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Research Article

SCREENING AND CHARACTERIZATION OF NAPHTHALENE DEGRADING BACTERIA

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ABSTRACT

Aim: Present investigation was carried out to study the screening and characterization of naphthalene degrading bacteria. Naphthalene is toxic to blood, kidneys, nervous system etc. Thus the aim of the study was to isolate cultures from oil spilled soil samples in Chennai and to study the naphthalene degrading bacteria present in sample. Methods: Samples were collected and isolated. Best grown isolates were selected and were maintained in M9 medium and stored at 4°C. Biochemical and physiological characterization were carried out. Biodegradation study with naphthalene adapted bacterial strains were done at optimized conditions. Results: A total of 8 strains were isolated and two strains P3 and P6 showed rich growth on 200 ppm naphthalene. These two strains were selected as naphthalene degrading bacteria and used for further studies. Discussion: These samples were extracted at 0 to 7 days for calculating the efficiency of both the strains. Strain P6 (*Pseudomonas* sp) was found to be more efficient to degrade naphthalene than strain P3. Genomic studies were carried out and the genetic relationship between the strains and known members of other species of *Pseudomonas* genus were estimated by parsimony analysis. Conclusion: Hence it was concluded that *Pseudomonas aeruginosa* is one of the most common strains found in oil spill region because soil is a rich source of microbes. These microbes have the ability to degrade the pollutants efficiently in the culture. PAH contaminated soil and sediments can be exploited further to study other bacterial strains capable of degrading PAHs and organic contaminants.

Keywords: Polycyclic aromatic hydrocarbons (PAH), Naphthalene, Biodegradation, *Pseudomonas* sp and M9 medium.

INTRODUCTION

Environmental protection and the prevention of pollution has become one of the main scientific, social and economic activities in this 21st century. However, the increasing concentrations of toxic substances in biota and their accumulation along food chains reported in many areas is a sign that degradation of the natural environment is still in progress[1]. Polycyclic aromatic hydrocarbons (PAHs) are widely distributed group of persistent organic pollutants (POPS). These are organic contaminants that are resistant to degradation and can remain and relocate in the environment for longer periods as a result of the incomplete combustion of organic matter. Being a major component of petroleum they are continuously released into natural environment and pose a serious risk to human health[2]. Many PAHs have the potential to cause adverse environmental effects and their epoxides are highly toxic, carcinogenic and mutagenic targeting the microorganisms as well as the higher order animals including humans. These PAH's have higher bioaccumulation and undergo biomagnification when they enter biotic entities. Many physico chemical methods have been applied to remove these compounds from the environment, but there are certain limitations. Xenobiotic degrading microorganisms have also found to have tremendous potential for biodegradation, but new modifications are required for these microorganisms to be effective and efficient in removing these compounds.

Many bacterial species are known to degrade PAHs and they are isolated from contaminated soils or sediments. *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Mycobacterium* sp, *Haemophilus* sp, *Rhodococcus* sp and *Paenibacillus* sp. are some of the frequently studied PAH degrading bacteria. Lignolytic fungi such as *Phanerochaete chrysosporium, Bjerkandera adusta* and *Pleurotus ostreatus* have shown the property of PAH degradation. It has been observed that Polycyclic aromatic hydrocarbons (PAHs) could be biodegraded under both aerobic and anaerobic conditions and the rate of biodegradation could be increased by physical and chemical pretreatment of contaminated soil. Further increase in bioavailability of PAH's and metabolic potential of the bacterial community could be done by the addition of biosurfactant producing bacteria and light oils. Compost materials when added to contaminated soils also enhance biodegradation without any long term accumulation of extractable polar and more available intermediates[3]. However, the field has expanded in recent years to encompass a wide variety of chemicals and a broad array of issues. Some technologies are being developed that markedly enhance microbial destruction or degradation of organic pollutants that otherwise would have persisted at the cleanup of many polluted groundwater and soils using the orthodox physical and chemical methods[4].

