

Biodegradation of benzo(a)pyrene by a newly isolated *Fusarium* sp.

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Abstract

Benzo(a)pyrene (BaP) is a five-ring polycyclic aromatic hydrocarbon produced by the incomplete combustion of organic materials. It is one of the priority pollutants listed by the US Environmental Protection Agency. This study describes a fungal isolate that is able to biodegrade benzo(a)pyrene. The filamentous fungus, isolated from leaves of *Pterocarpus macrocarpus* Kurz., was identified as a *Fusarium* sp. (strain E033). *Fusarium* sp. E033 was able to survive in the presence of benzo(a)pyrene concentrations up to 1.2 mM (300 mg L⁻¹). Biodegradation experiments using 0.4 mM (100 mg L⁻¹) benzo(a)pyrene demonstrated that *Fusarium* sp. E033 was able to degrade 65–70% of the initial benzo(a)pyrene provided, and two transformation products, a dihydroxy dihydro-benzo(a)pyrene and a benzo(a)pyrene-quinone, were detected within 30 days of incubation at 32 °C. The factors affecting biodegradation efficiency were also investigated. While increasing aeration promoted better fungal growth and benzo(a)pyrene biodegradation, increasing the glucose concentration from 5 to 50 mM had an adverse effect on biodegradation. Ethanol and methanol, provided at 5 mM to increase benzo(a)pyrene water solubility, increased the fungal biomass yield but did not promote degradation. The *Fusarium* sp. E033 isolated in this study can tolerate and degrade relatively high concentrations of benzo(a)pyrene, suggesting its potential application in benzo(a)pyrene bioremediation.

Introduction

Benzo(a)pyrene (BaP) is a five-ring polycyclic aromatic hydrocarbon (PAH) that is produced by the incomplete combustion of organic carbon-based material (Cerniglia, 1992). Benzo(a)pyrene is a hydrophobic compound having a low water solubility (0.0038 mg L⁻¹, at 25 °C), leading to its persistence in the environment. The US Environmental Protection Agency has listed benzo(a)pyrene as well as other 16 PAHs as priority pollutants because of their toxicity, mutagenicity and carcinogenic properties.

Although benzo(a)pyrene may undergo chemical oxidation and photolysis, microbial degradation has been reported as the major process affecting benzo(a)pyrene persistence and disappearance in nature (Cerniglia, 1993; Vidali, 2001). Such microbial degradation processes have been examined as providing an economic and efficient alternative treatment method compared with other chemical

and physical processes (Pothuluri & Cerniglia, 1998). However, the success of benzo(a)pyrene bioremediation projects has been limited by the failure to remove these high-molecular-weight PAHs due to the recalcitrance, poor bioavailability, the toxicity of benzo(a)pyrene as well as poor biodegradation abilities of microorganisms (Juhász & Naidu, 2000). Therefore, several biodegradation studies have focused on the isolation of microorganisms having a significant degradative ability for this high-molecular-weight compound (Wunch *et al.*, 1997).

Fungi have been reported to have biodegradation abilities for several recalcitrant organic compounds. Filamentous fungi may offer certain advantages over bacteria for bioremediation because of their rapid colonization of solid substrates and high tolerance of the toxin (Cerniglia *et al.*, 1985; Robinovich *et al.*, 2004). The majority of previous studies have focused on white rot fungi, particularly *Phanerochaete* sp.

(Barclay *et al.*, 1995; Field *et al.*, 1995; Pointing, 2001). These fungi are able to degrade benzo(a)pyrene and detoxify PAH-polluted soils as well as sediments through the production of extracellular lignin-degrading enzymes (Mester & Tien, 2000). Nonligninolytic fungi, such as *Cunninghamella elegans* (Cerniglia & Gibson, 1979) and *Penicillium janthinellum* (Launen *et al.*, 1995), also exhibit significant potential to metabolize PAHs. The ability of *Fusarium* spp. to degrade some recalcitrant substances has also been reported. *Fusarium* spp. are known to be able to degrade several high-molecular-weight substances such as cellulose, and coal hydrocarbons (Fakoussa & Frichter, 1999). However, little data have been produced on the ability of *Fusarium* spp. to degrade PAHs. *Fusarium solani* and *Fusarium oxysporum* isolated from contaminated soil have been reported to degrade PAHs including benzo(a)pyrene (Verdin *et al.*, 2004). In this study, a *Fusarium* sp. was isolated from plant leaves exposed to traffic emissions. It has a marked ability to degrade benzo(a)pyrene as well as tolerate high concentrations of BaP up to 1.2 mM (300 µg L⁻¹). Some of the factors involved in biodegradation of benzo(a)pyrene by the newly isolated *Fusarium* sp. (strain E033) are also described. As there has been an illustrative report of *Fusarium* sp. in soil bioremediation of PAHs (Li *et al.*, 2005), the results of this study demonstrating a relatively high benzo(a)pyrene biodegradability and tolerance of *Fusarium* sp. E033 suggested a potential use of this fungus in bioremediation of soil contaminated with benzo(a)pyrene.

