

Isolation, Characterization and Identification of Anthracene Degrading Bacteria Occurring in Oil Contaminated Soils of Mechanical Workshops

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ABSTRACT

Polyaromatic hydrocarbons (PAHs) released from various sources are accumulating in the environment. Some of the PAHs recommended by USEPA are highly toxic to living organisms. Anthracene is included in such toxic class of PAHs. When PAHs persist in the environment they cause deleterious effect to human beings and even they decrease crop productivity by polluting agricultural lands. Physical and chemical methods employed to remove PAHs from polluted soils are ineffective. Instead, microbes can be employed to metabolize PAHs occurring in contaminated soils. In the present paper, PAH selected for biodegradation studies is anthracene. Oil contaminated soil samples were collected from six mechanical workshops, processed in the laboratory and eighteen anthracene degrading bacteria were isolated and identified till genus level. Six bacterial isolates were identified as *Pseudomonas*, five were *Serratia*, four were *Bacillus* and remaining three was identified as *Acinetobacter* species. These bacteria can be improved and employed for bioremediation process.

Keywords: Polyaromatic hydrocarbons, Anthracene, microbes, biodegradation, oil contaminated soil

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are released into the environment due to various activities like refining of petroleum products, coal, industrial activities, fire accidents in forests etc. PAHs contain two or more fused benzene rings. They are highly stable in the nature and least soluble in water. Some of the PAHs are carcinogenic and mutagenic. When they persist in the environment they cause deleterious effects to humans and

environment. They cause skin allergy to humans and contaminate agricultural lands and decrease crop production.^[1] Environmental Protection Agency, US regarded sixteen PAHs as toxic to humans. Anthracene is one among those sixteen toxic PAHs. Many of the industrial sites are polluted with anthracene. Anthracene when reaches soil it will bind to soil particles and does not percolate deep into the soil.^[2] Anthracene can absorb ultraviolet radiation and undergo a series of chemical reactions. Such anthracene is highly toxic to aquatic life.^[3] The physicochemical techniques used to remove PAHs from contaminated sites are not effective. An alternative to physicochemical methods is use of microbes to degrade PAHs. This process of degradation of organic pollutants by employing microbes in the environment is called Bioremediation.^[4]

MATERIALS AND METHODS

Medium

Six automobile mechanical workshops of Autonagar of Kaman region, Karimnagar, Telangana state were selected for the study and they were designated as A, B, C, D, E and F. The soil samples polluted with petrol, diesel and used engine oil were collected and preserved in sterile polythene bags. One gram of each oil contaminated soil sample was transferred to 10 ml of Bushnell Haas broth (BHB) supplemented with anthracene (1 mg/ml) as sole carbon and energy source and incubated at 30°C for one week. After five sub cultivations each BH broth culture was serially diluted and spread on BH agar medium plates supplemented with 1 mg/ml anthracene and incubated at 30°C for two

days.^[5] After incubation period, different bacterial colonies were selected based colony characters.

The composition of BHB is magnesium sulfate-0.2 g, calcium chloride-0.02g, monopotassium phosphate-1.0 g, diammonium hydrogen phosphate-1.0 g, potassium nitrate-1.0 g and Ferric chloride-0.05 g, distilled water-1000 ml.

Identification of bacterial isolates

Microscopic examination

The bacterial isolates were observed under microscope after gram staining and endospore staining and motility test was also performed.

Biochemical tests

For the tentative identification of bacteria till genus level, Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Phenyl alanine test, Hydrogen sulfide test, Mannitol test, Urease test, Oxidase test, Catalase test, Starch hydrolysis, Gelatin hydrolysis and Casein hydrolysis tests were performed.

The anthracene bacteria isolated from Workshop, 'A' were designated as A1, A2, A3, A4....An, Workshop, 'B' as B1, B2, B3, B4.....Bn, Workshop 'C' as C1, C2, C3, C4....Cn, Workshop D as D1, D2, D3, D4....Dn, Workshop E as E1, E2, E3, E4...En and Workshop F as F1, F2, F3, F4... Fn.

RESULTS AND DISCUSSION

Eighteen anthracene degrading bacteria were isolated from oil contaminated soil samples of six automobile mechanical workshops located in Autonagar of Kaman region of Karimnagar town and identified till their genus level based on microscopic examination (Table-1) and biochemical characterization (Table-2). Six bacterial isolates (A3, B2, B4, C2, D1 and F1) were identified as *Pseudomonas*, five (A1, B1, B5, E1 and F2) were *Serratia*, four (A2, C1, D2 and E2) were *Bacillus* and remaining three (A4, B3 And D3) were identified as *Acinetobacter* species. Mohamed *et al.* (2018) isolated *Bacillus subtilis* and *Serratia liquefaciens* from industrial waste areas in Egypt and optimized their anthracene degrading parameters.^[6] *Acinetobacter johnsonii* was employed to degrade naphthalene and anthracene in the medium supplemented with naphthalene and anthracene in single and dual substrate systems.^[7] Rodrigo *et al.* (2005) evaluated the anthracene degradation ability of *Pseudomonas* species isolated from a fourteen year old landfarm contaminated with petrochemicals.^[8] In the present study majority of the anthracene degrading bacteria isolated were *Pseudomonas* species (six) and least (three) were *Acinetobacter* species.

