application of these bacteria for bioremediation and restoration of spent oil polluted environment should be done in mechanic workshops.

Key words: Spent engine oil · Pollution · Bacteria · Enzyme · Bioremediation · Mechanic workshops

INTRODUCTION

In Nigeria, oil spills at auto mechanic workshops have been left uncared for over the years and its continuous accumulation is of serious environmental concern, because of the hazard associated with it. For instance the spent motor oil disposed of improperly contains potentially toxic substances such as benzene (carcinogens), lead, arsenic, zinc and cadmium, which can seep into the water tables and contaminate ground water [1, 2].

One of the most significant impact associated with workshop seepage of used engine oil includes lose of soil fertility, water holding capacity, permeability and binding capacity [3]. It's a very costly approach to treat oil contaminated site by conventional methods such as use of chemicals or peat moss (a plant which absorbs hydrocarbons). Contamination of soil by petroleum hydrocarbon stimulates indigenous microbial populations, which are capable of utilizing the petroleum hydrocarbons as their carbon and energy source thereby degrading the contaminants. The ability to degrade hydrocarbon substrates is exhibited by a wide v*ariety* of bacteria genera [4,5] using culture dependent and independent isolation techniques different bacterial genera have been characterized from hydrocarbon polluted soils in different geographical and ecological contexts [6-8].

Bioremediation method is considered to be more economical and safe method for the treatment of oil contaminated site. It has been observed that micro-organism that grows on oil contaminated soil are much capable of degrading oil than those

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micro-organisms which are found on non-contaminated site of oil. This can be a very good example of adaptation. The natural process of biodegradation can be speed up if we add some nutrient to it which help in the growth of micro-organisms or we can isolate the microbes from contamination site inoculate in nutrient broth and mixed it in contaminated region [3,9].

MATERIALS AND METHODS

Sampling: Soils contaminated with mainly spent engine oil were collected from ten different locations within Kaduna metropolis. These sites were chosen on the basis of long period of existence (10 years above), level of activities and size. The selected study sites were located in Kurmin Mashi, Malali, Barnawa Railway Station, Kakuri (Artillery), Panteka, Television, Ori-Akpata, kawo, Nasarawa and Trikania. At each identified mechanic workshop 10 samples were taken from the top soil up to a depth of 0.02m using a soil auger. A total of 100 samples based on seasons in 2 replicates were aseptically taken using a sterilized spatula sampling each site soil was transferred into a sterilized label sample bottle and taken to the laboratory for analysis. Soil samples were similarly taken about 200m away from contaminated locations upstream of each mechanic workshop site studied as negative control.

Isolation of Bacteria: The bacteria were isolated by inoculating the soil samples on enrichment medium that contains the autoclaved Bushnell Haas Agar medium supplemented with single hydrocarbon compound as sole carbon source (1% used engine oil). The medium was also inoculated with 50 microgram per millilitre nystatin to suppress fungal growth. The isolates were further subcultured into nutrient agar medium preparatory for gram stain, morphological identification, biochemical characterization and DNA extraction [10].

Isolation of Genomic DNA from Bacteria: Microbe(s) with proven bioremediation capability (also efficient strains of these microbes) were selected and DNA isolated. *DNA* was extracted from 1ml of bacterial culture. the culture was pelleted by centrifuging at 12,000rpm for 2 min. the pellet was treated with lysis solution and proteinase k and incubated at 60°C for 30min. Nucleic acids were precipitated with isopropanol by centrifuging at 10,000 rpm for 10 min, washed with 1 ml of a 70% (v/v) ethanol solution and dissolved in 0.1 ml of a TE buffer. The purity and quantity of DNA were examined by recording its UV absorption spectrum and running on 1% agarose gel electrophoresis [10,11].

Polymerase Chain Reaction Amplification of 16s rRNA: The PCR reaction mixture contains 10XPCR buffer, 25 mM, Magnesium chloride, 2.5mM dNTP's, 10pm/µl primer concentrations template DNA tests were done for the isolated. PCR conditions were optimized using lab net thermal cycler. The PCR temperature Program began with an initial 5-min denaturation step at 94°C; 35 cycles of 94°C for 45sec, 55°C for 1 min and 72°C for 1 min; and a final 10-min extension step at 72°C. All reaction mixtures were held at 4°C until analyzed [12].