Materials and methods

Sample collection and isolation of fungi

Leaves and bark of *Pterocarpus macrocarpus* Kurz. located along heavily used roads in Bangkok, Thailand, were collected. Samples were taken from adult trees that showed no sign of disease. Fragments of leaves and bark were placed in an icebox and processed within 4 h after collection. Leaves were washed under running tap water and cut into circle-shaped pieces of *c.* 5 mm diameter. They were surface-sterilized by dipping successively into 70% ethanol for 1 min, 5% sodium hypochlorite for 3–5 min, dipped again for 1 min in 70% ethanol and finally rinsed twice in sterile distilled water (Petrini, 1995). Four leaf segments were placed in each Petri dish containing malt extract agar medium with 100 mg L⁻¹ streptomycin to suppress bacterial growth. Dishes were incubated in the dark at ambient temperature (*c.* 32 °C) for more than 15 days. The fungi growing out of the segments during the incubation period were collected.

Screening and identification of benzo(a)pyrene-degrading fungi

Fifty three fungi isolates collected from the isolation step were grown on malt extract agar with and without (a

control) 0.4 mM (100 µg L⁻¹) benzo(a)pyrene supplementation. Mycelial extension rate (growth diameter) was determined at intervals over 14 days. Three potential benzo(a)pyrene-degraders were selected depending on their greater growth rate compared with that of the control. The isolated fungal strain capable of benzo(a)pyrene degradation was then identified morphologically and genetically using the internal transcribed spacer (ITS) sequence comparison. The fungal spores were observed and pictures were taken using a Scanning Electron Microscope: an EDAX Genesis 4000 XMS SYSTEM 60 running under the ESEM mode, equipped with a Gaseous Secondary Electron (GES) detector at magnifications from 800 to 1200 × (EDAX, Mahwah, NY). The ITS sequence analysis was performed using the PCR with the ITS primers: ITS1F (5'-CTTGGTCATT TAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATT GATATGC-3') (White *et al.*, 1990; Gardes & Bruns, 1993). The 50 µL PCR mixture was prepared according to the manufacturer's instructions (Fermentas, CA) consisting of, at final concentration, *c.* 100 ng genomic DNA, 1X Taq DNA polymerase buffer, 0.25 mM of each dNTP, 1 µM of each primer, 1.5 mM MgCl₂ and 2.5 U of Taq DNA polymerase. DNA amplification was performed in a thermal cycler (TP 3000; Takara Shuzo, Tokyo, Japan) at 94 °C for 5 min, followed by 38 cycles of 94 °C for 1 min, 51 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 5 min. The PCR product was purified using the NucleoSpin[®] (Macherey-Nagel Inc., Easton) and cloned using the PCR-Script[™] Amp cloning kit according to the manufacturer's protocol (Stratagene). Plasmid DNA was extracted from positive clones and sequenced (Macrogen, Seoul, Korea) using the same primers for DNA amplification. The partial ITS sequence (up to 850 bp) of the isolate was compared with those available on the database using the BLAST program at the National Center for Biotechnology Information (NCBI), analyzed and submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB255352.

Growth conditions and determination of benzo(a)pyrene degradation ability

Five plugs of mycelia (6 mm diameter) taken from solid agar medium were inoculated into 250 mL Erlenmeyer flasks containing 50 mL of liquid culture media having the composition previously described (Verdin *et al.*, 2004). Glucose (5 mM) was provided as a carbon source unless stated otherwise. In order to determine the benzo(a)pyrene degradation ability of the fungal isolate, benzo(a)pyrene dissolved in a minimum volume of acetone was added to a final concentration of 0.4 mM (100 µg L⁻¹). The liquid cultures were grown at room temperature (*c.* 32 °C) with reciprocal shaking at 180 r.p.m. for 30 days. Abiotic controls were

