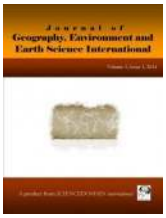

Journal of Geography, Environment and Earth Science International

11(2): 1-11, 2017; Article no.JGEESI.35447
ISSN: 2454-7352

Bioremediation of Polycyclic Aromatic Hydrocarbons by *Bacillus Licheniformis ATHE9* and *Bacillus Mojavensis ATHE13* as Newly Strains Isolated from Oil-Contaminated Soil

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Authors' contributions

All authors contributed to design the study, write, analysis and revise the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JGEESI/2017/35447
Editor(s):
(1) Anthony R. Lupo, Department of Soil, Environmental, and Atmospheric Science, University of Missouri, Columbia, USA.
Reviewers:
(1) Shruti Murthy, Bangalore University, India.
(2) Chioma Nwakanma, Michael Okpara University of Agriculture, Nigeria.
Complete Peer Review History: http://www.sciencedomain.org/review-history/20362

Original Research Article

Received 13th July 2017
Accepted 31st July 2017
Published 4th August 2017

ABSTRACT

Removal of Polycyclic Aromatic Hydrocarbons (PAHs) from the environment is important because of their potentially deleterious affect human health. Biological methods that was applied in the present study is typically contain microbial processes. It is the most innocuous and effective

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solution that transform pollutant to nontoxic or less toxic substances. The objectives of the present study are enrichment, isolation and identification of PAHs degrading bacteria from oil-contaminated soil by molecular detection methods. Eight indigenous bacteria were isolated from oil-contaminated soils by using the enrichment methods. Two isolates with the highest optical density potentials were selected in media with 12.8 mg. L^{-1} of 16 PAHs as the sole source of carbon. They were identified as *Bacillus licheniformis ATHE9* and *Bacillus mojavensis ATHE13* by subjected to polymerase chain reaction with 16SrDNA primer and biochemically characterized. The bacterium strain including *Bacillus licheniformis ATHE9* was isolated and removed by Acenaphtylene, Acenaphtene and Indeno pyrene in 72, 96 and 96 hours, respectively. The *Bacillus mojavensis ATHE13* could remove Naphthalene in 72 hours and Acenaphtene, Acenaphtylene, Benzo(ghi)prylene, Dibenzo(ah)anthracene and Indeno pyrene in 96 hours. The results of this study strongly indicate that some of the indigenous bacteria have the potential role to remediate polycyclic aromatic hydrocarbons from oil-contaminated sites. These bacterial strains were able to grow in media with 12.8 mg. L^{-1} of 16 PAHs and reach the stationary phase. The results showed that using native bacteria with PAH-utilizing capabilities in contaminated sites could be useful for removal of these compounds from the environment.

Keywords: Bacterial identification; gas chromatography; indigenous bacteria; pollution; remediation.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants generated from both natural and anthropogenic processes that remain for long periods in the environment and cause serious concerns for human health [1].

Recently, there is an interesting in development of biotechnologies to detoxify PAHs contaminated sites. The elimination of PAHs from the soil could be achieved through bioremediation strategies, where selected microorganisms transform them to CO_2, water, or at least, less dangerous compounds. Although attempts have been made since the 1950s to select microorganisms with the capacity of degrading PAHs from pure cultures, greater attention has been paid in the last few years to isolating microbes that effectively degrade PAHs [2]. Bioremediation, which is based on microbial transformation and degradation, is one of the most promising methods applied in the field of environmental biotechnology for cleanup of contaminated environments [3]. Considerable attention has been focused on the metabolic pathways and genetics of degradation of low and high molecular mass PAHs such as naphthalene, phenanthrene, anthracene, pyrene, benzo(a) pyrene and benzo(ghi)prylene, by bacteria [4]. Until now, various bacteria with the ability to degrade each PAHs have been isolated [5,6,7].

Microbial activity has been deemed the most influential and significant cause of PAHs removal [8]. Pollution by petroleum hydrocarbons may stimulate the growth of such organisms and cause changes in the structure of microbial communities in the contaminated area. Hydrocarbon-degrading microorganisms usually exist in very low abundance and diversity in the environment. Soil could receive considerable amounts of PAHs but microbial populations may not have the metabolic ability to degrade these compounds because of their structural complexity and low solubility in soil solution [9]. Therefore, identification of the organisms that play role in pollutant biodegradation is important for developing in situ bioremediation [10].

