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Research Article

Screening and Identification of Diesel Oil Degrading Bacterial Isolates from Petroleum Contaminated soil of Barmer

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ABSTRACT

In present world explosion and expansion of pollution is an important catastrophe. There has been an intense investigation on the impacts of pollution on environment which has led to find out the measures for their remediation. In the present work diesel degrading bacterial strains were isolated and screened by streaking a total of 21 bacterial isolates obtained from oil contaminated soil over Bushnell Haas Agar supplemented with 1% (v/v) filter sterilized Diesel as sole carbon source. Efficient diesel degrading bacterial strains 1A, 3E, 4F and 4H were found and identified as Bacillus coagulans, Bacillus cereus, Bacillus subtilis and Bacillus megaterium respectively based on phenotypic, biochemical and 16s rRNA phylogenetic analysis. Hydrocarbon degradation analysis of extracted diesel oil from contaminated soil was done using GCMS, to evaluate the degradation of various components with respect to time by different diesel degrading strains which showed almost total disappearance of the corresponding peak of each compound present in diesel oil. So this study reveals that microorganisms degrading hydrocarbon pollutants are ubiquitously present and can be isolated for bioremediation purpose and it also adds on in the pool of information regarding bioremediation by microorganisms.

Keyword: Hydrocarbon; diesel oil; bacterial isolates; GC MS; bioremediation

INTRODUCTION

Petroleum continues to be used as the principle source of energy in urban as well as in rural areas. However despite of its important uses, petroleum hydrocarbons also pose as a globally environmental pollutant [1]. Diesel oil is a common product of crude oil distillation with a very complex composition. It consists mainly of low molecular weight alkanes and polycyclic aromatic hydrocarbons (PAHs) [2]. It consists mainly of aliphatic hydrocarbons ranging from C9 to C23 and number of aromatic compounds [3]. The fate of the later compounds in nature may be of great human health importance, since PAHs have been considered toxic for plants and carcinogenic to people [2, 4, 5]. The spillage of petroleum hydrocarbon due to accidents during transportation, anthropogenic activities and various others reasons leads to contamination of air, soil and water. Uncontrolled releases of these compounds into soil and ground water are frequent as a result of accidents or poor control practices and attract public interest [6, 7, 8]. In case of an uncontrolled industrial leakage, diesel

oil and its constituents might act as a persistent water and soil pollutant. Petroleum compounds can decrease the availability of water, oxygen and nutrients in the soil [9].

Oil spills, especially in soil contamination have prompted research on cost-effective, environmentally benign cleanup strategies [10]. Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants [11].

Bioremediation processes have been found to be an efficient method for remediation of petroleum by-products, pesticides and other potential harmful chemical [12]. Biodegradation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contaminants, has been established as an efficient, economic, versatile and environmentally sound treatment [13]. Since hydrocarbons are natural products, it is not surprising to find organisms that are able to degrade these energy-rich substrates [14]. It is the subject of many research investigations and real-world applications and it is the basis for the emergent field of bioremediation [8].

The present study aims for the confirmation of hydrocarbon degrading activity in the contaminated soil samples and isolation, screening and identification of efficient diesel degrading bacterial strains from contaminated soil samples.

MATERIALS AND METHODS

Collection of Contaminated Soil Samples

Motor garage and Automobile repair shops in Barmer city of Marwar region of western Rajasthan (district is located between 24,58' to 26, 32'N Latitudes and 70, 05' to 72, 52' E Longitudes) was selected as soil sampling area. Samples were collected in sterile container and were transported by icebox to laboratory. All collected samples were air dried, sieved through 2mm mesh size and stored at 4°C.

Isolation and Screening of Diesel Degrading Bacteria

Enumeration of bacterial population was done by plating 0.1ml of serially diluted soil sample on nutrient agar. Diesel degrading bacterial strains were screened by streaking them over Bushnell Haas Agar supplemented with 1% (v/v) filter sterilized Diesel as a sole carbon source, that permit the growth of only hydrocarbon degrading bacterial population. [15].

