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Effect of Surfactant Brij 35 on Biodegradation of Polycyclic Aromatic Hydrocarbons by *Pseudomonas* sp. PSS6

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ABSTRACT

The effect of surfactant Brij 35 (1%) on biodegradation of various by polycyclic aromatic hydrocarbons (PAHs) by Pseudomonas sp. PSS6 was evaluated. The degrading capability of the test isolate was determined by Bushnell–Haas agar and Sprayed-plate method. Pseudomonas sp. PSS6 degraded PAHs forming an intensive clear zone in Bushnell–Haas agar. A modified mineral medium was employed for PAHs degradation along with Brij 35. The degradation of PAHs namely naphthalene, fluoranthene, phenanthrene and anthracene was influenced by 1% Brij 35. The anionic surfactant also increased the growth of the test isolate in the mineral medium.

Key words: Brij 35, *Pseudomonas*, Surfactant, Naphthalene, Fluoranthene, Phenanthrene, Anthracene.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated from both natural and anthropogenic processes and pose a serious concern to the health of aquatic life and humans through bioaccumulation [1,2]. Some bacteria are capable of oxidizing polyaromatic hydrocarbons. There are three types of microbial PAH degradation: complete mineralization, co-metabolic transformation and nonspecific oxidation [3]. To effectively decontaminate polluted sites it is desirable that there is complete mineralization to prevent accumulation of metabolites. Complete mineralization of different PAHs has been described for some bacterial strains belonging to different phylogenetic groups [4-6]. Bacterial degradation of low-molecular-mass PAHs such as naphthalene, phenanthrene and anthracene have been elucidated in detail [5]. The genus *Pseudomonas* has been the subject of much research as regards its ability to degrade PAHs [7–9].

The bioremediation of soils contaminated with PAHs is limited by the poor availability of these hydrophobic contaminants to microorganisms [10]. Surfactants can help, by solubilisation or emulsification, to release hydrocarbons sorbed to soil organic matter and increase the aqueous concentrations of hydrophobic compounds, resulting in higher mass transfer rates [11]. Contradictory results are found in the literature about the effects of addition of synthetic and biologically produced surfactants on PAH biodegradation [12]. However, recent studies indicate that they can enhance hydrocarbon biodegradation by increasing microbial accessibility to insoluble substrates [13,14]. Several researchers have investigated the addition of biosurfactants to enhance the biodegradation of hydrocarbons [15–17]. There are also several reports on improved hydrocarbon degradation by addition of

biosurfactant or chemical surfactant [18,19]. Against these backdrops, this study was aimed to determine the effect of Brij 35 (non-ionic surfactant) on biodegradation of PAHs by *Pseudomonas* sp. PSS6.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study namely naphthalene, fluoranthene, anthracene, phenanthrene and Brij 35 (polyoxyethylene lauryl ether) were purchased from Merck, India with 98% purity.

Source of Microorganisms

The bacterial strains were isolated from the soil sediments of local municipal waste dumping site near Chennai.

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 a colouration 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 [20]. 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 [21].

Characterization and Molecular Identification of Bacteria

The preliminary characterization of the isolated strain was done using Bergey's manual of systemic bacteriology [22]. 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 described by Ausubel *et al*. [23].

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 [24].

Effect of Brij 35 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 Brij 35 at 1%. The test isolate was studied for its growth on PAHs as sole carbon sources along with Brij 35. 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 Brij 35). 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) [20].

RESULTS AND DISCUSSION

Persistent organic pollutants (POPs) are among the most concerned environmental pollutants because they persist in the environment, bio-accumulate through the food web, and pose a risk of causing adverse effects to the environment and human health. POPs are also referred to as persistent, bio-accumulative and toxic chemicals (PBTs). POPs include PAHs which occur due to the combustion of organic matter, processing and use of fossil fuels [25]. A wide array of microorganisms including fungi, algae and bacteria are known to degrade PAHs. However, bacteria play by far the most important role in complete mineralization. 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 [26]. In this study, 15 bacterial strains were isolated from the soil sediments of municipal wastes and were tested for their ability to degrade PAHs (naphthalene, phenanthrene, fluoranthene and anthracene) 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 (Table 1).

