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ISOLATION OF NOVEL ORGANISM STENOTROPHOMONAS KOREENSIS strain AK-1 WITH POTENTIAL OF PAHs DEGRADATION.

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ABSTRACT

PAHs are an environmental concern because of their reduced bio-availability. Biodegradation via microorganisms who possess required enzyme systems for the breakdown of recalcitrant compounds is one of the best options available, as the end products are non toxic. Stenotrophomonas koreensis AK-1 (GENBANK:KP145677.1) a novel organism, was isolated from dust on dark leaves of Ficus religiosa (Peepal tree), which could aerobically degrade various aromatic hydrocarbons (Phenol, Benzene, Aniline, Xylene, Anthracene, Naphthalene). The degradation was estimated by analytical techniques like U.V visible spectroscopy, Reverse Phased HPLC and Phenol estimation titration. The key feature of the organism was its ability to degrade phenol and phenolic compounds aerobically. It was observed that genes for enzyme complex required for degradation were constitutive and not plasmid borne, as no plasmids were observed on Agarose gel electrophoresis. The organism, thus, holds potential for further application in field of biodegradation of PAHs.

KEYWORDS : Biodegradation, PAHs, aerobic, STENOTROPHOMONAS KOREENSIS strain AK-1

I. Introduction:

Polyaromatic hydrocarbons have been detected in sediments, soils, plants and animal tissues, and are known for their cytotoxicity and oncogenicity. Oil spills, use of iron and steel in industries, petroleum refining and other anthropogenic activities are responsible for environmental contamination with the same. They have reduced bio availability due to presence of high concentration of hydrophobic bonds. For the above reasons they are highly resilient in nature and tend to accumulate (Shuttleworth, Cerneglia, 1995). Only a few microorganisms like *Pseudomonas*, *Stenotrophomonas*(Yang et al.,2006), *Mycobacterium*, *Sphingomonas*(G J Zylstra, E Kim,1997) etc have the enzyme systems necessary to use complex compounds like PAHs as their sole carbon sources, which are often plasmid borne(Assinder,Williams,1990). The end production of bacterial degradation processes are often non toxic products like acetyl CoA and carbon dioxide,(Atlas ,1995). Thus, these microorganisms can be used as the key driving forces in bioremediation and bio-augmentation, which are highly cost efficient and energy efficient due to their simplicity. The aim of the current study was to find an efficient microbiological agent that would help eliminate these recalcitrant compounds from environment.

II. Materials: Aniline, Xylene, Benzene, Phenol, Anthracene and Naphthalene were provided as the sole carbon source. Chemicals were AR grade, obtained from Loba Chemicals. Filters for sterilization stock solutions, with pore size 0.25µm (HiMedia) were used.

III. Methods: Samples were collected from Petrol Pumps, Sewage water, surface of leaves (*Ficus religiosa)* plucked near petrol pumps. 1gm of soil/washed leave dust was enriched in sterile Nutrient broth (50ml). Sterile Streptomycin Nutrient agar plates with concentration of Streptomycin (Vanbroekhen et al, 2004) 200µg/ml were used for isolation of the desired organism and incubated for 24 hours at 37°C.

A. **Isolation:** Colonies with yellow pigmentation were selected and subjected to staining, biochemical tests and bacterial identification (16s rDNA sequencing).

B. Detection of Plasmid: Overnight 24 hour old culture was used for plasmid DNA extraction. Mini-prep method was used for isolation of Plasmid. The sample obtained was analyzed by Agarose Gel Electrophoresis.

C. Qualitative Studies: 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 sole carbon source from one of three selected aromatic hydrocarbons (0.4%v/v), were inoculated with the isolate for 8 days. To qualitatively determine the degradative ability of the isolate, turbidity and color change of the medium was observed.

D. Analytical techniques:

1. For degradation of Phenol, 6 Amino Antipyrene Method was used to determine the percentage of residual Phenol.

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

3. Reversed Phased HPLC was used for Benzene, filtered samples before and after degradation(8 days) were injected in LC C18 column of 250mm,5µm particle size),eluted with H_2O(0.1% formic acid) and acetonitrile (75:25), as the mobile phase ,flow rate of 1ml/min. Absorbance maxima: 254nm.

IV. Results and Discussions:

A. Isolation: The isolate was gram negative, non endospore forming and catalase, urease and oxidase (late) positive. On 16s rDNA sequencing, a 780 base pairs sequence was obtained and subjected to BLASTn analysis. The BLASTn search against *Stenotrophomonas* specific database revealed 99% *Stenotrophomonas koreensis* 16S ribosomal RNA gene, partial sequence. The annotated sequence was submitted to NCBI, and is available under the accession ID: KJ794190.1. (GenBank).

B. Detection of Plasmid: The gel electrophoresis result **Figure 1** of the isolated DNA from the isolated bacterial strain showed no plasmid DNA band when Mini-prep method was used. This indicates that absence of plasmid borne ability for PAHs degradation. Hence, it can be concluded, that the ability for degradation of recalcitrant compounds remain non plasmid borne, thus making *Stenotrophomonas koreensis AK-1*, novel organism for bioremediation.

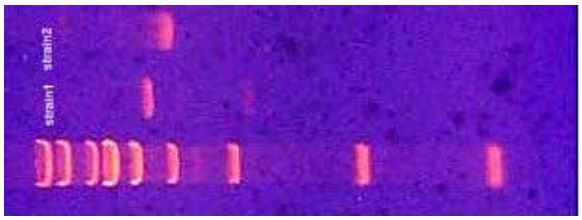

Figure 1: Detection of Plasmids. Lane 2 corresponds to *Stenotrophomonas koreensis AK-1*, where a band near 3 kilo bases was observed. No plasmid DNA band was observed.

C. Qualitative studies: Turbidity and color change were used as indicator of growth and degradation, which were observed with various aromatic hydrocarbons as the sole carbon source.

D. Analytical techniques:

1. 25% of Phenol was degraded after 10 days of incubation.

2. UV visible spectrophotometry analysis: After UV Visible spectral scan, the peaks observed at 0 time were absent after 15 days indicating degradation of Anthracene, Naphthalene and Benzene.

3. Reversed Phased HPLC analysis: Benzene's retention time at blank (0 Time) was 1.551 seconds and retention time after degradation was 1.861 seconds which indicates that the parent compound has been completely degraded.

The biochemical analysis for *Stenotrophomonas koreensis AK-1* revealed that the organism has the necessary enzyme system for the degradation of these recalcitrant PAHs. Further, analytical methods confirmed that the organism is a suitable agent for bioremediation of the environment from such aromatic pollutants.

V. Conclusion: PAHs are a potential and persistent threat to the environment. In situ degradation is an efficient alternative to limit these toxic chemicals from percolating in the environment. *Stenotrophomonas koreensis AK-1* is novel organism capable of degrading Aniline, Benzene, Phenol, Xylene, Anthracene and Naphthalene aerobically. Thus, this organism can be used to set up an in situ bioreactor and treat the toxic waste contaminated with PAHs at industrial site itself. The efficiency of the culture can be further improved with the help of recombinant DNA technology for enhanced rate of degradation.

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