conducted to ensure that the disappearance of benzo(a)pyrene was caused by biodegradation as well as to compensate for adsorption loss and photo-oxidation. Mycelial samples were collected every 5 days and lyophilized to determine the dry weight. benzo(a)pyrene in the filtrate, as well as BaP adsorbed to the surface of the mycelia, was extracted three times with an equal volume of dichloromethane (DCM). The extracted benzo(a)pyrene was analyzed using reverse phase HPLC (Agilent 1100 series) equipped with a UV detector (254 nm) and C18 column (Hewlett-Packard Hyposil column, 250 mm × 4 mm) using acetonitrile as the mobile phase at a flow rate of 1 mL min⁻¹. The benzo(a)pyrene degradation rate (%) was calculated as BaP of the abiotic control extraction (100%) – (benzo(a)pyrene from sample extraction + adsorption loss). All measurements are the means of six replicates.

Metabolites from benzo(a)pyrene biodegradation were determined using liquid chromatography (Agilent 1100) – mass spectrometer. Multiple-step tandem ESI-MS (ESI-MSⁿ) experiments were performed in the positive ion mode on an Iontrap HCT mass spectrometer (Bruker Daltonic, Bremen, Germany). The applied flow rate was 10 µL min⁻¹. All mass spectra were then analyzed by COMPASSTM. A culture-free extract sample was prepared from the fungal mycelium at the end of incubation. The mycelium was lyophilized, well ground and sonicated before being extracted with an equal volume of dichloromethane. Before liquid chromatography mass spectroscopy (LC-MS) determination, the samples (culture-free extract and culture filtrate) were prepared with 80% acetonitrile and 0.1% formic acid.

Factors involved in benzo(a)pyrene biodegradation

Some factors involved in BaP biodegradation by the fungal isolate were investigated. These included the following: (1) the aeration rate, (2) the initial benzo(a)pyrene concentration (which varied from 0.4 to 1.2 mM (100–300 µg L⁻¹)), (3) the concentration of glucose (5 and 50 mM), a cometabolic carbon source, and (4) the addition of a benzo(a)pyrene-solubility enhancer (methanol and ethanol). The benzo(a)pyrene biodegradation efficiency under each condition was then determined.

Results and discussion

Screening, isolation and identification of fungi capable of benzo(a)pyrene degradation

Emission of PAHs, including benzo(a)pyrene, as air pollutants and their deposition in the environment have been intensively reported (Motelay-Massei *et al.*, 2005). In fact, plant leaves contaminated by air pollutants have been used

as a specific index of environmental stress (Wen *et al.*, 2004). In this study, leaves of *P. macrocarpus* Kurz. plants located in a heavy traffic area were used as sources for screening of fungi. The isolated fungi were then screened for their abilities to tolerate and degrade 0.4 mM (100 mg L⁻¹) benzo(a)pyrene using a solid agar medium. The fungal isolates were selected on the basis of their greater growth rate in the presence of benzo(a)pyrene than that of the control in which benzo(a)pyrene was not provided. In order to exclude the effects of adsorption and auto-oxidation of benzo(a)pyrene, the biodegradation of benzo(a)pyrene was confirmed in liquid medium. Among the strains tested, one fungal isolate, designated E033, showed a relatively good growth rate on benzo(a)pyrene-malt extract agar and exhibited biodegradative ability for benzo(a)pyrene in the liquid medium. Consequently, this benzo(a)pyrene degrader was identified morphologically and genetically. The morphological characteristics of strain E033 including its fungal spores (Fig. 1) were compared with those of the known species of fungi (Barnette & Hunter, 1987) and it was strongly suggested that strain E033 belongs to the genus *Fusarium*. The analysis and comparison of the ITS of the fungal isolate showed 98% similarity to *Fusarium* sp., so the strain was designated as *Fusarium* sp. E033.

Biodegradation of benzo(a)pyrene by *Fusarium* sp. E033

The tolerance and ability of *Fusarium* sp. E033 to degrade 0.4 mM (100 mg L⁻¹) benzo(a)pyrene was investigated over 30 days at 32 °C in a liquid medium supplemented with 5 mM glucose as a carbon source. The growth of the fungal isolate was also estimated over the same incubation period. As degradation of PAHs is generally reported to be an oxidative reaction (Cerniglia *et al.*, 1985; Cerniglia, 1992,

