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Biotechnological procedures to select white rot fungi for the degradation of PAHs

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ABSTRACT

White rot fungi are essential in forest ecology and are deeply involved in wood decomposition and the biodegradation of various xenobiotics. The fungal ligninolytic enzymes involved in these processes have recently become the focus of much attention for their possible biotechnological applications. Successful bioremediation requires the selection of species with desirable characteristics. In this study, 150 taxonomically and physiologically diverse white rot fungi, including 55 species, were investigated for their performance in a variety of biotechnological procedures, such as dye decolorization, gallic acid reaction, ligninolytic enzymes, and tolerance to four PAHs, phenanthrene, anthracene, fluoranthene, and pyrene. Among these fungi, six isolates showed the highest (>90%) tolerance to both individual PAH and mixed PAHs. And six isolates oxidized gallic acid with dark brown color and they rapidly decolorized RBBR within ten days. These fungi revealed various profiles when evaluated for their biotechnological performance to compare the capability of degradation of PAHs between two groups selected. As the results demonstrated the six best species selected from gallic acid more greatly degraded four PAHs than the other isolates selected via tolerance test. It provided that gallic acid reaction test can be performed to rank the fungi by their ability to degrade the PAHs. Most of all, *Peniophora incarnata* KUC8836 and *Phlebia brevispora* KUC9033 significantly degraded the four PAHs and can be considered prime candidates for the degradation of xenobiotic compounds in environmental settings.

1. Introduction

Basidiomycetes are regarded as a very interesting group of fungi, given their exceptional adaptive abilities that allow them to grow under detrimental environmental conditions where they constantly act as natural degraders of lignocellulose (Choi et al., 2009). They are classified either as brown rot or white rot fungi (WRF) according to the way they degrade wood. Brown rot fungi efficiently degrade wood polysaccharides but are only slightly capable of altering lignins such as demethoxylate lignin, which leaves the wood brown, dry and weak. WRF can decompose all wood polymers, including lignin, which leaves the wood with a white, fibrous appearance (Blanchette, 1995). WRF inhabit the wood cell lumen, and the fungal hyphae move from cell to cell via bordered pits or directly through the cell wall.

WRF are active lignin degraders thanks to ligninolytic abilities conferred by an extracellular enzyme complex containing lignin peroxidase (LiP, EC 1.11.1.14), manganese-dependent peroxidase (MnP, EC 1.11.1.13), and laccase (EC 1.10.3.2). These enzymes are involved in the oxidation of the lignin present in wood and in oxidation of a wide range of xenobiotics with compound aromatic structures, including synthetic dyes and polycyclic aromatic hydrocarbons (PAHs).

The screening of fungi for ligninolytic enzymes usually involves monitoring the decolorization of dyes such as the polymeric/heterocyclic dye Remazol brilliant blue R (Lee H. et al., 2010). The ability of a fungus to decolorize this dye coincides with the onset of lignin metabolism and is regarded as predictive of its ability to degrade recalcitrant organopollutants such as PAHs (Anastasi et al., 2009; Barrasa et al., 2009; Zheng et al., 1999). PAHs are environmental pollutants generated from the incomplete combustion or pyrolysis of organic materials such as oil, petroleum gas, coal, and wood (Arun et al., 2008). PAH contamination has attracted both public and scientific attention due to the recalcitrant properties of PAHs and their mutagenic or carcinogenic characteristics (Lei et al., 2007). Many studies have been conducted regarding the removal of PAH compounds from contaminated environments. The use of WRF appears promising for the remediation of contaminant classes that include the PAHs (Brodkorb and Legge, 1992). It remains possible to identify many more strains of WRF that might be suitable for use as bioremediation agents, as hundreds of species of WRF have never been examined in this regard (Crawford, 2006).