Naphthalene, the simplest PAH, has long been used as a model compound in PAH Bioremediation studies. Common naphthalene-degrading bacteria include *Pseudomonas* sp, *Vibrio* sp, *Mycobacterium* sp, *Marinobacter* sp *and Sphingomonas* sp[5]. Naphthalene compound which has 2 fused aromatic rings is mostly used to isolate PAH catabolizing bacteria from freshwater and soils. The most common Naphthalene degrading bacteria from terrestrial environment include *Pseudomonas* and *Burkholderia* strains. Some types such as naphthalene-degrading

Pseudomonas sp, *Comamonas* sp, *Acinetobacter* sp and *Sphingomonas* sp strains have been isolated from soil enrichment cultures by using other PAHs[6,7]. Although many naphthalene degrading bacteria have been isolated, these bacteria may thrive in one environment but may not be able to compete with other micro-organisms in another environment as environmental conditions will impose a selection pressure on specific types of bacteria. Furthermore, indigenous bacteria have been shown to compete with artificially introduced strains in several bioremediation investigations[8]. Implementation of a successful bioremediation strategy should necessitate a detailed evaluation of the role of the indigenous bacteria[9]. Therefore, screening and characterization of naphthalene degrading bacteria were carried out to study the efficacy of bacteria by isolating the bacteria from oil contaminated soil samples.

MATERIALS & METHODS

Sample collection: The samples were collected from oil contaminated sites especially from fuel stations and motor garage at three different locations in and around Chennai. The sites include Guindy, Saidapet and Pallavaram. The oil contaminated soil samples were collected in clean and sterile covers and transported to the lab for the enumeration of microorganisms. Culture media such as Nutrient agar and Minimal medium (M9) for naphthalene degrading bacteria were used for initial screening.

Methods

The screening of the naphthalene degrading bacteria was done by dissolving l g of contaminated soil sample in the 100 ml nutrient broth (NB) containing 100ppm of naphthalene and incubated for a week at 37 °C in a rotary shaker at 1500rpm. After one week, 1 m1 of culture was inoculated to a 90 ml fresh new medium and incubated in shaker incubator with aeration at 30 °C for one week. The process was carried out until the environment became completely turbid. This turbidity probably results from bacterial growth, and not because of the turbidity of residues mixed with medium. The samples were further cultured on the naphthalene agar medium. After growth of consortia in nutrient broth, serial dilutions were made and 100ml of the liquid was inoculated on nutrient agar plates and plates were incubated at 37 °C for 72hrs.On the basis of morphology and colour, the microbial colonies were selected from nutrient agar plates containing naphthalene (100 ppm). Each colony was picked and streaked on nutrient agar plates with increased concentration of naphthalene[10].

Growth on M9 medium: The isolated colonies of bacterial strains in the nutrient agar with naphthalene was then inoculated on to the basal mineral medium with desired substrate and concentration to screen the best and most potent strains. Those bacterial colonies growing in minimum time and with the most turbidity was chosen as the sufficient bacterial strains. Out of these the best grown isolates were selected and maintained in M9 medium and stored at 4 °C for further work [10].

Identification of selected bacterial isolates: Selected isolates were characterized by colony morphology on nutrient agar, gram staining and the morphological characteristics were identified [11]. Biochemical tests such as catalase, oxidase, indole test, methyl red-voges proskauer (MR-VP), citrate utilization, carbohydrate utilization, triple sugar iron, amino acids utilization, gelatin liquefaction, lipase screening, casein hydrolysis, H_2S production, lipase test, xylanase test and pectinase screening and physiological testing such as Temperature, pH, salt concentration was carried out [12].

Biodegradation of Naphthalene in Soil: Biodegradation of naphthalene using F3 and S3 was evaluated in soil. Clean uncontaminated soil was collected, dried, sieved and sterilized by autoclaving. 100 ppm and 200 ppm of naphthalene dissolved in methanol were added to the clean soil and mixed thoroughly. Clean soil (5 g) and contaminated soil (5 g) collected from the fuel filling stations was placed in 150 ml conical flasks into duplicate vials. The samples were inoculated with 1 O.D (at 600 nm) of cultures in 50 ml of M9 medium. All vials were incubated at 37°C in a rotary shaker at 1500 rpm. Whole sample contained in the individual vials were extracted at 0 time, one week and two weeks for the purpose of measuring residual concentration[13]. Genomic DNA isolation, Agarose gel electrophoresis, Qualitative and quantitative determination of DNA by spectrophotometric method were carried out[14]. Polymerase chain reaction (PCR) was carried out[15]. Basic Local Alignment and Search Tool (BLAST), the 16s rRNA gene sequence was processed manually, analyzed at NCBI (National Centre for Biotechnology Information) using BLAST tool and compared to the corresponding neighbor sequences Gen-Bank-NCBI database and Construction of Phylogenetic Tree Consensus sequence (~744 bp) was imported into the Jalview and multiple alignment was performed with related *Pseudomonas* species (Gen-Bank NCBI database). The results obtained were further imported into MEGA software for the construction of a phylogenetic tree using Boot strap analysis with 1000 replicates using the Kimura2-Parametermodel substitution method and the Neighbor-joining statistical method.