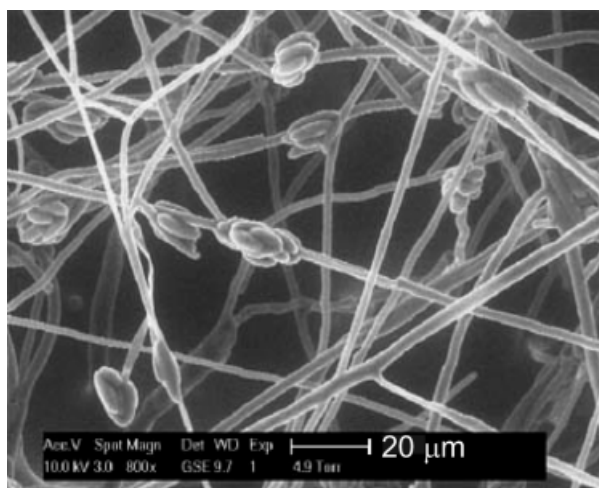


Fig. 1. Scanning micrograph of spore morphology of *Fusarium* sp. E033. Scale bar 20 µm.

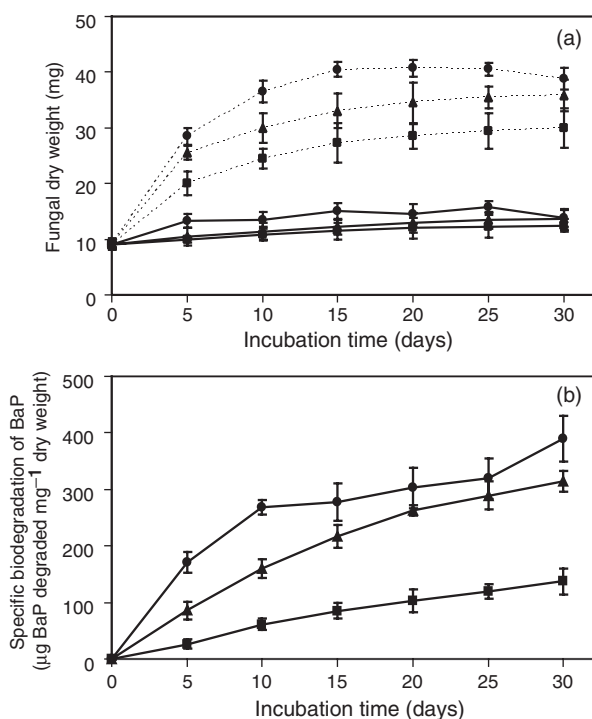


Fig. 2. Growth and biodegradation of *Fusarium* sp. E033 in glucose-containing liquid medium, in the absence of benzo(a)pyrene (---) or in the presence of 0.4 mM benzo(a)pyrene (—). (a) Growth of the fungi with reciprocatory shaking at 60 r.p.m. (■), 120 r.p.m. (▲) and 180 r.p.m. (●). (b) Biodegradation of benzo(a)pyrene at different shaking speeds: 60 r.p.m. (■), 120 r.p.m. (▲) and 180 r.p.m. (●). The biodegradation of benzo(a)pyrene was expressed as specific degradation per milligram cell dry weight at the time indicated.

1993), we optimized the aeration to investigate whether this affected the growth of *Fusarium* sp. E033 as well as its biodegradation ability (Fig. 2). Aeration obviously affected the growth of *Fusarium* sp. E033 in that the highest growth was obtained at the highest shaking speeds (Fig. 2a; without benzo(a)pyrene supplementation). However, it was found that, regardless of shaking speed, growth was significantly suppressed in the presence of 0.4 mM ($100 \mu\text{g L}^{-1}$) benzo(a)pyrene as the fungal biomass being reduced to 36–42% by weight compared to that in the absence of benzo(a)pyrene (Fig. 2a), although the biodegradation of benzo(a)pyrene was clearly observed after 30 days of incubation (Fig. 2b). This phenomenon was previously noted by Pineda-Flores & Mesta-Howard (2001) in that degradation of PAHs of four or more rings generally did not result in a remarkable increase in the biomass yield of the microbial consortia used, suggesting that these compounds were not preferentially used as a biosynthetic carbon source, but mostly as an energy source. As fungal growth under each of the shaking conditions tested was different, comparison of the biodegradation of benzo(a)pyrene is expressed as the specific degradation where the amount of benzo(a)pyrene degraded

was calculated per unit weight (mg) of dry fungal biomass. The results showed that the faster the shaking speed, the higher the aeration and biodegradation of benzo(a)pyrene (with *c.* 2.3–2.9 times increasing specific degradation at 120 and 180 r.p.m., respectively, compared with that at 60 r.p.m.) (Fig. 2b). This result agrees with previous reports in that the shaking conditions for PAH degradation not only increase oxygen availability but also increase PAH solubility into the aqueous phase for uptake by the organism (Johnsen *et al.*, 2005).

