Isolation and Characterization of a Phenanthrene-Degrading *Sphingomonas* sp. Strain P2 and Its Ability to Degrade Fluoranthene and Pyrene *via* Cometabolism

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ABSTRACT A bacterial strain P2 capable of using phenanthrene as a sole source of carbon and energy was isolated from a lubricant-contaminated soil sample collected from a garage in Prajinburi province, Thailand and identified by 16S rRNA gene sequence analysis to be in the genus of *Sphingomonas*. The *Sphingomonas* sp. strain P2 rapidly degraded phenanthrene from 100 mgl^{-1} to undetectable level by HPLC analysis within 72 h. Besides phenanthrene, this strain was able to degrade a wide variety of polycyclic aromatic hydrocarbons (PAHs) including naphthalene, acenaphthylene, acenaphthene, dibenzofuran, fluorene, and anthracene, meanwhile, it was also capable of co-metabolizing high molecular weight PAHs such as fluoranthene and pyrene in liquid minimum medium supplemented with phenanthrene.

KEYWORDS : *Sphingomonas* sp., polycyclic aromatic hydrocarbons, phenanthrene, degradation.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are classified as fused-ring aromatic compounds occur ubiquitously as environmental pollutants. PAHs are found as contaminant on certain industrial sites, particularly those associated with petroleum, gas production, and wood preservative industries.[1] The presence of these compounds in the environment should be seriously taken into consideration as evidenced by the toxicity exerted. Whereas some higher molecular weight compounds have mutagenic, teratogenic, and potential carcinogenic effects.[2]

Phenanthrene, a tricyclic PAH containing three fused benzene rings in angular arrangement, is a major constituent of coal derivatives and fossil fuels. Phenanthrene is one of the 16 PAHs in the list of priority pollutants compiled by the US Environment Protection Agency.[3] This compound being encountered regularly in environmental samples is not genotoxic but has a chemical structure found in several carcinogenic PAHs such as a benzo[*a*] pyrene.[4]

PAHs are widespread environmental contaminants and found at relatively high concentrations in various sites.[5] In Thailand, the contamination of PAHs in soil mostly arises from fossil fuel combustion and motor vehicle exhaust.[6] Since degradation of PAHs by microorganisms is well documented[7, 8] and microbial biotransformation is found to be a major environmental process affecting the fate of PAHs in both terrestrial and aquatic ecosystems, therefore, microbial remediation is a remarkable alternative in treating the hazardous waste, especially *in situ* bioremediation. Apart from the direct oxidation, microorganisms were found advantageously to detoxify PAHs which were recalcitrant compounds by means of co-metabolic oxidation.[9] Moreover, the use of microbes to convert toxic or persistent organic molecules into harmless products such as carbon dioxide and water as the end products is safe and economical.[10] To effectively clean-up these substances, it is particularly important to isolate and characterize microorganisms capable of degrading PAHs in many different geographic environments to accumulate the data about the PAHs-degrading microorganisms in each site in order to apply them back in their own environments.

In this paper, we report on the isolation and characterization of a *Sphingomonas* sp. strain P2 from a lubricant-contaminated soil in Thailand, which utilizes phenanthrene as the sole carbon and energy

source. In addition, the ability to degrade other PAHs and effects of phenanthrene on the degradation of high molecular weight PAHs, fluoranthene and pyrene as a mechanism of co-metabolic oxidation, are also reported.

Materials and Methods

Chemicals. All chemicals and solvents were of the highest purity. They were purchased as follows: naphthalene and phenanthrene from Sigma Chemical Co (St Louis, MO), acenaphthene, acenaphthylene, anthracene, fluorene, dibenzofuran, fluoranthene, and pyrene from Kanto Chemical Co, Inc.(Kyoto, Japan), bacteriological media and reagents from Difco Laboratories (Detroit, Mich), and solvents from Merck (Darmstadt, Germany) and Sigma Chemical.

Samples. Soil samples were collected from various petroleum-comtaminated sites in three provinces of Thailand, Bangkok, Saraburi, and Prajinburi and stored at 4°C until used.