The objectives of this study were to isolate and identify PAHs degrading bacteria from oil-contaminated soil, and the investigation of biodegradation ability of them.

2. MATERIALS AND METHODS

2.1 Sampling

Soil samples were collected from the Isfahan, Iran refinery (N, 51°30′27.04″ E, 32°46′53.41″), which had been contaminated for more than 10 years with oil sewage. These samples were placed into sterile plastic bags, kept at temperature of 4°C, and taken to the laboratory immediately. Some properties of the soil such as pH [11], EC [12], organic matter percentage [11], microbial respiration [13], and concentrations of 10 PAHs (Naphthalene, Acenaphthene, Acenaphthylene, Phenanthrene, Anthracene, Benzo(a)anthracene, Chrysene, Benzo(a)pyrene, Dibenzo(a,h)anthracene and Benzo(ghi) perylene) were measured [14].

2.2 Soil PAHs Determination

PAHs concentrations in the soil samples were determined by GC-FID using a soxhlet extraction system (5 g soil with 5 g Na_2SO_4 anhydrous) with 250 mL DCM-Acetone (1:1 v/v) mixture. The extract was concentrated by rotary and cleaned up by silica gel column. Then the solvent was changed with n-Hexane. The internal standard m-terphenyl was added to each vial before injected to the GC-FID. The Agilent 7090A Gas Chromatography used in this study with capillary column (30 m × 0.25 mm I.D. 0.25 µm film thickness, Agilent) was equipped with a FID model 6870 Agilent. The flow rate of carrier gas, N_2, was 1.5 mL.min^{-1}. The injector and detector temperatures were set at 260℃ and 270℃, respectively. The temperature program for target PAHs was 1 min hold at 70℃, ramp to 290℃ at 5℃.min^{-1}, ramp to 305℃ at 1℃.min^{-1}. The aliquot of 1 µL in 500 µL solvent was injected into the GC-FID [15].

2.3 Enrichment of PAH-degrading Bacteria

Nutrient agar plates were used for heterotrophic plate count of bacteria. Mineral salt medium (MSM) was used for the enrichment and isolation of PAHs-degrading bacteria. It contained the following compounds (g.L^{-1}): K_2HPO_4, 0.27; KH_2PO_4, 0.35; NH_4Cl, 2.7; $MgCl_2.6H_2O$, 0.1; $CaCl_2.2H_2O$, 0.1; $FeCl_2.4H_2O$, 0.009 and $MnCl_2.4H_2O$, 0.004. The pH was adjusted to 7.2, and the medium was autoclaved (121ºC for 15 min) prior to the addition of 16 PAHs as the sole source of carbon and energy. The solution of 16 PAHs contained: Naphthalene, Acenaphthene, Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(ghi)perylene, and Indeno(1,2,3-cd)pyrene in n-Hexane as 12.8 mg.L^{-1} concentration was added to a 100 mL conical flask. After the evaporation of n-Hexane, the mineral medium was added. Then, 1 gram of oil-contaminated soil was added to the conical flask and kept in the shaker incubator at 150 rpm and 37ºC. Upon observing the growth, enrichment culture of 5 mL was transferred to a fresh medium and incubated under the same conditions. Subsequently, the culture was transferred three times to enrich the degrading bacteria. The solid medium was prepared with 2% (w: v) agar [16].

2.4 Isolation of the Best PAH-degrading Bacteria

The isolation and purification of bacterial isolates were based on the colony morphology such as size, shape, color, margin and texture. The separate colonies of degrading bacteria on the solid medium plates were picked and cultured in MSM. After 24 hours, two isolates with the highest growth rate in presence of PAHs in 12.8 mg. L^{-1} were selected as the best bacteria and were streaked on to LB media plates for identification. Pre-selected isolates were repeatedly streaked on to agar plates in order to obtain a pure culture of bacteria [16].

2.5 Biochemical Analysis of Isolates

Gram staining of each bacterium was conducted before identification. The catalase activity, nitrate reduction, indole production, acid or gas production from D-glucose, D-monitol and sucrose, citrate utilization test and hydrogen sulfide production, anaerobic growth, growth temperature and salt tolerant were tested as described in Bergey's Manual of Systematic Bacteriology [17].

