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RESEARCH ARTICLE OPEN ACCESS

Isolation and Identification of *Pseudomonas aeruginosa* from Soil Contaminated with Petroleum Products

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Abstract

The amount of petroleum hydrocarbons affects biodegradation. Different classifications can be used to petroleum components Asphaltenes, ethers, fatty acids, porphyrins and resins saturated fatty acids. Twenty soil samples tainted with petroleum products and crude oil were gathered from the Basra oil fields and other parts of the Babylon governorate; of these twenty samples, seventeen showed *Pseudomonas aeruginosa* (*P. aeruginosa*) growth on the chromogenic agars, which manifested as purple colonies. Using 16S rRNA and PCR technology with a 505 bp PCR product size, *P. aeruginosa* isolated from soil contaminated with petroleum products was identified. The Kirby-Bauer disk diffusion susceptibility test, a method for testing antibiotic sensitivity, produced the following findings. The percentages of ciprofloxacin CIP, imipenem IPM, meropenem MEM, tobramycin TOB and amoxicillin + clavulanic acid AMC equal to 10 mg, ceftazidime CAZ (30 mg), and cefotaxime CTX (30 mg) are 59%, 70%, and 82% respectively. The goal of creating a minimum salt media and introducing diesel at 0.05% concentration is to ascertain how well bacteria may grow in petroleum product-containing settings by breaking down diesel. For a total of 21 days, the optical density in this test is measured every seven days. The results show a rise in the optical density, which suggests that the culture medium being used is growing more quickly. Additionally, it was noted that the center's color and turbidity was altered. *P. aeruginosa* can grow in soil contaminated with petroleum products, had the ability to degrade petroleum pollutant, highly resisted to antibiotic that used in this study.

Keywords: *Pseudomonas aeruginosa,* PCR, Antibiotic Sensitivity, Soil Bacteria, Petroleum, Biodegradation

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INTRODUCTION

Biodegradation is influenced by petroleum hydrocarbon concentration.[1,2] Different classifications can be used for petroleum components. Asphaltenes, ethers, fatty acids, porphyrins, and resins[3,4] saturated fatty acids. The following hydrocarbons have less effectiveness with microbial degradation when it comes to reducing structural complexity: the alkane-like compounds in a tree, Small-scale aromatic compounds, and Fragrant polymers. *Pseudomonas*, Nocardia, Xanthomonas, Bacterium, Corynebacterium, Mycobacterium, and Acinetobacter are among the rare bacteria that can completely oxidize aliphatic hydrocarbons.[5,6] Benzene rings and the structural complexity of aromatic hydrocarbons are factors that affect biodegradability.[7]

High molecular weight polycyclic aromatics can be inefficiently metabolized by certain bacteria.[8-10] Because microorganisms oxidize petroleum components preferentially, cleaning requires the presence of bacteria and fungi. Soil tainted with petroleum provides microbiological cultures. Four isolates from an oil mine in northern Ecuador, two fungal (*Geomyces* sp. strain HV) and two bacterial (*Bacillus thuringiensis* B3 and *B. cereus* B6), may be useful for bioremediation of soil contaminated by crude oil.[11] Biodegradation is influenced by microbial species, pH, temperature, aeration, water availability, and biogenic minerals. Petroleum breakdown by bacteria is influenced by temperature. The optimal temperature range for petroleum biodegradation in the soil is between 30 and 40 °C. This is hotter than the summertime 25-30 °C in soil from middle latitudes.[12,13] High temperatures kill a lot of bacteria by destroying their biological structures. Cellular metabolism is slowed by low temperatures. Petroleum biodegradation is slowed down if the temperature is not right[14] Enzymatic oxidation mediated by oxygenase is the first step in the breakdown of hydrocarbons.[15,16] Anaerobic environments, however, may also result in deterioration.[17] Electrons can be received by ferric ions and nitrate. Fuel oil[18] toluene[19] and even low temperatures[20,21] bioremediated petroleum-contaminated soils.[22]