In this work, 150 white rot fungal isolates representing a broad systematic and functional biodiversity were assessed for their ligninolytic

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enzyme activities as measured by their ability to decolorize dyes and degrade PAHs. The overall objective of this research was to characterize the abilities of these WRF and to select the most promising fungi for biotechnological applications. An integrated physiological perspective on WRF may provide the necessary knowledge for applying mycoremediation in environments contaminated with recalcitrant organic compounds.

2. Materials and methods

2.1. Fungal species

A total of 150 WRF, including 55 species obtained from Korea, were identified by the Korea University Culture collection (KUC). They were maintained as pure cultures. To specifically identify WRF, fungal DNA extraction and polymerase chain reaction (PCR) amplification were performed using the techniques described by Lim et al. (2005). To amplify the 28S rDNA sequences representing the partial large subunit (LSU) and the internal transcribed spacer (ITS) region, PCR was conducted using the fungal universal primers LR0R/LR3, and the resulting sequences were used to conduct a BLAST search of the GenBank database (Altschul et al., 1994).

2.2. Evaluation of optimal growth temperature and growth rate

A total of 150 WRF were cultured on 2% MEA (20 g malt extract, 15 g agar and 1 L distilled water) at four different temperatures (20, 25, 30 and 35 °C). After 7 days of incubation, the mean radial growth rate was determined by measuring the diameter of the expanding colonies in triplicate. The temperature that resulted in the highest growth rate was selected as the optimal growth temperature.

2.3. Dye decolorization test

A dye decolorization method was used as a screening method to determine if the WRF were capable of degrading PAHs. Remazol brilliant blue R (RBBR; Sigma Chemical Co., St. Louis, USA) is dramatically decolorized by lignin-degrading fungi (Pasti and Crawford, 1991). To use this method, fungi were inoculated onto 2% MEA containing 100 mg L^{-1} of RBBR and incubated at 27 °C. The petri dish was observed daily to determine the time to decolorize the full dish.

2.4. Gallic acid reaction

A strong correlation has been found between the ability of basidiomycetes to oxidize gallic acid to its brown-colored quinonic form in agar and the possession of ligninolytic ability (Leonard, 1971; Linderberg, 1948; Shleev et al., 2004). To characterize the fungi, oxidation of gallic acid was repeatedly measured. The fungi were inoculated onto 1.5% MEA supplemented with 5 g L^{-1} of gallic acid and incubated at 27 °C.

2.5. Assessment of ligninolytic enzyme activity

The activity of three ligninolytic enzymes, laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP), was assayed using a UV–vis spectrophotometer. Experiments were performed in triplicate on both 5-day-old and 10-day-old cultures in basal salts medium. The cultivation was performed according to the method described by Lee Y.M. et al. (2010). Four agar plugs were inoculated into 20 mL of the basal salts medium and cultivated at 27 °C with mixing at 150 rpm. After filtration of the mycelium using a syringe filter (0.45 μm) to remove the mycelium and spores, the enzymatic activity of the crude supernatant was measured. For laccase, 0.1 M sodium acetate (pH 4.5) and 1.5 mM 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were used. For MnP, 0.5 M sodium malonate (pH 4.5), 5 mM $MnSO_4$, 1 mM 2,6-dimethoxylphenol, and 1 mM H_2O_2 were used. For LiP, 0.25 M sodium tartrate (pH 2.5), 10 mM veratryl alcohol, and 5 mM H_2O_2 were used (Hadibarata et al., 2009; Novotný et al., 2004; Tien and Kirk, 1988; Wang et al., 2008). One unit of ligninolytic enzyme activity produced 1 μmol of reaction product per min at room temperature; activity was expressed in U/mL (Lee H. et al., 2010). All experiments were performed in triplicate.

