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Potential of a white-rot fungus *Pleurotus eryngii* F032 for degradation and transformation of fluorene

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ABSTRACT

The white-rot fungus *Pleurotus eryngii* F032 showed the capability to degrade a three fused-ring aromatic hydrocarbons fluorene. The elimination of fluorene through sorption was also investigated. Enzyme production is accompanied by an increase in biomass of *P. eryngii* F032 during degradation process. The fungus totally degraded fluorine within 23 d at 10-mg l^{-1} solution. Fluorene degradation was affected with initial fluorene concentrations. The highest enzyme activity was shown by laccase in the 10-mg l^{-1} culture after 30 d of incubation (1620 U l^{-1}). Few activities of enzymes were observed in the fungal cell at the varying concentration of fluorene. Three metabolic were detected and separated in ethylacetate extract, after isolated by column chromatography. The metabolites, 9-fluorenone, phthalic acid, and benzoic acid were identified using UV–vis spectrophotometer and gas chromatography–mass spectrometry (GC–MS). The results show the presence of a complex mechanism for the regulation of fluorene-degrading enzymes.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed persistent organic contaminants and concern for human health and natural ecosystems due to their characteristic of toxic, carcinogenic, and mutagenic. These organic compounds that can be biodegraded by a native microorganism capable of transforming and releasing enzymatic properties that is able to break down the strong structure molecular of these compounds. Lower Molecular Weight (LMW) PAHs such as a three ring fluorene is found abundantly in ecosystem (Arun & Eyini 2011; Bincova and Sram 2004; Bumpus 1989; Garcia-Junco *et al.* 2001). Fluorene is a known toxic and carcinogen compound, and its molecular structure is used as an indicator for investigating PAH-containing contaminant (Cerniglia 1992; Kweon *et al.* 2007).

The microbiological process of degradation and transformation of toxic organic pollutant are now considered as a promising method for the problem of environmental pollution. White-rot fungi have been extensively studied for pollutant removal because of the high ability to degrade wide range of pollutant. The fungi produce enzymes such as oxygenase (mono and dioxygenase), laccase, peroxidase (lignin peroxidase, manganese peroxidase, and versatile peroxidase), and

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hydrolase (lipase, cellulose, and protease) that able to degrade a variety of pollutants (Hadibarata *et al.* 2007a; Hofrichter 2002; Kristanti *et al.* 2011; Mouhamadou *et al.* 2013). Oxygenases participate in oxidation of reduced substrates by transferring oxygen from molecular oxygen. They play an important role in the metabolism of organic pollutants by increasing their water solubility or reactivity or bringing about cleavage of the aromatic structure (Fetzner 2003; Fetzner & Lingens 1994; Hadibarata *et al.* 2007b). Laccase is known to occur in multiple isoenzyme form each of which is encoded by a separate gene. They play a key role in the depolimerization of lignin, which results in a variety of phenol (Giardina *et al.* 1995; Kim *et al.* 2002). Peroxidases are ubiquitous enzymes that catalyze the oxidation of lignin and other phenolic compounds. These enzymes are effective for the degradation of aromatic compounds at the presence of hydrogen peroxidase and mediator (Hiner *et al.* 2002; Koua *et al.* 2009). Hydrolases disrupt major organic structure in toxic molecules and results in the elimination of their toxicity. These enzymes are useful for the removal of oil spill and organophosphate, organochlorine and carbamate insecticide (Lal & Saxena 1982; Williams 1977).

Although biodegradation of LMW PAHs has been widely observed, the microbial degradation and transformation of fluorene has not been extensively investigated, and therefore, is not well understood (Poonthrigpun *et al.* 2006; Stingley *et al.* 2004). The fungus *Pleurotus eryngii* F032 was selected for this investigation because it has been shown for the ability to degrade two-ring PAH naphthalene (Hadibarata *et al.* 2013). However, its capacity to degrade fluorene remains unexplored. The authors evaluated the ability of *Pleurotus* species to degrade fluorene in liquid cultures. The present study focused on biomass production, glucose consumption, and enzyme production during the degradation process and characterization of fluorene metabolites produced by *P. eryngii* F032.