2.6 Bacterial PAH Biodegradation

Each isolate was inoculated into test tube containing 25 mL of MSM media supplemented with 12.8 mg. L^{-1} of 16 PAHs as the sole source of carbon and energy. The incubation was done in a shaker incubator at 150 rpm and 37ºC. Every 24 hours, 5 mL of the media was extracted, and the cell density of each isolate was monitored spectrophotometrically by measuring the absorbance at 560 nm. An increase in turbidity indicated the ability of an isolate to utilize PAHs. This sample size (5 mL) was also used for the assay of PAHs biodegradation. After the addition of 15 mL dichloromethane, the sample was vortexed for 5 min and then centrifuged at 3000 rpm for 10 min. The extract was concentrated by rotary, cleaned up by silica gel column and dried with N_2 gas. The solvent was exchanging with n-Hexane and Phenanteren d_{10} was added in specific concentration as an internal standard to all samples. The supernatant of 1 µl was injected to GC-MS [18].

Analysis of polycyclic aromatic hydrocarbons was carried out by an Agilent GC Model 6890 coupled to a quadrupole mass spectrometer

(5975C). The system was operated in electron impact mode (EI, 70 eV). A HP-5 MS column (30 m × 0.25 mm I.D. 0.25 µm film thickness, Agilent) was used for separation of polycyclic aromatic hydrocarbons. In order to get the proper GC separation, a temperature programming was used. Temperature started from 80ºC with a 5 min hold time, it was then increased to 150ºC at a rate of 10ºC.min^{-1}. It was then increased to 300ºC at a rate of 5ºC.min^{-1}, keeping the final temperature for 5 min. Injection was performed in the split less mode. Helium gas was used as a carrier gas at a constant flow rate of 1.5 mL. min^{-1}. Injector, transfer line temperatures were 250 and 280ºC, respectively. Ions were selected after considering the total ion chromatogram of solution of compounds. The ions were divided into four groups. Peak detection and integration were carried out using Chemstation software and AMDIS (automated mass spectral deconvolution and identification system).

2.7 DNA Extraction and PCR Application

The genomic DNA extraction of best isolates was done using the DNA extraction kit (Sinaclon Bioscience Co.). The extraction procedures were carried out according to the manufacturer's instructions. In brief, 100 µL of overnight bacterial culture was pelleted by protease buffer; this was followed by the addition of 5 µL of protease to the mixed cells and then placed at 55℃ for 30 min. 100 µL of the obtained sample was mixed with a lysis solution, and a short vortexing at the lowest vibration setting was done to lyses the bacterium cell wall. The precipitation solution of 400 µL was added to precipitate the DNA. The DNA was washed with 1 mL washing buffer, dried, and dissolved with solvent buffer. The extracted DNA was analyzed by 1% agarose gel electrophoresis. The extracted and purified DNA was stored at -20℃ before use.

Standard PCR amplification was performed in 25 µL of reaction volume using smart-Taq DNA polymerase. The reaction mixture consisted of 2.5 unit smart-Taq polymerase, 2 mmol dNTP mix, 100 µL of 10X PCR reaction buffer, 2 mmol $MgCl_2$ and 2 µL of 100 pmole each of primers F27 (5-AGCGGTCCAGAGTTTTCCTGG-3) and R1492 (5-CTCTCTGCAGCCCTTGTTACG-3). The reaction mixture was subjected to an initial 5 min of incubation at 95℃ to denature the DNA. This was followed by 33 cycles of amplification, which included 20 seconds denaturalization at 94℃, 25 seconds annealing at 60℃ and 30 seconds extension at 72℃. 1min extension at 72℃ was performed at the end of the final cycle. The amplified products were stored at 4℃ prior to analysis by agarose gel 1% electrophoresis [19].

2.8 Statistical Analysis

The statistical analyses in this study were done with SAS and Excel software.

3. RESULTS

3.1 Chemical Soil Properties

Some properties of the contaminated soil, such as pH, EC, organic matter percentage, microbial respiration, and PAHs concentration, are shown in Table 1.