Media and supply of PAHs. The carbon-free minimum medium (CFMM)[11] contained (per liter) 3.0 g of NH_4NO_3, 2.2 g of Na_2HPO_4, 0.8 g of KH_2PO_4, 0.01 g of $MgSO_4 \bullet 7H_2O$, 0.005 g of $FeCl_3 \bullet 6H_2O$, 0.005 g of $CaCl_2 \bullet 2H_2O$. The final pH of the medium was adjusted to 7.5 with 0.1 N NaOH, and the medium was sterilized by autoclave (121^0C for 20 min) prior to the addition of PAHs substrates. Stock solutions of each PAH were prepared in dimethyl sulfoxide (DMSO) and sterilized by filtration. CFMM was supplemented with individual PAHs to achieve final concentrations of 100 mgl^{-1}. Solid medium was prepared by the addition of 1.5 % Bacto agar to the respective medium.

Enrichment cultures. Bacterial inocula were obtained by shaking 20 g (wet weight) of contaminated soils overnight in 100 ml of CFMM at 30 ^{0}C with agitation at 200 rpm. The bacterial cell suspension fluids (5ml) were used to inoculate into 45 ml of CFMM containing phenanthrene. After growth were visualized, 5 ml of enrichment cultures were then transferred to a fresh CFMM and incubated under the same conditions. Subsequent identical transfers were performed for three successive transfers in the respective phenanthrene-containing medium.

Isolation and identification of phenanthrene-degrading bacteria. Phenanthrene-degrading bacteria were isolated from the enrichment cultures by the spray-plate technique[12] using phenanthrene as the sole carbon and energy source. Phenanthrene degrading-bacteria were visualized by a distinct phenanthrene clear zone surrounding individual colonies. These representative colonies were aseptically removed and subcultured in liquid CFMM containing phenanthrene. The culture broths were analyzed by TLC and HPLC for phenanthrene degradation. The strain capable of rapid degradation of phenanthrene was selected. Species identification was performed by using bacteriological and biochemical tests under standard procedures as described by Bergey's Manual of Systematic Bacteriology.[13] In addition, sequence determination of 16S rDNA was also performed according to the method of Aman *et al*.[14]

Utilization of phenanthrene. Growth at the expense of phenanthrene was established by demonstrating an increase in bacterial number, and a decrease in concentration of phenanthrene. Phenanthrene-grown cells from the late-exponential growth phase were used as inoculum (10^6-10^7 cells ml^{-1}). Two replicate batch cultures were grown in 22-ml test tubes containing 5 ml of CFMM supplemented with 100 mg l^{-1} phenanthrene. Incubation was at 30 ^{0}C with agitation at 200 rpm. Two separate controls were included in the system namely "substrate control", basically CFMM with phenanthrene yet devoid of bacteria supplemented and "cell growth control" consisting of CFMM and bacteria without phenanthrene supplementation. Samples were taken daily for a period of 7 days. Cell number was determined by plate spreading on Luria-Bertani agar and incubated for 3 days at 30 ^{0}C. Phenanthrene residues remained in each sample was determined by HPLC.

Utilization of other PAHs. Utilization of 8 other PAHs as substrate for growth was tested by inoculating the selected isolate to two replicates 22-ml test tubes containing 5 ml of CFMM with individual compounds (100 mg l^{-1}) as follows: naphthalene, acenaphthylene, acenaphthene, dibenzofuran, fluorene, and anthracene, fluoranthene and pyrene. Incubation was at 30 ^{0}C with agitation at 200 rpm. Cultures were analyzed for the increase in bacterial cell number at 0, 2, 4, and 7 days. The remaining substrates in the culture broth were analyzed by HPLC of organic extracts.

