Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Chemica Sinica, 2012, 3(3):543-547

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Biodegradation of Polycyclic Aromatic Hydrocarbons by *Pseudomonas* sp. PSS6 Isolated from Municipal Wastes Sediment

Mukesh Kumar DJ[1,*], Amutha Magesh B[2], Devika S[3], Balakumaran MD[1] and Kalaichelvan PT[1]

[1]*Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, TN, India*
[2]*Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, TN, India*
[3]*Department of Biotechnology, Vivekanandha College of Engineering for Women, Tiruchengode, TN, India*

ABSTRACT

Polycyclic aromatic hydrocarbons are ubiquitous contaminants in environments impacted by fossil fuels directly or by the combustion of fossil fuels posing health hazards to human beings. This study investigated the ability of degrading PAHs (naphthalene, phenanthrene, anthracene and fluorene) by Pseudomonas sp. PSS6 isolated from municipal wastes collected from a waste dumping site. The strain PSS6 showed considerable growth over naphthalene, phenanthrene, anthracene, fluorene, as the sole carbon source with 1 mM concentration in Mineral Salt Medium (MSM) agar plates after 24 h. Pseudomonas sp. PSS6 showed greater degradation capability nearly around 100% against the PAHs tested.

Key-words: Polycyclic aromatic hydrocarbons, Naphthalene, Phenanthrene, Anthracene, Fluorene, *Pseudomonas.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are refers to a group of chemicals that consists of two or more fused aromatic rings arranged in a linear, angular, or cluster form [1]. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutant. That are generated from both natural and anthropogenic processes and it creates a serious concern to the health of aquatic life and humans through bioaccumulation [2,3]. PAHs are hydrophobic in nature and it gets readily adsorbed onto particulate matter and thus, coastal and marine sediments become the ultimate sinks for such compounds [4]. The marine organisms such as benthic, demersal and pelagic fishes, crustaceans and shellfishes are rigorously affected due to the carcinogenic nature of PAH'S [5].

Physical and chemical properties of PAHs varies according to the presence of number of rings and also increase in molecular weight decreases the chemical reactivity, aqueous solubility and volatility of PAHs [6]. Because of these reasons, PAHs differ in their transport, distribution and fate in the environment and their effects on biological systems [7]. The US EPA has identified 16 PAHs as main concerned pollutants and possibly some of these PAHs are considered to cause human carcinogens, and hence their distributions in the environment and possible exposure to humans have been of concerns [8]. Due to recalcitrant nature the high molecular-weight PAHs are paid particular attention, although PAHs are relatively stable and recalcitrant in soils and less easy to degrade than many other organic compounds [9]. The soils contaminated with more degradable or volatile organic compounds such as alkanes are treated successfully to remove those contaminants than the soil contaminated with PAH'S [9,10].

The abiotic and biotic processes, such as volatilization, photo-oxidation, chemical oxidation, bioaccumulation and microbial transformation are responsible for the outcome of PAHs into the environment. Microbial activity has been considered to play a significant role in cause of PAH removal [11–15]. Considerable attention has focused on the metabolic pathways and genetics of degradation of low molecular mass PAHs, such as naphthalene, phenanthrene and anthracene, by gram negative bacteria [16,17]. Against these backdrops this study was aimed to investigate the ability of degrading PAHs (naphthalene, phenanthrene, anthracene and fluorene) by *Pseudomonas* sp. PSS6 isolated from municipal wastes sediment collected from a waste dumping site near Perungudi, Chennai.

MATERIALS AND METHODS

Chemicals

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

Sampling Site

The municipal wastes sediment was collected from a waste dumping site near Perungudi, Chennai.

Mineral Salts Medium and Enrichment of Bacteria

The carbon free mineral salts medium (MSM) contained NH_4Cl–2.5g, KH_2PO_4–5.46 g, Na_2HPO_4–4.76 g, $MgSO_4$–0.20 g, NaCl–30.0 g and distilled water-1L 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 bacteria *Pseudomonas* sp. PSS6 isolated from the municipal waste was inoculated to the mineral medium containing PAH (phenanthrene) as sole carbon source. The inoculated conical flask was incubated at 37 °C with 150 rpm in shaker. After growth, the 5 mL of culture was transferred to a fresh medium and incubated under the above conditions mentioned. Subsequently the transfer of culture was performed in the respective PAH containing medium for enhancing the bacterial growth [5].

