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RESEARCH ARTICLE

"AN ANALYTICAL STUDY ON POLY AROMATIC HYDROCARBON (PAH) COMPONENTS FOR INDIGENOUS MICROBIAL CULTIVATION"

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ABSTRACT

The degradation pathways of a variety of petroleum hydrocarbons (e.g., aliphatics and polyaromatics) have been shown to employ oxidizing reactions; however, these pathways differ greatly because of the specific oxygenases found in different bacterial species. For instance, some bacteria can metabolize specific alkanes, while others break down aromatic or resin fractions of hydrocarbons. Many normal and extreme bacterial species have been isolated and utilized as biodegraders for dealing with petroleum hydrocarbons. This phenomenon is related to the chemical structure of petroleum hydrocarbon components. These organism are showing similarity to *Bacillus pumilus*, *B. subtilis*, *Micrococcus luteus*, *Alcaligenes faecalis, Enterobacter cloacae, Pseudomonas aeruginosa* respectively

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INTRODUCTION

Accordingly, there is a constant threat of contamination wherever oil is exploited when coupled with an insufficient ability to deal with oil-contaminated environments, especially in extreme or unique environments such as Polar Regions, deep sea areas, deserts, and wetlands. Although oil. Human exposure to PAHs occurs in three ways, inhalation, dermal contact and consumption of contaminated foods, which account for 88–98% of such contamination; (1). Both the World Health Organization and the UK Expert Panel on Air Quality Standards (EPAQS) have considered benzo(a)pyrene (BaP) as a marker of the carcinogenic potency of the polycyclic aromatic hydrocarbons (PAH) mixture Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in urban atmospheres (2). Petroleum oil is an important strategic resource for which all countries compete fiercely. Indeed, anthropogenic activity is reliant on oil to meet its energy demands, which causes the petrochemical industry to flourish. However, petroleum use results in environmental deterioration (3). During petroleum production, storage and transportation, refining and processing, as well as spills and discharges of petroleum hydrocarbons often occur as a result of blowout accidents during oilfield development, leakage from oil pipelines and storage tanks, oil tanker and tanker leakage accidents, oil well waxing, and during overhauls of refineries and petrochemical production equipment (4,5). Large spills should be recycled or eliminated to as great a degree as possible, but in some cases it is difficult to recover the spilled materials, resulting in its remaining in the affected area, and posing persistent risks to the environment. (6)

PETROLEUM HYDROCARBON-DEGRADING BACTERIA: Most petroleum hydrocarbons encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment (7).

The development of microbial biotechnology and high-throughput sequencing technology, such as microfluidic techniques (8), is beneficial for screening and identifying functional microorganisms from petroleum hydrocarbon contaminated environments. Indeed, many studies have revealed that there is a large number of hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill areas and oil reservoirs (9). Recent studies have identified bacteria from more than 79 genera that are capable of degrading petroleum hydrocarbons (10); several of these bacteria such as Achromobacter, Acinetobacter, Alkanindiges, Alteromonas, Arthrobacter, Burkholderia, Dietzia, Enterobacter, Kocuria, Marinobacter, Mycobacterium, Pandoraea, Pseudomonas, Staphylococcus, Streptobacillus, Streptococcus, and Rhodococcus have been found to play vital roles in petroleum hydrocarbon degradation (11). Similarly, some obligate hydrocarbonoclastic bacteria (OHCB), including Alcanivorax, Marinobacter, Thallassolituus, Cycloclasticus, Oleispira and a few others (the OHCB), showed a low abundance or undetectable status before pollution, but were found to be dominant after petroleum oil contamination (12). These phenomena suggest that these microorganisms are crucial to the degradation of petroleum hydrocarbons, and that they significantly influence the transformation and fate of petroleum hydrocarbons in the environment. Indeed, most bacteria can only effectively degrade or utilize certain petroleum hydrocarbon components, while others are completely unavailable (13). This can be attributed to the fact that different indigenous bacteria have different catalytic enzymes; thus, their roles in oil contaminated sites also vary widely. This also implies that the remediation of petroleum hydrocarbon contamination requires the joint action of multiple functional bacteria to achieve the best environmental purification effect (14).