Co-metabolism of high molecular weight PAHs. The selected isolate was tested for its ability to degrade high molecular weight PAHs in the presence of a growth-supporting PAH compound. Fluoranthene or pyrene was added to 5 ml. of CFMM containing phenanthrene (100 mg l^{-1}) to a final concentration of 100 mg l^{-1}. Phenanthrene-grown cell were used

as inoculum (10^6-10^7 cells ml^{-1}). Incubation was performed at 30 ^{0}C with agitation at 200 rpm. At each sampling period over 7 days, two replicate cultures were collected, bacterial cell number was determined by plate spreading on Luria-Bertani agar and incubating for 3 days at 30 ^{0}C. The remaining amount of PAHs in the culture broths was analyzed by HPLC.

Analytical methods. PAHs in the culture broth were extracted thrice with 1 volume of ethyl acetate after acidification to pH 2.5 with 6 N HCl. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary evaporator. The residue was dissolved in small volume of methanol. Thin-layer chromatography (TLC) analysis was performed on TLC Keisel gel 60 F_{254} (E. Merck, Germany) using toluene: dioxane: acetic acid at 90: 25: 4 as a solvent system. Phenanthrene and its metabolites were detected under the UV light. HPLC analysis was carried out on a Shimadzu model LC-3A chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with Shimadzu model SPD-2A detector and Pegasil ODS, (15 by 0.46 cm[inside diameter] column; Senshu Scientific Co., Ltd., Tokyo, Japan) heated in 40 ^{0}C column oven. Eluting solvent was 80% methanol in water (vol/vol) at a flow rate of 1 ml min^{-1}. The eluents were monitored by UV absorption at 275 nm.

RESULTS

Isolation and characterization of phenanthrene-degrading bacteria. Four different bacterial colonies (P1.1, P1.2, P2, and P3) were obtained from lubricant-contaminated soils collected from a garage in Prajinburi province, Thailand by the standard culture enrichment technique using phenanthrene as the sole source of carbon and energy. When these colonies were grown on phenanthrene-coated agar plates, clear zones were visualized indicating the phenanthrene degrading ability. Despite of this, when these strains were tested for their phenanthrene degrading ability in liquid medium, only strain P2 was able to degrade phenanthrene rapidly with no accumulation of degradative products. Morphologically the strain P2 is a rod-shape, gram negative bacterium with yellow pigmentation. Results of bacteriological and biochemical characteristics of this organism as shown in Table 1 suggested that the strain P2 belongs to the genus *Sphingomonas*. DNA sequence analysis of PCR-amplified 16S rDNA as compared to those of *Sphingomonas yanoikuyae* (Fig 1) confirmed the above result that strain P2 is indeed belong to the genus *Sphingomonas*.

Utilization of phenanthrene. When cultivated *Sphingomonas* sp. strain P2 on CFMM containing 100 mg l^{-1} of phenanthrene, utilization of phenanthrene as the sole source of carbon and energy was demonstrated by the increase in bacterial cell number with a concomitant loss of phenanthrene (Fig 2). After incubation for 36 h, only trace amounts of phenanthrene could be detected by HPLC analysis. Within 72 h, phenanthrene was completely disappeared from the culture medium and the cell number of *Sphingomonas* sp. strain P2 was increased from 5.77 to 8.85 log CFU ml^{-1}. No significant change in phenanthrene concentration was observed in uninoculated culture. The slight decrease (100 to 85 mg l^{-1}, over 7 days) in phenanthrene concentration seen in uninoculated culture was attributed to the loss by its volatility.

Substrate specificity. *Sphingomonas* sp. strain P2 could use naphthalene, acenaphthylene, acenaphthene,

Table 1. Biochemical and morphological characteristics of a phenanthrene-degrading *Sphingomonas* sp. strain P2

Characteristics	Results [a]
Bacteriological tests	
Motility	+
Gram reaction	Rod shape No endospore forming Yellow color colony
Growth tests	
Glucose	-
Galactose	-
Mannose	-
Fructose	-
Mannitol	-
Sucrose	-
Xylose	-
Simmon citrate	+
Biochemical tests	
Oxidase	+
Catalase	+
Nitrate reduction	-
O-F test[b]	0
Lysine decarboxylation	+
Urease	+
Esculin hydrolysis	+
Gelatinase	-
Hydrogen sulfide production	-