Characterization and Molecular Identification of Bacteria

The preliminary characterization of the isolated strain was done using Bergey's manual of systemic bacteriology [18]. 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*. [19].

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 [20].

PAHs Degradation

The PAHs were added in the medium at a concentration of 3 mg/L. The test isolate was studied for its growth on PAHs as sole carbon source. A qualitative assay by the spray-plate method was used to check the degradation of PAH. The clearing zones around the colonies indicating the utilization of PAH contained medium [21]. For the degradation study, the test isolate was inoculated in mineral medium containing PAH. Different compositions used in the degradation of PAH were (1) Medium + PAH + bacteria; (2) medium +PAH and (3) medium + bacteria where (2) and (3) served as controls .The percentage of naphthalene degradation was calculated against the values obtained from control. *Pseudomonas* sp. PSS6 was inoculated in the medium. 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 pH2.5 with 1 N HCl. The obtained extracts were filtered through anhydrous sodium sulphate and it was condensed into 1mL by using rotavapour unit (Buchi, Germany) and it was analysed by using high performance liquid chromatography (HPLC) technique [5].

RESULTS AND DISCUSSION

High concentrations of PAH'S was accumulated in environmental area's near to the coal gasification sites and tar oil distillation plants [22]. Major sources of PAHs released by the incomplete combustion of organic materials, gas production, wood treatment facilities, and waste incineration [23]. PAHs are formed naturally during thermal geologic reactions associated with fossil-fuel and mineral production, and during burning of vegetation in forest and

bush fires [24]. The strain PSS6 showed growth over important PAHs such as Naphthalene, Phenanthrene, Anthracene, Fluorene, as the sole carbon source with 1 mM concentration in MSM agar plates after 24 h (Table 1).

Table 1: Qualitative growth of the strain PSS6 on PAHs

PAHs	Qualitative growth in MSM agar plate
Naphthalene	++
Phenanthrene	+++
Anthracene	++
Fluorene	++
'+' indicates visible colony growth	