RESULTS & DISCUSSION

Environmental pollution is rising with the increase in industrial development all over the world. There also has been increasing pollution of hydrocarbon compounds, and many of these hydrocarbons are considered to be a potential health hazard. Some of the hydrocarbon compound pollutants are polycyclic aromatic hydrocarbons (PAHs) [16]. In the present study a total of 8 bacterial isolates (P1, P2, P3, P4, P5, P6, P7 and P8) were screened for their growth on naphthalene obtained from oil contaminated sites. Among them, 6 strains (P2 to P7) showed the best growth on nutrient agar plates with naphthalene (l00 ppm). These strains when tested for their growth in Minimal medium (M9) with naphthalene as sole source of carbon and energy, different levels of growth were observed. Majority of the isolates tested showed growth up to 100 ppm concentration of the naphthalene. Distribution and biodegradation of polycyclic aromatic hydrocarbons in contaminated sites of Hisar was reported[13]. Two soil samples were collected from various location of the Hisar city. These samples were analysed for six PAHs (naphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene and pyrene). Total mean concentration of six PAHs varied from 51.79 to 148.82 mg /kg dry weight of the soil. In the present study, strain P3 and P6 showed rich growth up to 200 ppm naphthalene. So these two strains were selected as naphthalene degrading bacteria for further study. The results of the morphological characterization and biochemical characterization of P3 and P6 bacterial isolates are presented in Table 1. Physiological characterization like optimum temperature, pH and salt concentration in M9 medium for the growth of both the strains was determined. The effect of temperature on the growth of P3 and P6 was evaluated in the M9 medium. The results showed that the maximum growth was observed at 37°C with O.D 600 of 0.186 after 48 hours and growth decreased at both 28°C and 44°C (OD 600's of 0.132 and 0.18).

Table 1: Morphological characterization and Biochemical characterization of P3 and P6 bacterial isolates

S.No	Morphological characterization and Biochemical characterization	Strains	
		P3	P6
1.	Grams stain	Negative	Negative
2.	Shape	Rod	Rod
3.	Spore stain	+	+
4.	Catalase	+	+
5.	Oxidase	+	+
6.	Indole test	-	-
7.	Methyl red	-	-
8.	Voges proskauer	-	-
9.	Citrate	+	+
10.	TSI	K/A	K/A
11.	Hydrogen sulphide production	-	-
12.	Sucrose	+	+
13.	Glucose	+	+
14.	Lactose	+	+
15.	Mannitol	+	+
16.	Pectinase	+	+
17.	Lipase	+	-
18.	Xylanase	-	-
19.	Starch	-	-
20.	Cellulase	-	-
21.	Gelatin	+	+
22.	Tyrosine	+	+
23.	Valine	+	+
24.	Glycine	+	+
25.	Arginine	+	+
26.	Alanine	+	+
27.	Leucine	+	+
28.	Threonine	+	+

+ - Positive, - - negative

Table 2: Quantification of isolated DNA

S.NO	Sample	OD at 260 nm	OD at 280 nm	Concentration (ng/ul)	Purity
1.	Blank	0.000	0.000	--	--
2.	P3	0.254	0.589	15029	1.75
3.	P6	0.325	0.179	16250	1.81

Table 3: 16s rRNA-based primer sets

S.NO	Primer	Sequence (5'-3')	Annealing Temperature	Product size
1.	PA-SS-Forward	CGACGATCCGTAACTGGTCT	$55^{O}C$	1450
2.	PA-SS-Reverse	CCGGTGCTTATTCTGTTGGT	$57\ ^{O}C$	1450

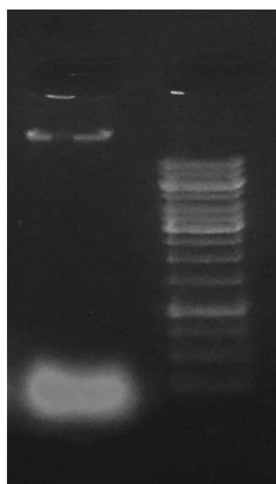

Figure 1: Genomic DNA of sample P6
Lane 1: Genomic DNA of sample P6
Lane 2: 1Kb Ladder (10,000 bp, 8000 bp, 6000 bp, 5000 bp, 4000 bp, 3000 bp, 2500 bp, 2000 bp, 1500 bp, 1000 bp, 750 bp, 500 bp, 250 bp.

