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Effect of Triton X-100 on Degradation of Polycyclic Aromatic Hydrocarbons by *Pseudomonas* sp. PSS6 Isolated from Municipal Wastes Sediment

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ABSTRACT

This study determined the effect of surfactant Triton X-100 (1%) on biodegradation of various by polycyclic aromatic hydrocarbons (PAHs) by Pseudomonas sp. PSS6. Sprayed-plate experiments were performed to find the degrading capability of the test isolate. The test strain efficiently degraded PAHs to a greater extent compared to other isolates forming an intensive clear zone in Bushnell–Haas agar. The degradation of PAHs was studied by inoculating the test isolate in mineral medium containing PAH with 1% Triton X-100.The growth of Pseudomonas sp. PSS6 and degradation of PAHs (naphthalene, fluoranthene, phenanthrene and anthracene) was influenced by 1% Triton X-100 in the mineral medium.

Key words*:* Surfactant, Triton X-100, Polycyclic aromatic hydrocarbons, *Pseudomonas.*

INTRODUCTION

Polycyclic aromatic hydrocarbons are common environmental pollutants with toxic, genotoxic, mutagenic and/or carcinogenic properties **[1]**. These compounds are normal products of organic matter combustion. However, they mainly occur in petroleum industry activities, coal-refining process, automobile exhausts as well as forest fires **[2,3]**. Oil spills because of pipeline breakages, tank leakages or storage and transportation accidents can be considered as the most frequent causes of hydrocarbon release, included PAHs, into soils and ground waters **[4]**.

A large number of bacteria with PAH degrading capabilities have been reported as able to either completely assimilate a defined range of compounds or carry out their transformation to different extents **[5,6]**. There are several mechanisms, or combinations thereof, by which microbial communities can adapt to the presence of PAHs in their environment. Firstly, there can be an increase in population size of those organisms that tolerate or even degrade the compound by induction of appropriate genes. Secondly, the cells can adapt through mutations of various kinds, such as single nucleotide changes or DNA rearrangements that result in resistance to or degradation of the compound. Thirdly, they may acquire genetic information from either related or phylogenetically distinct populations in the community by horizontal gene transfer. Horizontal gene transfer in natural habitats is largely mediated by mobile genetic elements such as plasmids and transposons **[7]**.

Hydrocarbons are commonly used as the substrate for the production of biosurfactants. It has been postulated that the biological function of surface-active compounds is related to hydrocarbon uptake, and therefore a spontaneous release occurs with these substrates **[8–11]**. Although it is agreed that surfactants can enhance the solubility and dissolution of hydrocarbons from contaminated soil, contradictory results have been reported on the ability of surfactants to enhance the biodegradation of hydrocarbons **[12–16]**. Tiehm and Frizsche **[17]** studied the biodegradation of both single and mixture of PAHs presolubilized by surfactant. Accelerated biodegradation rates

were found for both single and mixed PAHs presolubilized compared with the rate of PAHs in crystal form. This indicated that solubilization increased the bioavailability of PAHs.

Against these backdrops, this study was aimed to determine the effect of surfactant Triton X-100 on biodegradation of various PAHs by *Pseudomonas* sp. PSS6 isolated from soil sediments of municipal wastes.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study namely PAHs such as naphthalene, fluoranthene, anthracene and phenanthrene were purchased from Merck, India with high purity.

Source of Microorganisms

The bacterial strains were isolated from the soil sediments of local municipal waste dumping site near Chennai and the soil sample was placed in sterile polythene bags and brought to lab and stored at 4 °C.

Isolation of PAH-degrading Microorganisms

Isolates were plated on Bushnell–Haas agar (BH) (Difco) and sprayed (20, 37) with a 2% PAH stock solution in acetone. Presumptive PAH users were distinguished by formation of a clearing zone or coloration around the colonies. Sprayed-plate experiments were performed in duplicate. Naphthalene dioxygenase activity was detected by the formation of blue-indigo colonies when indole (1 mM) was added to the agar **[18]**. The organism producing high clearing zone was selected for further experiments.