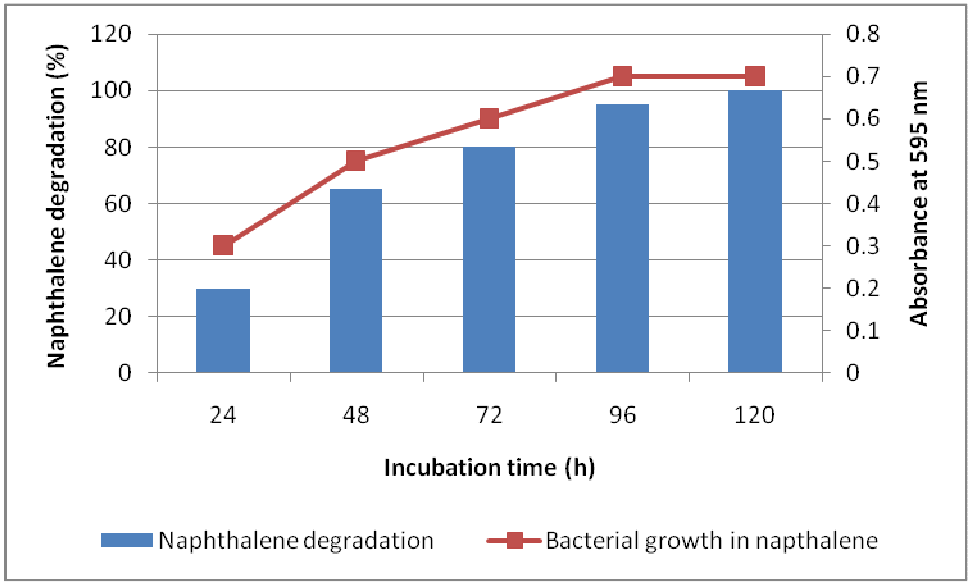

Figure 1: Growth of *Pseudomonas* sp. PSS6 and naphthalene degradation

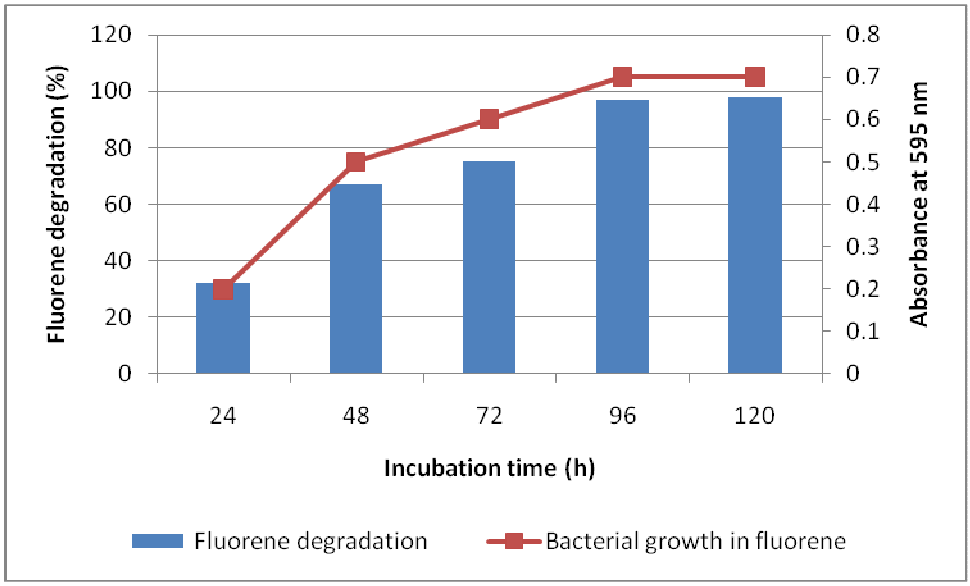

Figure 2: Growth of *Pseudomonas* sp. PSS6 and fluorene degradation

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 PSS6 was confirmed as *Pseudomonas* sp. And the sequence was submitted to Genbank (JQ838610).

Low molecular weight PAHs are compounds with less than three benzene rings and having a molecular weight in the range of 128–178 g/mol (eg : naphthalene, fluorene, phenanthrene and anthracene) [5].The test isolate *Pseudomonas* sp.PSS6 utilized naphthalene (3 mg/L) as sole carbon source. The growth of the test isolate was steady till 96h. Naphthalene was readily degraded by the consortium and nearly 95% of the compound was degraded in 96 h (Fig. 1).

Fluorene consists of two benzene rings coupled with a pentagonal ring (cyclopentane ring) and listed as a priority pollutant by Environmental Protection Agency (EPA) [25-27]. In this study, the test isolate utilized fluorene (3 mg/L) as the sole carbon source and showed a maximum degradation of 97% (Fig 2).