3.2 Isolation of Bacteria

From the contaminated soil samples, 8 bacterial strains were isolated and labeled as 1 2 3 4 5 6 7 8 and 9. Five isolates were gram negative, and the others were gram positive. Bacterial strains 1 and 8 experienced more growth than others in the presence of 16 PAHs as the sole source of carbon and energy (Fig. 1).

3.3 Identification of Bacteria

The more efficient isolate 1 and 8 were isolated in high purity and selected for more investigation

Table 1. Some properties of the oil-contaminated soil

Parameter	Amount	Parameter	Amount
Acidity	7.24±0.03	Anthracene (mg.L^{-1})	7±2
Electrical conductivity (dS/m)	3.4±0.2	Benzo(a)anthracene (mg.L^{-1})	31±4
Organic matter (%)	2.8	Chrysene (mg.L^{-1})	64±11
Naphthalene (mg.L^{-1})	32±5	Benzo(a)pyrene (mg.L^{-1})	6±2
Acenaphthene (mg.L^{-1})	37±9	Dibenzo(a,h)anthracene (mg.L^{-1})	8±4
Acenaphthylene (mg.L^{-1})	44±5	Benzo(ghi)perylene (mg.L^{-1})	7±0.5
Phenanthrene (mg.L^{-1})	10±5	Microbial respiration (mg CO_2.(g.d)$^{-1}$)	6648±50

of PAHs removal and strain identification. These isolates were subjected to PCR with 16S rDNA primer and biochemically characterized as according to Bergey's Manual of Determinative Bacteriology. The biochemical characteristics of the isolated bacteria (1 and 8) are shown in Table 2.

A gene sequence analysis was performed to further identify the bacteria and to support the results of the biochemical analysis. The percentage of maximum similarity and the gene bank accession numbers are shown in Table 2 too.

3.4 Growth Curve of Bacteria in Presence of PAHs

The PAHs degrading strains obtained a specific growth rate after 24 hours of incubation as seen in Fig. 2. Two bacterial isolates grew without any lag phase.

3.5 PAH Biodegradation

Biodegradation of PAHs by two isolated bacteria was investigated for 120 hours. The results are shown in Tables 3 and 4.

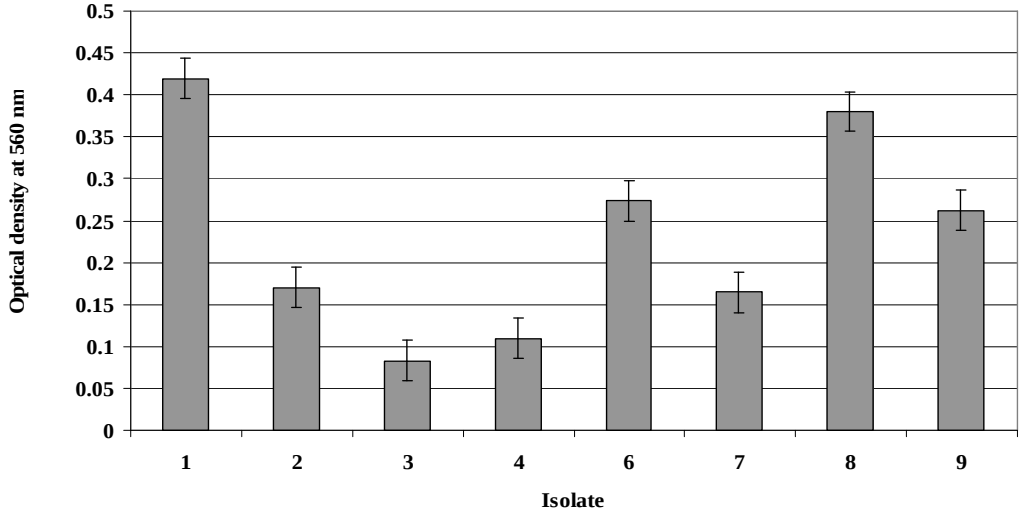

Fig. 1. Optical densities of isolated bacteria in 12.8 mg. L^{-1} PAHs after 24 hours