Table 1: Qualitative growth of the strains on PAHs

Bacterial strain	Naphthalene	Phenanthrene	Fluoranthene	Anthracene
PSS1	+	+	+	+
PSS2	–	+	+	–
PSS6	++	–	+	+
PSS4	_	++	–	+
PSS5	+	–	+	+
PSS6	+++	+++	+++	+++
PSS7	+	–	+	–
PSS8	++	+	–	–
PSS9	++	+	+	+
PSS10	–	+	–	+
PSS11	++	–	+	+
PSS12	+	+	–	–
PSS13	+	–	++	+
PSS14	–	++	+	–
PSS15	+	–	+	–
'+' indicates visible colony growth				

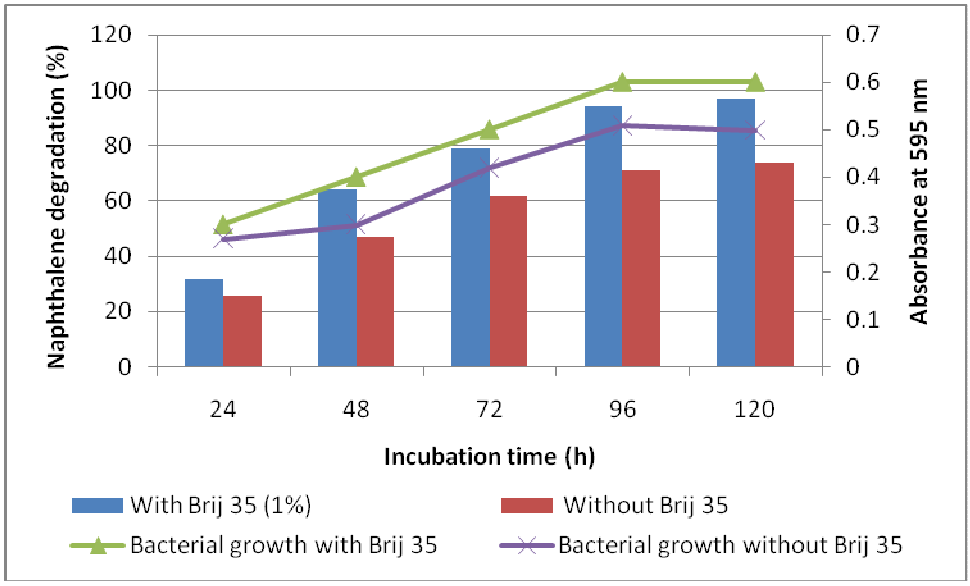

Figure 1: Effect of Brij 35 on naphthalene degradation by *Pseudomonas* sp. PSS6

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 (Accesion No. JQ838610).

The bacterial strain PSS6 was identified as *Pseudomonas* sp. by molecular sequencing. Most of the bacteria frequently isolated from hydrocarbon-polluted sites belong to the genera *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Alcaligenes*, *Micrococcus*, *Bacillus*, *Flavobacterium*, *Arthrobacter*, *Alcanivorax Mycobacterium*, *Rhodococcus* and *Actinobacter* [27, 28]. The biodegradation of hydrocarbons in polluted environment is mainly through the activities of bacteria and fungi. Typically, individual organisms degrade only a limited range of hydrocarbons. *Pseudomonas* sp. represents one of the most versatile groups of organisms involved in the degradation of hydrocarbons [29].