2.6. Tolerance to PAHs

The fungi were tested by culturing onto 2% MEA supplemented with 30 mg L^{-1} each of phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), and mixed PAHs. Analytical grade PAHs (Aldrich Chemical Co., St. Louis, USA) are almost completely insoluble in water and require dissolution in acetone (Samchun Chemical Co. Ltd., Pyeongtaek-si, Korea) prior to their addition to the MEA medium. Fungal isolates were inoculated onto a plate supplemented with the individual or mixed PAHs and incubated at 27 °C. After 7 days of incubation, the tolerance rate was calculated in triplicate using the following equation:

$$TR = FGR/GRC \times 100$$

Where FGR is the fungal growth rate and GRC is the growth rate of a control culture. Both FGR and GRC were determined by measuring the diameters of the expanding colonies. And TR is the tolerance rate.

2.7. Biodegradation of PAHs and ligninolytic enzyme assay during incubation

Experiments were performed using Erlenmeyer flasks (250 mL) containing 100 mL of 2% ME liquid medium. The fungal species that were selected for their tolerance to PAHs were aseptically inoculated into the flasks with ten agar plugs (7 mm diameter) of fungal preculture in 2% MEA. After the addition of the PAH stock solutions, the cultures were incubated on a rotary shaker at 150 rpm (27 °C). The concentration of the four individual PAHs (PHE, ANT, FLT, PYR) was 25 mg L^{-1}. An uninoculated abiotic negative control flask and a positive control flask containing *P. chrysosporium* KCTC 6293, an effective degrader of PAHs (Bamforth and Sigleton, 2005), were treated similarly. The liquid and solid parts of the fungal culture were extracted every other day for 10 days. Each sample was extracted three times with 100 mL of dichloromethane (Duksan, Korea) to obtain a final volume of 300 mL. Each extracted sample was concentrated to 5 mL using a vacuum rotary evaporator (Eyela N-1000 series, Japan). The concentrations of PAHs in the extracts were then determined by injecting 1 μL of the extract into a gas chromatograph-mass spectrometer (GC-MS, Agilent technologies, Model 7890, Santa Clara, CA, USA). Separation was achieved using a DB-5MS column (0.25 mm diameter by 30 m length, film thickness 0.25 μm). The GC-MS analysis was conducted according to the method described by Lee H. et al. (2010). And their ligniolytic enzymes were assessed every other day during incubation. The methods of ligninolytic enzyme activities were described above.

2.8. Data analysis

The degradation data were analyzed using a Statistical Analysis Systems (SAS 9.2, SAS Institute, Inc., Cary NC, USA) package. A one-way analysis of variance (ANOVA) followed by Tukey's test was used to assess the significance of different degradation values among the species. The mean values were compared using a significance level of $p < 0.05$.

3. Results

In this work, a taxonomically and physiologically diverse collection of 150 WRF, including 55 species belonging to 30 genera preserved at the KUC, was investigated. The ecological roles and potential biotechnological applications of these isolates were assessed by measuring the production of ligninolytic enzymes using a PAH tolerance test, an RBBR decolorization test and a gallic acid reaction assay. The results of their characteristics are reported in Table S1.

3.1. Evaluation of optimal growth temperature and growth rate

Usually WRF are grown in room temperature similar with the natural environment where they inhabit. To improve their characteristics for biotechnological remediation, their optimal growth temperature was investigated (Table S1). Among the four temperatures studied, 118 isolates (78.6%) had an optimal temperature of 30 °C; 20 isolates (13.3%) showed maximal growth at 25 °C. Fewer than 12 isolates (8%) showed optimal growth at 35 °C, and no isolate grew optimally at 20 °C. Among the isolates, *Phanerochaete sordida* KUC9201 displayed the highest growth rate, at 19.14 mm/day, while *Rhizochaete* KUC8391 had the lowest growth rate, at 0.94 mm/day. The mean growth rate of genus *Phanerochaete* was 11.08 mm/day as the fastest-growing genus.

