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Potential of the White-Rot Fungus *Pleurotus pulmonarius* F043 for Degradation and Transformation of Fluoranthene

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ABSTRACT

Fluoranthene, a four-ring polycyclic aromatic hydrocarbon that is possible genotoxic in nature, has been used as an indicator for assessing polycyclic aromatic hydrocarbon (PAH)-containing pollutants. Microbial degradation is one of the promising methods in removing up PAH-contaminated environments. White-rot fungi have showed the ability to degrade a wide range of PAHs. This study aimed to investigate enzyme production, fungal biomass, and glucose utilization during the biodegradation process of fluoranthene by a white-rot fungus *Pleurotus pulmonarius* F043 and to identify the metabolites produced in the degradation process. The extracellular ligninolytic enzyme system of the fungi, producing laccases and peroxidases, was directly linked to the biodegradation of fluoranthene. The production of ligninolytic enzymes during fluoranthene degradation was related to an increase in the biomass of *Pleurotus pulmonarius* F043. Fluoranthene removal decreased with an increase in fluoranthene concentrations. The highest biomass production of *Pleurotus pulmonarius* F043 ($\geq$ 4 400 mg L^{-1}) was found in the 10 mg L^{-1} fluoranthene culture after 30 d of incubation. Two fluoranthene metabolites, naphthalene-1,8-dicarboxylic acid and phthalic acid, were found in the process of fluoranthene degradation. Laccase was revealed as the major enzyme that played an important role in degradation process. Suitable conditions must be found to promote a successful fungal biotransformation augmentation in liquid culture.

Key Words: gas chromatography-mass spectrometer, ligninolytic enzymes, metabolites, naphthalene-1,8-dicarboxylic acid, phthalic acid

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) form a class of widespread persistent organic pollutants, generated as a consequence of pyrolysis of organic matter and as other by-products of industrial process, which contribute to the accumulation of toxic substance ingested and retained by organisms. Fluoranthene, a compound that consists of four fused-benzene rings, is abundant in PAHs (Zhong *et al.*, 2006; Arun and Eyini, 2011). Fluoranthene has been used as an indicator for assessing PAH-containing pollutants in the environment because of its molecular structures and carcinogen compound (Kweon *et al.*, 2007).

Fungi degradation of PAHs is an important natural process for the transformation of these contaminants. It represents an alternative method for the problems related to environmental pollution. White-rot fungi have been extensively used for their ability to degrade PAHs. These fungi produce extracellular enzymes such as laccase, manganese peroxidase (MnP), lignin peroxidase (LiP) and dioxygenases with very low substrate specificity. These enzymes are suitable for the transformation of a variety of organic contaminants (Kristanti *et al.*, 2011; Hadibarata *et al.*, 2012; Hadibarata and Kristanti, 2013). As well-known ligninolytic enzymes perform one-electron oxidation, produce cation radicals of pollutants, and lead to the formation of quinines (Vyas *et al.*, 1994). MnP, a common ligninolytic enzyme produced by white-rot fungi, is a heme containing glycoprotein that needs hydrogen peroxide as an oxidant and is capable of degrading the polymer *via* an oxidative process (Kuwahara *et al.*, 1984; Hofrichter, 2002). Laccase is a multicopper oxidase that plays an important role in the global carbon cycle and helps in the degradation of persistent com-

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pounds such as PAHs and synthetic dyes (Baldrian, 2004; Hadibarata *et al.*, 2013; Hadibarata and Kristanti, 2014). While biodegradation of lower-molecular-weight PAHs has been extensively discovered, the metabolite of PAHs containing four or more rings is less explored and, therefore, not well understood (Stingley *et al.*, 2004).

Pleurotus pulmonarius F043, a white-rot fungus isolated from tropical rain forest, has been extensively studied for its role in degradation of pyrene (Hadibarata and Teh, 2014). Its capacity to degrade and transform fluoranthene is not well known. Hence, this study aimed to investigate the enzyme production, fungal biomass and glucose utilization during the biodegradation process of fluoranthene by *Pleurotus pulmonarius* F043 and to identify the metabolites produced in the degradation process.