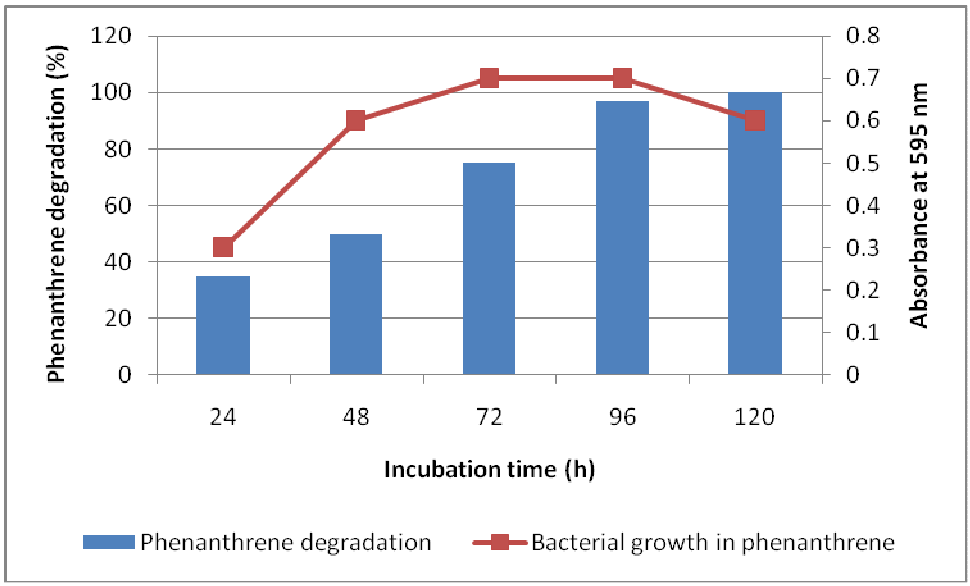

Figure 3: Growth of *Pseudomonas* sp. PSS6 and phenanthrene degradation

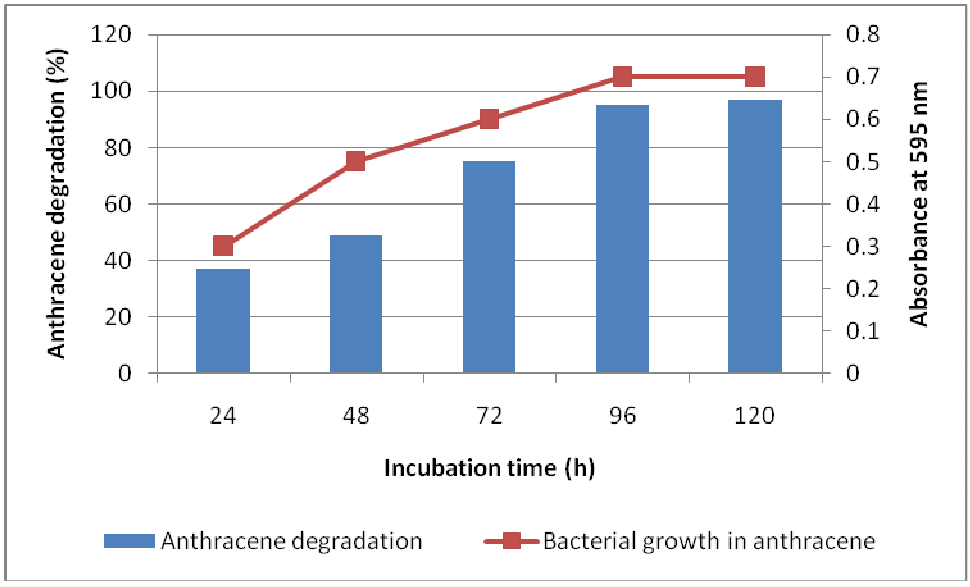

Figure 4: Growth of *Pseudomonas* sp. PSS6 and anthracene degradation

The bacterial growth was initially acclimatized on phenanthrene (3 mg/L). The growth of the test isolate was steady till 96 h.Nearly 50% of phenanthrene was degraded in 48 h and 100% degradation was recorded in 96 h. The

degradation of phenanthrene was 75% on the 3rd day (Fig. 3). The test isolate utilized anthracene (3 mg/L) and the growth increased steadily in 96 h. The consortium potently degraded anthracene 49% in 48 h and 95% in 96h (Fig. 4).The results were analysed for reproducibility of the PAHs degradation by the bacterial consortium using phenanthrene and fluorine showed no major change in the percent degradation (97–98%) was observed.

CONCLUSION

This study showed that a bacterium *Pseudomonas* sp. PSS6 had the ability to degrade the PAHs which are common environmental pollutants with toxic, genotoxic, mutagenic and/or carcinogenic properties. The strain PSS6 showed growth over naphthalene, phenanthrene, anthracene, fluorene, as the sole carbon source with 1 mM concentration in MSM agar plates after 24 h. *Pseudomonas* sp. PSS6 showed greater degradation capability nearly around 100% against the PAHs tested. To conclude, this isolate may be further exploited in the PAHs contaminated areas after careful investigations.