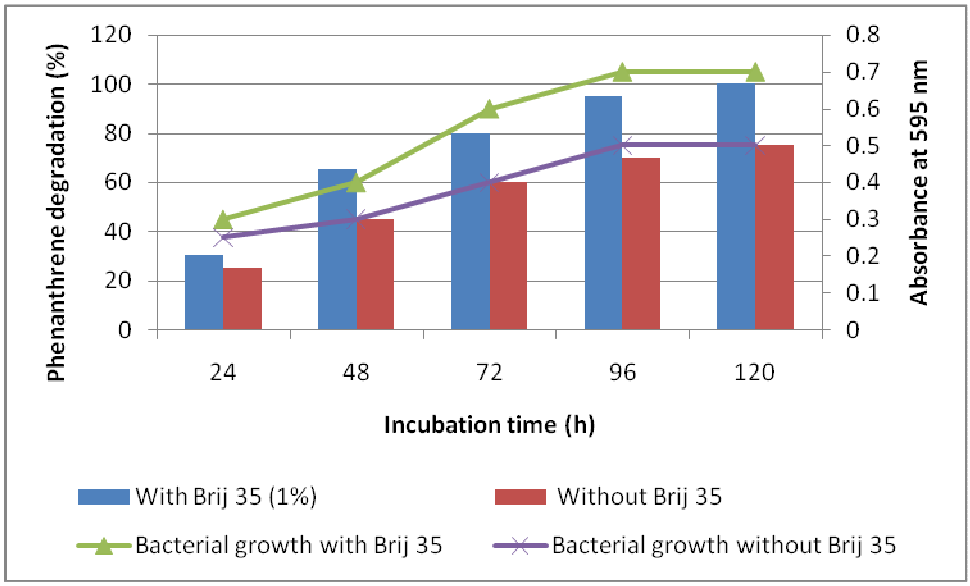

Figure 2: Effect of Brij 35 on phenanthrene degradation by *Pseudomonas* sp. PSS6

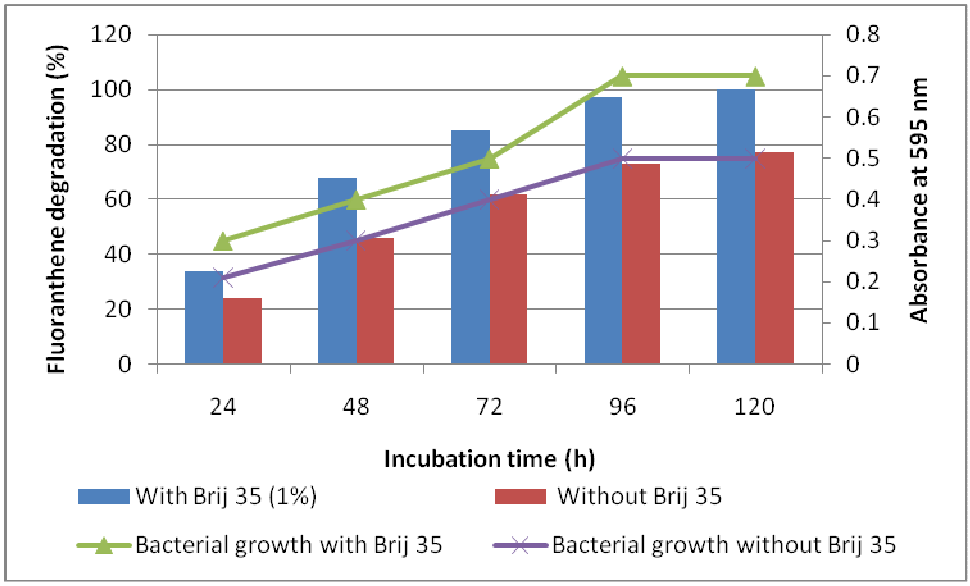

Figure 3: Effect of Brij 35 on fluoranthene degradation by *Pseudomonas* sp. PSS6

Biosurfactants are known to enhance hydrocarbon solubility and/or improve affinity of microbial cells for the substrate to facilitate their bioavailability and degradation in aqueous and soil system [18]. In this study the effect of Brij 35, a non-ionic surfactant at 1% was tested for PAHs (Naphthalene, Phenanthrene, Fluoranthene and Anthracene) degradation by *Pseudomonas* sp. PSS6. It was noted in Fig. 1 that Brij 35 influenced the degradation percentage of naphthalene. The growth of the test isolate was also high compared to control (without Brij 35). The degradation percentage nearly reached 98% after 96 h of incubation in Brij 35 by *Pseudomonas* sp. PSS6. Liu *et al.* found an increase in naphthalene degradation when Brij 35 is used as surfactant in his study [30].