Confirmation of Hydrocarbon Degrading Activity of Bacterial Isolates and Their Identification

For confirmation of hydrocarbon degrading activity of microorganisms in petroleum contaminated soil sample extraction of oil from soil sample was done by mixing 5gm of soil sample with 50ml of dichloromethane in separating funnel. The organic phase was passed through Na_2SO_4 concentrated to 0.2ml. It was analyzed by GCMS. GC-MS was performed with Thermo GC 1300 and "TSQ 8000 "Triple Quadrupole GC-MS MS SYSTEM with auto sampler Al 1310. Gas Chromatography 1300 fused with a GC column TG-5MS AMINE. The column length was 30 m with internal diameter 250 µm; coated film 0.25µm. The conditions were as follows: PTV Temp. Program: 70 °C, hold 2.00 min, 10 °C/min to 270 °C, hold 10 min. Carrier gas helium flow rate 1ml/min, split ratio 1:50. GC is equipped with auto-sampler AI 1300 and sample volume was 1µ litre. The Elutes were automatically passed into a mass spectrometer. GC mass Spectrum analysis was conducted using TSQ8000 with transfer line temperature 280°C and ion source temperature 230°C in EI mode. Mass scan time was 4 min with full Scan MS. The mass spectrum was also equipped with a computer fed NIST mass Spectra data library. Chemical constituent components of the extracts were identified by matching the peaks with Computer NIST MS libraries and confirmed by comparing mass spectra of the peaks of literature.

Bacterial isolates were identified by 16S rRNA sequencing and phylogenetic analysis. Bacterial Genomic DNA was isolated by using the InstaGeneTM Matrix Genomic DNA isolation kit.

Using below 16S rRNA Universal primers gene fragment was amplified using MJ Research Peltier Thermal Cycler. The Universal primers 27F and 1492R (27F, AGAGTTTGATCMTGGCTCAG; 1492R, TACGGYTACCTTGTTACGACTT) were used to obtain a PCR product of approximately 1,400bp in the case of bacteria. Include a positive control (E.coli genomic DNA) and a negative control in the PCR. The PCR product was sequenced using the 518F and 800R (518F,

5'CCAGCAGCCGCGGTAATACG -3'; 800R, TACCAGGGTATCTAATCC -3') primers. Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The full multiple sequences were aligned using the program MUSCLE 3.7 [16]. The resulting aligned sequences were cured using the progam Gblocks 0.91b. [17]. Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model. The program Tree Dyn 198.3 was used for tree rendering [18].

