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RESEARCH ARTICLE

ISOLATION, SCREENING AND MOLECULAR IDENTIFICATION OF NATIVE HYDROCARBON DEGRADING BACTERIA FROM OIL CONTAMINATED SOIL, KRI, IRAQ

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ABSTRACT

There is little information on native hydrocarbon degrading bacterial species, for restoration (bioremediation) of KRI oilfield wastes, Iraq. Thus, an attempt was made to screen, isolate then identify native bacterial isolates, from oil contaminated environments, over five major oilfields. The identification was mainly based on traditional methods and recognized by molecular techniques as confirmatory/comparative analysis. Dynamics of bacterial association was assessed by application of polymerase chain reaction (PCR) method, then reinforcement process of 16S rRNA gene fragment, which occurs right after denaturing gradient gel electrophoresis (DGGE). Out of 55 isolated bacterial strains; three genera (i.e. Bacillus sp., Kocuria sp. and Pseudomonas spp.) were identified as the most potent for hydrocarbon degradation. DNA sequence and phylogenetic analysis confirmed the validity of the species as; Bacillus subtilies-MK000710; Kocuria rosea-MK648258; Pseudomonas fluorescens-LR134300.1 and Pseudomonas putida-GQ303714.1. The four bacterial isolates followed an equal order of occurrence at different geographical locations within the oilfields.

KEYWORDS

Hydrocarbon degrading bacteria 1; Molecular identification 2; oil-laden soil 3; KRI oilfields 4, Iraq 5.

1. INTRODUCTION

Adequate management of all waste streams produced during the production life cycle, from the initial planning of projects and operations to the decommissioning and restoration of the site, is necessary for the sustainable development of petroleum resources.These days, bioremediation of hydrocarbon-contaminated soils is a proven technique (Al-Hawash et al., 2018). By introducing exogenous microbial populations or promoting native ones, bioremediation—an economical and environmentally friendly technique—aims to greatly increase the rates of natural degradation. Numerous bacteria that degrade oil have been identified, and research has been done on their capacity to degrade oil. The majority of research on bioremediation has been conducted on pure cultures, and it is still unclear what functions these bacteria would play in the natural world .Microbiologists have found it particularly difficult to analyze the microbial communities involved in in-situ hydrocarbon biodegradation processes (Alsohail and Bani-Hasan, 2018). The quick development of molecular ecology techniques has sparked interest in this field, molecular identification methods based on total DNA extraction offer a distinctive barcode for the identification and determination of various isolates of microorganisms up to the species level. Phylogenetic analysis and partial 16S rRNA gene sequencing via PCR amplified part can provide a more accurate molecular identification of bacteria. It is a great effort to isolate strong native haydrocarbon-degrading bacterial strains that have been identified at the species level through molecular strategies in order to restore hydrocarbon-contaminated soils through bioremediation (Al-Hawash et al., 2018). Thus, the purpose of this study was to screen, isolate, and identify the native (indigenous) bacterial species that degrade hydrocarbons from oil-laden soils, or oilfields, across a range of geographical locations in the Kurdistan Region of Iraq (KRI), Iraq.

2. MATERIALS AND METHODS

2.1 Sample collection

Samples of soil contaminated by oil were collected from 41 drilling waste pits. The pits were dispersed throughout five significant oil and gas fields in KRI, Iraq, each of which was situated in a different geographic location. A tiny hand auger was used to gather the sample collection. 100 g representative samples that ranged in depth from 0 to more than 15 cm were extracted (Ahmad and Ganjo, 2020). Three to four samples collected over several square meters were homogenized to create each sample, which was then put into sterile nylon bags.

2.2 Enrichment and isolation of petroleum hydrocarbon degrading bacteria

In order to isolate bacterial populations from oil-contaminated soils, 10 g of each soil were diluted in 90 ml of Basal Saline Medium (BSM), which contained the following amounts of grams per liter: Na2HPO4, 4.3; K2HPO4, 4.2; MgSO4.7H2O, 5.5; CaCl22H2O, 0.06; (NH4)2HPO4, 2.7. For 40 days, cultures were incubated at 30°C and 200 rpm of continuous agitation. The culture supernatant, about 100 µl, was used to inoculate BSM plates that followed (Tamura et al., 2013). Individual colonies were removed from the plates and placed onto fresh BSM plates for molecular identification and characterization after the plates were incubated at 30°C for an additional 4 days. Gas chromatography mass spectrophotometer (GC-MS) analysis of control samples (i.e. (i.e. oil contaminated soil without bacterial isolates) was performed.

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2.3 Molecular identification and characterization of bacterial isolates

The Genomic DNA extraction kit (Promega, USA) was utilized to extract genomic DNA from bacterial isolates. Using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-CTACGGCTACCTTGTTACGA-3′) to amplify a 1,450-bp fragment of the bacterial 16S rRNA gene, isolates were identified by 16S rRNA sequencing. The products of the polymerase chain reaction (PCR) were purified using the Column-pure DNA Gel Recovery Kit (ABM inc., Canada) and analyzed by agarose gel electrophoresis. Using oligonucleotides 27F/1525R as sequencing primers and an Applied Biosystems ABI 3100 genetic analyzer, BigDye Terminator v3.1 Cycle Sequencing Kit (Invitrogen, USA) was used to perform DNA sequencing.