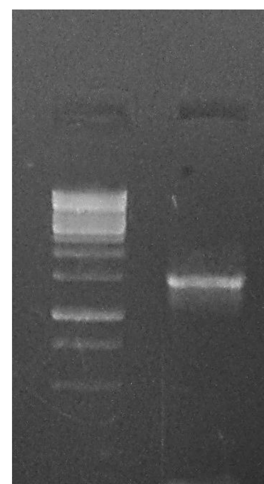

Figure 2: PCR Amplification of sample P6
Lane 1: 1Kb Ladder (10,000 bp, 8000 bp, 6000 bp, 5000 bp, 4000 bp, 3000 bp, 2500 bp, 2000 bp, 1500 bp, 1000 bp, 750 bp, 500 bp, 250 bp
Lane 2: PCR Amplicon of sample P6.

>*Pseudomonas aeruginosa* strain DSM 50071
GAACTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCAGCAGGGGCCTTCAACACATGCAAGTCGAGCTTATGAAGGGAGCTTG
CCTTGGATTCAGCGGCGGACGGGTGAGTAATCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGCCGCTAATACCGC
ATACGTCCTGAGGGAGAAAGTCGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAG
GCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT
AAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTGAGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGC
GGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAAGTGGTTCAGCAAGCTTGAGTGAAATCCCCGG
GCTCAACCTGGGAACTGCATCCAAAAGCTACTGAGCTAGAGTACGGTAGAGGTGGTAGAATTTCCTGTGTAGCGGTGAAATGCGTAG
ATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGTACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGA

Figure 3: 16s rRNA sequence *Pseudomonas aeruginosa* strain DSM 50071

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
JQ665313.1	Pseudomonas aeruginosa strain CSMCRI-1009 16S ribosomal RNA gene, partia	1516	1516	100%	0.0	100%	
JQ613503.1	Pseudomonas aeruginosa strain CSMCRI-1005 16S ribosomal RNA gene, partia	1516	1516	100%	0.0	100%	
NR_026078.1	Pseudomonas aeruginosa strain DSM 50071 16S ribosomal RNA, complete seq	1516	1516	100%	0.0	100%	
HQ616311.1	Uncultured Pseudomonas sp. clone T00223 16S ribosomal RNA gene, partial se	1456	1456	96%	0.0	99%	
CP003149.1	Pseudomonas aeruginosa DK2, complete genome	1409	5638	100%	0.0	98%	
AP012280.1	Pseudomonas aeruginosa NCGM2.S1 DNA, complete genome	1409	5638	100%	0.0	98%	
CP002496.1	Pseudomonas aeruginosa M18, complete genome	1409	5638	100%	0.0	98%	
FM209186.1	Pseudomonas aeruginosa LESB58 complete genome sequence	1409	5633	100%	0.0	98%	
CP000744.1	Pseudomonas aeruginosa PA7, complete genome	1409	5629	100%	0.0	98%	
CP000438.1	Pseudomonas aeruginosa UCBPP-PA14, complete genome	1409	5638	100%	0.0	98%	
AE004091.2	Pseudomonas aeruginosa PAO1, complete genome	1409	5638	100%	0.0	98%	E
JN000304.1	Pseudomonas aeruginosa strain T-1 16S ribosomal RNA gene, partial sequence	1404	1404	100%	0.0	98%	
JX502184.1	Pseudomonas aeruginosa strain CIAH-M 16S ribosomal RNA gene, partial sequ	1398	1398	92%	0.0	100%	
JN003625.1	Pseudomonas aeruginosa strain BS8 16S ribosomal RNA gene, partial sequenc	1397	1397	99%	0.0	98%	
JF513146.1	Pseudomonas aeruginosa strain S164S 16S ribosomal RNA gene, partial seque	1397	1397	99%	0.0	98%	
HM593867.1	Bacterium enrichment culture clone DT3-61 16S ribosomal RNA gene, partial se	1397	1397	99%	0.0	98%	
GU586317.1	Pseudomonas aeruginosa strain IR-222 16S ribosomal RNA gene, partial sequ	1397	1397	99%	0.0	98%	
