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Azotobacter species-An efficient napthalene degrader

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ABSTRACT

Many polycyclic aromatic hydrocarbons (PAHs) are known to be toxic and their contamination of soil and aquifers is of great environment concern. In the present study the degradation of naphthalene a model PAH by nitrogen fixing bacteria was investigated and an efficient naphthalene degrader sp. was screened. Twenty isolates degrading naphthalene as a sole source of carbon and energy were isolated from petroleum contaminated sites. They were identified by using standard microbiological methods. Isolates were screened on the basis of growth in terms of O.D. at 610 nm, CFU mL^{-1} and degradation efficiency. Screened isolates were– *Azotobacter chroococcum* and *Azotobacter vinelandii*, confirmed by molecular identification using 16 S r RNA gene analysis .They showed 0.28, 0.22, O.D. at 610 nm respectively at 1 m mol concentration of naphthalene after 72 hr. of incubation. HPLC analysis of diethyl ether extract obtained from cultures of *Azotobacter chroococcum*, *Azotobacter vinelandii* showed 98%, 97.7%, degradation of naphthalene respectively at the end of 72 h. HPLC elution profile of extract indicated presence of intermediate metabolites of Napthalene degradation at different retention time.

KEYWORDS

PAHs;
Napthalene;
Biodegradation;
Nitrogen fixing bacteria;
Azotobacter species.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic pollutants that have accumulated in the environment due to both natural and anthropogenic activities[1-4]. Human exposure to PAHs can take place through multiple routes including air, soil, food, water and occupational exposure[5,6]. They have cytotoxic, mutagenic and in some case carcinogenic effects on human tissue[7-9]. Based on their ecotoxicity, the US Environment Protection Agency (EPA) has listed 16 PAHS as priority pollutants for remediation[10,11].

The International Agency for Research on Cancer (IARC) has identified 15 PAHs including 6 of 16 US EPA regulated PAHS as potential carcinogens and naphthalene as possibly carcinogenic to humans and animals (Group 2B)[12]. PAH released in to the environment could be removed by many processes, including volatilization, photo oxidation, chemical oxidation and adsorption on soil particles[13]. However, the principal process for successful removal and elimination of PAHs from environment is microbial transformation and bio-

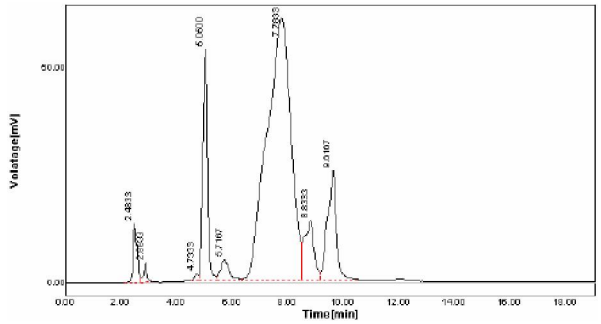

Figure 1 : HPLC elution profile of the metabolites formed from naphthalene by Azotobacter chroococcum

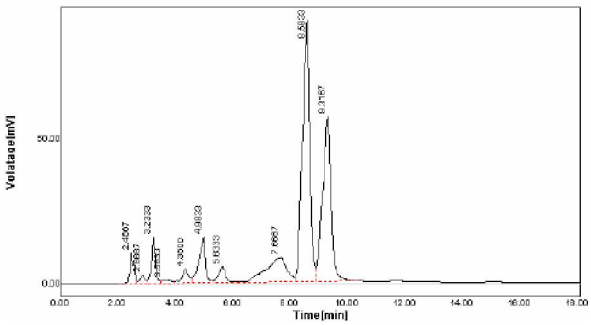

Figure 2 : HPLC elution profile of the metabolites formed from naphthalene by Azotobacter vinelandii (R.T. 5.6 min-naphthalene)

degradation[14,15].

Number of factors affects the process of biodegradation. The PAH degradation significantly increases in the presence of other nutrients. The addition of inorganic nitrogen and phosphorous stimulated the degradation of PAH in top soil and aquifer sand[16-18]. Several bacterial genera including species of *Pseudomonas*, *Alcaligenes*, *Mycobacterium*, *Rhodococcus*, *Sphingomonas*, and *Cycloclasticus* are capable of degrading PAHs[9,19-21]. PAH degradation by nitrogen fixing bacteria still not studied in detail. Hence it is desired to study ability of nitrogen fixing bacteria to degrade PAHs. Degradation of some PAHs by free-living nitrogen fixing bacteria was investigated by Chen et al.[24] they reported that free living nitrogen fixing bacteria have ability to grow and fix nitrogen in presence of aromatic compounds. Dissimilation of aromatic compounds with reference to nitrogenase activity was investigated[26]. Following study was aimed to screen out efficient naphthalene degrading free living nitrogen fixing bacteria from polluted sites.

