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Isolation and Identification of Bacillus Species from Soils Contaminated with Petroleum Derivatives and Detection of Their Role in the Biodegradation of Kerosene

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Annotation: The current study aimed to determine the biodegradation rate of crude oil using some different Bacillus species isolated from soil. 100 mg of contaminated and uncontaminated soil samples used in bioremediation experiments were collected from three areas in Tikrit city, including generator soil, refinery soil, and normal uncontaminated soil. The soil samples (weight: 100 g and depth: 3-12 cm) were collected using dark counters and then transferred to the laboratory. Hydrocarbon materials were added with the prepared nutrient agar medium and left to solidify, then both contaminated and normal soils were placed and incubated for 24 hours at 37 °C. the result showed that 86.7% of the bacterial isolates gave positive growth after being cultivated on blood and MacConkey agar media. While 13.3% of the soil samples showed negative bacterial growth when grown on the same media. the result showed that the most isolated bacteria was B. subtilis, which represented 28.6%, followed by B. tropicus, which represented 19.0%, while the lowest isolation percentage was for Bacillus taxi and B. flexus, which represented 4.8% of the total 21 isolates. The high degradation percentage by B. thuringiensis was 75% for a concentration of 1% kerosene. The high degradation percentage by B. subtilis was 82% for a concentration of 1% kerosene. The high degradation percentage by B. firmus was 69% for a concentration of 1% kerosene. The high degradation percentage was 83% for a concentration of 1% Kerosene. The high degradation percentage by B. taxi reached 73% for a concentration of 1% of kerosene. The high degradation percentage by B. muralis reached 93% for a concentration of 1% kerosene. The high degradation percentage by B. megaterium was 72% for a concentration of 1% kerosene. The results of the current study showed the ability of the studied Bacillus to biodegrade contaminated kerosene in soil. The most efficient Bacillus species in biodegrading kerosene was Bacillus muralis.

Keywords: kerosene, B. thuringiensis, contaminated soil, B. firmus.

Introduction

One of the biggest problems of our time is oil pollution, which has drawn the interest of numerous researchers to investigate environmental pollution caused by the presence of hydrocarbons derived from petroleum, particularly in the wake of significant disasters that have impacted the soil and resulted in oil leaks or spills [1,2]. According to Sivagamasundari and Jeyakumar [3], oil pollution is one of the most significant environmental pollution problems now recognized because it is difficult to regulate, particularly in soil, and because it affects every area of life. They believed that the presence of large molecular weight branches in molecules with twenty carbons or more was the primary cause of petroleum hydrocarbon contamination. According to Husain [4], these compounds have a high resistance to decomposition since they are not soluble in water, which means they stay in the soil for a long time. The most crucial elements that affect the toxicity of petroleum hydrocarbons are their concentration, nature, content, and biodegradability, all of which affect how long they remain in soil [5]. An ecosystem becomes unbalanced as a result of prolonged persistence in soil [6]. According to Barnier et al. [7], oil pollution has an impact on biodiversity because hydrocarbon supplies in soil diminish over time; for this reason, oil pollution of soil is regarded as pollution. Leaks or spills of oil have an impact on the soil, seriously harming living things and the ecosystem [8]. As noted by Ikuesan [9], oil pollution of soil results in sterility and consequent alterations to its composition and microbiological, physical, and chemical characteristics, which delays plant growth by reducing soil fertility and its capacity to absorb and hold water [10]. Reduced agricultural output as a result of these oil spill effects has a detrimental economic impact on people's livelihoods [11]. Many petroleum hydrocarbons are biodegradable by microorganisms such as bacteria, fungi, yeast, and microalgae [12]. Biodegradability depends on the chemical composition of the molecule, the presence of viable microorganisms capable of degrading the material, and environmental conditions [13]. So, the current study aimed to determine the biodegradation rate of crude oil using *Bacillus* species isolated from soil.

Materials & Methods

Soil Sample Collection

100 mg of contaminated and uncontaminated soil samples used in bioremediation experiments were collected from three areas in Tikrit city, including generator soil, refinery soil, and normal uncontaminated soil. The soil samples (weight: 100 g and depth: 3-12 cm) were collected using dark counters and then transferred to the laboratory. Hydrocarbon materials were added with the prepared nutrient agar medium and left to solidify, then both contaminated and normal soils were placed and incubated for 24 hours at 37 °C.

Measuring the percentage of quantitative loss of Kerosene oil

The rate of Kerosene oil degradation was measured using the gravimetric method by measuring the difference between the weight of the amount of Kerosene oil added to the culture medium used before and after the development of the bacterial isolates used, as followed in Wehner and Teschner [14]. The percentage of the rate of Kerosene oil degradation was measured by each isolate separately.