3.2. Degradation assessment using dye decolorization and gallic acid reaction tests

The results from the RBBR test and the gallic acid reaction test can be used to determine whether fungi are capable of oxidizing xenobiotics. All the tested fungi were able to decolorize RBBR, and 71.3% of the isolates decolorized the full dish within ten days. Among these, 45 isolates (30%) belonging to ten genera *Bjerkandera*, *Ceriporica*, *Fomes*, *Hypochnicium*, *Irpex*, *Perenniporia*, *Phanerochaete*, *Phlebia*, *Porostereum*, and *Schizopora*, decolorized RBBR within five days. For the gallic acid reaction test, 22.7% of the fungi had a positive reaction as indicated by a brown color. Six isolates belonging to the genera *Peniophora*, *Phanerochaete*, and *Phlebia* oxidized gallic acid to a dark brown color. And they were selected to degrade PAHs if they have a strong oxidation to degrade recalcitrant compounds (Table 1).

3.3. Assessment of ligninolytic enzyme activity

Three white rot fungal ligninolytic enzymes, LiP, MnP and laccase, were tested in this study. Most of the fungal isolates generated these three main ligninolytic enzymes under nitrogen-limited conditions. Despite producing the enzymes with high efficiency, they did not dramatically decolorize RBBR, oxidize gallic acid or have a high tolerance to PAHs. There was no correlation between enzyme production

Table 1
Fungal characterization and selection of fungi for degradation of PAHs.

KUC ID	Fungal species	Optimal Temp. (°C)	Growth rate (mm/day)	LiP (U/mL)	MnP (U/mL)	Lac (U/mL)	ANT	PHE	FLT	PYR	Mixture	RBBR	Gallic acid reaction	Selection
KUC8836	*Peniophora incarnata*	30	6.4	48.2	6.4	1.8	+++++	+++++	+++++	+++++	+++++	B	DB	*
KUC9140	*Peniophora cinerea*	25	3.3	195.9	0.9	0.8	+++++	+++++	+++++	+++++	+++++	E	YB	*
KUC8073	*Phanerochaete sordida*	30	5.8	4.3	256.7	0.7	+++++	+++++	+++++	+++++	+++++	B	BR	*
KUC9130	*Trichaptum abietinum*	30	7.7	6.5	1.3	0.6	+++++	+++++	+++++	+++++	+++++	B	N	*
KUC8201	*Mycoaciella bispora*	30	3.8	62.7	4.3	0.0	+++++	+++++	+++++	+++++	+++++	C	YE	*
KUC9161	*Phlebia tremellosa*	30	7.4	1.1	2.6	0.0	+++++	+++++	+++++	+++++	+++++	B	BR	*
KUC8391	*Rhizochaete* sp.	25	0.9	6.5	7.2	0.0	++++	+++++	+++++	+++++	+++++	C	N	
KUC8371	*Rhizochaete* sp.	25	1.6	5.0	11.5	0.3	+++	+++++	+++++	+++++	+++++	C	N	
KUC8613	*Dentipellis dissita*	30	4.4	255.9	16.6	3.0	+++++	++++	+++++	+++++	+++++	C	YE	
KUC9207	*Phlebiella* sp.	25	4.7	14.2	35.3	1.9	+++++	++++	+++++	+++++	+++++	C	YE	
KUC8320	*Heterobasidion annosum*	25	5.6	364.7	8.5	0.6	+++++	+++	+++++	+++++	+++++	C	N	
KUC8364	*Rhizochaete* sp.	30	2.5	9.7	35.8	0.1	+++++	+++	+++++	+++++	+++++	C	YE	
KUC9110	*Pseudochaete tabacina*	25	4.3	7.0	14.0	0.2	+++++	++	+++++	+++++	+++++	E	YB	
KUC8891	*Phyllotopsis nidulans*	25	2.2	92.5	4.7	0.8	+++++	+	+++++	+++++	+++++	C	YE	
KUC8204	*Bjerkandera adusta*	25	7.2	12.4	1.3	0.0	+++++	++++	+++++	+++++	+++++	C	N	
KUC8606	*Ceriporia lacerata*	25	3.7	12.5	2.6	0.8	++++	+++++	++++	++++	+++++	C	N	
KUC8040	*Phanerochaete calotricha*	30	11.5	61.1	2.1	0.5	+++++	++++	++++	++++	++	A	DB	*
KUC8003	*Phanerochaete calotricha*	30	11.3	7.3	2.6	0.3	+++++	++++	++++	++++	++	A	DB	*
KUC9033	*Phlebia brevispora*	30	11.7	3.2	83.8	6.1	+++++	+++++	++++	+++++	++	B	DB	*
KUC9045	*Phlebia brevispora*	30	10.8	2.9	101.7	9.4	+++	+++++	+++++	+++++	++	A	DB	*
KUC8323	*Phanerochaete* sp.	35	11.5	23.3	347.7	1.2	+++++	+++	++++	+++++	+	A	DB	*