The processes involved in microbial bioremediation are intricate and multifaceted. Numerous advantages come from the wide variety of microorganisms and their various hydrocarbon detoxification techniques. Numerous pollutants in the soil are eliminated by bioremediation. To protect the ecosystem, petroleum and its byproducts must be removed from contaminated soil. According to this summary, bioremediation of petroleum-contaminated soil is effective and has a lot of promise. Alkanes can be broken down by many bacteria; however, the process is highly dependent on branch topologies and the length of the carbon chain. More research is required on alkene and aromatic degradation. Biosurfactants are of practical interest in bioremediation. Plants and bacteria together have potential. Due to the complexity of microbial bioremediation and the ways in which environmental factors and pollutant types influence their effectiveness, many study findings are not used. We must get past this obstacle. The study and uses of petroleum bioremediation are the main emphasis of applied ecology, or environmental science. This study aims to isolate *Pseudomonas aeruginosa* present in soils contaminated with petroleum products, and to evaluate the efficiency of these isolates in eliminating contamination.

MATERIALS AND METHODS

Sample collection

Using sterile instruments, twenty soil samples were taken at a depth of roughly 10 cm. Approximately 100 cc of soil was taken and

Table 1. MSM content

No.	Per liter	Weight
1.	$FeSO_4$	1 mg
2.	$MgSO_4.7H_2O$	200 mg
3.	Na_2HPO_4	210 mg
4.	NaH_2PO_4	90 mg
5.	$CuSO_4.5H_2O$	5 µg
6.	H_3Bo_3	10 µg
7.	$MnSO_4.5H_2O$	10 µg
8.	$ZnSO_4.7H_2O$	70 µg
9.	MoO_3	10 µg
10.	$CoSO_4$	10 µg
11.	KCl	40 mg
12.	$CaCl_2$	15 mg
13.	NH_4Cl	500 mg
14.	$NaNO_3$	2 mg
15.	0.05% (v/v) diesel	

stored in sterilized containers. Samples of soil were collected from several areas inside the Hilla Governorate as well as from the Basra crude oil fields, as shown in Table 1.

Culture cultivation

One gram of soil was collected from each sample, mixed with nine milliliters of brain heart infusion media, and incubated for twenty-seven hours at thirty-seven degrees Celsius. Next, a loop complete transfer was obtained from each sample, plotted on the chromogenic agar medium for *P. aeuroginosa*, and cultured for twenty-seven hours at thirty-seven degrees Celsius.

Antibiotic susceptibility

We completed the disk diffusion of susceptibility tests in accordance with (CLSI 2023) requirements. Ciprofloxacin CIP (10 mg), Imipenem IPM (10 mg), Meropenem MEM (10 mg), Tobramycin (10 mg), Amoxicillin + clavulanic acid (10 mg), Ceftazidime CAZ (30 mg), and Cefotaxime CTX (30 mg) were the antibiotics disc potency that were administered. Every test was conducted on the Muller-Hinton Agar platform.

Identifying the activity of microorganisms in diesel

Inoculate colonies of different bacteria (previous experiment) into 50 ml mineral salts medium (MSM).

Table 2. Primer sequence 5'-3'

Gene	Direction	Sequence 5'-3'	Product size
16S rRNA	F	TGCCTGGTAGTGGGGGATAA	505 bp
	r	GGATGCAGTTCCCAGGTTGA	

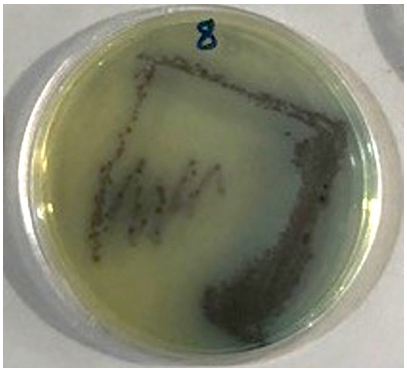

Figure 1. *P. aeruginosa* on Chromogenic agar

The growth response of each of the above isolated bacteria on diesel can initially determine at 7 days intervals by physical appearance (turbidity) and measuring the optical density (O.D.) at 540 nm, after an incubation period of 12 days at 28 °C.