GU296674.1	Pseudomonas aeruginosa strain ANSC 16S ribosomal RNA gene, partial sequer	1397	1397	99%	0.0	98%	
EU221383.1	Pseudomonas aeruginosa strain Y2P7 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
GQ180117.1	Pseudomonas aeruginosa strain MW3A 16S ribosomal RNA gene, partial seque	1397	1397	99%	0.0	98%	
FJ864676.1	Pseudomonas aeruginosa strain pp1a 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
FJ655794.1	Pseudomonas aeruginosa isolate D5AO3 16S ribosomal RNA gene, partial sequ	1397	1397	99%	0.0	98%	
EU331416.1	Pseudomonas aeruginosa strain pY11T-3-1 16S ribosomal RNA gene, partial se	1397	1397	99%	0.0	98%	
EU221384.1	Pseudomonas aeruginosa strain Y2P8 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
EU221382.1	Pseudomonas aeruginosa strain Y2P5 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
EU221381.1	Pseudomonas aeruginosa strain Y2P3 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
EU221380.1	Pseudomonas aeruginosa strain Y2P2 16S ribosomal RNA gene, partial sequen	1397	1397	99%	0.0	98%	
EU037096.1	Pseudomonas aeruginosa strain CMG860 16S ribosomal RNA gene, partial seq	1397	1397	99%	0.0	98%	
DQ420635.1	Pseudomonas aeruginosa strain PGSL 03 16S ribosomal RNA gene, partial seq	1397	1397	99%	0.0	98%	
AY548952.1	Pseudomonas aeruginosa strain Z5 16S ribosomal RNA gene, complete sequer	1397	1397	99%	0.0	98%	
AJ387904.1	Pseudomonas sp. 16S rRNA gene, strain OLB-1	1397	1397	99%	0.0	98%	
FN826322.1	Uncultured bacterium partial 16S rRNA gene, clone UK15.36_V02193E_089	1395	1395	99%	0.0	97%	
AF094713.1	Pseudomonas aeruginosa strain ATCC 10145 16S ribosomal RNA gene, partial	1395	1395	99%	0.0	98%	
JX035794.1	Pseudomonas aeruginosa strain N002 16S ribosomal RNA gene, partial sequen	1393	1393	98%	0.0	98%	
FM995966.1	Uncultured bacterium partial 16S rRNA gene, clone 16sps16-2g10.p1k	1393	1393	98%	0.0	98%	
FN826560.1	Uncultured bacterium partial 16S rRNA gene, clone US17.14_V02193C_054	1393	1393	99%	0.0	97%	
FN826327.1	Uncultured bacterium partial 16S rRNA gene, clone UK15.39_V02193E_083	1393	1393	99%	0.0	97%	
FN826318.1	Uncultured bacterium partial 16S rRNA gene, clone UK15.34_V02193E_093	1393	1393	99%	0.0	97%	
AF193514.2	Pseudomonas aeruginosa 16S ribosomal RNA gene, partial sequence	1393	1393	99%	0.0	97%	
JX514417.1	Pseudomonas aeruginosa strain BM6 16S ribosomal RNA gene, partial sequenc	1391	1391	99%	0.0	97%	
JQ927220.1	Pseudomonas aeruginosa 16S ribosomal RNA gene, partial sequence	1391	1391	99%	0.0	97%	
JQ773431.1	Pseudomonas aeruginosa strain RI-1 16S ribosomal RNA gene, partial sequen	1391	1391	99%	0.0	97%	
JF926712.1	Uncultured bacterium clone 136 16S ribosomal RNA gene, partial sequence	1391	1391	99%	0.0	97%	
JF926708.1	Uncultured bacterium clone 106 16S ribosomal RNA gene, partial sequence	1391	1391	99%	0.0	97%	
JN882043.1	Uncultured bacterium clone A27 16S ribosomal RNA gene, partial sequence	1391	1391	99%	0.0	97%	