Materials and methods

Microorganism

Pleurotus eryngii F032 cultured on malt extract agar slants was stored at 4 °C. The fungus was activated at 25 °C for 7 d prior to use. Three plugs (5 mm) of mycelia were transferred into fluorene (Sigma–Aldrich, Milwaukee, WI) media supplemented with mineral nutrient (pH 4.3) in 100-mL Erlenmeyer flasks with agitation 80 rpm. Correlation between biomass production, fluorene utilization, and concentration of fluorene was performed in solution of Tween 80, dimethylformamide, and benomyl. At the incubation stage, a desired amount of fluorene (10, 20, and 30 mg l^{-1}) was aseptically added to the Erlenmeyer flasks, and fungal cell and liquid medium were assayed for enzyme activity at 7 d intervals. All the cultures were incubated on a rotary shaker in the dark at 25 °C for 7–30 d.

Enzyme assays and biomass

Culture samples were harvested every week, homogenized and filtered to remove suspended biomass, and examined for enzyme activity. The metabolites derived from fluorene were extracted with ethylacetate, separated with column chromatography and preliminary detected with TLC. Finally, the identification of the sample was performed with gas chromatograph mass spectrometer. Metabolites were derivatized by silylation reagents to transform functional groups of the thiol, phosphate, hydroxyl, carboxylic acid, and amine prior to gas chromatography–mass spectrometry (GC–MS) injection (Hadibarata *et al.* 2012).

The culture samples were homogenized (15 000 rpm, 10 min) at room temperature, rapidly rinsed thrice with phosphate buffer (pH 7), and then the mycelia were separated by sonication with a sonic oscillator for 12 min. Laccase and MnP were assayed by determination of absorbance of the extracted sample using UV spectrophotometer. Laccase activity was determined by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) oxidation at 420 nm. MnP activity due to oxidation of malonate and dimethoxyphenol in $MnSO_4$ solution was assayed by determining the changes in the absorbance at 270 nm (Hadibarata *et al.* 2012). The enzyme activities were expressed in Unit/liter (U l^{-1}).

The increase in biomass during the degradation process was investigated. For this, 20 ml of the fungal culture was centrifuged at 1000 rpm for 45 min, followed by filtration through a Whatman No. 1 filter paper. The biomass was washed with 15 ml of distilled water, dried in an oven at 40 °C for 5 h, and kept in a desiccator until it reached constant weight. The control experiment was carried out without fluorene in acetone under the same conditions. The biomass of the fungi was expressed in mg l^{-1}.

GC–MS analyses

A gas chromatograph mass spectrometer (Agilent 5975E FID GC–MS) was used to determine the degradation rate and metabolic product. The GC–MS has a DB-1 capillary column with 0.25 mm diameter, 0.25 μm film thickness, and 30 m length. The temperature conditions were: 70 °C for 1 min, increased at 18 °C min^{-1}–150 °C, and then at 28 °C min^{-1}–330 °C, which was maintained for 10 min. The injector and interface temperatures were maintained at 260 °C with a splitless time of 2 min. The solvent delay time was set to 3 min, and the injection concentration was 1 μl in the splitless condition. The flow rate of the carrier gas was set to 1 ml min^{-1} using electronic pressure control. The GC–MS was operated in electron impact (EI) ionization mode with electron energy of 1.3 eV and a mass range of 50–500 amu (atomic mass unit). The mass spectra from the samples were identified by comparison with standard compounds and the Wiley 275 L mass spectra database (Hadibarata & Kristanti 2012).

Results and discussion

Biodegradation of fluorene by Pleurotus eryngii *F032*

The effect of initial concentration was evaluated by adding different concentrations (10–30 mg l^{-1}) of fluorene to the *P. eryngii* F032 culture. *Pleurotus eryngii* F032 degraded a 100 % of a 10-mg l^{-1} fluorene solution within 23 d, and degradation decreased with increasing concentration. Degradation of fluorene reached 86.5 % and 65.9 % in initial concentration of 20