Microorganism identification and homology searching were done using the Basic Local Alignment Search Tool (BLAST) (Huerta-Cepas et al., 2016). The MEGA-6 software package was utilized for conducting phylogenetic analysis. The international Robinson pipette method, as detailed in a study, was used to determine the soil texture (Dilmi et al., 2017). Using a 1:1 soil to water ratio, the glass electrode pH meter (Adwa pH-Adwa Microprocessor pH meter) measured the pH of soil samples. The methodology used to determine the chloride content was that of (Haddadi and Shavandi, 2013). The TPH concentration was determined by gas chromatography (EPA method number 8015) with an HP 6890 GC-MS equipped with a flame ionization detector (FID). As per researchers, counts of colony forming units (cfu) were kept track of (Al-Mailem et al., 2013). Using the formula described in the frequency, occurrence, and incidence of bacterial isolates as well as the percentage of hydrocarbon degradation (%) were determined (Okparanma et al., 2009).

3. RESULTS

3.1 Oil contaminated soil properties

Table 1 presents the properties of an oil-contaminated soil sample, which may give an on-land image of the preferred habitat used by bacterial communities that break down hydrocarbons. The pH had an average of 6.9 and ranged from 6.5 to 9.3. Chloride concentrations reached as high as 4.0%. It was observed that the level of total petroleum hydrocarbon (TPH), which accounted for up to 31.3%, exceeded the 10,000 mg/kg threshold previously set by the Iraqi Department of Petroleum Resources. The soil samples' texture ranged from fine to coarse particulates (4 µm to 15 mm), with sand, silt, and clay predominating. The water content of the samples was between 18 and 36%, while the liquid limits were between 31 and 68%.

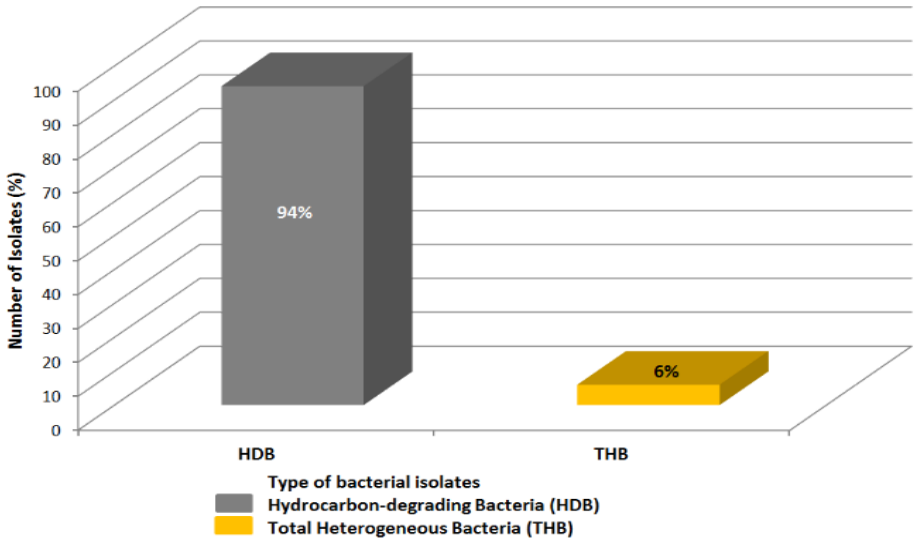
Table 1: Value and range of properties of the oil contaminated soils

Property	Value*	Texture	Value
pH	6.5 – 9.3	Particle size	4 µm – 15 mm
Chloride	Up to 4.0%	Gravel	1 – 4%
Hydrocarbons (TPH)	Up to 31.3%	Sand	34 – 59%
Water content (%)	18 – 36%	Silt	16 – 42%
Liquid limit	31 – 68%	Clay	18 – 28%

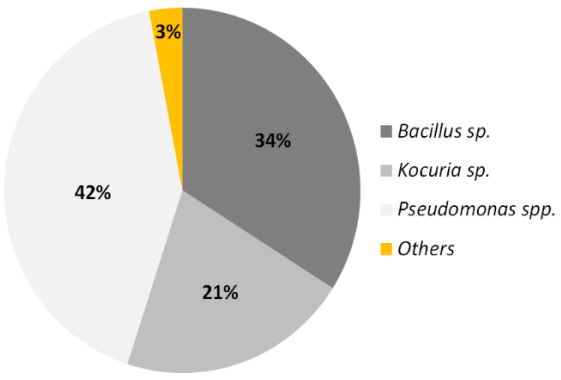
* The value is mean from 150 samples, collected at 5 major oilfields

3.2 Isolation and morphological features of petroleum hydrocarbon degrading bacteria

Among the 55 bacterial isolates, the majority hydrocarbon-degrading genera were Bacillus sp., Kocuria sp., and Pseudomonas spp. Conversely, the majority of the heterogeneous isolates were Aeromonas, Staphylococcus, Micrococcus, and Corynebacterium species, among others. In general, the hydrocarbon-degrading bacteria's (HDB) incidence and frequency of occurrence (%)in the oilfields were greater than those of total heterogeneous bacteria (THB), with 94% of HDB and only 6% of THB occurring frequently (Figure 1A). As illustrated in Figure (1B), the mean percentage occurrence of Bacillus sp., Kocuria sp., Pseudomonas spp., and other isolates was 34%, 21%, and 42%, respectively, out of the 94% of HDBs.



(A)



(B)

Figure 1: Frequency of occurrence % (A), along mean percentage occurrence (B) of HDB and THB isolates in the oil-laden soil samples.

The four (4) bacterial isolates that showed positive growth on hydrocarbon compounds and crude oil were identified as Bacillus subtilies, Kocuria rosea, Pseudomonas fluorescens, and Pseudomonas putida and are shown in the self-explanatory Plate (1) (a, b, c, and d), respectively. The isolated bacteria were first examined based on cultural, microscopic, and morphological characteristics.