Mineral Salts Medium and Enrichment of Bacteria

The carbon free mineral salts medium (MSM) contained NH_4Cl–2.5 g, KH_2PO_4–5.46 g, Na_2HPO_4–4.76 g, $MgSO_4$–0.20 g, NaCl–30.0 g and distilled water–1 L at pH–7.4 ± 0.2. The final pH of the medium was adjusted to 7.4 with 0.1N NaOH, and the medium was autoclaved (121 °C for 15 min) prior to the addition of PAHs. Stock solutions of each PAH (300 mg/L) were prepared in ethyl acetate and stored. PAH dissolved in ethyl acetate was added to 250 mL conical flask and after the evaporation of ethyl acetate, the mineral medium (100 mL) was added. The test strain was inoculated to the mineral medium containing PAH (phenanthrene) as sole carbon source. The conical flask was kept in shaker at 150 rpm with 37 °C as incubation temperature. After growth was visualized under microscope, 5 mL of enrichment culture was transferred to a fresh medium and incubated under the same conditions. Subsequent identical transfer of culture was performed in the respective PAH containing medium to enrich the bacteria **[19]**.

Characterization and Molecular Identification of Bacteria

The preliminary characterization of the isolated strain was done using Bergey's manual of systemic bacteriology **[20]**. The identity of the isolate was determined by sequence analysis of the 16S rDNA gene. The overnight cultured bacterial cells were lysed with lysozyme and the DNA was extracted by the phenol: chloroform (1:1) extraction method **[21]**. The 16S rDNA was amplified in PCR with the primer pair 16s FP: (5′-AGAGTRTGATCMTYGCTWAC-3′), 16s RP: (5′-CGYTAMCTTWTTACGRCT-3′). The amplified region was then sequenced and subject to BLAST analysis for analyzing its phylogeny **[22]**.

Effect of Triton X-100 on PAHs Degradation

The PAHs (naphthalene, fluoranthene, phenanthrene and anthracene) were added in the medium at a concentration of 3 mg/L along with surfactant Triton X-100 at 1%. The test isolate was studied for its growth on PAHs as sole carbon sources along with Triton X-100. For the degradation study, the test isolate was inoculated in mineral medium containing PAH along with the respective surfactants. The percentage of naphthalene degradation was calculated against the values obtained from control (without Triton X-100). The culture prepared in duplicates were incubated at 37 °C in shaker at 150 rpm and extracted at every 24 h time interval for 5 days. The culture samples were extracted twice with ethyl acetate (v/v) after acidification to pH 2.5 with 1 N HCl. The extracts were filtered through anhydrous sodium sulphate and condensed to 1mL using rotavapour unit (Buchi, Germany) and analysed in a high performance liquid chromatography (HPLC) **[19]**.

RESULTS AND DISCUSSION

Polycyclic aromatic hydrocarbons (PAHs) are unique contaminants in the environment because they are generated continuously by the inadvertently incomplete combustion of organic matter, for instance in forest fires, home heating, traffic, and waste incineration. PAH contaminated sites are mostly found in or near cities, thus representing a considerable public health hazard. The fate of PAHs in the environment is associated with both abiotic and biotic processes, including volatilization, photo-oxidation, chemical oxidation, bioaccumulation and microbial

transformation. Microbial activity has been deemed the most influential and significant cause of PAH removal **[23–27]**. The bacterial strains isolated from the soil sediments of municipal wastes were tested for their ability to degrade PAHs by Sprayed-plate experiments. It was found that a bacterial strain PSS6 efficiently degraded PAHs to a greater extent compared to other isolates forming an intensive clear zone (data not shown).