[a]Days to decolorize the full dish within; A, 5 days; B, 6 to 10 days; C, 11 to 15 days; D, 16 to 20 days; and E over 21 days. [b]Gallic acid reaction columns indicate; DB, dark brown; BR, brown; YB, yellowish brown; and YE, yellow of brown-colored quinonic form. [c]PAH tolerance was determined by the percentage of mycelial growth inhibition (% MGI) on MEA media amended with anthracene, phenanthrene, fluoranthene, pyrene and mixed four PAHs were expressed as +++++ ($0 \le$ % MGI < 10), ++++ ($10 \le$ % MGI < 30), +++ ($30 \le$ % MGI < 50), ++ ($50 \le$ % MGI < 70), + ($70 \le$ % MGI < 100). [d]Definition of all abbreviations, LiP, lignin peroxidase; MnP, manganese dependent peroxidase; Lac, laccase; ANT, anthracene; PHE, phenanthrene; FLT, fluoranthene; PYR, pyrene; Mixture, mixture of four PAHs; RBBR, remazol brilliant blue R.

and favorable degradation properties because the enzymes were produced in nitrogen-limited conditions without any substrate. Thus, the selection of excellent species for PAH biodegradation requires testing beyond the determination of ligninolytic enzyme activities without any substrate. For example, supplementing a medium with a xenobiotic substrate as a sole carbon source may induce ligninolytic enzyme activity.

3.4. Tolerance to PAHs

We investigated the level of tolerance of these fungal isolates to 3- or 4-ring PAHs (phenanthrene, anthracene, fluoranthene, and pyrene). We found that 137 (91.3%) and 127 isolates (84.7%) demonstrated a high tolerance (more than 70%) to anthracene and pyrene, respectively. Additionally, 93 (62%) and 59 (39.3%) of the fungal isolates were able to tolerate fluoranthene and phenanthrene, respectively. It indicates that most of WRF inhabiting in Korea have a tolerance against respective PAH compound, while they cannot survive in contaminated soil with PAHs mixed. 24 isolates (16%) showed a high tolerance of over 70%. 39 isolates (26%) demonstrated high tolerance to four PAHs individually. Among them all strains belonging to *Ceriporia lacerata*, *Cerrena consors*, *Dentipellis dissita*, *Peniophora cinerea*, *Peniophora incarnata*, *Phlebia subochracea*, *Phlebia tremellosa*, *Trametes pubescens*, showed their high tolerance to four PAHs. The fungi with the highest tolerance for the PAHs might be valuable as a useful resource for the biodegradation of PAHs and a variety of other biotechnological applications. And among these fungal isolates, six isolates were selected to degrade PAHs due to their high tolerance against both four and mixture PAHs more than 90%.