(a): *Bacillus Subtilies* LC6-B **(b): *Kocuria rosea* GL11**

(c): *Pseudomonas* **(d): *Pseudomonas putida* W30**

Plate 1: Morphological characters/ gram stain for the isolated hydrocarbon degrading bacteria, grown on BSM agar with crude oil

3.3 Molecular identification and DNA extraction from isolated bacteria

Using PCR and Low-Stringency Single Specific Primer PCR, the genes involved in hydrocarbon degradation were found and characterized molecularly. Figures (2A) and (2B) display the amplified product of 16S rRNA and the DNA extraction for the four strong bacterial isolates of concern along with the negative control. The Bioscience Bacteria DNA preparation kit (Jena Bioscience GmbH 07749 Jena Germany) was used to isolate the genomic DNA. It is intended to facilitate the quick and easy isolation of genomic DNA from gram-positive and gram-negative bacterial samples. A 1% Agarose gel was used for the electrophoresis of the isolated DNA (Figure 2B). The primers were able to produce a band that was approximately 1500 bp in size. 1.5% Agarose gel was used to electrophorese and visualize the PCR product. It was shown that the primers produced~up to 1000 bp band as shown in Figure (2B).

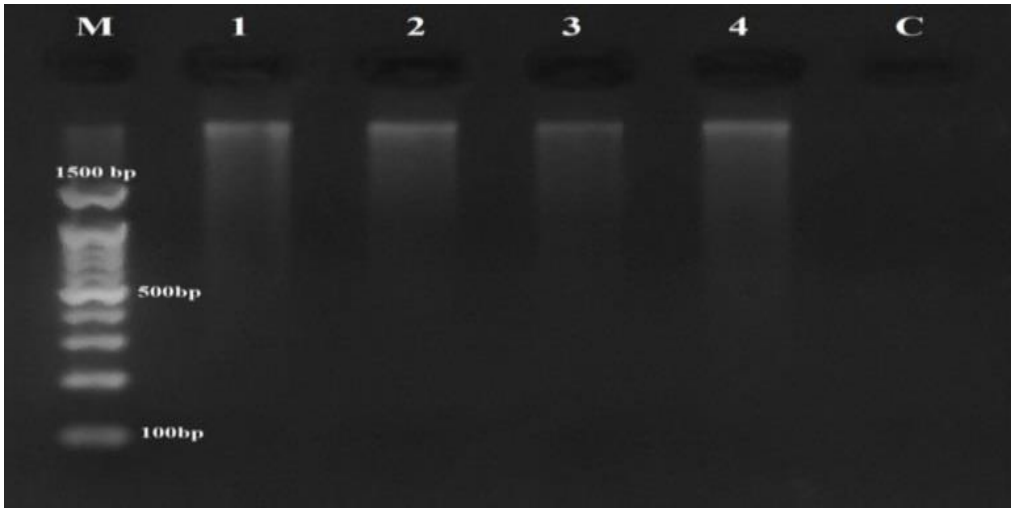


Figure (2A): Genomic DNA isolated from HDBs, DNA extraction product from bacterial isolates (1, 2, 3 and 4), M = markers, and C is control.

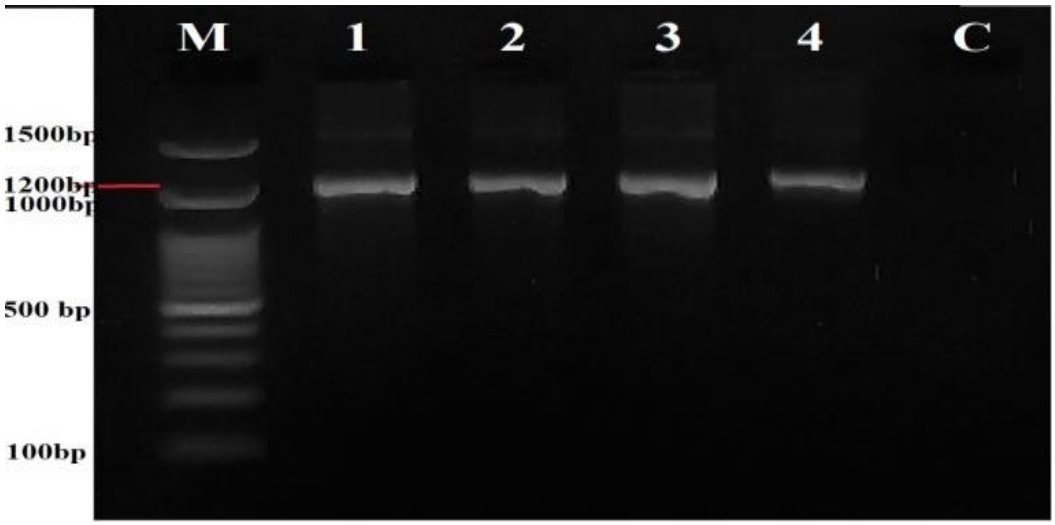


Figure (2B): Agarose gel electrophoresis of PCR amplification product using 16S rDNA primers: lane M: Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific). M = markers; Lane 1= *Bacillus subtilies*-MK000710.1, Lane 2= *Kocuria rosea*-MK648258.1, Lane 3= *Pseudomonas fluorescens*-LR134300.1, Lane 4= *P. putida*-GQ303714.1 and C is control.