Figure 4: Graphical representation of 16s rRNA gene sequence in BLAST

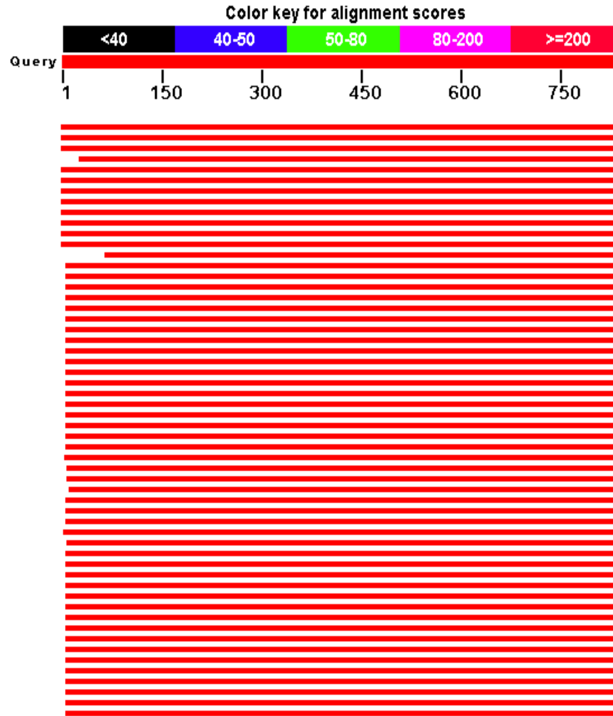

Figure 5: Tabular representation of 16s rRNA gene sequence in BLAST

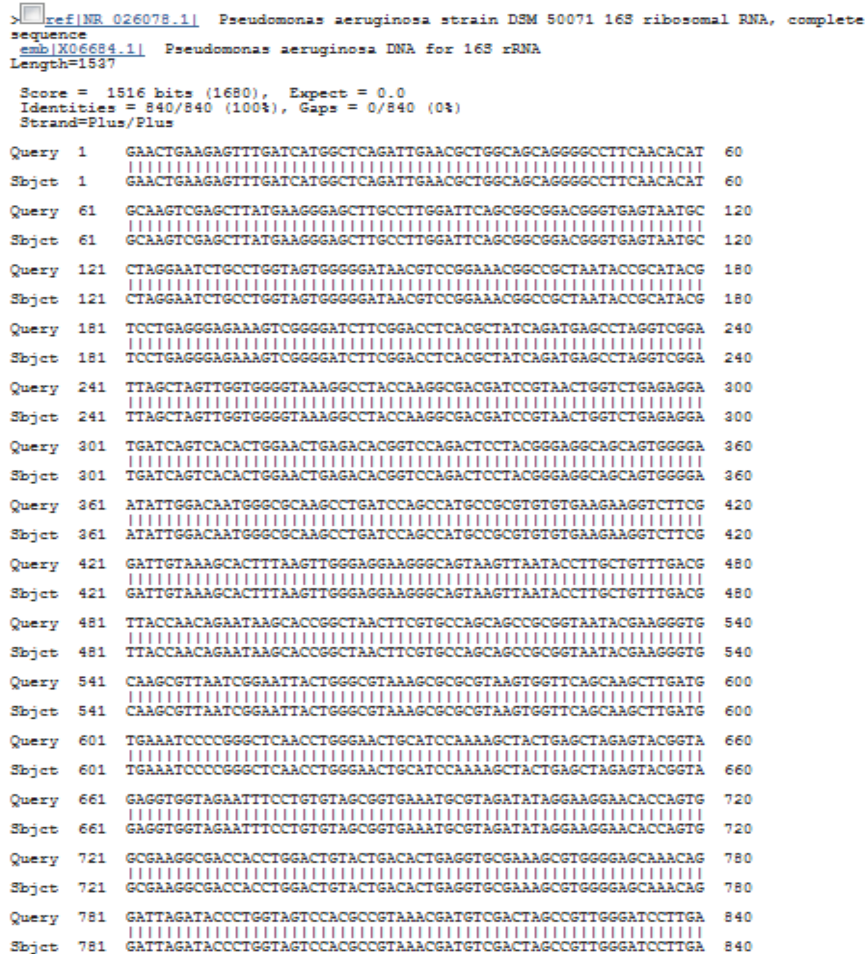

Figure 6: Alignment representation of 16s rRNA gene sequence in BLAST

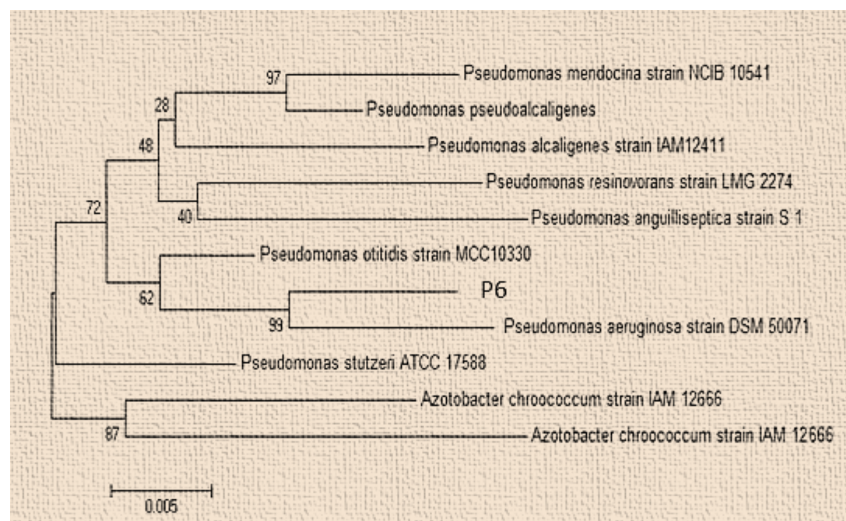

Figure 7: Average distance tree of *Pseudomonas aeruginosa* – 16s rRNA gene sequence