3.5. Biodegradation of PAHs

11 species exhibited a high tolerance to PAHs as indicated by the low percentage of mycelial growth inhibition (<10%) or showed dark brown color in gallic acid oxidation. These species were evaluated for their ability to degrade PAHs. Approximately, most of isolates selected from gallic acid reaction degraded PAHs at a higher rate than *P. chrysosporium* KCTC6293 (Fig. 1). Specifically, the highest degradation rates for PHE (95.3%), FLT (95.0%), and PYR (97.9%) were observed with *Peniophora incarnata* KUC8836 after two weeks of incubation. *Phlebia brevispora* KUC9033 showed the highest degradation rate for ANT (80.4%). As PAH structures are cleaved by enzymatic catalysis, *P. brevispora* KUC9033, *P. incarnata* KUC8836 extracellularly produced laccase and MnP with higher efficiencies (Fig. 2a), while LiP were remarkably produced from *P. incarnata* KUC8836 and *Phanerochaete* sp. KUC8323 (Fig. 2b). Thus *P. incarnata* KUC8836 have a great ability to degrade several PAHs at once with three ligninolytic enzymes.

4. Discussion

The aim of this study was to develop a procedure for selecting and evaluating fungal isolates that show excellent PAH degradation for biotechnological applications. We characterized 150 WRF by a variety of experiments to select such species. Most of the fungi evaluated in this study showed optimal growth at 30 °C. However, these fungi can also be grown rapidly at 27 °C in Korea, which represents a special case (Cho et al., 2004; Kim et al., 2012). For this reason, all our experiments were performed at 27 °C as the optimal temperature. For some species, multiple isolates were tested to evaluate intra-species differentiation among strains of a single species. Casieri et al. (2010) reported that fungal diversity was apparent in ligninolytic enzyme activity and physiological properties. Thus, the high variability in the dye decolorization capability observed among different species of the same genus, and often between different strains of the same species, underlines the usefulness of including several strains of each species in a search for optimal species. Moreover, the genera *Phanerochaete* and *Trametes* occupied 34.6% of all fungi in this study and may be valuable to biotechnological industries because of their rapid growth rates, high tolerance, and different characteristics among isolates.

In the RBBR dye decolorization assay in solid medium, 107 fungal isolates decolorized the full dish within ten days, belonging to the groups A and B. The growth rates of the fungal isolates were significantly correlated with the number of days required to completely decolorize the RBBR. Many studies selected desirable fungal isolates for their ability to decolorize RBBR (Lee H. et al., 2010). Indeed, while group A isolates decolorized RBBR more rapidly than other groups because of their growth rates, they did not exhibit greater tolerance than the fungi in other groups. It means that the ability of isolates to decolorize RBBR is not a greatly useful characteristic for selecting desirable species for PAH degradation. In addition, the fungi that completely decolorized