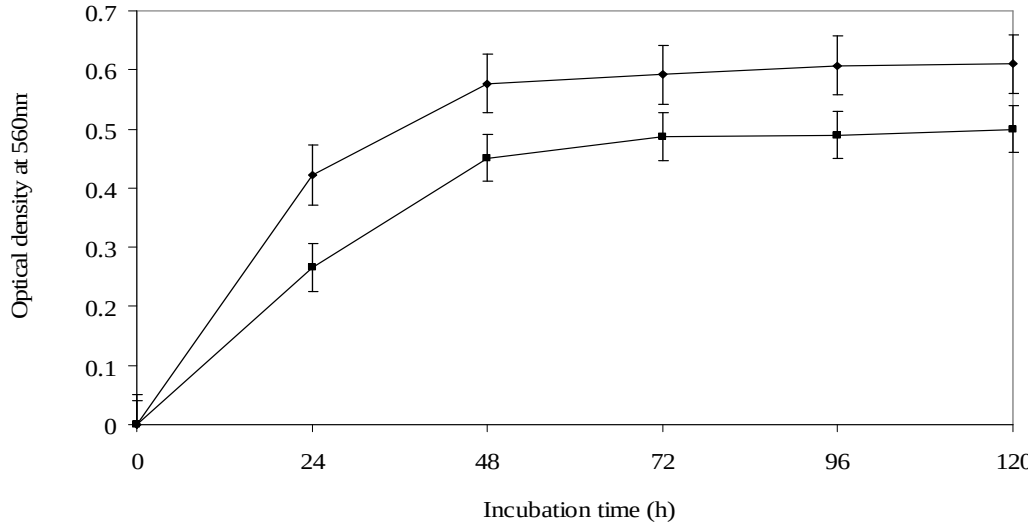

Fig. 2. Growth carves of *Bacillus licheniformis ATHE9* (—♦—) and *Bacillus mojavensis ATHE13* (—■—) at 12.8 mg.L^{-1} of PAHs

Table 2. Biochemical characteristics of the isolates

	Isolate 1	Isolate 8
Morphological characteristic		
Type of colony	Roud, 2-4 mm diameter, fimbriate margins, whitish color, rough surface.	Circular, 2 mm diameter, smooth margins, white Opaque color
Endospore	+	+
Motility	+	+
Gram nature	+	+
Biochemical characteristics		
Catalase	+	+
Oxidase	+	+
Maximum temperature	50ºC	50ºC
Growth at pH=5.6	+	+
Growth at NaCl 5%	+	+
Growth at 7%	+	+
Growth at 10%	+	+
Acid from Glucose	-	+
Monnitol	+	+
Lactose	-	+
Citrate utilization	+	+/-
Nitrate reduction	+	+
Indol production	-	+
H_2S production	-	+
Anaerobic growth	+	-
Growth at 5ºC	-	-
Growth at 10ºC	-	+
Growth at 55ºC	-	-
Growth at 65ºC	-	-
Isolate	*Bacillus licheniformis*	*Bacillus mojavensis*
Identity	99%	98%
Accession No.	KC329470.1	KC469987.1
Strains name of organism on NCBI site	*Bacillus licheniformis ATHE9*	*Bacillus mojavensis ATHE13*

Table 3. Removal percentage of 16 hydrocarbons by *Bacillus licheniformis ATHE9* during 120 hours

Time (h) / PAH (%)	0	24	48	72	96	120
Naphtalene	0	53	67	83	83	100
Acenaphtylene	0	54	68	100	100	100
Acenaphtene	0	35	55	71	100	100
Flourene	0	51	68	69	69	69
Phenanteren	0	30	66	67	73	73
Antracene	0	33	65	67	73	75
Floranthene	0	5	59	70	69	83
Pyrene	0	3	25	42	42	56
Benzo(a)antracene	0	23	55	61	65	66
Chrysene	0	22	22	72	73	74
Benzo(b)floranthene	0	10	58	58	68	65
Benzo(k)floranthene	0	10	58	59	69	69
Benzo(a)pyrene	0	12	59	62	71	72
Benzo(ghi)prylene	0	35	55	55	100	100
Dibenzo(ah)anthracene	0	28	55	55	56	100
Indeno pyrene	0	27	54	55	100	100

Table 4. Removal percentage of 16 hydrocarbons by *Bacillus mojavensis ATHE13* during 120 hours