[a] +: positive; -: negative

[b] O-F: oxidation-fermentation

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S.sp.P2      : -------GATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGATCTTCGGATCTAGTGG :   73
S.yanoikuyae : AGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGATCTTCGGATCTAGTGG :   80

S.sp.P2      : CGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTCGGAATAACTTCTGGAAACGGAAGCTAATACCGGATGATGA :  153
S.yanoikuyae : CGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTCGGAATAACTTCTGGAAACGGAAGCTAATACCGGATGATGA :  160

S.sp.P2      : CGTAAGTCCAAAGATTTATCGCCCAAGGATGAGCCCGCGTAGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGA :  233
S.yanoikuyae : CGTAAGTCCAAAGATTTATCGCCCAAGGATGAGCCCGCGTAGGATTAGCTAGTTGGTGGGGTAAAGGCCCACCAAGGCGA :  240

S.sp.P2      : CGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA :  313
S.yanoikuyae : CGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA :  320

S.sp.P2      : GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTT :  393
S.yanoikuyae : GGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTT :  400

S.sp.P2      : TACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAG :  473
S.yanoikuyae : TACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAG :  480

S.sp.P2      : CTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTATTCAAGTCAGAGGTGAAAGCCCGGGGCTCAACC :  553
S.yanoikuyae : CTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTATTCAAGTCAGAGGTGAAAGCCCGGGGCTCAACC :  560

S.sp.P2      : CCGGAACTGCCTTTGAAACTAGATAGCTTGAATCCAGGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGA :  633
S.yanoikuyae : CCGGAACTGCCTTTGAAACTAGATAGCTTGAATCCAGGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGA :  640

S.sp.P2      : TATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACA :  713
S.yanoikuyae : TATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACA :  720

S.sp.P2      : GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCAGGGCACATGGTGTTTTGGTGGCGCAGCTA :  793
S.yanoikuyae : GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCAGGGCACATGGTGTTTTGGTGGCGCAGCTA :  800

S.sp.P2      :  CGCATTAAGTTATCCGCCTGGGGAGTTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGT :  873
S.yanoikuyae : ACGCATTAAGTTATCCGCCTGGGGAGT-ACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGT :  879

S.sp.P2      : GGAGCATGTGGTTTAATTCGAAGCAACGCGCAAAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACACTT :  953
S.yanoikuyae : GGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACACTT :  959

S.sp.P2      : TCCTTCAGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG : 1033
S.yanoikuyae : TCCTTCAGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG : 1039

S.sp.P2      : CAACGAGCGCAACCCTCGCCTTTAGTTGCCAGCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAA : 1113
S.yanoikuyae : CAACGAGCGCAACCCTCGCCTTTAGTTGCCAGCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAA : 1119

S.sp.P2      : GGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGCGTTGGGCTACACACGTGCTACAATGGCGACTACAGTGGGCAGCC : 1193
S.yanoikuyae : GGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGCGTTGGGCTACACACGTGCTACAATGGCGACTACAGTGGGCAGCC : 1199

S.sp.P2      : ACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCGTCTCAGTTCGGATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAAT : 1273
S.yanoikuyae : ACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCGTCTCAGTTCGGATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAAT : 1279

S.sp.P2      : CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTT : 1353
S.yanoikuyae : CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTT : 1359

S.sp.P2      : GGATTCACTCGAAGGCGTTGAACTAACCGTAAAGAAGC---------------------------------- : 1391
S.yanoikuyae : GGATTCACTCGAAGGCGTTGAGCTAACCGTAAGGAGGCAGGCGACCACAGTGGGTTTAGCGACTGGGGTGA : 1430
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Fig 1. 16S ribosomal DNA sequence of *Sphingomonas* sp. strain P2 comparing with *Sphingomonas yanoikuyae*.