The bacteria were identified using the homology index, and the NCBI's blast tree view was used to build the phylogenetic tree (Table 2) and (Figure 3). Based on the 16S rRNA nucleotide sequence, phylogenetic analysis grouped the four bacteria species under investigation along expected lines. Using phylogeny construction and sequence divergence similarity data, it was possible to determine how closely related the species in each genera were to one another. Bacillus Subtilies LC6-B, Kocuria rosea GL11, Pseudomonas fluorescens NCTC10783, and Pseudomonas putida W30 from the NCBI blast program were the four HDBs that were clustered together in a single cluster with high similarity (Figure 3).

Table 2: Identification of most potent isolated HDBs, along with GenBank accession number and GenBank species identification.

Query Cover (%)	Identity (%)	GenBank Accession Number	GenBank Identification	Country Identification
96	99.53	MK000710	*Bacillus Subtilies* LC6-B	Sri Lanka
97	93.46	MK648258	*Kocuria rosea* GL11	India
86	93.93	LR134300.1	*Pseudomonas fluorescens* NCTC10783	United Kingdom
91	94.94	GQ303714.1	*Pseudomonas putida* W30	China

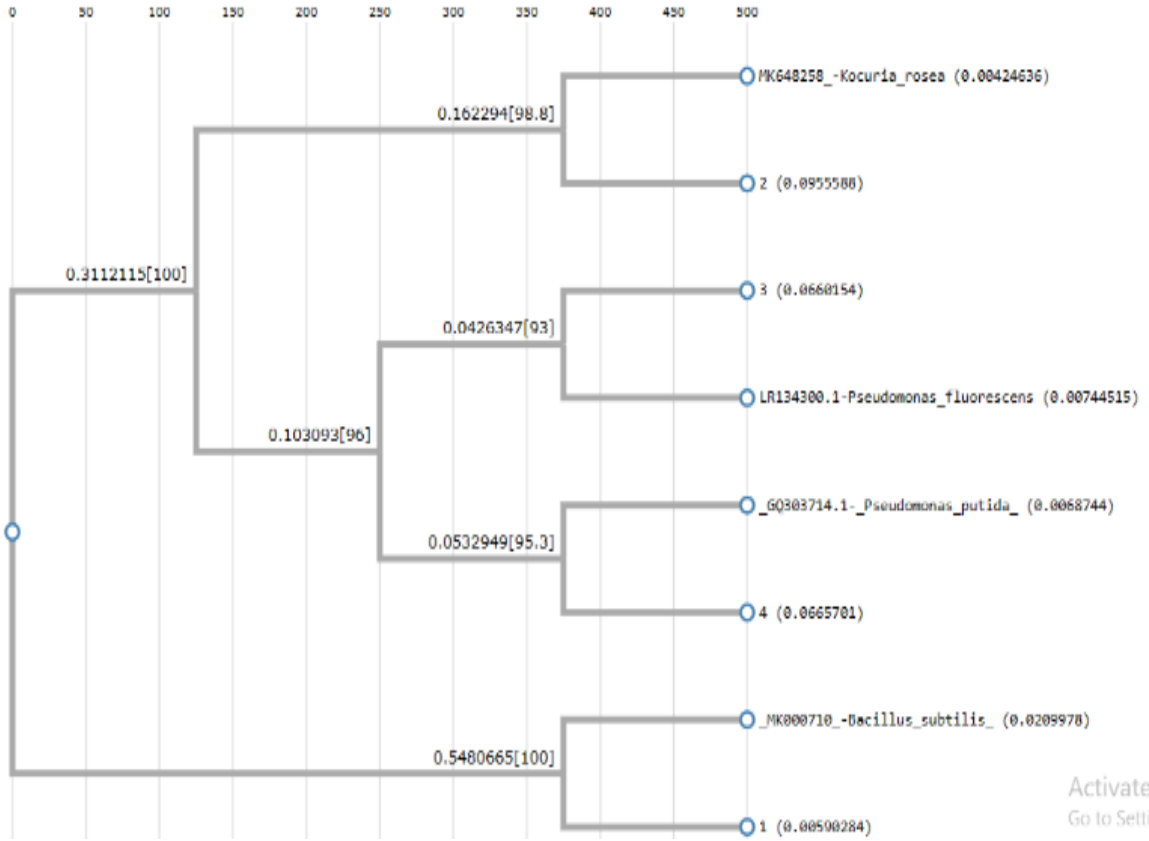
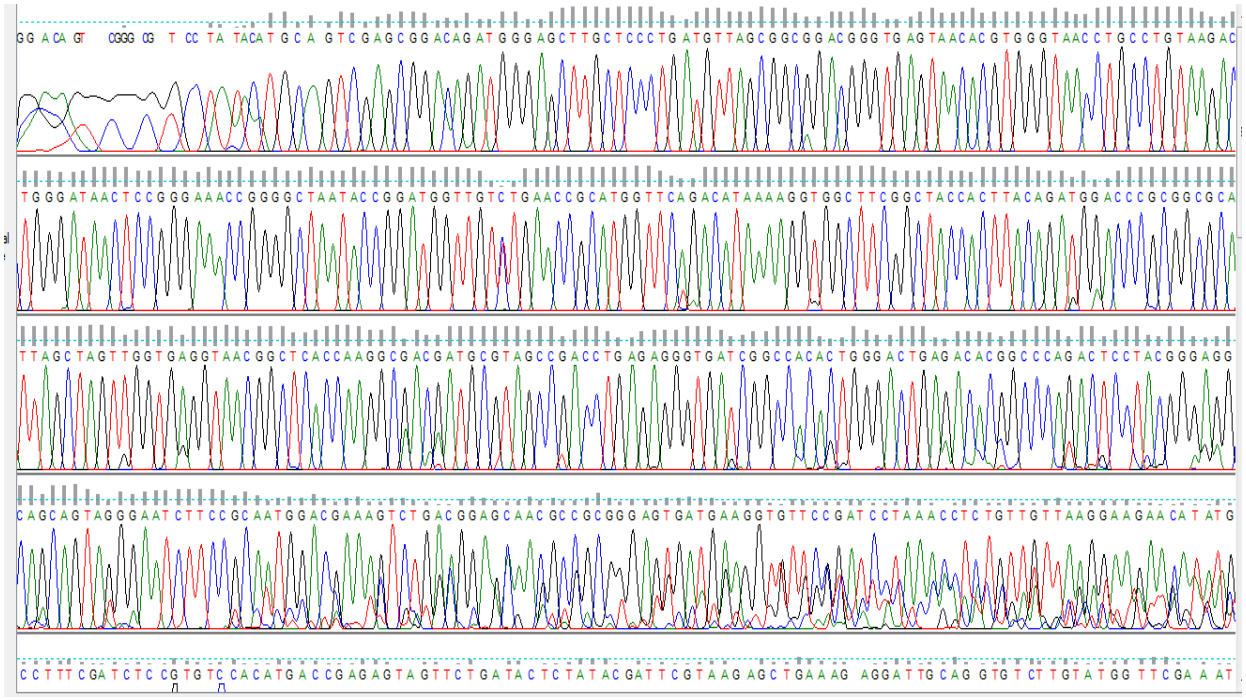