Time (h) / PAH (%)	0	24	48	72	96	120
Naphtalene	0	53	68	100	100	100
Acenaphtylene	0	54	59	72	100	100
Acenaphtene	0	35	41	60	100	100
Flourene	0	33	65	67	73	75
Phenanteren	0	30	41	74	87	93
Antracene	0	33	41	74	89	92
Floranthene	0	5	18	59	58	64
Pyrene	0	3	18	34	34	35
Benzo(a)Antracene	0	23	23	43	69	71
Chrysene	0	22	22	53	59	70
Benzo(b)floranthene	0	10	23	55	70	70
Benzo(k)floranthene	0	10	16	40	55	55
Benzo(a)pyrene	0	12	25	34	40	40
Benzo(ghi)prylene	0	35	54	55	100	100
Dibenzo(ah)anthracene	0	28	56	56	100	100
Indeno pyrene	0	27	53	61	100	100

4. DISCUSSION

The biodegradation of PAHs has been extensively studied and microbial strains have been isolated for their ability to metabolize PAHs at varying degradation rates. It has been known that some soil bacteria are able to degrade toxic organic compounds [20]. Many soil microorganisms having the ability to degrade chemicals, including herbicides, pesticides, solvents and other organic compounds have been found. They provide a reasonable and effective way to remove chemical wastes. The degradation of PAHs in soil has been successfully demonstrated for large scale remediation [9]. Microorganisms capable of degrading PAHs exist in most environments [21].

Polymerase Chain Reaction (PCR) and DNA hybridization have also proven useful in the detection of polycyclic aromatic hydrocarbon degradation genes in different soil bacteria. Different strains of *Mycobacterium, Sphingomonas, Rhodococcus* and *Xanthomonas* which degrade polycyclic aromatic hydrocarbons have been characterized for gene encoding degradation enzymes for PAH [22,23].

In this study between eight isolated bacteria, two gram positive bacilli belonging to the genus *Bacillus* were found to be more efficient in the biodegradation of PAHs than other isolates. The strains were identified as *Bacillus licheniformis ATHE9* and *Bacillus mojavensis ATHE13* with accession Nos. KC329470.1 and KC469987.1 respectively (Table 2).

The ability of the *Bacillus* species to degrade PAHs has been reported by a number of investigators. The *Bacillus* spp. are more tolerant to high concentrations of poly cyclic aromatic hydrocarbons in soil due to their resistant endospores so the isolates belonging to the *Bacillus* sp. could be effective in removal of PAHs in the contaminated soils [24].

The results show that, the *Bacillus* species were able to grow in the presence of 12.8 mg. L^{-1} PAHs. Similarly, there are several reports of pollutant bioremediation by the action of *Bacillus sp.* occurring in extreme environments [25]. Recent studies have shown that PAHs can be used by *Bacillus* and especially *B. licheniformis* as carbon sours [26].

Ijah et al. [27] reported that *Bacillus spp.* are the predominant isolates of all crude oil–utilizing bacteria characterized from highly polluted soil samples.

Two isolates in this study also showed a very fast growth rate and high growth capacity, as evidenced, in the fact that they showed a very high exponential phase. The results provide evidence of the usefulness of the bacteria *B. licheniformis ATHE9* and *B. mojavensis ATHE13* for bioremediation of PAH-contaminated environments (Fig. 2).

According to Tables 3 and 4, the present study has demonstrated that the bacterium *Bacillus mojavensis ATHE13* carry out rapid degradation of PAHs from the liquid medium rather than *B. licheniformis ATHE9.*

In the study by Kumar et al. [5], *Bacillus sp.* strain isolated from oil-contaminated soil grew and produced biosurfactant in the presence of crude oil. It was capable of utilizing PAHs as a sole carbon and energy source across a wide range of temperatures and salinity levels. Through 16S rDNA analysis, this strain was related to *Bacillus licheniformis*.

One bacterium was isolated from petroleum-contaminated soil from the Shanghai oil field in northern China. This bacterium was used to treat oily sludge. Based on morphological and physiological characteristics and molecular identification, it was identified as *Bacillus amyloliquefaciens* [6].

Cunha et al. [7] isolated seven spore-forming, gram-positive bacteria from the rock of an oil reservoir located in a deep-water production basin in Brazil. These strains were identified using polymerase chain reaction (PCR) as belonging to the genus *Bacillus*. The strains were tested for oil degradation ability in microplates, and they showed positive results.