The ability of *Fusarium* sp. E033 to survive and degrade benzo(a)pyrene at various concentrations [$100 \mu\text{g L}^{-1}$ (0.4 mM), $200 \mu\text{g L}^{-1}$ (0.8 mM) and $300 \mu\text{g L}^{-1}$ (1.2 mM)] was also investigated. It was shown that *Fusarium* sp. E033 could survive at higher concentrations of benzo(a)pyrene (0.8 and 1.2 mM) but growth was significantly limited (with *c.* 25% reduction by weight) when compared with that occurring in 0.4 mM benzo(a)pyrene (Fig. 3a). This limited growth at higher concentrations of benzo(a)pyrene could result from higher toxicity and lower water solubility, meaning that benzo(a)pyrene uptake was limited. Consequently, a slower initial specific benzo(a)pyrene degradation rate at a higher concentration was detected. In 0.4 mM benzo(a)pyrene, the degradation rate of benzo(a)pyrene was rapid over the first 5 days of incubation in which $34 \mu\text{g}$ benzo(a)pyrene was degraded mg^{-1} fungal dry weight day^{-1} , after which time the degradation continued at a slower rate (Fig. 3b). Although loss of benzo(a)pyrene through physical adsorption and photo-oxidation was observed after 10 days of growth, it was found to be low and *c.* 10% and 20% over 30 days of incubation, respectively. After subtracting the abiotic loss of benzo(a)pyrene, the total biodegradation of benzo(a)pyrene after 30 days of incubation was *c.* 65–70% of the initial amount. The growth and substrate depletion kinetics revealed in this study are similar to the classical pattern of benzo(a)pyrene degradation in that degradation only occurred rapidly at the early stages of incubation previously reported for *F. solani* (Veignie *et al.*, 2004). However, *Fusarium* sp. E033 demonstrated a greater biodegradation efficiency than that of *F. solani*, which degraded 6.8% benzo(a)pyrene over 15 days of incubation (Veignie *et al.*, 2004). Even at a higher concentration of benzo(a)pyrene, it was observed that *Fusarium* sp. E033 could maintain a gradual degradation rate to finally reach 55–60% degradation of the initial amount of benzo(a)pyrene after 30 days of incubation (Fig. 3b). This indicates a good benzo(a)pyrene degradation ability of *Fusarium* sp. E033 when compared with other benzo(a)pyrene-degrading fungi. For instance, benzo(a)pyrene degradation studies using white-rot fungi were generally carried out at a lower range of benzo(a)pyrene concentrations (0.08–0.1 mM equivalent to $20\text{--}25 \mu\text{g L}^{-1}$) (Kotterman *et al.*, 1998; Capotorti *et al.*, 2004).

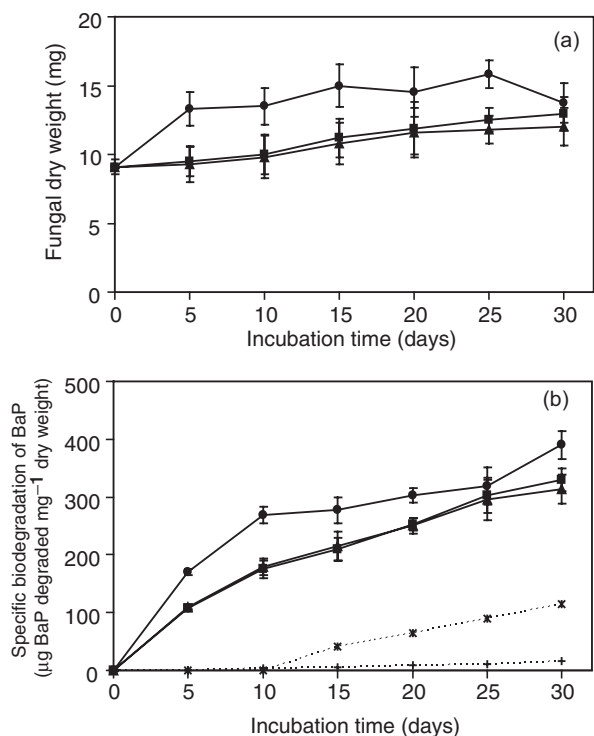


Fig. 3. Growth (a) and biodegradation ability (b) of *Fusarium* sp. E033 in glucose-containing liquid medium at various concentrations of benzo(a)pyrene: 0.4 mM (●), 0.8 mM (■) and 1.2 mM (▲). The biodegradation of benzo(a)pyrene was expressed as specific degradation mg^{-1} cell dry weight at the time indicated. The effects of physical adsorption (+) and photo-oxidation (*) of benzo(a)pyrene are also shown. Some error bars are not present because they are smaller than the diameter of the symbol.