Figure 3: Phylogenetic tree for the four potent HDBs based on 16S rDNA sequence. Mega blast program of NCBI blast shows phylogenetic positioning of the four samples according to sequences of 16S rRNA employing maximum likelihood available in GenBank sequence.

3.4 Partial 16S rRNA gene and molecular identification of genus and species of HDBs

DNA sequencing, using forward and reverse primer; UNIBACF (AGAGTTTGATCCTGGCTCAG) UNIBACR (CTACGGCTACCTTGTTACGA) was performed separately by ABI 3130X genetic analyser (Applied Biosystem). Sequence-specific PCR amplification used the PCR products of the four bacterial strains as a source of DNA template. The BLAST program from GenBank (http://blast.ncbi.nlm.nih.gov/) provides the four 16S rRNA sequence samples with sizes ranging from 100 to 1500, which are used to compare our amplified sequences with other species of bacterial sequences that are stored. The BLAST was used to gather the results.

Figures 4(a and b), 5(a and b), 6 and 7(a and b) show the chromatography sequence results of 16S rRNA sequence (a) and NCBI blasting pairwise alignment of 16S rRNA query sequence (b) for the bacterial species of concern, respectively. The corresponding author can provide full details on the tabular overview of BLASTN result, including hits identifiers and scoring 16S rRNA sequences for the bacterial species of concern.



(a)

Score	Expect	Identities	Gaps	Strand
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Sbjct  5    GGCGTGCTATACATGCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCG  64

Query  529  GACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGG  588
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  65   GACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGG  124

Query  589  GGCTAATACCGGATGGTTGTCTGAACCGCATGGTTCAGACATAAAAGGTGGCTTCGGCTA  648
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  125  GGCTAATACCGGATGGTTGTCTGAACCGCATGGTTCAGACATAAAAGGTGGCTTCGGCTA  184

Query  649  CCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC  708
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  185  CCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC  244

Query  709  GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG  768
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  245  GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG  304

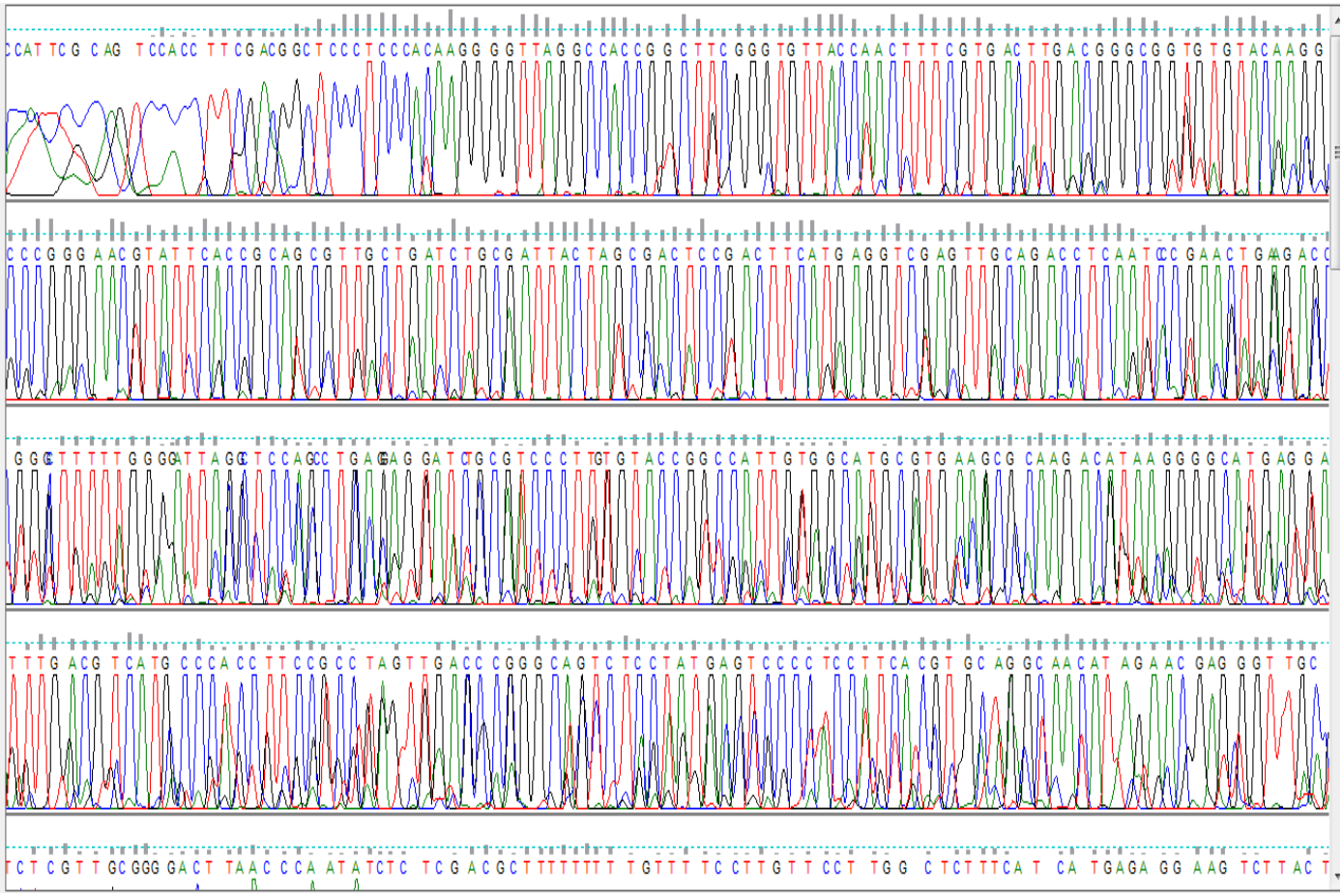
Query  769  ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA  828
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  305  ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA  364