Temperature is another important variable that influences petroleum biodegradation. Optimum temperature dictates the rate of PAHs metabolism by microorganisms and also the pattern of the microbial community. Temperature also has direct effect on the physical nature and chemical composition of the PAHs constituents. When temperatures are low, PAHs tend to be more viscous and their water solubility is greatly reduced. The optimum temperature is typically in the range of 30 to 40°C. At temperature above this, normal enzymatic activities are inhibited as proteins denature[17]. The effect of pH on growth of P3 in M9 medium was evaluated and the study revealed maximum growth at pH 7 with an O.D 600 of 0.981 after 48 hours. The effect of pH on growth of P6 in M9 medium was evaluated with maximum growth observed at pH 7 with an O.D 600 of 1.220 after 48 hours. Most important PAHs degrading heterotrophic bacteria and fungi perform best when pH is neutral. At pH 7, the mineralization of oily sludge in soil is also improved, thus, enhancing the overall biodegradation process[18]. The effect of salt concentration was studied and maximum bacterial growth occurred at 12% of the salt (NaCl) concentration for P3 and at 20% salt concentration for P6. Studies have shown that there are generally positive correlations between salinity and rates of mineralization of PAHs such as phenanthrene and naphthalene as reported[17].

However, hyper salinity will result in the decrease in microbial metabolic rates. Biodegradation studies of Naphthalene was conducted with naphthalene adapted bacterial strains P3 and P6 at optimized conditions. Biodegradation was done in M9 medium using standard (100ppm) naphthalene soil samples and contaminated soil samples. The samples were extracted at 0 to 7 days for calculating the biodegradation efficiency of both the strains. It showed more efficiency of strain P6 (*Pseudomonas* sp) to degrade naphthalene as compared to strain P3 whose biodegradation efficiency was less than strain P6 (*Pseudomonas* sp). Thus from this study it is summarized that at optimum conditions strain P6 (*Pseudomonas* sp) may be better for the degradation of naphthalene after 7 days of incubation. A study showed an increase in PAH concentrations in soil samples from local auto market whereas a low concentration was observed in agriculture soils. The effect of pH (5.0 to 9.0), Temperature (20° to 40°C) and concentration of polycyclic aromatic hydrocarbons (PAHs) compounds (5 to 20 mg/kg) on biodegradation were optimized. Two acclimatized microbial strains *Pseudomonas putida* and *Pseudomonas paucimobilis* were used to evaluate the biodegradation of phenanthrene (3-ring) and pyrene (4-ring). Biodegradation was found to be maximum in oil spiked phenanthrene (5 mg/kg) and pyrene (5 mg/kg), pH was 7.0 and temperature 30°C (at optimized conditions) in 42 days of incubation period with *Pseudomonas putida* and *Pseudomonas paucimobilis* than the native unsterilized contaminated soil (without optimized conditions) Phenanthrene was degraded and disappeared without any trace after 28 days with *Pseudomonas putida* and 35 days with *Pseudomonas paucimobilis* whereas Pyrene disappeared upto 97.4% with *Pseudomonas putida* and 95.5% with *Pseudomonas paucimobilis* after 42 days incubation period at optimized conditions. The results of the Molecular characterization of P6 bacterial isolate are presented in figure 1 & 2 .Molecular characterization of bacterial samples were subjected for whole genomic DNA isolation[14]. The isolated nucleic acids were subjected for qualitative and quantitative analysis using Agarose gel electrophoresis. Out of the two isolates P3 and P6, the latter was subjected to PCR analysis and sequencing of 16s rRNA gene and presented in figure 3. 16s rRNA sequence has long been used as a taxonomic "gold standard" in determining the phylogenies of bacterial Species. Selective amplification of 16s rRNA was carried out by PCR[19]. Based on alignment of 16s rRNA sequences available in GenBank, two primer pairs were designed. Primers PA-F and PA-R was intended to amplify *Pseudomonas* species. While the pair PA-SS-F and PA-SS-R was designed to amplify only *Pseudomonas aeruginosa*. Amplified PCR product was visualized at 1450bp for 16s rRNA gene for the isolated P6 sample. The resulting sequence were used to identify species and similarity was analyzed using the BLAST network service for 16s rRNA is presented in figure 4, 5 & 6. Isolates subjected to molecular characterization was identified as *Pseudomonas aeruginosa* strain DSM 50071. Phylogentic analysis of 16s rRNA sequence obtained was subjected to MSA analysis using ClustalW tool for *Pseudomonas aeruginosa* strain DSM 50071. Phylogenetic tree was constructed for the MSA output using Jalview for *Pseudomonas aeruginosa* strain DSM 50071 presented in figure 7. Novel molecular techniques have been extremely valuable in exploring the diversity of microbiota despite culture-based microbiological methods and have provided important information about the microbial diversity. The isolates were phenotypically identified and bacterial strains which exhibited relatively higher degradation were selected as most active strains. The tentative taxa and phylogenetic affiliation of the 16s rRNA of purified bacterial isolates were amplified by PCR and the bacterial 16s rRNA sequences were aligned with

Blast search of NCBI databases. Partial 16s rRNA gene sequencing and database homology search for the isolates revealed their tentative close relationship to members of *Achromobacter* sp. and *Pseudomonas aeruginosa*[20].