Sequence Determination of 16s rRNA: The DNA isolated was amplified using 16s rRNA universal primers and sequenced for the identification of microbial strains at molecular level. Amplification of the PCR products of expected size was confirmed by electrophoresis. The sequence of the 16S rRNA was determined with a Dye terminator sequencing kit (Applied Biosystems) and the product was analyzed with an ABI Prism DNA sequencer (ABI). The gene sequences of each isolate obtained in this study were compared with known 16s rRNA gene sequences in the GenBank database [10]. Basic local alignment search tool (BLAST) was used to sequence similarities against the entire DNA of the indigenous bacteria and the existing database.

Bacterial Biodegradative Activity by Turbidometry: Eight set of 250ml flasks were set for the work. The broth was sterilized in an autoclave at 121°C for 15 minutes. 120ml of nutrient broth was inoculated with 1% used engine oil concentration into each of the seven flasks. The first flask was left uninoculated with bacteria isolates but the second, third, fourth, fifth sixth and seventh were inoculated with the isolated bacteria which are *Enterobacter*, *Escherichia, klebsiella, shigella*, *Wesiella*, *lactobacillus sp* respectively. The eighth flask was inoculated with a mixed culture consortium of the six bacteria isolates the experimental set up was left to stand for 14 days. The growth of the bacteria was measured by taking the Optical Density (O.D) readings at 595nm from 0hrs- 14 days at regular intervals of 2 days against mineral salt medium as blank [10].

RESULTS AND DISCUSSION

Isolated Bacteria from Spent Engine Oil in Sampled Locations: Bacterial counts ($x10^4$) of spent engine oil soil collected from Kaduna Metropolis are presented in Table 1. The bacterial loads of the soil ranged between 12.82±1.03 CFU/ml in Trikania and 44.04±1.50 CFU/ml (Television Garage). The bacterial load of Television Garage was highest (44.04±1.50 CFU/ml) and that

Table 1: Bacterial Isolates from Ten Sampled Mechanic Workshops in Kaduna Metropolis

Location	Bacterial Count ($\times 10^4$ CFU/ml)
Artillery	29.45±1.64
Barnawa Railway	20.16±1.24
Kawo	26.56±0.64
Kurmi Mashi	28.24±0.96
Malali	16.93±1.78
Ori-Akpata	38.64±2.04
Panteka	35.45±3.45
Sabo/Tasha	23.33±1.32
Trikania	12.82±0.52
Television Garage	44.04±1.50
Control	9.45±1.03

of the Trikania polluted with spent engine oil lowest (12.82±0 CFU mL^{-1}). The sample collected from unpolluted soil 9.45±1.03 CFU/ml. The Six bacterial isolates obtained from engine oil–contaminated soil in this study were found to be similar with the study which was carried out by Kafizadeh, *et al.* [13] were 80 bacteria strains belonging to 10 genus were isolated and identified as follows; *Baccilus, Corynebacterium, Staphalococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella and Enterobacter*. In the same manner research carried out by Survery *et al.* [14] on soil near different petrol pumps of Karachi recorded the presences of the following bacteria genera as follows; *Staphalococcus, Corynebacterium, Bacillus Proteus, Pseudomonas, Klebsiella, Escherichia* while *Proteus* and *Ecoli* had the highest rate of degradation [14]. Bacteria are known to hydrolyze long chain hydrocarbons to release the element utilizable as nutrients for their growth and to display their phases of growth. It is observed from this study that when the environment was contaminated with spent engine oil components the proportion of hydrocarbon-degrading microorganisms' increases rapidly. High numbers of certain hydrocarbon-degrading microorganisms from an environment implies that those organisms are the active biodegraders of these compounds in that environment [15]. The presence of oil-degrading organisms in the polluted soil suggests that the indigenous microbes were carrying out their metabolic activity. The activities of these microorganisms could be responsible for the bioremediation of the environment.