Query  829  CGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGT  888
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  365  CGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGT  424

Query  889  GCCGTT  894
            ||||||
Sbjct  425  GCCGTT  430
```

(b)

Figure 4: The chromatography sequence result of 16SrRNA sequence (a) and NCBI blasting pairwise alignment of 16SrRNA query sequence (b) for *Bacillus Subtilies* LC6-B



(a)

Score	Expect	Identities	Gaps	Strand
745 bits(403)	0.0	403/403(100%)	0/403(0%)	Plus/Minus

```
Query  1     GTCCACCTTCGACGGCTCCCTCCCACAAGGGGTTAGGCCACCGGCTTCGGGTGTTACCAA  60
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Sbjct  1407  GTCCACCTTCGACGGCTCCCTCCCACAAGGGGTTAGGCCACCGGCTTCGGGTGTTACCAA  1348

Query  61    CTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1347  CTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGC  1288

Query  121   TGATCTGCGATTACTAGCGACTCCGACTTCATGAGGTCGAGTTGCAGACCTCAATCCGAA  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1287  TGATCTGCGATTACTAGCGACTCCGACTTCATGAGGTCGAGTTGCAGACCTCAATCCGAA  1228

Query  181   CTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGTATCGCAACCCTTTGTACCGGCC  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1227  CTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGTATCGCAACCCTTTGTACCGGCC  1168

Query  241   ATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCATCCCCACC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1167  ATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCATCCCCACC  1108

Query  301   TTCCTCCGAGTTGACCCCGGCAGTCTCCTATGAGTCCCCACCATCACGTGCTGGCAACAT  360
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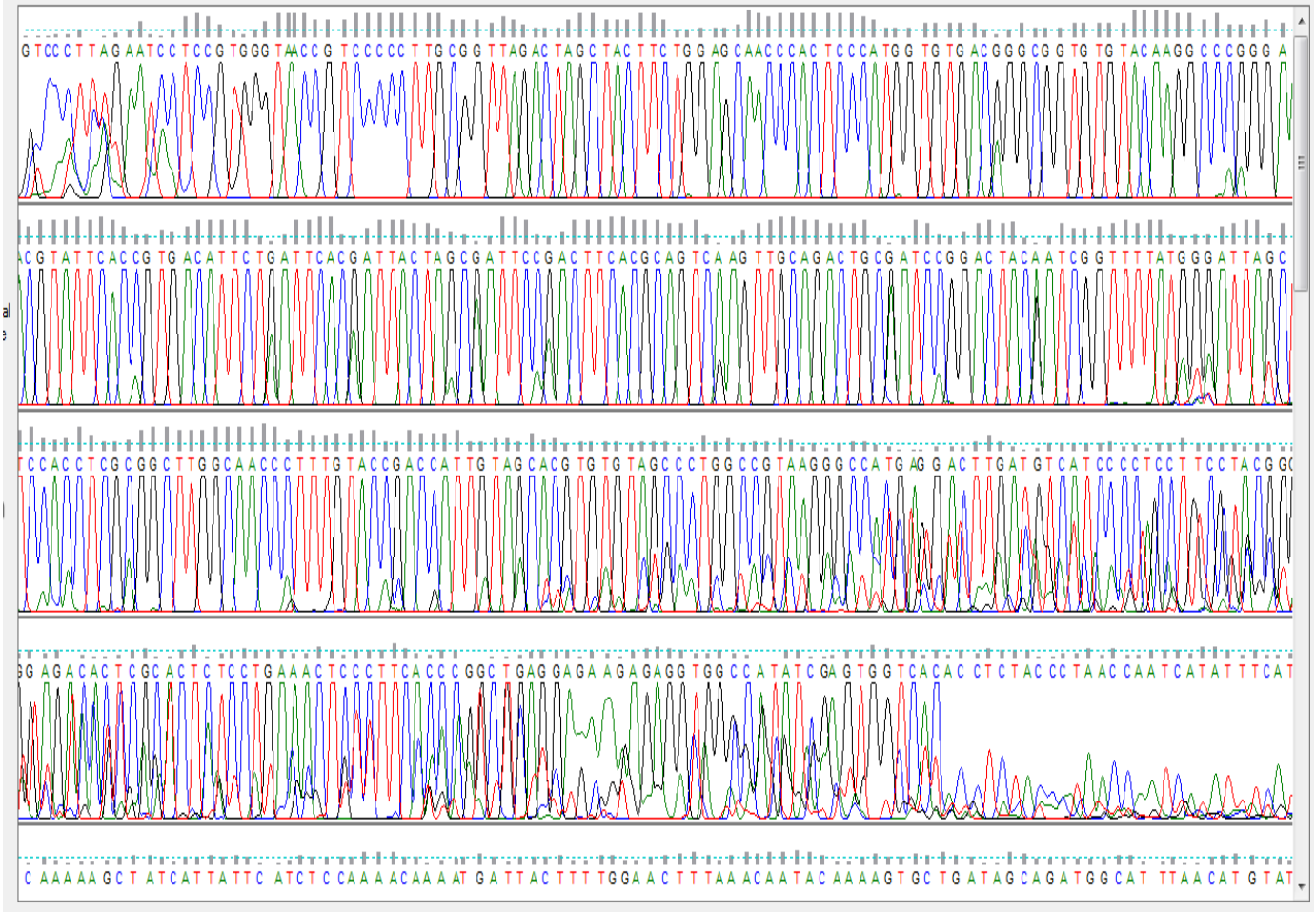
Sbjct  1107  TTCCTCCGAGTTGACCCCGGCAGTCTCCTATGAGTCCCCACCATCACGTGCTGGCAACAT  1048