METHODOLOGY

SAMPLING SITES: Soil and sludge samples were collected from deposited areas near by Rewa(M.P.) Gasoline and samples of used motor oil, Castrol Syntec, Servo oil residues were obtained from a local gas station such as Transport Nagar, Fort road, Khannachowk , Bus stand, RTO Road, University Stadium Road Rewa (M.P), for the performance of incubation test, hydrocarbons (C10-C16) and 2,6,10,14-tetramethyl pentadecane were also purchased from SIGMA.

MICROORGANISMS AND ISOLATION: Microorganisms used in all experiments were isolated by selective enrichment technique from the selected areas from which we have taken 12 samples from Transport Nagar, 06 samples from Sirmour Chowk, 06 from Bus Stand ,06 samples from Fort Road, 06 samples from Khanna Chowk , 06 samples from RTO Road , 06 samples from University Stadium Road, Rewa (M.P). Bushnell-Haas Broth was used in the enrichment technique supplemented with 2 % v/v hydrocarbon substrates (15). The hydrocarbons substrates used in enrichment methods represent: equivalent mixture of hexadecane, heptadecane and 2,6,10, 14-tetramethylpentadecane (pristane); motor oil (Quaker State); equivalent mixture of motor oil from local gas station; equivalent mixture of organic waste and used motor oil (16). After 20days of incubation on rotary shaker incubator at 30^{O}C, 1 ml of sample from primary enrichment was transferred to a fresh Bushnell-Haas Broth containing the same hydrocarbon mix as primary culture and continued to incubate. Unless otherwise stated, after 2nd enrichment, 0.1 ml of media was plated after appropriate dilution on PCA agar and incubated at 26^{0}C (17). After 48 hour incubation, pure colonies were isolated by using a single colony isolation procedure from each enrichment. Isolated colonies were stored at 4^{0}C and re-plated at PCA agar plates at 3- week intervals. Bacterial isolates not used in biodegradable experiments were mixed with 40 % glycerol and stored at -70^{0}C for future use (**18**). The initial number of total viable cells in the original sample (before enrichment) was determined by serial dilution-agar plating procedure (0.1 ml of series of dilutions 10^{2}-10^{8} was spread on PCA agar plates and incubated at 26^{0}C for 48 hours) (**19**). All soil samples and sludge of waste water were collected from 5 to 20 cm below the surface with sterilized soil layers and the top 5 cm of the samples were discarded. The soil layers were placed in sterile poly bags and stored at 4^{0}C to be used within 4-6 hours. Water samples were collected in 100ml screw capped sterile glass tubes and transported to lab(**20**).

RESULTS

GROWTH OF DIFFERENT INDIGENOUS BACTERIAL COMMUNITIES ON DIFFERENT PAHs: The soil and sludge samples collected from sludge wastes and from agriculture soil which were used to isolate their microbial communities (Indigenous mixed bacteria) to investigate their ability to grow and degrade the chosen Polycylic Aromatic

Table no 1. Shows the collection sites of soil samples

Sampling sites (No.)	Type of sample	Depth	Distance from the origin deposit	Tenure of exposure	pH of sample
1	Soil contaminated with oil	surface	Zero m *	Chronic soil	4.99
2	Soil contaminated with oil	surface	Zero m *	Chronic soil	4.56
3	Soil contaminated with oil	30 cm	Zero m *	Recent soil	5.40
4	Soil contaminated with oil	30 cm	100 m	Chronic soil	4.90
5	Soil contaminated with oil	30 cm	100 m	Chronic soil	5.20
6	Sludge waste of petroleum	surface	100 m	Recent soil	5.11
7	Agriculture soil	surface	100 m	Chronic soil	5.26

Selected Compound	Log Indigenous Bacterial Communities Count													
	1		2		3		4		5		6		7	
	Log I_1	Log I_1/log Io	Log I_2	Log I_2/log Io	Log I_3	Log I_3/log Io	Log I_4	Log I_4/log Io	Log I_5	Log I_5/log Io	Log I_6	Log I_6/log Io	Log I_7	Log I_7/log Io
Napthalene	5.987	1.0	4.873	0.8	6.0	0.9	702.4	1.0	6.602	1.0	6.00	1.0	5.903	1.0
Phenanthrene	6.602	1.1	7.447	1.3	7.041	1.0	7.204	1.0	6.778	1.0	5.778	0.9	6.301	1.0
Anthracene	7.079	1.2	5.954	1.0	7.079	1.0	6.698	0.9	6.602	1.0	6.301	1.0	6.602	1.1
Acenapthalene	6.845	1.2	6.447	1.0	6.6.2	1.0	6.903	0.9	6.602	1.0	5.778	0.9	6.477	1.0
Fluoranthene	5.602	1.0	4.301	0.7	6.954	1.0	7.278	1.0	6.698	1.0	5.477	0.9	6.000	1.0
Pyrene	7.531	1.3	5.602	0.9	6.903	1.0	6.903	0.9	6.301	0.9	5.602	0.9	6.778	1.1