CONCLUSION

PAH contaminated environments like soil and sediments can be exploited for other bacterial strains capable of degrading PAHs. These isolates can also be checked for their ability to degrade other organic contaminants like pesticides, TNTs and PCBs etc. These bacterial isolates need to be applied in fields for bioremediation studies of PAHs and other organic compounds and identification of metabolites from PAHs biotransformation to elucidate the complete pathways by selected isolates.

REFERENCES

1. Baumard P, Budzinski H, Garrigues P. Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean sea. Environmental Toxicology and Chemistry, 1998; 17:5, 765- 776.
2. Sack U, Martin H, Wolfgang F. Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of *Nematoloma frowardii*. FEMS Microbiology Letters, 1997; 152:2, 227–234.
3. Prince RC. Petroleum spill bioremediation in marine environments. Critical Reviews in Microbiology, 1993; 19:4, 217-240.
4. Alexander M. Biodegradation and Bioremediation. Academic Press, New York: 1994. p.147-158.
5. Hedlund BP, Geiselbrecht AD, Bair T, Staley JT. Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. Applied and Environmental Microbiology, 1999; 65:251-259.
6. Mueller JG, Devereux R, Santavy DL, Lantz SE, Willis SG, Pritchard PH. Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. Antonie van Leeuwenhoek, 1997;71:329–343.
7. Zylstra GJ, Kim E, Goyal AK. Comparative molecular analysis of genes for polycyclic aromatic hydrocarbon degradation. Genetic Engineering, 1997; 19:257–269.
8. Iwabuchi, T, Inomata Y, Yukie, Katsuta, Atsuko, Harayama S. Isolation and characterization of marine *Nocardioides* capable of growing and degrading phenanthrene at 42°C. Journal of Marine Biotechnology, 1998;6: 86-90.
9. Piehler MF, Swistak JG, Pinckney JL, Paerl HW. Stimulation of diesel fuel biodegradation by indigenous nitrogen fixing bacterial consortia. Microbial Ecology, 1999;38:69-78.
10. Kafilzadeh F, Rafiee S, Tahery Y. Evaluation of Bioremediation of naphthalene using native bacteria isolated from oil contaminated soils in Iran., Annals of Biological Research, 2011; 2:6, 610-616.
11. Cappuccino JG, Sherman N. Microbiology a Laboratory Manual. 5th ed. The Benjamin/Cummings Publishing Company Inc, California: 1996. p. 125-178.
12. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology. 4th ed. New Age International (P) Ltd., Publishers, New Delhi: 2003. p.122- 127.
13. Bishnoi K, Sain U, Kumar R, Singh R, Bishnoi NR. Distribution and biodegradation of polycyclic aromatic hydrocarbons in contaminated sites of Hisar (India). Indian Journal of Experimental Biology, 2009; 47: 3, 210-7.
14. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory Manual. 2nd ed. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press: 1989. p. 545-547.
15. Mullis KB. The unusual origin of the polymerase chain reaction. Scientific American. 1990; 262: 4:56–61.
16. Abd-Elsalam H, Hafez E, Hussain AA. Isolation and identification of tree-rings polyaromatic hydrocarbon (anthracene and phenanthrene) degrading bacteria. American- Eurasian Journal of Agricultural Environmental Sciences, 2009; 51: 31-8.
17. Leahy JG, Colwell RR. Microbial degradation of hydrocarbons in the environment. Microbiology Reviews, 1990;54(3):305-15.
18. Van Hamme JD, Singh A, Ward ONW. Recent advances in petroleum microbiology. Microbiological and Molecular Biology Review 67, 2003;4: 503-549.
19. Spilker T, Coenye T, Vandamme P, LiPuma JJ. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. Journal of Clinical Microbiology, 2004; 42:5:2074-9.
20. Demnerova K, Mackova M, Spevakova V, Beranova K, Kocha L. Two approaches to biological decontamination of ground-water and soil polluted by aromatics characterization of microbial populations. International Microbiology, 2005; 8: 205-211.

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