Isolation and identification of bacteria

Isolation

1 g of soil was diluted using test tubes containing 9 ml of distilled water and then 1 ml of the fourth and fifth dilutions were transferred to Petri dishes. After that, nutrient agar containing 1% Kerosene oil was added as a carbon source. Petri dishes were incubated at 37 °C for 24-48 hours [15].

Bacterial Identification

Bacteria were identified based on the following aspects

Pheteromorphic diagnosis and cultural characteristics

Bacterial colonies were identified based on the cultural characteristics of the colonies growing on MacConkey agar and blood agar. The growing colonies were isolated and purified by taking a single colony and re-cultivating it using the Streaking method on MacConkey agar again to ensure the purity of the isolated colonies [16].

Microscopic Examination

Based on the morphological features of the bacterial cells observed under a microscope and the way they reacted with Gram stain, which indicates the kind of response, the bacterial cell's form, and its arrangement, the bacterial colonies were diagnosed.

Biochemical Tests

The following biochemical tests were conducted as stated in [16].

Results and Discussion

Table (1) shows the bacterial isolates that were isolated and identified from the soil, where it is noted that 86.7% of the bacterial isolates gave positive growth after being cultivated on blood and MacConkey agar media. While 13.3% of the soil samples showed negative bacterial growth when grown on the same media.

Table (1): Number and percentages of bacterial isolates from the soil

Results	No. of isolates	Percentage of isolates
+ve	13	86.7%
-ve	2	13.3%
Total	15	100%

Table (2) shows the number and percentages of each type of bacteria that were isolated from the soils collected from three regions. It is noted that the most isolated bacteria were *B. subtilis*, which represented 28.6%, followed by *B. tropicus*, which represented 19.0%, while the lowest isolation percentage was for Bacillus taxi and *B. flexus*, which represented 4.8% of the total 21 isolates.

Table (2): Number and percentages of each species of Bacillus

Results	No. of isolates	Percentage of isolates
Bacillus thuringiensis	2	9.5%
Bacillus subtilis	6	28.6%
Bacillus firmus	3	14.3%
Bacillus tropicus	4	19.0%
Bacillus taxi	1	4.8%
Bacillus muralis	2	9.5%
Bacillus megaterium	2	9.5%
Bacillus flexus	1	4.8%
Total	21	100%

The results of the current study also showed that the highest isolated genera were Bacillus spp. If it reached 80.8%, the species Bacillus subtilis was the highest among the Bacillus spp. genera, where its percentage reached 23.1%. The results of the current study agreed with the study of Ibrahim and Neihaya [17], where they indicated that the isolation percentage of Bacillus spp. reached 73.1% of different soil samples, and they also indicated that the isolation percentage of Bacillus subtilis reached 28.9%, and they indicated that the diagnosis of the bacillus bacteria was done through morphological characteristics, microscopic diagnosis and biochemical tests, and then it was confirmed using the Vitek 2 system.

Biodegradation of Kerosene

Biodegradation of kerosene was carried out by different types and genera of bacteria according to the ideal conditions for each type.

Bacillus thuringiensis

Table (3) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by Bacillus thuringiensis. The high degradation percentage was 75% for a concentration of 1% Kerosene, while the lowest degradation percentage was for a concentration of 5% Kerosene, which reached 27%.

Table (3): Percentage of Kerosene oil degradation by *Bacillus thuringiensis*

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene oil sample
Control	0.702	0.702	0.0	0.0%
1%	0.702	0.173	0.529	75%
3%	0.702	0.236	0.466	66%
5%	0.702	0.51	0.192	27%

Bacillus subtilis

Table (4) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by Bacillus subtilis. The high degradation percentage was 82% for a concentration of 1% Kerosene, while the lowest degradation percentage was 5% for a concentration of 5% Kerosene.

Table (4): Percentage of Kerosene degradation by Bacillus subtilis

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene oil sample
Control	0.7228	0.7228	0.0	0.0%
1%	0.7228	0.13	0.5928	82%
3%	0.7228	0.615	0.1078	14%
5%	0.7228	0.683	0.0398	5%

Bacillus firmus

Table (5) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by Bacillus firmus. The high degradation percentage was 69% for a concentration of 1% Kerosene oil, while the lowest degradation percentage was 32% for a concentration of 5% Kerosene.