Table No. 2. Shows ability of indigenous isolated strains to grown on different available PAHs

Isolated samples	Napthalene	Anthracene	Phenanthelene	Acenapthalene
SC -1	+	+	+	+
SC -2	+	+	+	-ve
SC -3	+	+	+	+
BS-1	+	+	+	-ve
BS-2	+	++	+++	-ve
BS-3	++	+++	++	++
TN-1	+	+	++	+
TN-2	++	+++	++	+
TN-3	++++	+++	+++	++++
KC-1	+	+	+	+
KC-2	++	-ve	++	+
KC-3	++++	++	++	+
FR-1	++	+++	++	++
FR-2	+	-ve	-ve	+
FR-3	++	++	+	++
RT-1	++++	+++	+++	++++
RT-2	+	+++	+	+
RT-3	+++	+++	++	+++
US-1	+	++	+	+
US-2	+	-ve	+	-ve
US-3	+	+	+	+

Table No 3. shows the Biochemical Identification Test of potential HDB species

Sn.	Biochemical Test	SC-3	KC-3	BS-2	BS-4	TN-2	US-1
1	Grams Stain	+ve	+ve	+ve	-ve	-ve	-ve
2	Catalase Test	+ve	+ve	+ve	+ve	+ve	+ve
3	Oxidase Test	-ve	-ve /+ve	+ve	+ve	-ve	-ve
4	MacConkey's agar	-ve	-ve	-ve	+ve	+ve	+ve
5	Dnase Test	-ve	-ve	+ve	-ve	-ve	-ve
6	Pigment Test	+ve	-ve	-ve /+ve	-ve	-ve	+ve
7	TSI test	-ve	+ve	-ve	+ve	+ve	-ve
8	ONPG test	-ve	-ve	+ve	+ve	+ve	-ve
9	MR-VP test	-ve	-ve /+ve	+ve	-ve	-ve /+ve	-ve
10	Citrate Utilization Test	-ve	+ve	+ve	+ve	+ve	+ve

Hydrocarbons(PAH) [naphthalene (Naph.), Phenanthrene (Phen.), anthracene (Anth.), Acenaphthene (Ace.), Fluroanthen (Flu.), Pyrene (Pyr.) (21) as a sole carbon and energy source. The seven different indigenous microbial (bacterial) communities' samples as indicated in Table No.7 were isolated from recent and chronic soils contaminated with petroleum at different depths and distances. The chronic soil had a 20-30 years exposure history for deposition of petroleum wastes. (**22**)

ISOLATION AND DETERMINATION OF STRAINS HAVING THE ABILITY TO DEGRADE DIFFERENT PAHs: The ability of different indigenous isolated stains to grow on different concentrations of PAHs had been indicated in Table (2). It is clear that isolates of code SC-3, KC-3,BS-2,BS-4,TN-2 and US-1 are the best isolates having the abilities to grow on different PAHs as a sole carbon and energy source. These six most potent strains were used for further studies by using each isolate with different concentrations of different PAHs.

CONCLUSION

We had found several species that are able to grow slightly and some are very efficiently able to grow on the selected medium and also on the carbon sources that are provided to them. Among from all those species we have finally found some of the most prominent species that may grow efficiently on every concentration of the provided carbon sources in the medium. In this study we have found the totally six strains that are showing the better potential of hydrocarbon degradation potential.

These organism are showing similarity to *Bacillus pumilus*, *B. subtilis*, *Micrococcus luteus*, *Alcaligenes faecalis, Enterobacter cloacae, Pseudomonas aeruginosa* respectively were able to grow on mineral liquid media amended with Naphthalene, Fluaranthene, Acenapthelene, phenanthrene, fluoranthene and pyrene as sole carbon and energy source.

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