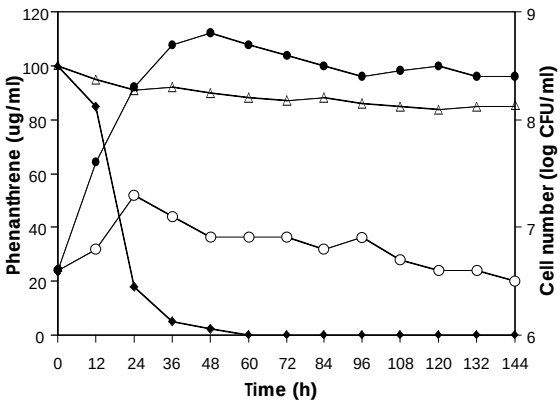

Fig 2. Growth profiles of *Sphingomonas* sp. strain P2 culturing in CFMM with (●) and without (o) phenanthrene supplemented, and the phenanthrene concentrations in the cultures with (♦) and without (Δ) bacterial inoculation.

dibenzofuran, fluorene, and anthracene as a growth substrate as shown in Table 2 and Fig 3. During growth on these substrates, the visible yellow-orange metabolites obtained indicate the accumulation of certain metabolites from naphthalene, dibenzofuran, fluorene, and anthracene while no activity were observed in case of fluoranthene and pyrene.

Co-metabolism of high molecular weight PAHs. The ability of this strain in degrading four-ring PAHs, fluoranthene and pyrene, to serve as sole carbon and energy source for growth was examined. Neither fluoranthene nor pyrene could be used by the bacterium as no growth was observed (Table 2 and Fig 3). To induce its ability to degrade these compounds, phenanthrene was added to fluoranthene- and pyrene-containing media. The results depicted in Fig 4 and 5 showed that strain P2 was able to degrade fluoranthene and pyrene (100 mg l^{-1}) in the presence of phenanthrene (100 mg l^{-1}), respectively. Phenanthrene was degraded to trace amount after 72 h and the cell number was increased from 5.5 to 7.7-8.7 log CFU ml^{-1} during this period. Significant concentrations of fluoranthene and pyrene were decreased over 7 days of incubation. It was also observed that degradation of the four-ring PAHs by strain P2 took place rapidly when phenanthrene was present in the culture medium but ceased immediately when phenanthrene was completely degraded.

Discussion

The phenanthrene-degrading bacteria strain P2 was isolated from a lubricant-contaminated soil collected from a garage in Prajinburi province, Thailand. Its ability in complete removal of phenanthrene in CFMM was demonstrated, which

Table 2. Activities of *Sphingomonas* sp. strain P2 on single PAHs.

Substrate	Growth	Color
Naphthalene	+	Yellow
Acenapthylene	+	Pale yellow
Acenaphthene	+	Pale yellow
Dibenzofuran	+	Orange
Fluorene	+	Yellow
Anthracene	+	Brown
Fluoranthene	-	NC[a]
Pyrene	-	NC

[a] NC: no change; +: growth; -: no growth

led to a corresponding increase in bacterial cell number (Fig 2). On the basis of morphological, growth, biochemical tests, (Table 1) and a phylogenetic analysis based upon 16S rRNA (Fig 1), this strain was classified within genus *Sphingomonas*.

During growth on phenanthrene in batch culture, the water-soluble intermediates causing a change in color of the medium from clear to yellow was detected. No attempt was made to identify the materials responsible for the color change however, the accumulation of catechol-like compounds and their meta-ring clevage products may be attributed to this color change.[9]

Sphingomonas sp. strain P2 showed broad versatility in its action on polycyclic aromatic substrates. In addition to phenanthrene, it was also able to utilize naphthalene, acenaphthylene, acenaphthene, dibenzofuran, fluorene, and anthracene as a growth substrate since yellow-orange metabolites were formed during its growth in the medium containing these compounds. Production and accumulation of meta-clevage products may also be considerable as in the metabolism of these PAHs by different bacteria.[15, 16]

Co-metabolism as mean to enhance the degradation of high molecular weight PAHs in particular, those with four or more aromatic rings has been observed.[17] Only few studies have observed the improved degradation of recalcitrant PAHs when more labile PAHs are present.[18] It has recently been reported that stimulation of dibenz[*a,h*]anthracene and benzo[*a*]pyrene degradation by *Burkholderia cepacia* was achieved by the addition of small quantities of phenanthrene to culture containing these compounds.[19] In contrast, the degradation of fluoranthene and pyrene by a *Rhodococcus* sp. was inhibited when phenanthrene was added to cultures containing either fluoranthene or pyrene [18], this