Query  361   AGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTC  403
             |||||||||||||||||||||||||||||||||||||||||||
Sbjct  1047  AGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTC  1005
```

(b)

Figure 5: The chromatography sequence result of 16SrRNA sequence (a) and NCBI blasting pairwise alignment of 16SrRNA query sequence (b) for *Kocuria rosea* GL11



(a)

Score	Expect	Identities	Gaps	Strand
501 bits(271)	1e-137	300/314(96%)	1/314(0%)	Plus/Minus

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Sbjct  1436  ATCCACCGTGGTACCGTCCTCCCGAAGGTTAGACTAGCTACTTCTGGTGCAACCCACTCC  1377

Query  71    CATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATTCTGATT  130
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1376  CATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATTCTGATT  1317

Query  131   CGCGATTACTAACGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACG  190
             |||||||||||  ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1316  CGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACG  1257

Query  191   ATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGCCAACCCTCTGTACCGACCTTTGT  250
             |||||||||||||||||||||||||||||||||||  ||||||||||||||||||  ||||
Sbjct  1256  ATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTCTGTACCGACCATTGT  1197

Query  251   AGCATGTGTGTAACCCAGGACGTAATGGCCATGATGACTTGTCCTCATAACCACCTTCCT  310
             ||||  |||||||  ||||||  |||||  ||||||||||||||  |  ||||  ||||||||||
Sbjct  1196  AGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCT  1137

Query  311   TGGGTTTGTC-CCG  323
               ||||||||  |||
Sbjct  1136  CCGGTTTGTCACCG  1123
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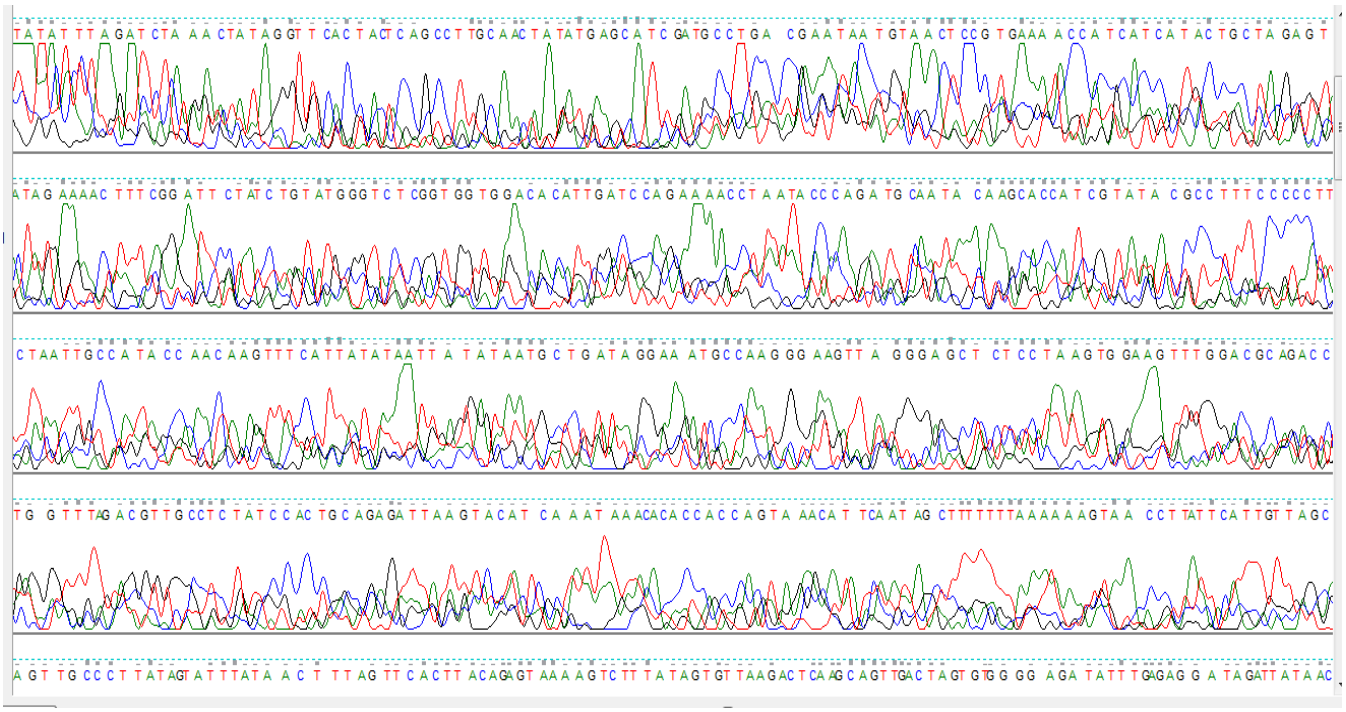
(b)

Figure 6: The chromatography sequence result of 16SrRNA sequence (a) and NCBI blasting pairwise alignment of 16SrRNA query sequence (b) for *Pseudomonas fluorescens* NCTC10783



(a)

Score	Expect	Identities	Gaps	Strand
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Query  6     ATGATCACACCGTGGTACCGTCCTCCCAGAAGGTTAGACTAGCTACTTCTAGGTGCAACC  65
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Query  66    CACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATT  125
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Query  126   CTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCAAGTTGCAGACTGCGATCCGG  185
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Sbjct  1321  CTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGG  1262

Query  186   ACTACAATCGGTTTTGTGAGATTAGCTCCACCTCGCGGCTTGTCGACCCTCGGTCCCGCC  245
             ||||| |||||||||||||||||||||||||||||||| | |||||| || ||| |
Sbjct  1261  ACTACGATCGGTTTTGTGAGATTAGCTCCACCTCGCGGCTTGGCAACCCTCTGTACCGAC  1202

Query  246   CTTTGTTGCATCTGTGT  262
             | |||| |||  |||||
Sbjct  1201  CATTGTAGCACGTGTGT  1185
```