Average Colonial Count of Bacteria Isolates from Ten Mechanic Workshops in Kaduna Metropolis: Table 2 shows the result of bacteria Colonial count from the cultured soil sampled obtained from ten investigated mechanic workshops in Kaduna Metropolis. Bacteria loads had 70% in *Entrobacter* and *Shigella* while *Lactobacillus* and *Wiesella* had the lowest percentage occurance of 40% respectively. Similarly, *Aspergillus spp* had the highest percentage occurance of 70% while *Penicilium spp* had the lowest percentage occurance of 30%. The frequency of occurrence for bacteria (Table 2) showed that *Enterobacter* and *Shigella* spp. had the highest frequency of occurrence while *Lactobacillus* and *Weisiella* were the lowest. The report is contrary to the findings made by Jesubunmi [16] who stated in her research that *Pseudomonas* and *Micrococcus* had the highest frequency of occurrence while *Klebsiella* and *Bacillus s*pp. were the lowest. According the report made by Ugoh and Moneke [17] the ability to isolate high numbers of certain oil degrading micro organism from oil polluted environment is commonly taken as evidence that these micro organisms are the active degraders in the environment. These findings correspond favorably with Usman *et al.* [18] whose micrococcus *Luteus* exhibited exponential, stationary and death phase while *Bacillus spp* experience exponential and stationary phase only. The results observed in *Lactobacillus plantarium* which experienced all four phase of growth contradict with the finding of Ojuma *et al*. [19] and Usman *et al*. [18] reports on their bacteria isolates which did not exhibit lag phase of growth. The reason for the higher counts of bacteria during its earlier growth may be as a result of the presence of high quantity of Nitrogen and phosphorus in the mineral salt medium which are necessary for

Table 2: Average Colonial Count of Bacteria from Ten Mechanic Workshops in Kaduna Metropolis

Isolates	AR	BR	KW	KM	ML	OR	PT	SB	TK	TV	Frequency (%)
E. Coli	-	-	+	+	-	+	+	-	-	+	50
Enterobacter	+	-	-	+	+	+	+	+	-	+	70
Lactobacillus	-	+	-	+	-	-	+	-	-	+	40
Shigella	+	-	+	-	+	+	+	-	+	+	70
Wiesella	-	+	-	-	+	-	-	+	+	-	40
Klebsiella	+	-	+	+	-	+	+	--		+	60

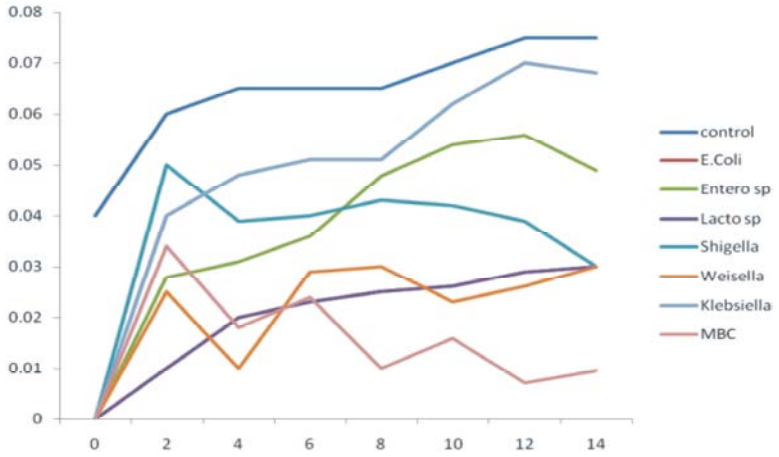

Fig. 1: Bioremediation Potential of each Bacteria isolates in 1% used engine oil Mineral Salt Medium

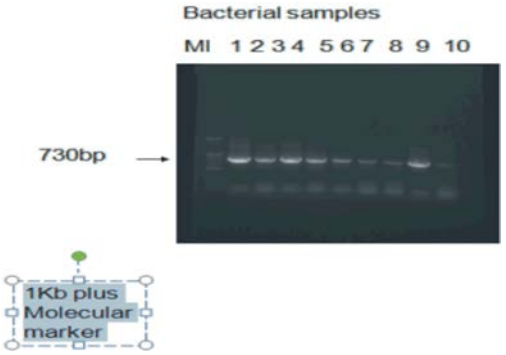

Fig. 2: PCR Agarose Gel Electrophoretic Analysis of the Amplified 16S rRNA for the Bacterial Isolates from the Ten Study Site in Kaduna Metropolis
Lane 1-DNA marker(ladder), lane 2,*enterobacter sp, lane 3 Escherichia coli, lane 4 klebsiella sp, lane 5 Shigella flexneri lane 6 Cronobacter sp, lane 7 enterobacter sp,lane 8 klebsiella sp, lane 9 Lactobacilla sp, lane 10 Weisella ciberia, lane 11 Cronobacter sp*

