Research Paper

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Biodegradation of Polyaromatic Hydrocarbons by Novel Organism Sphingomonas Anadarae Pp1.

Microbiology

KEYWORDS : Sphingomonas anadarae strain PP1, bioremediation, PAHs, aerobic.

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ABSTRACT

Aromatic Hydrocarbons are recalcitrant in nature and are known for their toxicity and oncogenecity. They resist degradation because of the alternating hydrogen bonds present in the hydrocarbon rings. Microorganisms are suitable agents for bioremediation, as they can breakdown complex compounds to non toxic end products. The isolated organism, Sphingomonas anadarae PP1,(GENBANK:KJ794190.1) was isolated from soil collected near petrol pump and could aerobically degrade various aromatic/ polyaromatic hydrocarbons (Aniline,Xylene, Phenol, Benzene, Naphthalene and Anthracene). Due to presence of required enzyme system for the breakdown of PAHs, this organism has potential to be agent for bioremediation of such persistent compounds in the environment.

I. Introduction: Poly aromatic hydrocarbons (PAHs) are a pressing issue, as they persist in the environment for a very long time due to the alternating double bonds present in the hydrocarbon rings, reducing their bio-availability for degradation. They are toxic to all life forms, and are known carcinogens. Microorganisms are the key components in the breakdown of many carbon sources, including PAHs (Shuttleworth, Cerniglia, 1995). Bioremediation of these, lead to production of harmless end products i.e. acetyl CoA and Succinate. These organisms possess monodioxygenase, dehydrogenase enzyme systems, which are plasmid borne (Assinder et al., 1990). Thus, microorganisms like *Sphingomonas (*Zhencheng, Jian Wang et al. 2013) *spps, Psuedomonas,* Stenotrophomonas (Yang, 2006), Phanerochaete *chrysosporum* can prove to be a major work force in bio remediation and bio augmentation. They are promising alternative for the recovery of the environment from existing pollutants. The above mentioned processes are highly cost and energy efficient, thus making it a suitable choice for environmental remediation. The aim was to find a suitable microbial mediator for the degradation of these harmful and recalcitrant chemicals.

II. Materials:
Aromatic hydrocarbons used were xylene, Naphthalene, Phenol, Benzene, Aniline. PAHs such as Anthracene, Naphthalene (AR grade) obtained from Loba chemicals were used .For purification of stock solutions, filters with 0.25µm pore size were used (Hi-Media).

III. Methods:
Samples were collected from soil near Petrol Pumps, industrial effluents, Sewage water. 1gm of soil was inoculated into 50ml of sterile Nutrient broth. Dilutions of the same were streaked on sterile Streptomycin Nutrient agar plates with concentration (Vanbroekhen) 200µg/ml incubated at 37˚C for 24 hours.

A. Isolation: Yellow pigmented colonies were observed and used for staining, biochemical tests and bacterial identification by 16s rDNA sequencing.

B. Qualitative Studies: The isolate was inoculated in Minimal Salt Medium no.9 ($Na_2HPO_4$6g,$KH_2PO_4$3g,NaCl3g,NH_4Cl1g ,$CaCl_2$10mM,$MgSO_4.7H_2O$0.1M) with Xylene, Phenol, Benzene Anthracene and Naphthalene(0.4%v/v) as a sole carbon source and incubated for 8 days at 37˚C. Turbidity/change in color (Fig.1) of the medium was used in order to qualitatively determine degradative abilities of the isolates.

C. Analytical techniques:
1. 6 Amino Antipyrene Method was used to calculate residual phenol percentage.

2. UV Visible spectrophotometery was used for estimation of Anthracene, Naphthalene, Benzene (following extraction from the medium) before and after degradation.

3.Reversed Phased HPLC was used for detection of Benzene, wherein filtered samples were injected in LC C18 column(250mm,5µm),eluted with H_2O(0.1%formic acid and acetonitrile(75:25),as the mobile phase at a flow rate of 1ml/min. The absorbance maxima was at 254nm.

IV. Results and discussions:

A. The isolate was found to be gram negative, non-endospore forming, non-fermentative, oxidase, catalase, urease positive. With reference to Bergey's manual, the isolate was categorized under the genus Sphingomonas. The isolate was sequenced; a 723 base pair sequence was obtained, subjected to BLASTn analysis. The annotated sequence with information was submitted to NCBI. The sequence is now available under the accession ID: KJ794190.1 and the name *Sphingomonas anadarae PP1.*

B. Turbidity/color change was observed with various aromatic hydrocarbons as the sole carbon source, indicative of growth of isolate and degradation of the compounds.



Figure 1. Change in color and turbidity was observed after 8 days of incubation in Minimal salt medium with Xylene as the

sole carbon source.

C. Analytical techniques:

1. The organism was found to aerobically degrade 40% of Phenol after 10 days of incubation.
2. UV visible spectrophotometry analysis: The peaks observed after UV Visible spectral scan at 0 time were absent after 15 days of incubation, indicating degradation of Anthracene, Naphthalene and Benzene.
3. Reversed Phased HPLC analysis: The retention time for the Benzene at 0 time (blank reading) was 1.551 seconds whereas the retention time after incubation of 8 days was 1.643 seconds. Indicating the parent compound has been completely utilized by the organism.

The biochemical analysis for *Sphingomonas anadarae strain PP1* proves that it has a versatile enzyme system which can break down an array of aromatic and polyaromatic hydrocarbons. The analytical methods employed further confirmed our claim of this organism being capable of bioremediation or various pollutants which originate from PAHs.

V. Conclusion: Aromatic hydrocarbons are abundantly found in environment, specially soil and water. *Sphingomonas anadarae strain PP1* was isolated from top soil near petrol pump. Hence this organism can be used for degradation of aromatic hydrocarbons present in top soil, oils spills and thus restrict their percolation into ground water, preventing these toxic compounds from being persistent in the environment. Therefore, Sphingomonas anadarae strain PP1 is a potential agent for bioremediation of PAHs and can be applied for various contaminated sites. Using this organism in-situ bioreactors can be installed at industry site to free its effluents from PAHs aerobically.

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