(b)

Figure 7: The chromatography sequence result of 16SrRNA sequence (a) and NCBI blasting pairwise alignment of 16SrRNA query sequence (b) for *Pseudomonas putida* W30

4. Discussion

Numerous authors have described how hydrocarbon-degrading bacterial strains prefer a pH range of 7 to 8, a moderate chloride content, and the ability to use crude oil as their only source of carbon and energy (Faso, 2010; Wang et al., 2016; Cerqueira et al., 2011). Additionally, sandy and loamy textures help to circulate fluids, which carry nutrients and oxygen that microorganisms in the medium can access (Das and Adholeya, 2012). Sam can be concluded for the native bacterial communities isolated from KRI oilfields that degrade hydrocarbons.

Generally speaking, it can be said that HDB isolates can use crude oil as their only source of energy and carbon, unlike THBs. Nevertheless, the separated HDBs demonstrated that when cultured in an environment with a high hydrocarbon concentration, the number of their colonies increased (data not provided here). When compared to colonies grown on BSM supplemented with 0.5% crude oil as the sole source of carbon and energy, the number and size of colonies grown on BSM media alone (i.e., without hydrocarbon) appeared to be significantly lower. Previous research has led to similar conclusions from other authors (Das and Adholeya, 2012).

Conversely, the HDB isolates occurred in the same order at each of the oilfields' various geographic locations. Many authors have addressed a variety of factors that frequently affect the degradation efficiency of petroleum hydrocarbons by different microorganism communities in the soil environment (Das and Chandran, 2011; Diaz, 2008; Fatihah et al., 2014).

First, the soil bacterial fraction is separated from soil debris and fungal mycelia as part of the DNA isolation protocol. It was guaranteed that the extracted DNA was bacterial in origin and not fungal or from another source by first separating the bacterial fraction from the soil. Additionally,

it was intended to shield the DNA from direct soil contact because nucleic acids in the soil are easily broken down by microorganisms that produce nuclease (Liu and Voigt, 2010). Given that alkali extraction is the most popular technique for recovering humic and fulvic acids from soil, it is also probable that direct DNA extraction techniques involving alkaline hydrolysis would contaminate the DNA with these substances (Mohamed et al., 2006). The National Centre for Biotechnology Information (NCBI)'s BLAST program was used to perform a homology search on the sequences (Liu and Voigt, 2010).

The majority of taxonomy researchers concur that DNA coding is a helpful method for identifying and classifying different species of microorganisms (Koshlaf et al., 2016). It has been demonstrated that molecular methods, like PCR and DNA sequencing, are extremely sensitive and specific for identifying different species of organisms (Madigan et al., 1997). Molecular biology and the molecular diagnosis of microorganisms have been transformed by molecular techniques, especially the PCR technology. The findings demonstrated that Bacillus subtilies (MK000710), Kocuria rosea (MK648258), Pseudomonas fluorescens (LR134300.1), and Pseudomonas putida (GQ303714.1) could be successfully identified using morphometric data and molecular methods.

5. Conclusion

Using conventional techniques, the most effective genera for hydrocarbon degradation were found to be Bacillus sp., Kocuria sp., and Pseudomonas spp. out of 55 isolated bacterial strains. The validity of the following species was confirmed by DNA sequencing and phylogenetic analysis: Bacillus subtilies (MK000710), Kocuria rosea (MK648258), Pseudomonas fluorescens (LR134300.1), and Pseudomonas putida (GQ303714.1).

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