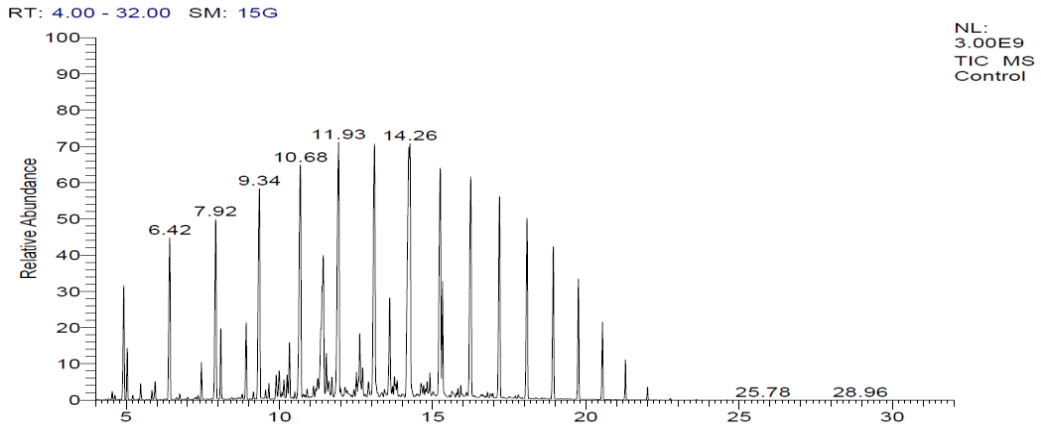
RESULTS

Isolation and Screening of Diesel Degrading Bacteria

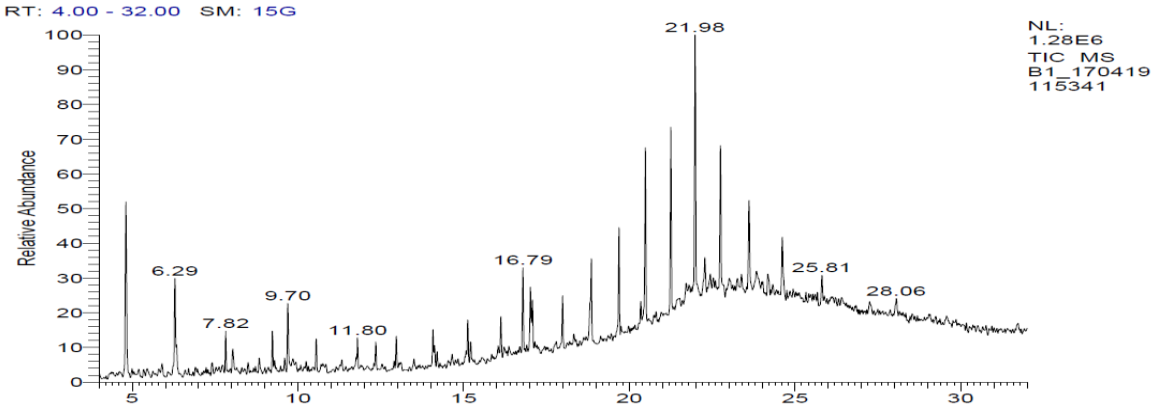
Total 8 hydrocarbon degrading bacterial strains were isolated from the contaminated soil samples using Buhsnell Haas medium with diesel oil as sole source of carbon. Out of 8 bacterial isolates 4 predominant bacterial isolates 1A, 3E, 4F and 4H were identified as potential hydrocarbon degraders and used for further investigation.

For confirmation of hydrocarbon degrading activity in contaminated soil GC-MS analysis of control i.e. Diesel oil (without microorganisms) was done which showed it was a mixture of different hydrocarbons and further it was compared with GCMS results of oil extracts from contaminated soil. Bacterial micro flora were found actively able to degrade total mixture of hydrocarbons present in diesel oil contaminated soil samples collected from motor workshop area of Barmer. The result was confirmed by almost total disappearance of the corresponding peak of each compound (shown in Fig 1. B, C, D, E).

A.



B.