Table-1: Microscopic examination of bacterial isolates

S.No.	Bacterial isolate	Gram staining	Morphology	Endospore staining	Motility
1	A1	Gram negative	Rod shaped	Negative	Positive
2	A2	Gram positive	Rod shaped	Positive	Positive
3	A3	Gram positive	Rod shaped	Negative	Positive
4	A4	Gram negative	Rod shaped	Negative	Negative
5	B1	Gram negative	Rod shaped	Negative	Positive
6	B2	Gram positive	Rod shaped	Negative	Positive
7	B3	Gram negative	Rod shaped	Negative	Negative
8	B4	Gram positive	Rod shaped	Negative	Positive
9	B5	Gram negative	Rod shaped	Negative	Positive
10	C1	Gram positive	Rod shaped	Positive	Positive
11	C2	Gram positive	Rod shaped	Negative	Positive
12	D1	Gram positive	Rod shaped	Negative	Positive
13	D2	Gram positive	Rod shaped	Positive	Positive
14	D3	Gram negative	Rod shaped	Negative	Negative
15	E1	Gram negative	Rod shaped	Negative	Positive
16	E2	Gram positive	Rod shaped	Positive	Positive
17	F1	Gram positive	Rod shaped	Negative	Positive
18	F2	Gram negative	Rod shaped	Negative	Positive

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

S.No.	Bacterial Isolate	Indole test	Methyl red test	Voges Proskauer	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>Serratia</i>
2	A2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
3	A3	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
4	A4	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
5	B1	-	-	-	+	-	+	+	+	+	-	-	+	+	<i>Serratia</i>
6	B2	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
7	B3	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
8	B4	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
9	B5	-	-	-	+	-	+	+	+	+	-	-	+	+	<i>Serratia</i>
10	C1	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
11	C2	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
12	D1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
13	D2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
14	D3	-	-	-	+	-	-	+	-	-	+	-	+	-	<i>Acinetobacter</i>
15	E1	-	-	-	+	-	+	+	+	+	-	-	+	+	<i>Serratia</i>
16	E2	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Bacillus</i>
17	F1	-	-	-	+	-	-	-	-	+	+	-	-	+	<i>Pseudomonas</i>
18	F2	-	-	-	+	-	+	+	+	+	-	-	+	+	<i>Serratia</i>

Indication of Symbols: - for negative the test; + for the positive test

CONCLUSION

Oil contaminated soil samples of six mechanical workshops were analyzed and eighteen anthracene degrading bacteria were isolated and identified till genus level. *Pseudomonas* species were isolated more in number. These anthracene degrading bacteria can be identified till species level and efficient strains can be identified among them. The efficient strains can be improved and employed for bioremediation process.

REFERENCES

- Zahra F, Golamhossein E. Isolation of Polycyclic Aromatic Hydrocarbons (PAHs) Degrading Bacteria from Arak Petrochemical Wastewater. *Journal of Microbiology, Biotechnology and Food Sciences*. 2018; 7(5): 499-504.
- Abidin ANZ, Talib SA, Alias S, Tay SS. Occurrence and Bioremediation of Anthracene in the Environment. *Journal of Fundamental and Applied Sciences*. 2017; 9(6S): 214-226.
- Ceng G, Xinin Z, Ningning X, Xuei T. Toxic effects of combined effects of anthracene and UV radiation on *Bronchionus plicatilis*. *International Symposium on Resource Exploration and Environmental Science IOP Publishing IOP Conf. Series: Earth and Environmental Science* 64. 2017; 1-11.
- Umar ZD, Nor AAZ, Syaizwan ZZ, Muskhazli M. Identification of Phenanthrene and Pyrene degrading Bacteria in used Engine Contaminated Soil. *International Journal of Scientific & Engineering Research*. 2016; 7(3): 680-686.
- Mahesh K, Tukaram K, Hemalata B, Kanchanprabha M. Characterization of Anthracene Degrading bacteria from Drug Industry Effluent Polluted Soil. *Archives of Applied Science Research*. 2015; 11:16-22.
- Mohamed TS, Hamdy AA, Gehad HZ. Studies on Utilization and Degradation of Anthracene by Bacteria Isolated from Industrial Waste Water Areas in Egypt. *Journal of Biochemistry, Microbiology and Biotechnology*. 2018; 6(1): 35-40.
- Jiang Y, Qi H, Zhang XM. Co-biodegradation of anthracene and naphthalene by the bacterium *Acinetobacter johnsonii*. *Journal of Environmental Science and Health*. 2018; 53(5): 448-456.
- Rodrigo JSJ, Eder CS, Fatima MB, Maria CRP, Pedro AS, Enilson LSSa, Flavio AOC. Anthracene biodegradation by *Pseudomonas* sp. isolated from a petrochemical sludge landfarming site. *International Biodeterioration & Biodegradation*. 2005; 56(3): 143-150.

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