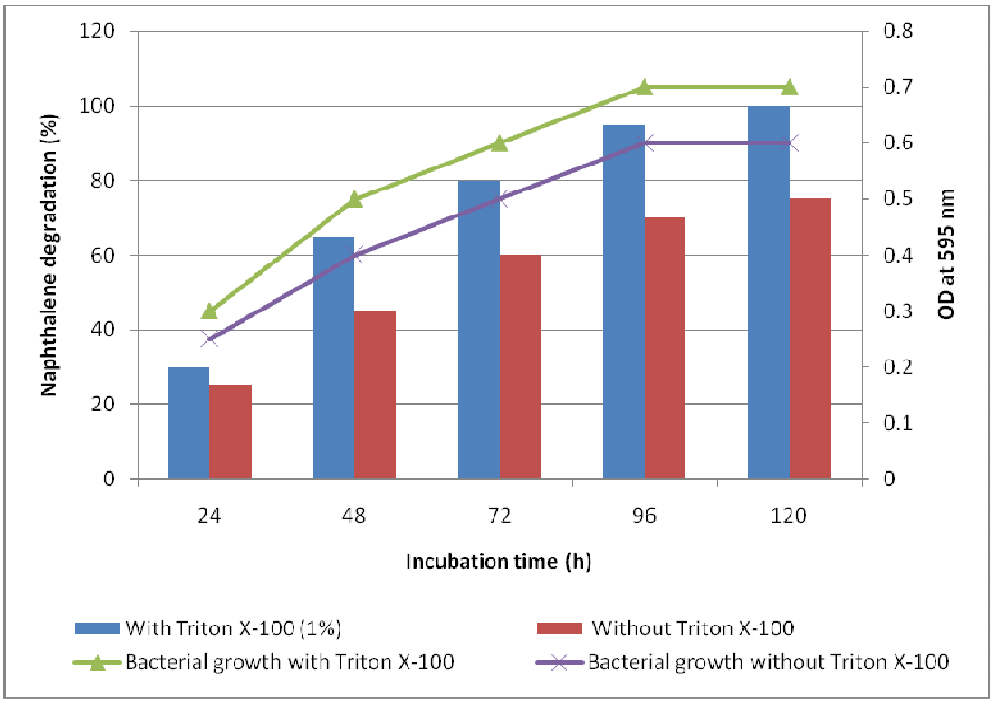

Figure 1: Effect of Triton X-100 on naphthalene degradation by *Pseudomonas* sp. PSS6

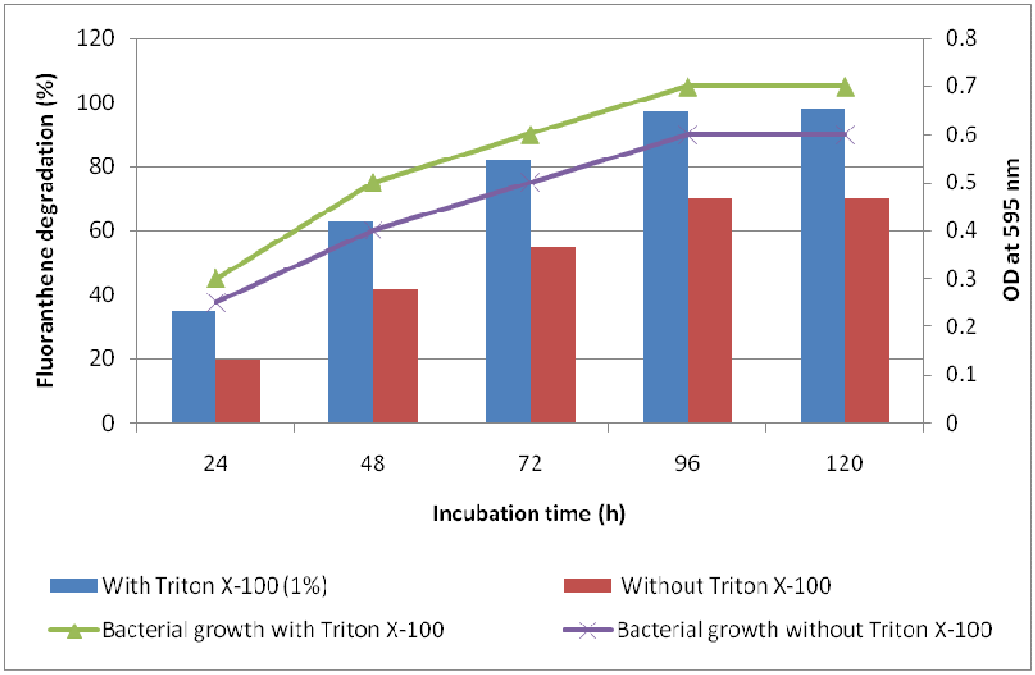

Figure 2: Effect of Triton X-100 on fluoranthene degradation by *Pseudomonas* sp. PSS6