Fig. 2 depicts the degradation percentage of phenanthrene by *Pseudomonas* sp. PSS6with and without Brij 35. It was noted that the degradation percentage reached 95% at the end of 96 h incubation with Brij 35. The degradation percentage of fluoranthene and anthracene also reached around 95–98% with Brij 35 after 96 h (Figs. 3 and 4).

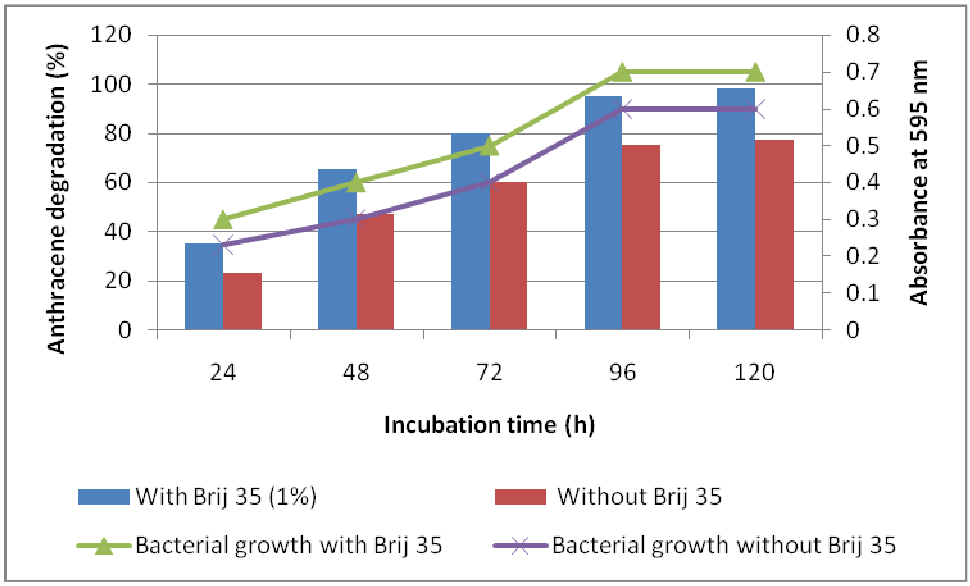

Figure 4: Effect of Brij 35 on anthracene degradation by *Pseudomonas* sp. PSS6

It is agreed that surfactants can enhance the solubility and dissolution of hydrocarbons. The enhanced biodegradation in the micellar solution by Brij 35 can be attributable to the increased solubility and bioavailability of substrate to bacteria [31], surfactant-enhanced substrate transport through the microbial cell wall [32], increased interfacial area in the presence of surfactant , enhanced contact of bacteria with the hydrocarbon–water interface [33], facilitated direct contact between cells and non-aqueous liquid phase [34], and decreased diffusion path length between the site of adsorption and site of bio-uptake by the microorganism due to enhanced adsorption of cells to hydrocarbon occupied soil particles in the presence of surfactant. The effects of surfactant on PAH biodegradation and vice versa were also studied by some other researchers [34-36]. It was observed that the biodegradation of naphthalene and phenanthrene were dependent on the surfactant used, and the presence of naphthalene and phenanthrene also influence the biodegradation of different surfactants to different degrees.

CONCLUSION

Pseudomonas sp. PSS6, an organism isolated from soil sediments of municipal wastes showed its ability to degrade PAHs to a greater extent. It showed high degradation percentage of PAHs influenced by Brij 35 which is used as a surfactant. For a successful field application, the selection of surfactants is probably the most important step. Such a surfactant should be non-toxic to the microorganism and pose no environmental concerns, also should have a good solubilisation capacity for the targeted contaminants. All these factors together with the bioavailability of the compounds solubilized in the micelles of the surfactant should be examined before field applications.

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