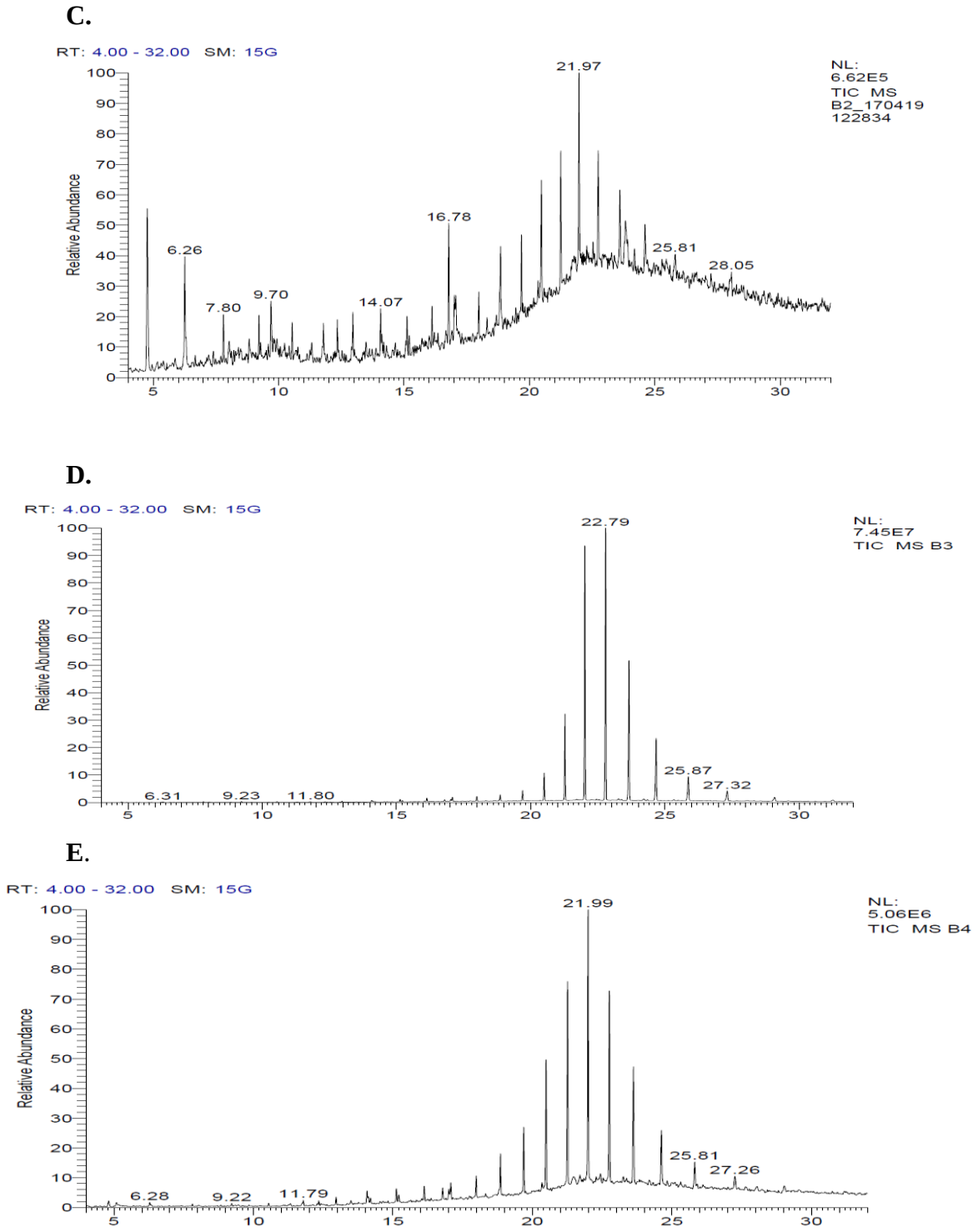


Fig. 1. GC-MS profiles of degraded oil extracts from Diesel contaminated soil – A.- Control (Pure Diesel), B.- B1, C.- B2, D.- B3 and E.- B4 soil samples

The complete 16S rRNA gene sequence (1475 bp) of strain 1A, 3E, 4F,4H were determined. Phylogenetic tree were constructed (Fig. 2) that was based on 1475 unambiguous bp. The 16s rRNA gene sequence of strains were deposited in the NCBI gene bank under Accession no. 1A(KX061101), 3E(KX061098), 4F(KX061099) and 4H(KX061100). The phylogenetic analysis

showed that strain 1A, 3E, 4F and 4H are a member of genus bacillus and closely related to *Bacillus subtilis*(FJ532063), *Bacillus pseudomycoides*(KF439816), *Bacillus cereus*(KF862935), *Bacillus licheniformis*(KM246409) respectively.