The ability of a number of fungal isolates to oxidize benzo(a)pyrene has been shown to mainly occur cometabolically with an alternative carbon source and only a few isolates have been reported to solely degrade benzo(a)pyrene as a carbon and energy source (Juhász & Naidu, 2000). Glucose, at a concentration ranging from 5 to 50 mM, has been widely used as a carbon source for fungal growth concomitant with benzo(a)pyrene biodegradation. Glucose is a rapidly metabolized growth substrate (Tanzer *et al.*, 2003) and the higher the concentration used, the greater the biomass yield of the fungus. In this study, the glucose concentration was increased from 5 to 50 mM in order to promote fungal growth, and in turn the increase of the biodegradation rate would be expected. In the absence of benzo(a)pyrene, the growth of *Fusarium* sp. E033 with 50 mM glucose was enhanced and the fungal dry weight increased five times over the first 5 days of growth compared with that occurring in the presence of 5 mM glucose (Fig. 4a). The presence of 0.4 mM ($100 \mu\text{g L}^{-1}$) benzo(a)pyrene obviously inhibited the growth of *Fusarium* sp. E033 in the medium supplemented with 5 mM glucose. In contrast,

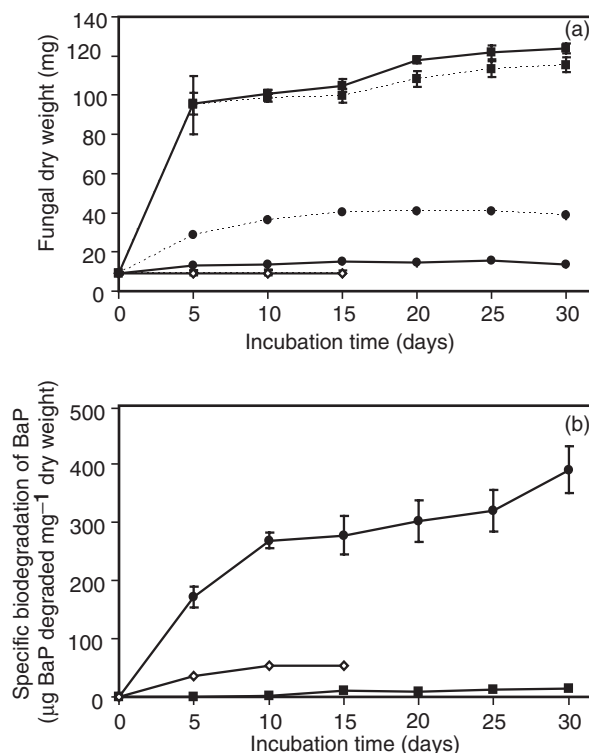


Fig. 4. Growth (a) and biodegradation ability (b) of *Fusarium* sp. E033 in liquid medium containing various concentrations of glucose: 0 (\diamond), 5 mM (●) and 50 mM (■). Fungal growth was determined in the absence (---) or in the presence (—) of $100 \mu\text{g mL}^{-1}$ benzo(a)pyrene. Some error bars are not present because they are smaller than the diameter of the symbol.

growth inhibition was not observed to a great extent when a higher concentration of glucose, i.e. 50 mM, was provided. benzo(a)pyrene biodegradation by *Fusarium* sp. E033 in the presence of two different glucose concentrations was also determined. It was found that at 50 mM glucose, benzo(a)pyrene biodegradation was almost completely repressed (Fig. 4b). Carbon catabolite repression in this organism may be responsible for this phenomenon in which the catabolism of a less-preferred carbon source is repressed if a more favorable growth substrate is available (Ilyes *et al.*, 2004). Benzo(a)pyrene degradation by *Fusarium* sp. E033 was also examined in the absence of glucose (Fig. 3a), although the fungal isolate was non-viable after 15 days of incubation. While there was no increase in biomass, it was found that benzo(a)pyrene was degraded at a rate of c. $50 \mu\text{g}$ benzo(a)pyrene mg^{-1} dry weight over 15 days of incubation.

