



Isolation and identification of Phenanthrene degrading bacteria occurring in oil contaminated soils of mechanical workshops

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Abstract

Polyaromatic hydrocarbons (PAHs) are the most deleterious and toxic compounds which adversely affect the living beings. Sixteen PAHs were regarded as carcinogenic by US Environmental Protection Agency. One among them is phenanthrene. PAHs are present in fossil fuels (petroleum products and coal). From different sources PAHs reach the environment and cause pollution. The present study was conducted to isolate and identify the phenanthrene degrading bacteria occurring in oil contaminated soils of automobile mechanical workshops using M9 minimal medium supplemented with phenanthrene as sole carbon and energy source. Fourteen phenanthrene degrading bacterial isolates were recovered from oil contaminated soils of six mechanical workshops. Eight were identified as *Pseudomonas* species, three were *Bacillus* species and remaining three were identified as *Acinetobacter* species. *Pseudomonas* species were predominantly present and they were occurring in all oil contaminated soil samples of six mechanical workshops. These bacteria can be exploited for bioremediation of polluted sites.

Keywords: Polyaromatic hydrocarbons, Phenanthrene, carcinogenic, phenanthrene degrading bacteria, oil contaminated soils, bioremediation

Introduction

The polyaromatic hydrocarbons (PAHs) are the most deleterious, carcinogenic, and mutagenic compounds entering in high levels into the environment from various sources ⁽¹⁾. Crude oil (petroleum products) contains alicyclic, aliphatic and different aromatic hydrocarbons. PAHs are the complex aromatic heterocyclic molecules made up of two or more aromatic rings ⁽²⁾. PAHs are highly stable and sparingly soluble in water due to which they are highly recalcitrant and persistent in the environment ⁽³⁾. Due to leakage of underground petroleum storage tanks ⁽⁴⁾, accidents during transport of fuels, and industrial processes, PAHs enter into the surrounding environment ⁽⁵⁾. Even engine oil is the one of the major sources of PAHs. US environmental protection agency categorized sixteen PAHs as most hazardous and

carcinogenic compounds which include phenanthrene ⁽³⁾. Phenanthrene is composed of three aromatic rings and it is highly toxic to living beings ⁽⁶⁾. Phenanthrene is a photosensitizer allergen of human skin. It induces the exchange of sister chromatids and inhibits the intercellular communication in humans ⁽²⁾. Phenanthrene adversely affects the invertebrates and fishes when enters into the aquatic environment. The effects include increase of respiration rate, reduced growth, impairment of larval development, decreased feeding and offspring formation ⁽⁶⁾. Many physical and chemical methods are being employed for the removal (degradation) of PAHs from environment but most of them are ineffective, costly and not ecofriendly. Bioremediation is an alternative to physical and chemical methods for removing PAHs from

contaminated sites. Bioremediation employs microbes for degrading (metabolizing) PAHs occurring in polluted sites⁽⁵⁾. Many bacterial, fungal and algal representatives have the ability to metabolize PAHs but bacterial species are most widely employed for degradation of PAHs. In recent years microbes have been exploited for the degradation of PAHs and information pertaining to biodegradation pathways, genes and enzymes related PAHs metabolism were revealed. Phenanthrene, naphthalene and anthracene are regarded as representatives of PAH pollution due to their wide occurrence in the nature. Structure of many carcinogenic PAHs is similar to phenanthrene and anthracene. Phenanthrene is the simplest PAH exhibiting both bay and K regions and hence, it is considered as model compound for biodegradation (metabolism) studies of carcinogenic PAHs⁽⁷⁾. The present study deals with the isolation and identification of phenanthrene degrading bacteria occurring in oil contaminated soils of various automobile mechanical workshops of Kaman region (Autonagar) of Karimnagar town, Telangana, India.

Materials and Methods

Collection of soil samples

Oil contaminated soil samples were collected from six different automobile mechanical workshops of Kaman region of Karimnagar town. The workshops were designated as A, B, C, D, E and F. Soil samples were collected from workshop sites contaminated with used engine oil, petroleum and diesel. Spatulas sterilized with ethanol (70%) were used for soil collection and collected soil samples were stored in sterile tubes⁽⁸⁾.