MATERIALS AND METHODS

Chemicals and microorganisms

Organic solvents used in this experiment were supplied by Qrec from New Zealand. The main compound used for the degradation study, fluoranthene, was obtained from Sigma-Aldrich, Milwaukee, USA. The structure and physico-chemical properties of fluoranthene are shown in Table I. Malt extract, nutrients, agar, and polypeptone were supplied by Difco, Detroit, USA. *Pleurotus pulmonarius* F043 was cultured on 2% (weight:volume) malt agar slants at 4 °C and was then activated at 25 °C for 5 d. This fungus was selected for this study due to its ability to produce extracellular ligninolytic enzymes during the degradation of PAHs such as pyrene (Hadibarata and Teh, 2014). After incubation for several times, the mycelium was harvested with a sterile NaCl solution. It was then inoculated into 20 mL of modified mineral salt broth (MSB, pH 4.5) in 100-mL Erlenmeyer flasks keeping at 25 °C and totating at 120 r min^{-1}. The compositions of modified MSB are shown in Table II. To correlate fungal biomass production with fluoranthene utilization, sufficient fluoranthene was dissolved in tween 80, dimethylformamide (DMF), and benomyl (300 mg L^{-1}) to inhibit bacterial growth. At the beginning of the 5th day of incubation, dissolved fluoranthene was added aseptically, at desired concentrations, to the 100-mL flasks containing 20 mL of liquid medium. Aliquots were assayed for laccase and MnP activities. All the cultures were incubated at 25 °C on a 120 r min^{-1} rotary shaker in the dark for 30 d. All the treatments were performed in triplicate.

TABLE I

Structure and physico-chemical characteristics of fluoranthene

Characteristic	Result
Molecular formula	$C_{16}H_{10}$
Appearance	Yellow to green needles
Molecular weight	202.26
Melting point (°C)	110.8
Boiling point (°C)	375
Aqueous solubility at 15 °C (mg L^{-1})	0.265
Octanol-water partition coefficient (logL kg^{-1})	4.933

TABLE II

Concentrations of constituents of mineral salt broth (MSB) medium

MSB constituent	Concentration	Trace element[a)]	Concentration
	g L^{-1}		mg L^{-1}
Malt extract	20	$FeSO_4{\cdot}7H_2O$	10
Polypeptone	5	$MnSO_4{\cdot}7H_2O$	2
$MgSO_4{\cdot}7H_2O$	1	$ZnSO_4{\cdot}7H_2O$	2
KH_2PO_4	1	$CoSO_4{\cdot}7H_2O$	1
$CaCl_2{\cdot}2H_2O$	1	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	1
Ammonium tartrate	1		

a) Trace elements of 10 mL are also included in MSB medium.

Experimental determination

At the end of the incubation, the liquid medium was acidified with 1.0 mol L^{-1} HCl and then ethyl acetate was added to extract fluoranthene remaining in the liquid medium. Samples were centrifuged and filtrated to remove suspended biomass, and ligninolytic (laccase and MnP) activity in supernatants was determined. All the treatments were performed in triplicate. Extraction and analyses of the fluoranthene metabolites were performed as described in our previous study (Hadibarata *et al.*, 2007).

To determine fungal biomass during the process of fluoranthene degradation, 20 mL of culture broth was centrifuged at 1 000 r min^{-1} for 45 min, then filtered using Whatman No. 1 filter paper, washed with 10 mL of distilled water, dried at 40 °C for 5 h, and placed in a desiccator until a fixed weight was achieved. A control treatment with addition of acetone without fluoranthene was also studied. The biomass of fungi was defined in mg L^{-1}.

The liquid cultures were centrifuged at 10 000 r min^{-1} for 15 min at room temperature and the cells were rapidly washed 3 times with 50 mmol L^{-1} phosphate buffer (pH = 7). An enzymatic analysis of laccase and MnP was undertaken in accordance with our previous study (Hadibarata and Kristanti, 2013). Laccase production was assayed by oxidation of 2,2′-azino-

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