Molecular identification of *Candida* species

The PCR test was performed using the primer in Table 2 and in accordance with the guidelines listed in Table 3 to identify the isolated species from the petroleum product-contaminated soil.

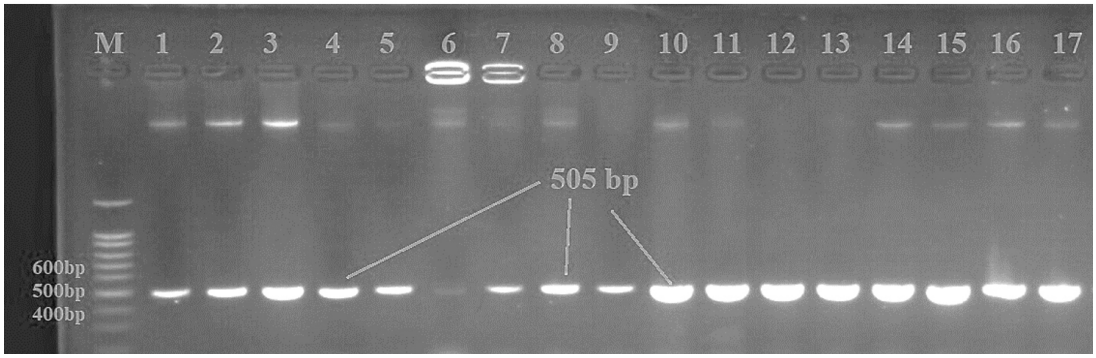

Figure 2. Agarose gel electrophoresis of PCR for 16S rRNA gene; M: Marker DNA ladder; lane 1-17 samples; 1.5% agarose gel with safe red stain, at 80 V/cm, it took sixty minutes

The mixture of PCR was 2 µl of DNA sample, 1.5 µl of each forward and reverse primer, and 15 µl of green master mix (Promega).

RESULTS

Isolation

Twenty soil samples tainted with petroleum products and crude oil were gathered from the Basra oil fields and various parts of the Babylon governorate; of these twenty samples, seventeen showed *P. aeruginosa* growth on the chromogenic agars, manifesting as purple colonies (Figure 1).

Table 3. PCR condition for amplifying C and gene

No.	Steps	Temp. (°C)	Time	Cycles
1.	Initial Denaturation	95	3 min.	
2.	Denaturation	95	30 sec.	35 cycle
3.	Annealing	66	30 sec.	
4.	Extension	72	45 sec.	
5.	Final Extension	72	5 min.	
6.	Storage	4		

Table 4. Antibiotic susceptibility of *P. aeruginosa*

No.	Antibiotic	Resistance % according to CLSI 2023
1.	Ciprofloxacin CIP (10 mg)	59
2.	Imipenem IPM (10 mg)	70
3.	Meropenem MEM (10 mg)	70
4.	Tobramycin TOB (10 mg)	82
5.	Amoxicillin + clavulanic acid AMC (10 mg)	35
6.	Ceftazidime CAZ (30 mg)	35
7.	Cefotaxime CTX (30 mg)	35

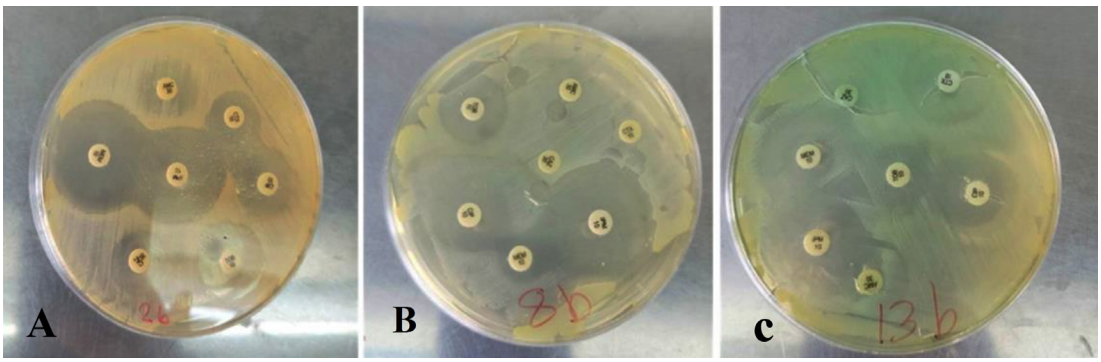

Figure 3. Antibiotic susceptibility of *P. aeruginosa.* (A) *P. aeruginosa* isolate No. 2 which was sensitive to all antibiotics used, (B) *P. aeruginosa* isolate No. 8 which was resistant to three types of antibiotics, (C) *P. aeruginosa* isolate No. 13 resistant to two types of antibiotics that used