Table (5): Percentage of Kerosene degradation by Bacillus firmus

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene sample
Control	0.7113	0.7113	0.0	0.0%
1%	0.7113	0.215	0.4963	69%
3%	0.7113	0.356	0.3553	49%
5%	0.7113	0.48	0.2313	32%

Bacillus tropicus

Table (6) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by Bacillus tropicus. The high degradation percentage was 83% for a concentration of 1% Kerosene, while the lowest degradation percentage was 43% for a concentration of 5% Kerosene.

Table (6): Percentage of Kerosene degradation by Bacillus tropicus

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene oil sample
Control	0.7316	0.7316	0.0	0.0%
1%	0.7316	0.12	0.6116	83%
3%	0.7316	0.215	0.5166	70%
5%	0.7316	0.41	0.3216	43%

Bacillus taxi

Table (7) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by *Bacillus taxi*. The high degradation percentage reached 73% for a concentration of 1% of Kerosene, while the lowest degradation percentage was for a concentration of 5% of Kerosene, reaching 47%.

Table (7): Percentage of Kerosene oil degradation by *Bacillus taxi*

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene sample
Control	0.6899	0.6899	0.0	0.0%
1%	0.6899	0.18	0.5099	73%
3%	0.6899	0.27	0.4199	60%
5%	0.6899	0.361	0.3289	47%

Bacillus muralis

Table (8) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by *Bacillus muralis*. The high degradation percentage reached 93% for a concentration of 1% Kerosene, while the lowest degradation percentage was for a concentration of 5% Kerosene, reaching 58%.

Table (8): Percentage of Kerosene degradation by *Bacillus muralis*

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene sample
Control	0.7432	0.7432	0.0	0.0%
1%	0.7432	0.05	0.6932	93%
3%	0.7432	0.19	0.5532	74%
5%	0.7432	0.3067	0.4371	58%

Bacillus megaterium

Table (9) shows the percentages of Kerosene degradation at different concentrations (1%, 3%, 5%) by *Bacillus megaterium*. The high degradation percentage was 72% for a concentration of 1% Kerosene, while the lowest degradation percentage was 41% for a concentration of 5% Kerosene.

Table (9): Percentage of Kerosene oil degradation by *Bacillus megaterium*

Conc.	Unanalyzed	Untreated	Weight of Kerosene decomposed	% of degradation of Kerosene sample
Control	0.82	0.82	0.0	0.0%
1%	0.82	0.38	0.44	72%
3%	0.82	0.49	0.33	59%
5%	0.82	0.63	0.19	41%

Bacillus and its genus are the major microbial groups that degrade hydrocarbons. Carmela et al. [18] studied the biodegradation capacity of aromatic hydrocarbons (PAH5) by Bacillus, and revealed its ability to biodegrade aromatic hydrocarbons. Shahidi Rizi et al. [19] demonstrated the ability of Bacillus to biodegrade high-grade crude oil and convert it into simpler derivatives. The findings demonstrated that a number of chemicals, including benzo-alpha-pyrene, anthracene, and naphthalene, underwent biodegradation and saw a slight drop in concentration. It was demonstrated that bacteria were better at biodegrading aromatic chemicals than aliphatic ones. The rise in the rate of kerosene degradation found in this investigation is in line with patterns of bacterial hydrocarbon consumption. The elevated activity of bacteria that use hydrocarbons at this degree of oil contamination is responsible for the high rates of biodegradation [20]. The majority of the contaminated oil appears to be used as an organic carbon source by microorganisms in a process known as kerosene biodegradation, which breaks down the petroleum components into smaller molecular weight molecules [21]. According to Abioye et al. [22], the levels of total petroleum hydrocarbons could be considerably decreased by lengthening the incubation period when treating soil that has been contaminated by spent oil. These researchers' findings are in line with the crude oil deterioration rate's ongoing rise. From samples of desert dirt, Sorkhoh et al. [23] identified 368 isolates of the species Bacillus. These Bacillus species are more resilient to the negative effects of high soil hydrocarbon levels and temperatures. This could be because members of this genus can produce endospores that proliferate quickly when favorable conditions return, allowing them to survive periods of unfavorable environment. This study's isolation of Bacillus species (the most effective bacterial isolates for crude oil degradation) from petroleum hydrocarbon-contaminated areas supports findings that Bacillus species are widely distributed in environments associated to petroleum [24, 25, 26]. Additionally, 20 bacteria that break down hydrocarbons were recovered from tropical soils by Malatova [27]. Eight isolates were classified as Bacillus species and ten as Pseudomonas species based on their morphological characteristics.

Conclusions

The results of the current study showed the ability of the studied Bacillus to biodegrade contaminated kerosene in soil. The most efficient Bacillus species in biodegrading kerosene was Bacillus muralis.

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