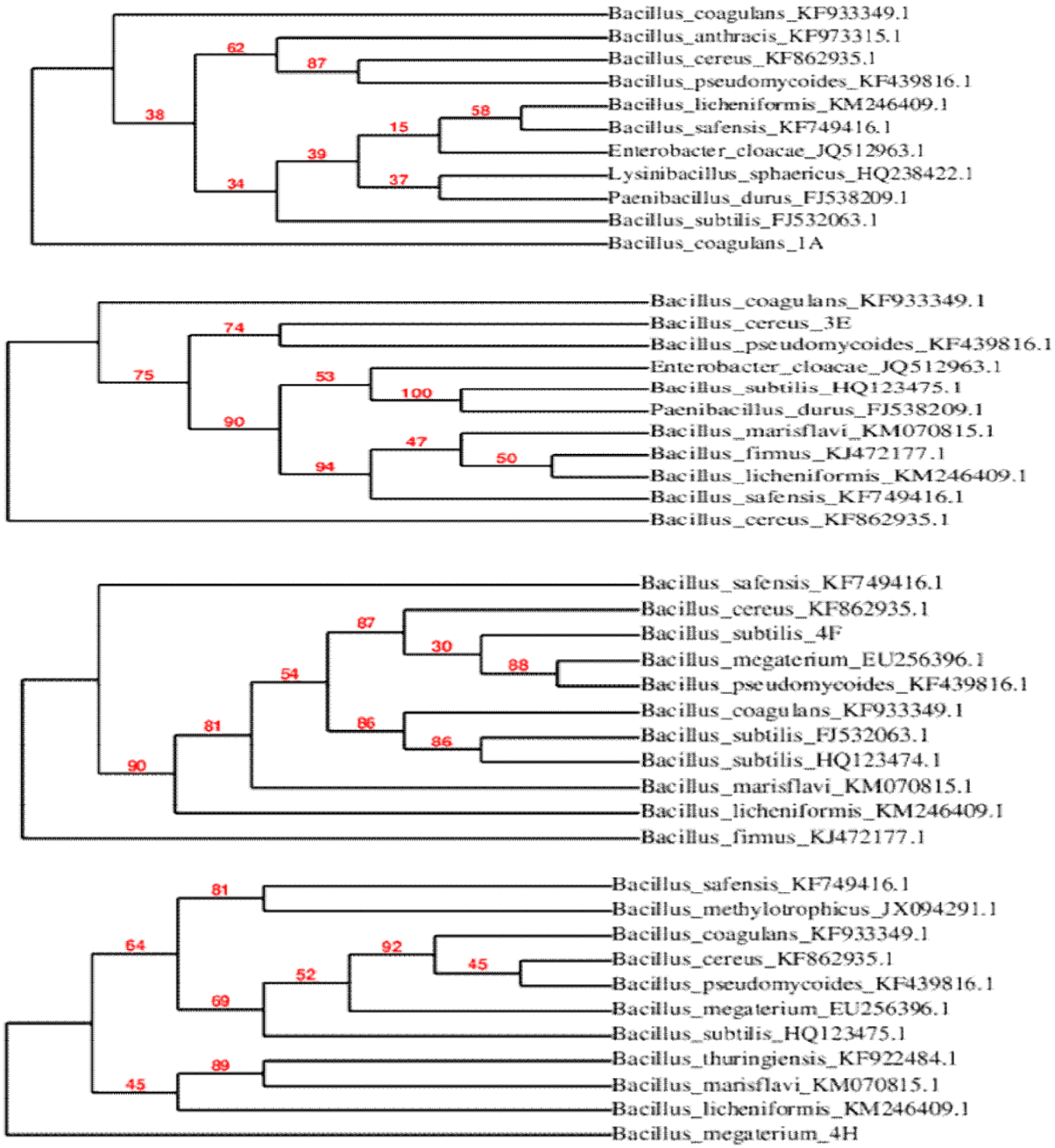


Fig. 2: Phylogenetic tree based on 1475 unambiguous nucleotides of the 16S rRNA sequence, showing the position of strain 1A(KX061101), 3E(KX061098), 4F(KX061099) and 4H(KX061100) related to genus Bacillus. Bootstrap values, expressed as percentage of 100 replications, are shown in (red colour) middle of each branch.

Biochemical characteristics of Diesel degrading strains 1A, 3E, 4F and 4H are given in table 1.

Table1: Biochemical characteristics of Diesel degrading strains 1A, 3E, 4F and 4H

Character	1A	3E	4F	4H
Gram Stain	+	+	+	+
Shape	Rods	Rods	Rods	Rods
Spore	+	+	+	+
Motility	+	+	+	+
Catalase	+	+	+	+
Oxidase		-	Variable	+
Citrate	-	+	+	+
Indole	-	-	-	-
Glucose	+	+	+	+
VP	+	+	+	-
Gelatin	+	-	+	+
Methyl Red	+	-	-	+
Starch	+	+	+	+

DISCUSSION

The present study clearly revealed that microorganisms inhabiting petroleum contaminated soil could rapidly degrade mixture of hydrocarbons. Researchers have been focused to find out bioremediation potential of hydrocarbon degrading bacteria inhabiting contaminated soil. Bacterial strains were isolated having capability of hydrocarbon degradation. Subsequently Palanisamy *et al*. (2014) have reported the isolation of bacteria from diesel contaminated soil and identified by 16S rRNA gene sequence analysis to be *Acinetobacter baumannii*. Diesel oil biodegradation by A. *baumanii* was further studied at initial pH 7, 35°C and initial hydrocarbon concentration at 4% and the biodegradation products were analyzed by GC-MS. Similarly F. J. Márquez-Rocha *et al*. (2000) have also performed Biodegradation of diesel oil using a diesel oil-degrading bacterial consortium in which concentration of diesel in contaminated soil was reduced to <15% of the initial concentration within a duration of five weeks in comparison with controls (without bacterial consortium).