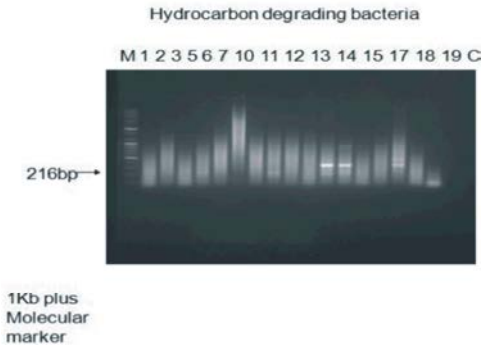

Fig. 3: Amplification of Catechcol 2,3 dioxygenase from both Bacteria Isolates
Lane 1-DNA marker(ladder) lane 2 –*Aspergillus niger*, lane 3 Rhizopus sp., lane -4 *Penicellium sp.,* Lane 5 *rhizopus sp.,* lane 6 *Aspergillus niger,* lane 7 *Penicellium sp, Lactobacilla sp.,* lane 10 *Weisella ciberia,* lane 11 *Cronobacter sp.,*. lane 13, *Enterobacter sp.,* lane 14 *Escherichia coli,* lane 15 *klebsiella sp.,* lane 17 *Shigella flexneri,* lane 18 *Cronobacter sp.,* lane 19- *klebsiella sp. sp.,*

bacterial biodegradative activities [20,21]. According to Onuoha *et al*. [21] the decrease could be attributed to decline in the availability of readily metabolizable hydrocarbons and exhaustion of nutrient in the medium.

16s rRNA GENE Amplification from Bacteria Isolates: The isolates result were further confirmed by 16s rRNA Sequencing. Based on DNA extracts of isolates (Fig. 2). 16s rRNA which amplified by PCR using 35 cycles and primers 16sF and 16sR was got sequence result and listed in Table 3-8.

Amplification Of Catechol 2,3, Dioxygenase Gene in Identified Hydrocarbon Degrading Microbes: Catechcol 2, 3 dioxygenase enzyme band size of 216bp was detected in *Enterobacter hormaechei, Escherichia coli* and *Shigella flexneri* (Fig. 3).

16s rRNA Sequences Result Were Aligned with BLAST Search of NCBI Data Bases: The bacterial 16s rRNA sequences result were aligned with BLAST search of NCBI d ata bases. The sequences aligned, gave 99%

Table 3: SEQUENCE AND BLAST RESULT OF *Enterobacter sp.*

CGGTAACAGGAANCANGCTTGCTGCTTCGCTGACGAGTGGNGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAAC
TACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGG
GATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA
CGGTCCANACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGTCC

Query 1 CGGTAACAGGAANCANGCTTGCTGCTTCGCTGACGAGTGGNGGACGGGTGAGTAATGTCT 60
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 2 CGGTAACAGGAAGCA-GCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCT 60

Query 61 GGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGT 120
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 61 GGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGT 120

Query 121 CGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATT 180
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Sbjct 121 CGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATT 180

Query 181 AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATG 240
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Sbjct 181 AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATG 240

Query 241 ACCAGCCACACTGGAACTGAGACACGGTCCANACTCCTACGGGAGGCAGCAGTGGGGAAT 300
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 241 ACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 300

Query 301 ATTGCACAATGGGCGCAAG 319
| | | | | | | | | | | | | | | | | | |
Sbjct 301 ATTGCACAATGGGCGCAAG 319

Enterobacter hormaechei strain B.Pat.50 16S ribosomal RNA gene, partial sequence
Sequence ID: gb|KF862936.1|Length: 860Number of Matches: 1
Related Information
Range 1: 2 to 319GenBankGraphics Next Match Previous Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
556 bits(616)	4e-155	315/319(99%)	1/319(0%)	Plus/Plus

Table 4: SEQUENCE AND BLAST RESULT OF *Escherichia sp.*

TCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCA
TAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGC
TCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCANC
GGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGA

Query 1 TCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATA 60
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 11 TCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATA 70

Query 61 ACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG 120
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 71 ACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG 130

Query 121 GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC 180
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 131 GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC 190

Query 181 CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG 240
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 191 CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG 250

Query 241 GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG 300
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Sbjct 251 GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG 310

Query 301 CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCANCGGGGAGGA 360
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 311 CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGA 370

Table 4: Cont.