Concurrent with the growth curve of each isolate at a specified time, it was attempted to investigate their ability to remove hydrocarbons. Every 24 hours, one sample taken from the culture and the hydrocarbons concentrations in it were measured. The results are presented in Tables 3 and 4. The bacterium strain *Bacillus licheniformis ATHE9* was isolated in this study removed acenaphtylene in 72 hours, acenaphtene and indeno pyrene in 96 houres, and Dibenzo (ah) antracene and naphthalene in 120 hours. This isolate also removed about 70% of flourene, antracene and phenantheren, 83% of flourantene, and 72% of benzo (a) pyrene in 120 hours as shown in Table 3.

The results shown in Table 4 indicate that the bacterium strain *Bacillus mojavensis ATHE13* could remove naphthalene from media in 72 hours and acenaphtene, acenaphtylene, benzo (ghi) prylene, dibenzo (ah) anthracene and indeno pyrene from media in 96 hours. This isolate could also remove 64% flouranthene, 75% flourene, 92% antheracene and phenenteren in 120 hours (Table 4).

Maiti et al. [28] were isolated and identified one strain of *Bacillus* from oil contaminated soil in India that it could mineralized antheracene, Fluoranthene, pyrene and benzo (a) pyrene, [29,30,31,32].

Toledo [29] isolated fifteen bacterial strains from solid waste oil samples for their capacity to grow in the presence of naphthalene, phenanthrene, fluoranthene, or pyrene as the sole carbon source. The isolates were identified by PCR, and results showed that the strains belonged to the genera *Bacillus*, *Bacillus pumilus* (eight strains), and *Bacillus subtilis* (two strains) [33,34,35].

Velayutham et al. [30] isolated two efficient PAH-degrading strains and identified them as *Pseudomonas stutzeri* and *Bacillus subtilis*. These bacterial strains were able to degrade within a period of 6 days 95.1% and 99.4% of naphthalene (100 mg. L^{-1}) and 99.5% and 94.6% anthracene (50 mg. L^{-1}), respectively, as the sole source of carbon and energy in the liquid phase [36,37,38,39].

Abd-Elsalam [31] identified some bacterial strains isolated as *Escherichia coli (EF105548), Soil bacterium (EF105549), Alcaligenes sp. (EF105546),* and *Thiobacter subterraneus (EF105547)* that have the ability to degrade PAHs*.* The average degradation rates of anthracene by these bacteria were 28.6, 30.2, 26.6 and 32.1%, while those of phenanthrene were 42.45, 48.44, 34.35 and 40.45% for these strains, respectively [40,41,42,43].

In another study, Eman et al. [32] were isolated three different bacterial strains (*Klebsiella oxytoca, Klebsiella pneumonia,* and *Acinetobacter sp*.) from crude petroleum oil samples that were able to utilize four different PAHs (phenanthrene, fluoranthene, pyrene, and benzene). *Klebsiella oxytoca* is the most efficient utilizer of PAHs compared with *Klebsiella pneumonia* and *Acinetobacter.* It can utilize almost 83% benzene after 48 hours of shaking, if supplemented with a nitrogen source [44,45]. The isolates have been molecularly identified by partial sequencing of the 16S rDNA gene (approximately 900 bp), and the results demonstrate they have a high degree of homology to *Klebsiella oxytoca* (up to 99% similarity), *Klebsiella pneumonia* (from 97 to 99% similarity), and *Acinetobacter sp* (up to 99% similarity) [46,47].

Individual bacterial strain can metabolize only a limited range of hydrocarbon substrates due to specific enzymatic capacities. Because of this behavior, any special bacteria have special ability to degrade some PAHs.

5. CONCLUSION

Bioremediation is a method used to transform compounds to less hazardous or non-hazardous forms with less input of chemicals, energy, and time. There are a number of bacterial species capable of degrading PAHs that have been isolated from different environments. In this study two degrading bacterial strains were isolated from contaminated soil and identified as *Bacillus licheniformis ATHE9* and *Bacillus mojavensis ATHE13*. These bacterial strains were able to grow in media with 12.8 mg. L^{-1} of 16 PAHs and reach the stationary phase. These bacterial strains were also able to remove some PAHs in the media. The results show that using native bacteria with PAH-utilizing capabilities in contaminated sites could be useful for removal of these compounds from the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20362