ISOLATION AND IDENTIFICATION OF NAPHTHALENE DEGRADING BACTERIA OCCURRING IN OIL CONTAMINATED SOILS

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***Abstract*: Polyaromatic hydrocarbons (PAHs) are the most hazardous compounds which damage the environment and human health. They are released from various industries, mechanical workshops and oil refineries. Naphthalene is one the prominent hazardous polyaromatic compounds. Bioremediation which uses microbes for cleaning up the environment is the most suitable method for the removal of PAHs. In the present study oil contaminated soils of four mechanical workshops were processed in the laboratory for the isolation of naphthalene degrading bacteria. Fourteen bacteria were isolated and identified till genus level. Eight bacterial isolates were identified as *Bacillus,* three were *Micrococcus* and another three were identified as *Pseudomonas* species.**

Keywords*: Polyaromatic hydrocarbons, Naphthalene, Oil contaminated soils, Bacteria, *Bacillus, Pseudomonas* and *Micrococcus.

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) commonly occur in the in petroleum products and crude oils. These compounds enter into the environment from industries, automobile mechanical workshops and through other manmade activities. Soil composition and texture is disturbed due to the presence of PAHs. These compounds affect the biogeochemical cycles, vegetation, food production etc. On the other hand PAHs are highly persistent, mutagenic, toxic and carcinogenic. Hence, soils polluted with PAHs are of human health concern. Naphthalene is one of the prominent PAHs which occur commonly in oil contaminated soils [1, 2]. Therefore, removal or detoxification of these PAHs is essential to protect the environment and human health. Bioremediation is the best suitable, eco-friendly, economical and safe approach for the removal of such pollutants. Bioremediation is the process which employs microbes to clean polluted environment. There are numerous bacteria in nature which have the ability to degrade PAHs especially naphthalene which is one of the most hazardous polyaromatic pollutant [3]. Many bacteria which have the ability to degrade naphthalene were isolated. They include the genera, *Micrococcus, Rhodococcus, Pseudomonas* etc [1]. This paper deals with the isolation and identification of naphthalene (a model PAH compound) degrading bacteria occurring in oil contaminated soils of mechanical workshops of Kaman region of Karimnagar town, Telangana State.

II. MATERIALS AND METHODS

A. Soil samples

Oil contaminated soil samples were collected as per standard procedure from four mechanical workshops of Kaman region of Karimnagar town. The five workshops were designated as A, B, C, and D. The soil samples were processed in the laboratory for the isolation and identification of naphthalene degrading bacteria.

B. Medium and procedure for the isolation of naphthalene degrading bacteria

One gram of each oil contaminated soil sample is added to 100 ml Basal salt mineral (BSM) medium (K_2HPO_4, 0.38g; $MgSO_4.7H_2O$, 0.2g; NH_4Cl, 1.0g; $FeCl_3$, 0.05g; Distilled water, 1.0 liter with Ph, 7.0) liquid medium supplemented with 1.0g peptone and 0.1% naphthalene. All the flasks were incubated at 30^0 C for one week. The only carbon source available for the bacteria in the medium is naphthalene. After incubation period the bacteria grown in BSM broth were streaked on BSM agar medium (supplemented with peptone and naphthalene) plates [2]. Then each bacterial colony is isolated separately on BSM agar slants (supplemented with naphthalene). The colony characteristics of each colony were recorded. All the bacterial isolates were observed microscopically by gram staining, endospore staining and motility test. Biochemical tests were performed for each bacterial isolate to identify them to till genus level. The biochemical tests performed were IMViC tests, Phenylalanine, Hydrogen sulfide, Mannitol salt, Urease Oxidase, Catalase tests and Starch hydrolysis, Gelatin hydrolysis and Casein hydrolysis [4]. Abundance of bacterial isolates was examined.

III. RESULTS AND DISCUSSION

Soil samples of five mechanical workshops were processed in the laboratory for the isolation of naphthalene degrading bacteria using BSM broth supplemented with peptone and naphthalene. Fifteen predominantly growing bacterial isolates were identified till

genus based on colony characteristics (table-1), microscopic examination (table-1) and biochemical tests (table-2). The bacterial isolates workshop A were designated as A1, A2, A3……., workshop B as B1, B2, B3….. workshop C as C1, C2, C3…….., and workshop D as D1, D2, D3…….. Eight bacterial isolates were identified as *Bacillus* species, three were *Pseudomonas* and remaining three were identified as *Micrococcus* species. The *Bacillus* species are occurring in all the oil contaminated soils of four mechanical workshop. Navdeep *et al.*, (2013) isolated *Micrococcus* species from oil contaminated soils of Guru Gobind Singh refinery, Bhatinda, Punjab and Panipat oil refinery, Haryana [5]. Recent studies reported that *Bacillus* and *Pseudomonas* species are able to degrade naphthalene [6]. From the present study it is clear that *Bacillus* species are predominantly occurring in oil contaminated soils. Further these bacteria can be identified till species level and their naphthalene degrading ability may be improved. The bacteria isolated in the present study can be used for bioremediation.

Table-1: Colony characteristics and Microscopic examination

Sl.No	Bacterial isolate	Colony characteristics					Microscopic examination		
		Shape	Surface	Elevation	Margin	Colour	Gram Staining	Endospore Staining	Motility test
1	A1	Round	Smooth	Flat	Entire	Green	Gram positive cocci	Negative	negative
2	A2	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
3	A3	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
4	B1	Round	Smooth	Flat	Entire	Green	Gram positive cocci	Negative	negative
5	B2	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
6	B3	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
7	C1	Circular	Smooth	Flat	Entire	Cream	Gram positive rod	Negative	Positive
8	C2	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
9	C3	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
10	D1	Circular	Smooth	Flat	Entire	Cream	Gram positive rod	Negative	Positive
11	D2	Circular	Smooth	Flat	Entire	Cream	Gram positive rod	Negative	Positive
12	D3	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
13	D4	Round	Smooth	Flat	Entire	Milky white	Gram positive rod	Positive	Positive
14	D5	Round	Smooth	Flat	Entire	Green	Gram positive cocci	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	-	+	-	+	+	+	-	+	+	+	-	+	+	*Micrococcus*
2	A2	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
3	A3	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
4	B1	-	+	-	+	+	+	-	+	+	+	-	+	+	*Micrococcus*
5	B2	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
6	B3	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
7	C1	-	-	-	+	-	-	+	-	-	+	-	+	-	*Pseudomonas*
8	C2	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
9	C3	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
10	D1	-	-	-	+	-	-	+	-	-	+	-	+	-	*Pseudomonas*
11	D2	-	-	-	+	-	-	+	-	-	+	-	+	-	*Pseudomonas*
12	D3	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
13	D4	-	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus*
14	D5	-	+	-	+	+	+	-	+	+	+	-	+	+	*Micrococcus*

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