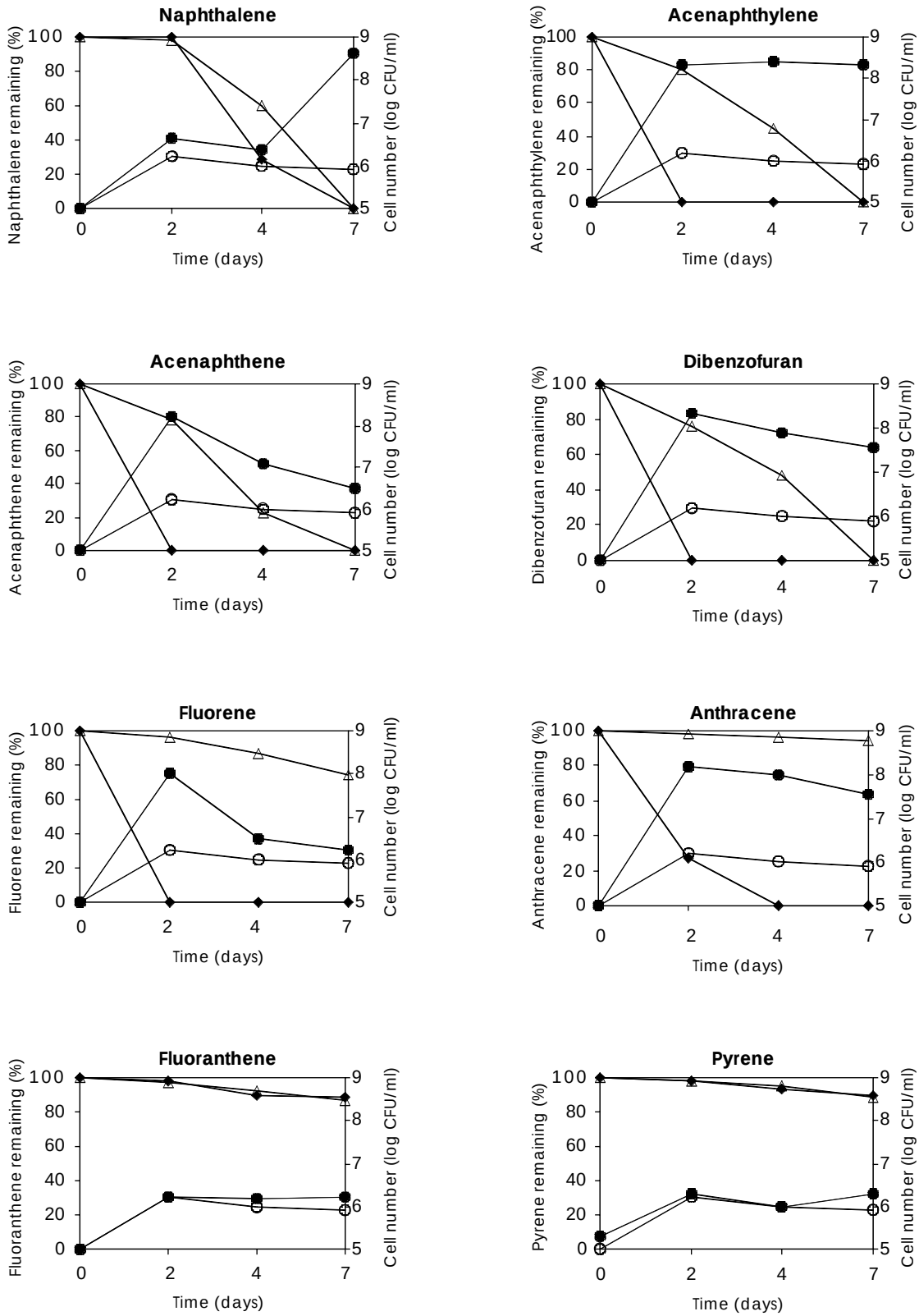

Fig 3. Growth profiles of *Sphingomonas* sp. strain P2 culturing in CFMM with (●) and without (o) various PAH supplemented as indicated and the PAH concentrations in the cultures with (♦) and without (Δ) bacterial inoculation.