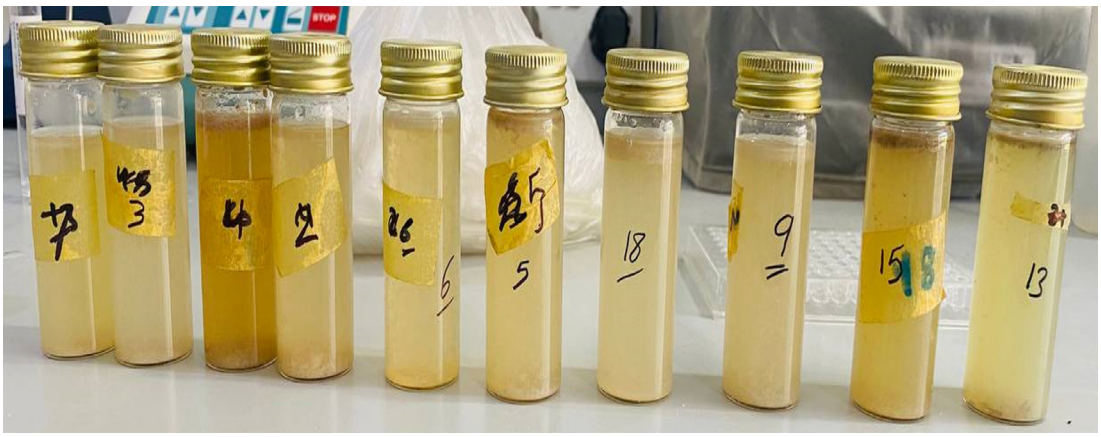

Figure 4. Change in turbidity and color

Molecular identification of *Candida* species

Using PCR technology and a 16S rRNA Table 1, *P. aeruginosa* isolated from soil contaminated with petroleum products was identified in this study. The PCR product size was 505 bp (Figure 2). It was possible to isolate a highly efficient hydrocarbon-degrading strain from a lake wetland. When peptone and beef extract are present, this strain develops swiftly. The colonies in Figure 1 have boundaries that expand unevenly and are colored yellow brown. In addition, this strain gives the MSM solution a subtle pink hue. According to SEM analysis, this strain has a length and width of around 0.4 and 1.0 meters, and it is Gram-negative. The 16S rRNA gene sequence has 1389 base pairs and a G+C content of 54.2%. About 100% of the 16S rRNA gene segment resembles *Pseudomonas aeruginosa.*

Antibiotic susceptibility

The isolates' resistance rates were found to be extremely high when an antibiotic sensitivity test utilizing disc (Kirby-Bauer disk diffusion susceptibility test) was performed, as indicated in Table 4 and Figure 3.

Table 5. The change in optical density over three weeks for different isolates

Candida species	After 7 days	After 14 days	After 21 days
0.05% msm with desal	1.694	-	-
Isolate 1	0.967	1.394	1.894
Isolate 2	0.62	1.439	1.939
Isolate 3	0.227	1.294	1.794
Isolate 4	0.815	1.305	1.805
Isolate 5	0.675	1.431	1.931
Isolate 6	0.395	1.166	1.666
Isolate 7	0.284	1.194	1.694
Isolate 8	0.347	0.352	1.349
Isolate 9	0.103	1.388	0.852
Isolate 10	0.091	1.401	1.888
Isolate 11	0.865	1.302	1.901
Isolate 12	0.967	1.394	1.802
Isolate 13	0.613	1.439	1.894
Isolate 14	0.967	1.294	1.939
Isolate 15	0.62	1.305	1.794
Isolate 16	0.227	1.431	1.805
isolate 17	0.815	1.166	1.931

Identifying the activity of microorganisms in diesel

The purpose of making a minimum salt media and adding 0.05% diesel to it is to test how well bacteria can grow in petroleum product-containing settings by breaking down desal. For a duration of 21 days, the optical density in this test is evaluated every seven days. The results show a rise in the optical density, which suggests that the culture medium being used is growing more quickly (Table 5). Additionally, Figure 4 showed that the center's color and turbidity had changed.