Query 361 AGGGAGTAAAGTTAATACCTTTGCTCATTGA 391

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Sbjct 371 AGGGAGTAAAGTTAATACCTTTGCTCATTGA 401

Download GenBankGraphics Next Previous Descriptions Escherichia coli strain CICC 10667 16S ribosomal RNA gene, partial sequence Sequence ID: gb|KJ643937.1|Length: 1371Number of Matches: 1 Related InformationRange 1: 11 to 401GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score Expect Identities Gaps Strand

702 bits(778) 0.0 390/391(99%) 0/391(0%) Plus/Plus

Table 5: SEQUENCE AND BLAST RESULT OF *Lactobacillus sp.*

GGAANCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTA
GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGA
CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT
TGTAAAG

Query 1 GGAANCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTG 60

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Sbjct 71 GGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTG 130

Query 61 CCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACC 120

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Sbjct 131 CCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACC 190

Query 121 AAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAG 180

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Sbjct 191 AAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAG 250

Query 181 GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCAC 240

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Sbjct 251 GTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCAC 310

Query 241 ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA 300

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Sbjct 311 ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA 370

Query 301 TGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAG 359

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Sbjct 371 TGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAG 429

GenBankGraphics Next Previous Descriptions Lactobacillus plantarum strain AU5-800R 16S ribosomal RNA gene, partial sequence Sequence ID: gb|KF023220.1|Length: 760Number of Matches: 1

Related Information Range 1: 71 to 429GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score Expect Identities Gaps Strand

645 bits(714) 0.0 358/359(99%) 0/359(0%) Plus/Plus

Table 6: SEQUENCE AND BLAST RESULT OF *Shigella sp.*

CGGTAACAGGAANCAGCTTGCTGCTTCGCTGACGAGTGGNGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACT
ACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGG
ATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGC
CTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACNTtaCcCGCaGAAGAA

Query 1 CGGTAACAGGAANCAGCTTGCTGCTTCGCTGACGAGTGGNGGACGGGTGAGTAATGTCTG 60

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Sbjct 12 CGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTG 71

Query 61 GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTC 120

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Sbjct 72 GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTC 131

Query 121 GCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTA 180

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Sbjct 132 GCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTA 191

Table 6: Cont.

Query 181 GCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA 240
Sbjct 192 GCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA 251

Query 241 CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA 300
Sbjct 252 CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA 311

Query 301 TTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT 360
Sbjct 312 TTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT 371

Query 361 TGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACNTT 420
Sbjct 372 TGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTT 431

Query 421 ACCCGCAGAAGAA 433
Sbjct 432 ACCCGCAGAAGAA 444

Download GenBankGraphics Next Previous Descriptions

Shigella flexneri partial 16S rRNA gene, isolate 2P1A12 Sequence ID: emb|HF936916.1|Length: 776Number of Matches: 1 Related Information Range 1: 12 to 444GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
771 bits(854)	0.0	430/433(99%)	0/433(0%)	Plus/Plus

Table 7: SEQUENCE AND BLAST RESULT OF *Weissella sp.*

TCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGANGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGA
TAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGA
GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAG
AAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGNGNTAAGGTTAATANCNTTGNNNATTGACGTTACCCGCAGAAGAAGC
ACCGGCTAACTCCGTGCCANCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTTTGT

Query 1 TCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGANGGGTGAGTAAT 60
Sbjct 53 TCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAAT 112

Query 61 GTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATA 120
Sbjct 113 GTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATA 172

Query 121 ACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGG 180
Sbjct 173 ACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGG 232

Query 181 GATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG 240
Sbjct 233 GATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG 292

Query 241 GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG 300
Sbjct 293 GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG 352

Query 301 GAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT 360
Sbjct 353 GAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTT 412

Query 361 CGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGNGNTAAGGTTAATANCNTTGNNNATTG 420
Sbjct 413 CGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTG 472

Table 7: cont.