REFERENCES

[1] P.Y. Cheung, B.K. Kinkle, *Appl. Environ. Microbiol.*, **2001**, 67, 2222–2229.
[2] E.C. Okafor, K. Opuene, *Int. J. Environ. Sci. Technol*., **2007**, 4(2), 233–240.
[3] E.O. Fagbote, E.O. Olanipekun, *Int. J. Environ. Sci. Technol.*, **2010**, 7(3), 561–570.
[4] S.H.Yu, L. Ke, Y.S. Wong, N.F.Y. Tam, *Environ. Int.*, **2005**, 31(2), 149–154.
[5] P. Arulazhagan, N. Vasudevan, I.T. Yeom, *Int. J. Environ. Sci. Technol.*, **2010**, 7(4), 639–652.
[6] K.H. Lee, S.H. Byeon, *Int. J. Environ. Res*., **2010**, 4(3), 439–442.
[7] L.C. Osuji, P.E. Ezebuiro, *Int. J. Environ. Sci. Technol*., **2006**, 3(3), 313–320.
[8] C.A. Menzie, B.B. Potochi, J. Santodonato, *Environ. Sci. Technol.*, **1992**, 26, 1278–1284.
[9] J.B. Hughes, D.M. Beckles, S.D. Chandra, C.H. Ward, *J. Ind. Microbiol. Biotechnol*., **1997**, 18(2–3), 152–160.
[10] P. Pitter, J. Chudoba, Biodegradation of Organic Substances in the Aquatic Environment, (CRC Press: Boca Raton, FL), **1990**.
[11] C.E. Cerniglia, J.C. Morgan, D.T. Gibson, *Biochem. J.*, **1979**, 180, 175–185.
[12] C.O. Nwuche, E.O. Ugoji, *Int. J. Environ. Sci. Technol.*, **2008**, 5(3), 409–414.
[13] S. Haghighat, A. Akhavan Sepahy, M. Mazaheri Assadi, H. Pasdar, *Int. J. Environ. Sci. Technol*., **2008**, 5(3), 385–390.
[14] I.E. Agbozu, K. Opuene, *Int. J. Environ. Res.*, **2009**, 3(1), 117–120.
[15] R. Atlas, J. Bragg, *Microbiol. Biotechnol.*, **2009**, 2(2), 213–221.
[16] P.A. Williams, J.R. Sayers, *Biodegradation*, **1994**, 5(3–4), 195–217.
[17] O.A.Opasola, S.O.Adewoye, A.O.Adewoye, A.S.Bolaji, *Euro. J. Exp. Bio.*, **2011**, 1(2), 189-197.
[18] N.J. Palleroni, N.R. Krieg, J.G. Holt, Bergey's manual of systematic bacteriology, (Williams and Wilkins, Baltimore), **1984**, 141–198.
[19] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl K (Eds.), Current Protocols in Molecular Biology, (Wiley & Sons, New York), **1987**.
[20] V. Strubel, K.H. Engesser, P. Fischer, H.J. Knackmuss, *J. Bacteriol.*, **1991**, 173, 1932–1937.
[21] H. Kiyohara, K. Nagao, K. Yana, *Appl. Environ. Microbiol*., **1982**, 43(2), 454–457.
[22] G. Capotori, P. Digianvincenzo, P. Cesti, A. Bernardi, G. Guglielmetti, *Biodegradation*, **2004**, 15, 79–85.
[23] T.J. Kim, E.Y. Lee, Y.J. Kim, K.S. Cho, H.W. Ryu, *World J. Microbiol. Biotechnol.*, **2003**, 19, 411–417.
[24] A.L. Juhasz, R. Naidu, *Int. Biodeter. Biodegr.*, **2000**, 45, 57–88.
[25] R.C.B. Gomes, R. Nogueira, J.M. Oliveira, J. Peixoto, A.G. Brito, Kinetics of fluorene biodegradation by a mixed culture, Proceedings of the Second IASTED International Conference Advanced Technology in the Environmental Field, (Lanzarote, Canary Island, Spain), **2006**, 84–87.
[26] L. N. Ukiwe, U. U. Egereonu, *Adv. Appl. Sci. Res.*, **2012**, 3(2),1132-1136.
[27] L. N. Ukiwe, U. U. Egereonu, *Der Chemica Sinica*, **2012**, 3(2), 435-439.