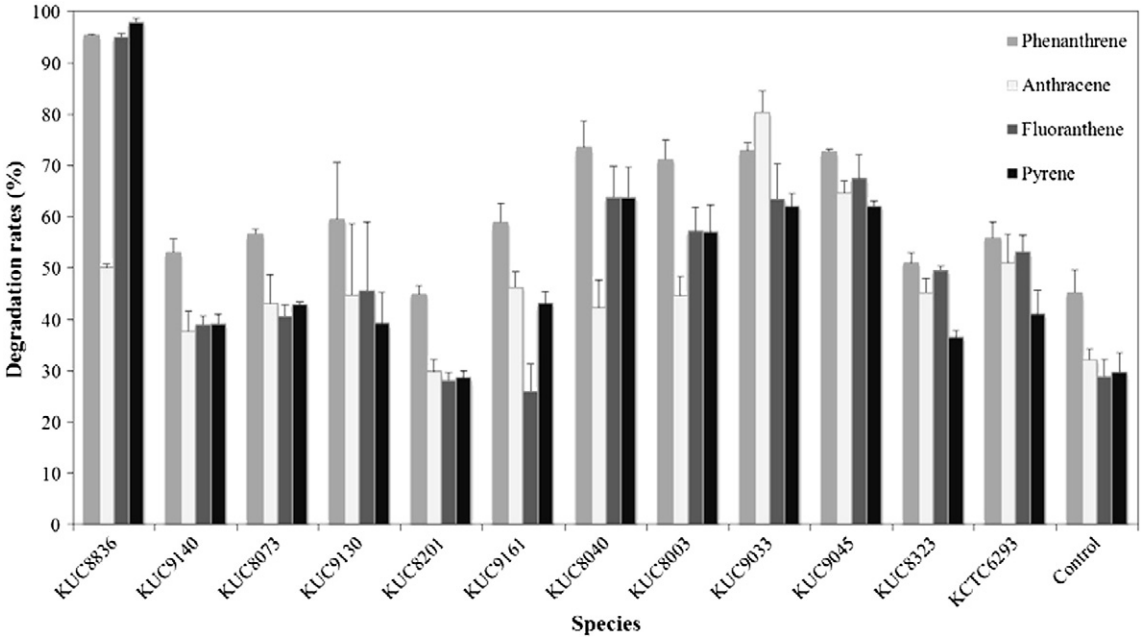

Fig. 1. Biodegradation of four PAHs by selected white rot fungi.