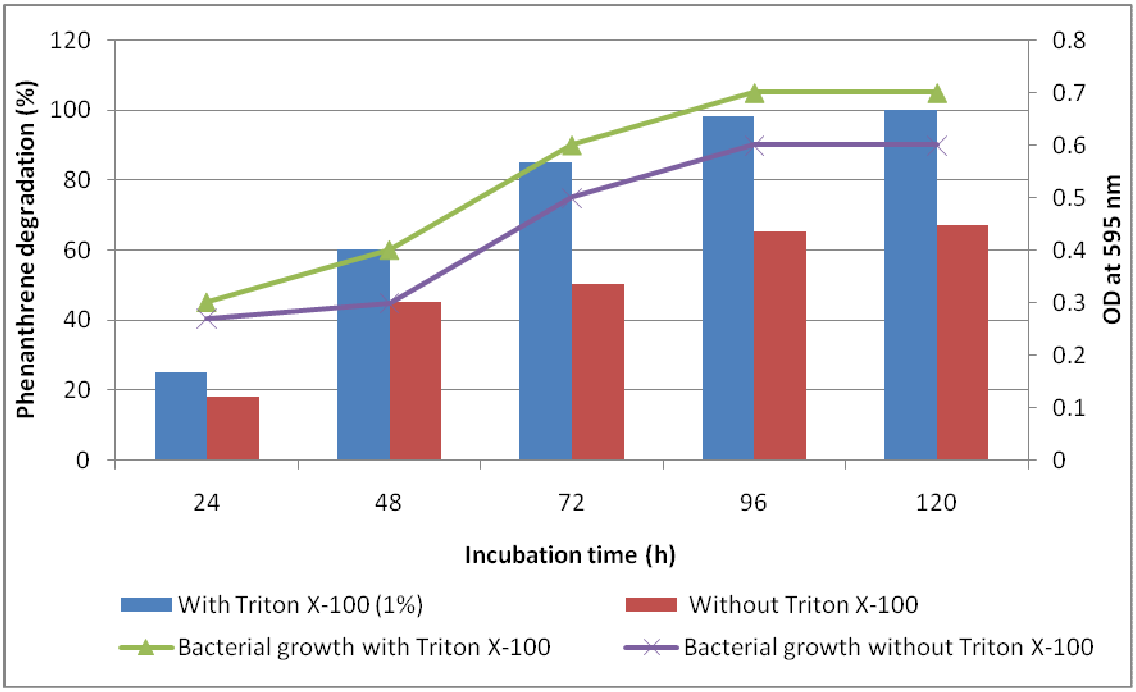

Figure 3: Effect of Triton X-100 on phenanthrene degradation by Pseudomonas sp. PSS6

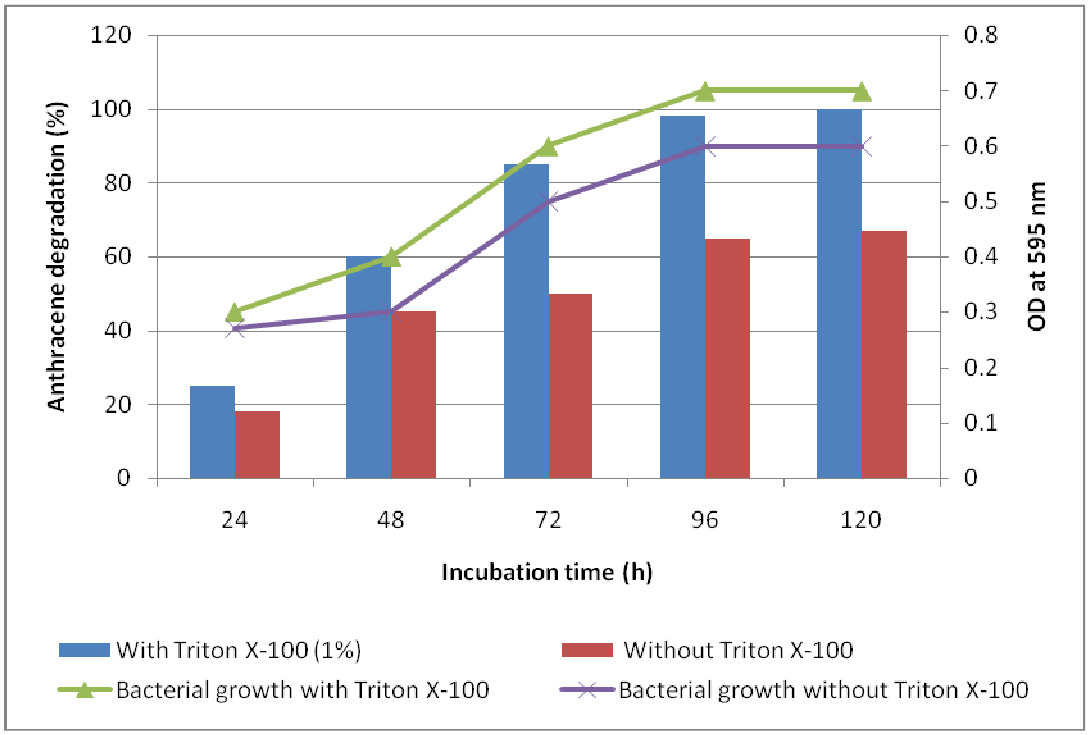

Figure 4: Effect of Triton X-100 on anthracene degradation by *Pseudomonas* sp. PSS6

From microscopic appearance and the biochemical tests, the isolate was identified as *Pseudomonas* sp. PSS6 and further confirmation was done by sequencing the 16S rDNA gene and compared with the GenBank databases using the BLASTN program. The 16S rDNA sequence of the isolate revealed a close relatedness to *Pseudomonas* sp. with 95% similarity. Hence the strain was confirmed as *Pseudomonas* sp. PSS6 and the sequence was submitted to Genbank (Accession No.: JQ838610).