Query 421 ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCANCAGCCGCGGTAATACGGAGG 480
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 473 ACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGG 532

Query 481 GTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCACGCGGTTTGT 532
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 533 GTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGT 584

Download GenBankGraphics Next Previous Descriptions Weissella cibaria strain AU2-800R 16S ribosomal RNA gene, partial sequence Sequence ID: gb|KF023260.1|Length: 752Number of Matches: 1 Related Information Range 1: 53 to 584GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
919 bits(1018)	0.0	521/532(98%)	0/532(0%)	Plus/Plus

Table 8: SEQUENCE AND BLAST RESULT OF *Klebsiella sp.*

TACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGG GATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGG CCTTCGGGTTGTAAAGTACTTTCANCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGA

Query 1 TCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATA 60
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 60 TCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATA 119

Query 61 ACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG 120
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 120 ACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCG 179

Query 121 GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC 180
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 180 GGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCAC 239

Query 181 CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG 240
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 240 CTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG 299

Query 241 GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG 300
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 300 GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG 359

Query 301 CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCANCGGGGAGGA 360
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 360 CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGA 419

Query 361 AGGGAGTAAAGTTAATACCTTTGCTCATTGA 391
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 420 AGGGAGTAAAGTTAATACCTTTGCTCATTGA 450

Klebsiella pneumoniae. clone HL-B65 16S ribosomal RNA gene, partial sequence
Sequence ID: gb|KR149406.1|Length: 863Number of Matches: 1
Related Information
Range 1: 60 to 450GenBankGraphics Next Match Previous Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
702 bits(778)	0.0	390/391(99%)	0/391(0%)	Plus/Plus

result similarity with *Enterobacter hormaechei, Escherichia coli, Lactobacillus plantarum, Shigella flexneri* while *Weissella cibaria* had 98% similarity (Table 3-8). These results highlight the different species of bacteria strains involve in hydrocarbon degradation.

The Growth Potential of Hydrocarbon Utilizing Bacteria (Hydrocarbons by Turbidometry): The results in Fig.1 demonstrated that the bacteria *Enterobacter, Escherichia, Klebsiella, Shigella, Wesiella* and *Lactobacillus spp.* had the ability to degrade hydrocarbon. The result of analysis shows that there is a significant difference in the overall growth rate readings at 595nm for 14 days of incubation. The overall result also indicated that growth rate increase significantly from the 4th to 12th day. *Lactobacillus plantarium* growth rate had lag, exponential, stationary and death phases. MBC, *shigella flexneic, Weisella cibaria* and *kleibsiella spp* growth rate observed was exponential,

stationary and death phases while *Enterobacteria hormaechei* and E.coli exhibited exponential and stationary growth phase (Fig. 1). The test on the degrading activity of isolates on hydrocarbon from engine oil contaminated samples revealed that bacteria genus *Escherichia coli, Enterobacter* spp., *lactobacillus* spp*., Shigella* spp*., Wesiella* spp*., Klebsiella* spp *and* mixed bacteria consortium (MBC) were potent degraders of hydrocarbons with 6.5%, 19.4%, 6.5%, 21.9%, 10.7%, 17.2% and 70.3% biodegradation rate respectively within the days of the study. The above analysis shows that the MBC has significantly ($p<0.05$) lower optical density, implying that it has the highest growth. While *Klebsiella* spp. has significantly ($p<0.05$) higher optical density, implying that it has the least growth. Mixed bacterial consortium observed high bioremediation potential (70.3%) compared to single isolates whose bioremediation potential observed in decreasing order were E.coli (6.5), *Shigella spp* (21.9) *Enterobacter spp* (19.4) *Klebsiella spp (*17.2), *Weisella spp* (10.7) and *Lactobacillus spp* (6.5). MBC showed high percentage degradation of hydrocarbon which might be attributed to the synergistic effect between the catabolic enzymes in the six bacteria isolates. These findings correspond with the result obtained by Abdullah *et al*. [22] whose research reported biodegradation rates at 97% degradation by MBC.

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