DISCUSSION

Water and soil samples from several hydrocarbon-contaminated sites in Basrah were found to contain *Bacillus* spp.*, Pseudomonas* spp.*,* and *Micrococcus* spp.[23] *P. aeruginosa* was one of the nine bacterial strains that Kridi and her colleagues were able to recover. Based on biochemical testing and unique morphological features, *P. aeruginosa* was identified.[24]

In a molecular study, it was possible to isolate a highly efficient hydrocarbon-degrading strain from a lake wetland. When peptone and beef extract are present, this strain develops swiftly. The colonies in Figure 1 have boundaries that expand unevenly and are colored yellow brown. In addition, this strain gives the MSM solution a subtle pink hue. According to SEM analysis, this strain has a length and width of around 0.4 and 1.0 meters, and it is Gram-negative. The 16S rRNA gene sequence has 1389 base pairs and a G+C content of 54.2%. About 100% of the 16S rRNA gene segment resembles *Pseudomonas aeruginosa*, indicating a stable genetic clade with previously identified *Pseudomonas* strains.[25]

Antibiotic sensitivity, Sedighi's research showed that *P. aeruginosa* populations that produce MBL are a major hazard to therapeutics. The rate at which MBL is causing imipenem resistance has skyrocketed. According to Kadivarian et al.[26] early detection and infection control measures are the most effective antimicrobial methods for this bacterium.[26]

Baban's work aims to limit the overuse of carbapenem antibiotics by developing antimicrobial stewardship. Early identification of

isolates resistant to carbapenem is essential to reducing the possibility of these isolates spreading to patients in critical condition. Active surveillance and rigorous adherence to infection prevention and control measures may be useful tactics for reducing the emergence of carbapenemase resistance because of Baban's work.[27]

Liu and colleagues obtained a novel strain of *P. aeruginosa* from a contaminated freshwater marsh by using crude oil as its only carbon source. In addition, under both dynamic and static culture conditions, the isolated strain shown encouraging results in the degradation of important components of crude oil, including as n-alkanes, alkylcyclohexane, alkylbenzene, and alkyltoluene, in addition to several PAHs and aromatic chemical families. Since these wetlands are a major source of hydrocarbon-degrading bacteria, screening native bacteria with high degradation efficiency and the ability to break down a wide variety of crude oil components may provide a rich microbial resource for wetland restoration.[25]

Five different bacterial strains were found in soil samples from five different oil-polluted areas. The ability of the microorganisms to degrade diesel fuel was examined by researchers. By using phenotypic analysis, the bacterial colonies were shown to be *Pseudomonas* species, specifically *P. putida*, *P. maltophilia*, and *P. mallei*. They discovered a variety of bacteria, such as *Acinetobacter lowffi* and *Enterobacter cloacae*. *Pseudomonas* members were discovered to be the most common in oil-polluted Kuwaiti desert soil samples that were treated in several methods, along with *Bacillus, Streptomyces*, and *Rhodococcus*.[28] Further as an add up scientific decipher, *P. aeruginosa* remains the subject of recent researchers e.g., evaluation of antibacterial activity of disinfectants,[29] drug resistanxe[30,31] and medical research tool.[32,33]

CONCLUSION

Pseudomonas aeruginosa is a kind of bacteria that can live in soil that has been polluted with oil products. The results of this experiment showed that the *P. aeruginosa* that was isolated could break down oil spills and can use in petroleum biodegradation. The *P. aeruginosa* isolates that were evaluated were very resistant to the medications that were utilized in this investigation.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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