According to previous studies on benzo(a)pyrene biodegradation, there are several limiting factors that influence the rate and extent of benzo(a)pyrene degradation. Therefore, studies on such factors involving the biodegradation of the fungus are necessary if soil bioremediation will be applied. Besides the growth substrate and oxygen availability

effects described in this study, substrate bioavailability is also an important limiting factor. As benzo(a)pyrene has a low water solubility (0.0038 mg L^{-1} , at 25°C) (Mackay & Hickie, 2000), the addition of surfactants as well as organic solvents may enhance solubility and increase bioavailability of benzo(a)pyrene to the organism. However, there have also been reports that addition of surfactants can have inhibitory and toxic effects (Volkering *et al.*, 1995) and can also result in a significant decrease in contaminant degradation due to extensive micellation (Graves & Leavitt, 1991; Smith *et al.*, 1997). On the other hand, organic solvent-enhanced PAH biodegradation has been successfully reported in laboratory tests and in soil remediation (Jimenez & Bartha, 1996; Kilbane, 1997; Lee *et al.*, 2001). In this investigation, two common organic solvents (methanol and ethanol), used in solubilization of hydrophobic contaminants, were chosen to enhance the solubility of benzo(a)pyrene in the liquid medium. Methanol has been described as one of the most effective PAH-extracting agents (Bergknut *et al.*, 2004; Chen *et al.*, 2005) whereas ethanol has been demonstrated to not only enhance PAH solubility (Chen *et al.*, 2005) but also to increase the degradation rate of anthracene in aqueous medium (Field *et al.*, 1995). Methanol and ethanol provided in the liquid medium at 5 mM served as a benzo(a)pyrene solubility enhancer as well as a carbon source for *Fusarium* sp. E033. It was found that the growth of *Fusarium* sp. E033 grown in either methanol or ethanol without benzo(a)pyrene was slightly less than that shown in glucose indicating that glucose, is a better carbon source for this strain (Figs 4a and 5a). A comparison of cell growth with each solubility-enhancing agent was investigated in the presence and absence of 0.4 mM benzo(a)pyrene (100 mg L^{-1}). Growth was significantly decreased in the presence of 0.4 mM benzo(a)pyrene in glucose-containing medium, but growth was markedly increased in the medium supplemented with either methanol or ethanol, indicating that more benzo(a)pyrene was solubilized and more available for uptake (Fig. 5). However, when the biodegradation of benzo(a)pyrene was taken into consideration, it was found that methanol and ethanol, although provided at a low concentration (5 mM, i.e. $< 1\%$, v/v), showed an adverse effect, in that biodegradation was almost completely inhibited as the specific biodegradation after 30 days of incubation was decreased from $390 \text{ } \mu\text{g}$ benzo(a)pyrene degraded mg^{-1} fungal dry weight in the absence of the alcohols (Fig. 2b) to 14 and $62 \text{ } \mu\text{g}$ benzo(a)pyrene degraded mg^{-1} fungal dry weight, respectively (Fig. 5b). As the concentration of methanol or ethanol used in this study was low and was not toxic, alcohol toxicity could not be the reason for the inhibition of benzo(a)pyrene biodegradation. Although the significance of methanol and ethanol in fungal carbon catabolite repression was not clearly demonstrated, it cannot be ruled out in this study.