effect was thought to be a result of the blocking of the induction of PAH-degrading enzymes. In this work, we observed that phenanthrene could stimulate microbial degradation of fluoranthene and pyrene when added to the P2 cultures containing these compounds (Fig 4 and Fig 5). Comparison with phenanthrene as a sole carbon source, growth of *Sphingomonas* sp. strain P2 in cultures containing fluoranthene and pyrene in the presence of phenanthrene decreased slightly (Fig 2, Fig 4 and Fig 5) and the degradation of fluoranthene and pyrene were stopped immediately after all of

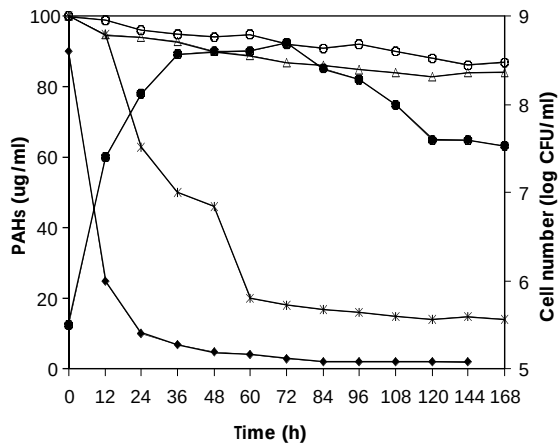

Fig 4. Growth profiles of *Sphingomonas* sp. strain P2 culturing in CFMM supplemented with phenanthrene and fluoranthene (●), and the phenanthrene concentrations in the cultures with (♦) and without (Δ) bacterial inoculation and the fluoranthene concentrations in the cultures with (*) and without (o) bacterial inoculation.

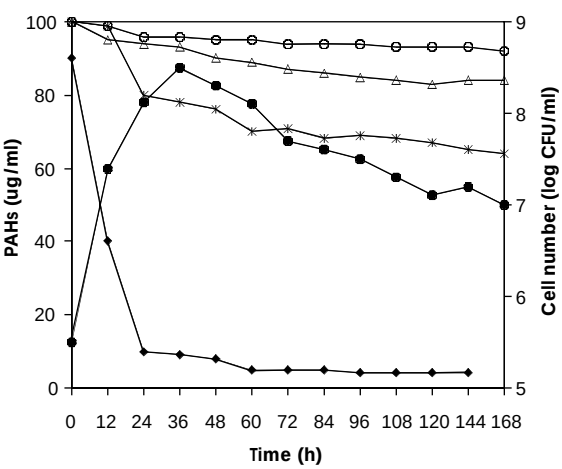

Fig 5. Growth profiles of *Sphingomonas* sp. strain P2 culturing in CFMM supplemented with phenanthrene and pyrene (●), and the phenanthrene concentrations in the cultures with (♦) and without (Δ)bacterial inoculation and the pyrene concentrations in the cultures with (*) and without (o) bacterial inoculation.

phenanthrene in the culture medium had been consumed. This could be due to the accumulation of toxic intermediates formed from biodegradation of the four-ring compounds since many PAHs intermediates have been identified as carcinogenic, mutagenic and teratogenic[20] agents and some of them also affected the survival and activity of micro-organism.[21, 22]

Owing to the fact that the present study has demonstrated the ability of *Sphingomonas* sp strain P2 in rapid degradation of phenanthrene from the liquid medium, having wide-range of substrate specificity and ability to degrade high molecular weight PAHs in the present of more labile PAH. It, therefore, provides concrete evidence of the usefulness of this *Sphingomonas* sp. strain P2 for bio-remediation of PAHs-contaminated environments.

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