Isolation of phenanthrene degrading bacteria

M9 minimal broth supplemented with phenanthrene (0.01%) as sole carbon and energy source was used to isolate phenanthrene degrading bacteria. Ten grams of each soil sample was added to 100 ml of M9 minimal medium supplemented with phenanthrene and incubated at 30°C for 7 days. After incubation period, each soil broth culture was serially diluted and plated on agar plates for the development of phenanthrene degrading bacterial colonies⁽⁹⁾.

Identification of phenanthrene degrading bacteria

Microscopic observation

Each phenanthrene degrading bacterial isolate was examined under microscope after gram staining and

endospore staining. Further each bacterial isolate was tested for motility.

Biochemical tests for identification of bacterial isolates

Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Phenyl alanine test, Hydrogen sulfide test, Mannitol salt test, Urease test, Oxidase test, Catalase test, Starch hydrolysis, Gelatin hydrolysis and Casein hydrolysis tests were performed for each bacterial isolate for tentative identification till genus level. The abundance different bacterial species was observed⁽¹⁰⁾.

Results and Discussion

In the present study, oil contaminated soil samples of six automobile mechanical workshops were processed in the laboratory for the isolation and identification of phenanthrene degrading bacteria by employing M9 minimal medium supplemented with phenanthrene as sole carbon and energy source. Fourteen bacteria were isolated from the oil contaminated soil samples. The phenanthrene degrading bacteria isolated from mechanical workshop A were designated as A1, A2, A3....., workshop B as B1, B2, B3..... and bacteria isolated from soil samples of remaining workshops were similarly designated. Microscopic examination (Table-1) and biochemical tests (Table-2) of bacterial isolates revealed that 8 bacterial isolates were identified as *Pseudomonas* species, 3 were *Acinetobacter* species and remaining 3 were identified as *Bacillus* species. Farshid *et al.*, (2014) isolated phenanthrene degrading bacterial strains from soil samples near oil Company, Andimeshk, Iran and identified them as *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Microluteus* species⁽¹¹⁾. Alejandro *et al.*, (2017) isolated *Pseudomonas* strain from Antarctic soil samples collected from sites of King George Island, South Shetland Islands⁽⁸⁾. Fazilah *et al.*, (2018) isolated phenanthrene degrading *Acinetobacter* strain from soils polluted with PAHs along the East Coast Peninsular of Malaysia in the State of Pahang, Terengganu and Kelantan⁽¹²⁾. In the present work *Pseudomonas* species were found to be present predominantly and they were occurring in all the soil samples. *Bacillus* species were occurring only in mechanical workshops B, D and F whereas *Acinetobacter* species were found in mechanical workshops B, C and F.

Table-1: Microscopic examination of bacterial isolates

S.No.	Bacterial isolate	Gram staining	Endospore staining	Motility
1	A1	Gram positive rods	Negative	Positive
2	A2	Gram positive rods	Negative	Positive
3	B1	Gram positive rods	Positive	Positive
4	B2	Gram negative rods	Negative	Negative
5	B3	Gram positive rods	Negative	Positive
6	B4	Gram positive rods	Negative	Positive
7	C1	Gram negative rods	Negative	Negative
8	C2	Gram positive rods	Negative	Positive
9	D1	Gram positive rods	Positive	Positive
10	D2	Gram positive rods	Negative	Positive
11	E1	Gram positive rods	Negative	Positive
12	F1	Gram positive rods	Negative	Positive
13	F2	Gram positive rods	Positive	Positive
14	F3	Gram negative rods	Negative	Negative

Table-2: Biochemical characters and identification bacteria till genus level

S.No.	Bacterial Isolate	Indole test	Methyl red test	Voges Proskauerr test	Citrate test	Phenylalanine test	Hydrogen sulfide test	Mannitol test	Urease test	Oxidase test	Catalase test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Identified bacterium
1	A1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
2	A2	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
3	B1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
4	B2	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
5	B3	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
6	B4	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
7	C1	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
8	C2	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
9	D1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
10	D2	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
11	E1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
12	F1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
13	F2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
14	F3	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>

Symbols: + and - indicate positive and negative respectively

Conclusion

The fourteen phenanthrene degrading bacteria isolated from oil contaminated soils were identified till genus level. They can be identified till species level by 16S rRNA gene sequencing. Majority of the phenanthrene bacterial species occurring in oil contaminated soils were found to be *Pseudomonas*. Further the phenanthrene degrading ability of the bacteria can be improved, optimized and exploited in the area of bioremediation.

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