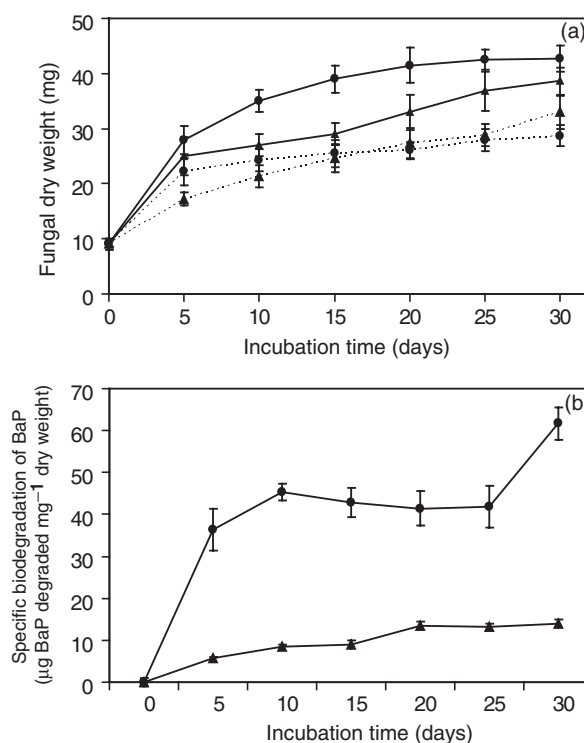


Fig. 5. Growth (a) and biodegradation ability (b) of *Fusarium* sp. E033 in the liquid medium supplemented with 5 mM methanol (\blacktriangle) or 5 mM ethanol (\bullet). Fungal growth was determined in the absence (---) or in the presence (—) of 0.4 mM benzo(a)pyrene. Some error bars are not present because they are smaller than the diameter of the symbol.

Transformation products from benzo(a)pyrene degradation by *Fusarium* sp. E033

LC-MS analysis of the intracellular metabolite from the oxidation of benzo(a)pyrene by *Fusarium* sp. E033 showed a molecular ion at m/z 285.2, suggesting the formation of a dihydroxy dihydro-benzo(a)pyrene (Fig. 6a) whereas a molecular ion at m/z 282 was observed from the extracellular extractable sample, suggesting formation of benzo(a)pyrene-quinone (Fig. 6b). These results agree with two transformation products formed from the benzo(a)pyrene degradation pathway proposed in *F. solani*, which involves an intracellular cytochrome P-450 mono-oxygenase and the extracellular lignin peroxidase enzymes (Verdin *et al.*, 2004), and confirm the degradation of benzo(a)pyrene by our new isolate, *Fusarium* sp. E033.

Conclusions

A *Fusarium* sp. E033 isolated from leaves of *P. macrocarpus* Kurz. plants exposed to traffic emissions had the ability to degrade benzo(a)pyrene with a degradation efficiency of up to 65–70% of the initial amount supplied and formed two transformation products, a dihydroxy-dihydro-

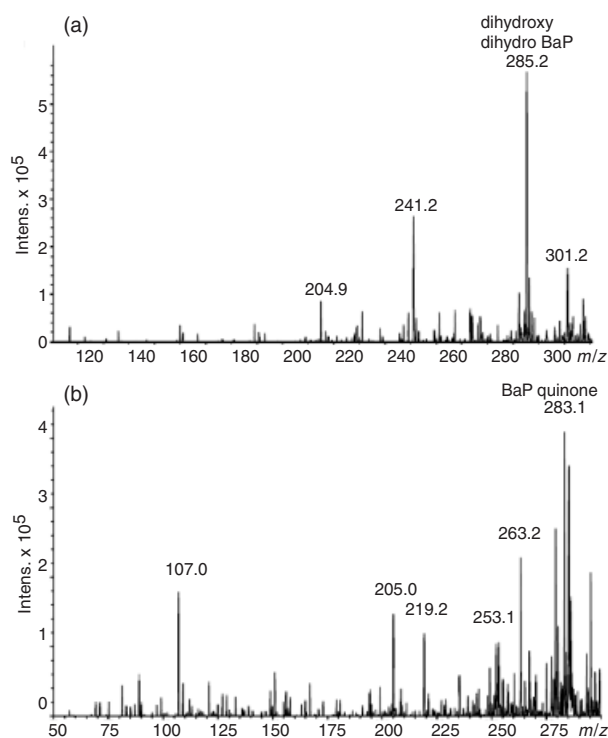


Fig. 6. Mass spectra of transformation products from benzo(a)pyrene oxidation by *Fusarium* sp. E033. The samples were (a) intracellular cell-free extract and (b) extracellular extractable sample (from culture filtrate).

benzo(a)pyrene and a benzo(a)pyrene-quinone, over 30 days of incubation at 32 °C. While a relatively high aeration increased the yield of fungal biomass as well as biodegradation, a high glucose concentration (50 mM) increased the fungal biomass but repressed the biodegradation of benzo(a)pyrene. Attempts to increase benzo(a)pyrene bioavailability using a low concentration of solubility-enhancing solvents, i.e. methanol and ethanol, successfully increased biomass production but failed to increase the benzo(a)pyrene biodegradation rate by *Fusarium* sp. E033. Nonetheless, due to the significant ability of *Fusarium* sp. E033 to degrade benzo(a)pyrene up to 1.2 mM, the potential use of this newly isolated fungus in soil remediation has been suggested. Further studies on its biodegradation mechanisms as well as benzo(a)pyrene biodegradation in soil using this fungal isolate will be necessary for a practical application in soil remediation.

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