EXPERIMENTAL

Enrichment, isolation and identification of nitrogen fixing bacteria

For the isolation of naphthalene degrading nitrogen fixing bacteria, soil samples were collected from petroleum contaminated sites. Soil Samples (1gm) were inoculated in to flask containing Burk's medium[22] for enrichment. Flasks were incubated at 150 rpm and 30°C for 96 hours. One mL of enriched culture transferred to flask containing Burk's medium incorporated with 0.128 g, 1mmol of naphthalene as a sole carbon and energy source and incubated on rotary shaker at 150 rpm and 30°C for 72 hours. After two successive sub culturing in the same medium, isolates were obtained by plating dilutions of enrichment broth on Burk's agar medium containing 1 m mole naphthalene as sole carbon and energy source. Napthalene degrading isolates were identified by using standard microbiological methods[22].

Growth study of the isolates

Flasks containing Burk's medium incorporated with 1 m mol of naphthalene were inoculated with 1 ml of inoculums in exponential growth phase and having O.D. 0.3 at 610 nm. Conditions were same as previously described. Amount of growth was measured in terms of O.D. at 610 nm and cell count as $CFUmL^{-1}$ estimated by direct plate count method at an interval of 24 h, up to 72 hours. Selected isolates were used for studies on biodegradation.

16 S r RNA analysis

Two isolates with significant growth were subjected to 16 S r RNA gene analysis for molecular identification. The 16 S r RNA gene was amplified by PCR and the nucleotide sequence was determined and analysed using BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST).

Study on biodegradation of napthalene

Screened isolates were inoculated in 50 mL of Burk's medium with 0.128; 1m mole of Napthalene as sole source of carbon. At the end of 72 h of incubation period, medium subjected to liquid-liquid extraction method[23]. It was acidified to pH 2.5 and extracted with diethyl ether. Solvent layer was separated, allowed to evaporate.

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TABLE 1 : Physiological and biochemical characteristics

Test	*Azotobacter chroococcum*	*Azotobacter vinelandii*
cell	Ovoid rods	Straight rods
Gram staining	–	–
Pigment	Black	–
Motility	+	+
Cyst formation	+	+
Starch	+	–
Mannitol	+	+
Rhamnose	–	+
Meso-inositol	–	+

HPLC

Residue obtained was dissolved in methanol and analysed using reversed phase HPLC. Analysis was done by using Youngling Isocratic liquid chromatography system. Model No. ACME 9000 equipped with rheodyne injector 20 µL fixed loop, UV/visible detector model Sp 930 d. the C-18 column Varian (250×4.6 mm, particle size 5µ) was used as stationary phase.

RESULTS AND DISCUSSION

Twenty isolates were selected and identified by using standard microbiological method. These isolates were found to be of the genera. *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Azomonas* and *Derxia*. On the basis of growth, the efficient Napthalene degraders were screened. They were Azotobacter *chroococcum*, *Azotobacter vinelandii*, (TABLE 1).They produced O.D. O.28, 0.22 at 610 nm at the end of 72 h respectively incubation period. Cell count was 2×10^{10}, 3×10^{9} CFU mL^{-1} respectively at the end of 72 h. of incubation. Molecular identification of isolates degrading 98%, 97.7% naphthalene by using 16 S r RNA analysis confirmed that they were *Azotobater chroococcum* strain 10006 and *Azotobacter vinelandii* strain ISSDS-386.

HPLC elution profile of residues obtained from *Azotobacter chroococcum, Azotobacter vinelandii* showed 98%, 97.7%, degradation of naphthalene and presence of several degradation products. HPLC elution profile of *Azotobacter chroococcum* showed presence of metabolites at R.T. 2.4, 5.0, 7.7, 9.8 min. respectively (Figure 1) .HPLC elution profile of *Azotobacter vinelandii* showed presence of metabolites at R.T. 2.4, 8.5, 9.2 min (Figure 2).

R.Thavasi et.al.[25] reported hydrocarbon degrading potential of a marine nitrogen fixing bacterium, *Azotobacter chroococcum* with reference to emulsification activity. Expression of catechol 1,2-dioxygenase in nitrogen fixing bacteria was reported by Chen et.al.[24]. Results of present study confirmed that nitrogen fixing bacteria have ability to degrade naphthalene. *Azotobacter chroococcum and Azotobacter vinelandii* are efficient Napthalene degraders.

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