However, In the present work diesel degrading bacterial strains were isolated and screened by streaking isolates obtained from hydrocarbon contaminated soil over Bushnell Haas Agar supplemented with 1% (v/v) filter sterilized Diesel as sole carbon source. Efficient diesel degrading bacterial strains *Bacillus coagulans*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* were found and identified based on phenotypic, biochemical and 16s rRNA phylogenetic analysis. Hydrocarbon degradation analysis of extracted diesel oil from contaminated soil was done using GCMS to evaluate the degradation of various components by different diesel degrading strains which showed almost total disappearance of the corresponding peak of each compound present in diesel oil.

CONCLUSION

Microbial analysis of Petroleum contaminated soil indicates the presence of diesel degrading bacterial population. GCMS analysis of extracted oil showed that diesel pollutants were degraded by the existing microbial fauna confirmed by disappearance of peaks related to hydrocarbon compared to pure diesel. Bioremediation is a rapidly growing area of environmental biotechnology which is used for cleaning up of pollutants. It is apparent from this study that microorganisms degrading hydrocarbon pollutants are ubiquitously present and can be isolated for bioremediation purpose. So this study adds on in the pool of information regarding bioremediation by microorganisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Plohl K et al. Biological degradation of motor oil in water. Acta Chim Slovenica 2002; 49: 279-289.
2. Bona C et al. Effect of soil contaminated by diesel oil on the germination of seeds and the growth of Schinus terebinthifoilus. Brazilian Arch Biol Technol 2011; 54(6): 1379-1387.
3. Ciric L et al. Hydrocarbon utilization within a diesel-degrading bacterial consortium. FEMS Microbiol Lett 2010; 303: 116-122.
4. Alkio M et al. Stress responses to polycyclic aromatic hydrocarbons in Arabidopsis include growth inhibition and hypersensitive response like symptoms. J Exper Bot 2005; 56(421): 2983-2994.
5. Reynoso-Cuevas L et al. In vitro evaluation of germination and growth of five plant species on medium supplemented with hydrocarbons associated with contaminated soils. Bioresource Technol 2008; 99(14): 6379-6385.
6. Mishra S et al. Evaluation of inoculums addition to stimulate in situ bioremediation of oily-sludge contaminated soil. Appl Environ Microbiol 2001; 67(4): 1675-1681.
7. Roling W F M et al. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Appl Environ Microbiol 2002; 68(11):5537-5548.
8. Atlas R M, Philp J. Bioremediation: applied microbial solutions for real-world environmental cleanup. Washington D C: ASM Press; 2005, p1.
9. Nogueira L et al. Phytotoxicity of petroleum contaminated soil and bioremediated soil on Allphylus edulis. Rodriguiesia 2011; 62(3): 459-466.
10. Margesin R, Schinner F. Bioremediation (natural attenuation and biostimulation) of diesel-oil contaminated soil in an Alpine glacier skiing area. Appl Environ Microbiol 2001; 67(7):3127-3133.
11. Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnol Res Int 2011; 941810.
12. Castro-Gutiérrez V M et al. Hydrocarbon Degrading Microflora in a Tropical fuel-contaminated aquifer: assessing the feasibility of PAH Bioremediation. Int J Environ Res 2012; 69(1): 345- 352.
13. Mehrashi M R et al. Biodegradation of petroleum hydrocarbons in soil. Iranian J Public Health 2003; 2(3):28-32.
14. Delille D et al. Effectiveness of bioremediation of crude oil contaminated subantarctic intertidal sediment: the microbial response. Microb Ecol 2002; 44:118-126.
15. Barathi S, Vasudevan N. Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from a petroleum contaminated soil. Environ Int 2001; 26: 413-416.
16. Edgar R C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004; 32(5):1792-1797.
17. Talavera G, Castresana J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systemat Biol 2007; 56: 564-577.
18. Dereeper A et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 2008; 1: 36.
19. Palanisamy N et al. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. J Environ Health Sci Eng 2014; 12:142.
20. Márquez-rocha F J et al. Biodegradation of Diesel Oil in Soil by a Microbial Consortium. Water Air and Soil pollution. 2001; 128(3):313-320.

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