There are numerous bacteria which are able to degrade PAHs belong to the genera *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Alcaligenes*, *Micrococcus*, *Bacillus*, *Flavobacterium*, *Arthrobacter*, *Alcanivorax Mycobacterium*, *Rhodococcus* and *Actinobacter***[28-30]**. The bacterial strain was identified as *Pseudomonas* sp. PSS6 by 16S rDNA sequencing.

The low solubility and high hydrophobicity of many hydrocarbon compounds make them highly unavailable to microorganisms. Release of bio-surfactants is one of the strategies used by microorganisms to influence the uptake of PAHs and hydrophobic compounds in general**[31, 32]** . In this study the effect of Triton X-100 at 1% were tested for PAHs (naphthalene, fluoranthene, phenanthrene and anthracene) degradation by *Pseudomonas* sp. PSS6. Surfactant biodegradability is one factor that determines its applicability for *in-situ* bioremediation applications. If the surfactant is highly degradable to the microorganism, it may become a competitive carbon source, which influences the degradation of the primary substrate. Inhibited degradation of PAHs due to the preferable degradation of surfactant was reported**[33]**.

Naphthalene has often been used as a model compound to investigate the ability of bacteria to degrade PAHs because it is the simplest and the most soluble PAH **[34]**. From Fig. 1 it is noted that Triton X-100 at 1% enhanced the degradation percentage of naphthalene and the growth of the bacteria compared to control. The findings are in accordance with Liu *et al.* **[35]** who noticed similar effects when he used Triton X-100 as surfactant in his study. There are conflicting results that have been reported concerning the effect of surfactants/emulsifiers on biodegradation of hydrocarbons **[30,36–38]**.

Fluoranthene, a four-ring PAH, is one of the principal PAHs in the environment**[39,40]**. Fig. 2 depicts the effect of Triton X-100 on the degradation of fluoranthene. It was noted that an increase in degradation rate is noticed after 96 h of incubation in Triton X-100. Phenanthrene, a three aromatic ring system, is found in high concentrations in PAH-contaminated sediments, surface soils, and waste sites**[41]**. With reference to phenanthrene the degradation rate of it almost reached 100% when incubated with Triton X-100 (Fig. 3). Allen *et al.* **[31]** found that Tritox-100 increased the rate of phenanthrene degradation in *Pseudomonas* strain. Anthracene was degraded to 97–100% by Triton X-100 incubated *Pseudomonas* sp. PSS6 after a duration period of 96 h (Fig. 4). The results were similar with the findings of Das et al. **[42]** who indicated that Triton X-100 influenced the degradation rate of anthracene in *Bacillus circulans.*

The biodegradation of Triton X-100 can lead to the release of more PAHs from the micellar phase into the aqueous phase, which increases their bioavailability. However, surfactants that can be readily degraded will quickly lose their solubilization capacity and render them ineffective for solubilization purpose **[18]**. Therefore, in practical applications, balance has to be found between the biodegradability of the surfactants and their influence on the biodegradation of PAHs. Suitable surfactants have to be pre-screened before an *in-situ* bioremediation process to be carried out **[42]**. The ability of pH, sulphate masse and sodium phosphate on PAH degradation and vice versa were also studied by some other researchers **[43,44].** Parameters to be considered include the solubilization capacity of the surfactants for the desired contaminates, physical properties of the surfactants such as its stability (clouding point, etc), and a suitable degree of biodegradability.

CONCLUSION

Conclusively, this study has shown that *Pseudomonas* sp. PSS6, an organism isolated from soil sediments of municipal wastes had the ability to degrade PAHs to a greater extent. In *Pseudomonas* sp. PSS6 showed high degradation percentage of PAHs influenced by Triton X-100 which is used as a surfactant. This little piece of investigation may be used to find out the effect of other surfactants on the degradation of toxic PAHs in the contaminated sites.

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