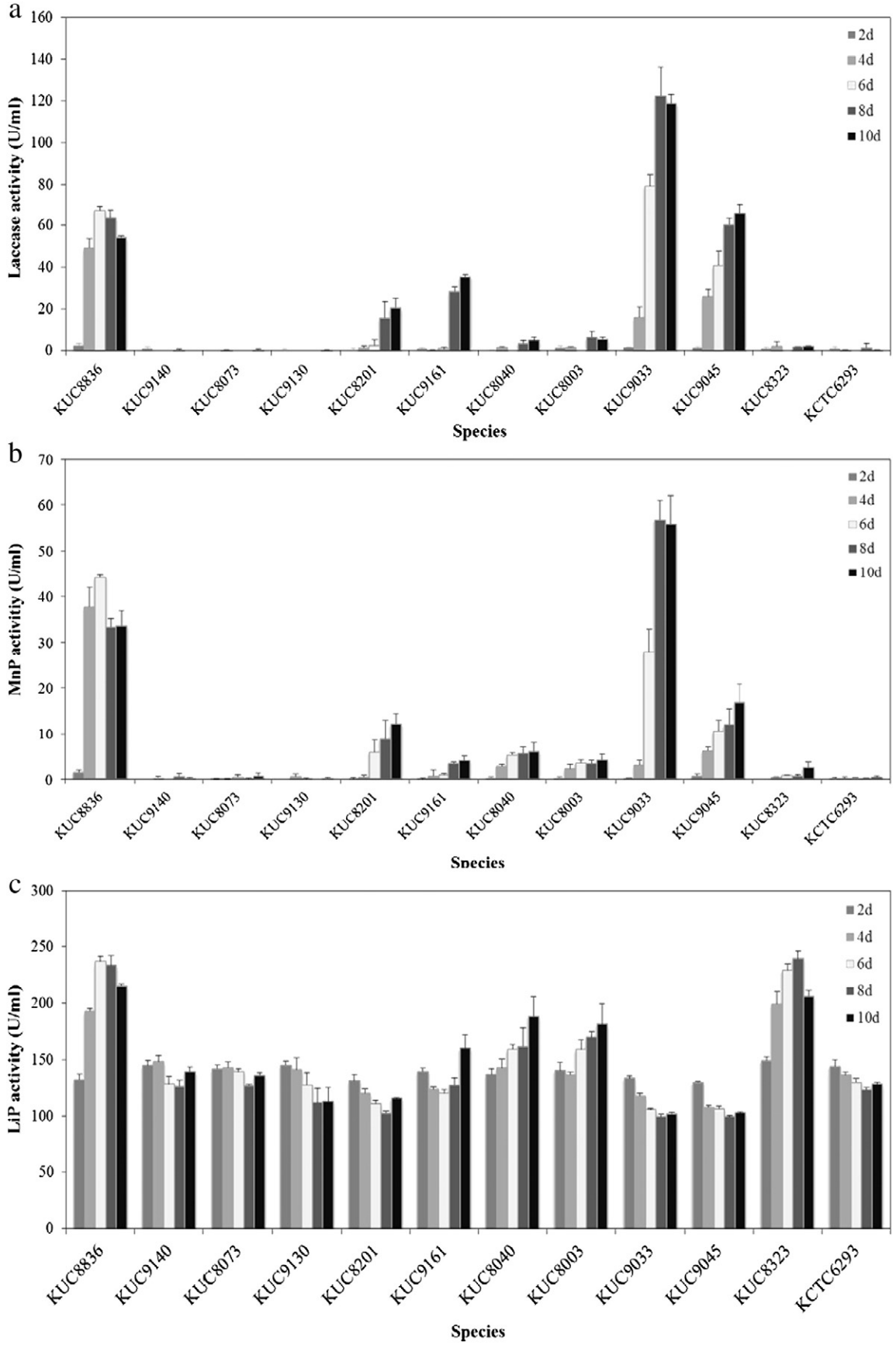

Fig. 2. Time courses of ligninolytic enzymes produced from white rot fungi during degradation of PAHs (a, Laccase; b, MnP; c, LiP).

RBBR within ten days did not show a good result in a gallic acid reaction. Among the 107 full-dish RBBR decolorizers, fewer than 30% (32 isolates) of the fungi oxidized gallic acid to a dark brown or brown color (groups DB and BR). Moreover, these gallic acid oxidizers did not show a significantly higher tolerance for PAHs than other isolates that decolorized the full RBBR plate after ten days. The results of the gallic acid reaction did not always match the results of an RBBR decolorization test, yet all but six isolates showing a dark brown-colored positive gallic acid reaction rapidly decolorized RBBR within ten days. And among the tested fungal isolates, 77.3% did not show the positive brown color in the gallic acid reaction test. However, the fungi in group DB (positive for gallic acid oxidation) demonstrated higher tolerance than other groups, suggesting that a positive gallic acid reaction is related to tolerance for PAHs. Thus these isolates were considered to be useful for anthraquinone dye decolorization in industrial bioremediation. Nevertheless these fungi could not greatly tolerate the four tested PAHs excluding *Peniophora incarnata* KUC8836, their degradability of PAHs was carried out as they showed the greatest oxidation of anthraquinone dye and gallic acid (Table 1).

All the fungi in this study produced three ligninolytic enzymes, LiP, MnP, and laccase. However, because the ligninolytic enzyme activities of the fungi were evaluated in basal salts medium which is a nitrogen-limited condition, high enzyme activity did not correspond to high performance in the RBBR and gallic acid tests. Ligninolytic enzymes can be regulated by aromatic compounds, including various dyes and PAHs (Yang et al., 2011), so that the fungi can consume such aromatic compounds as the sole carbon source. To screen for species with excellent PAH biodegradation, it might be inadvisable to assay for ligninolytic enzyme activity in the absence of substrate. The use of xenobiotic compounds as substrates can induce ligninolytic enzyme activity. This approach is feasible for fungal isolates that produce ligninolytic enzymes with high efficiency (e.g., *Bjerkandera adusta* KUC9107 and *Skeletocutis perennis* KUC8514 for Lip; *Phanerochaete velutina* KUC8366 and *Phanerochaete* sp. KUC9015 for MnP; and *Cerrena consors* KUC8416 and 8421 for laccase, Ding et al., 2012; Moreira et al., 2006).

In a tolerance test against four PAHs, we found the fungal isolates that were able to degrade PAHs. Among 24 fungal isolates with high (>90%) tolerance to mixed PAHs, 29.1% of them completely decolorized an RBBR plate within ten days and three isolates positively oxidized gallic acid with brown color (Table 1). Most importantly, a PAH tolerance test should be the primary factor in identifying desirable PAH-degrading fungal species for biotechnological procedures (Carrera, 2010). Desirable species can then be further identified and characterized using other analysis methods.

We primarily selected desirable isolates by the results of the tolerance test (Table 1). Six fungal isolates demonstrated the highest tolerance to four PAHs both individually and in a mixture as indicated by the percentage of mycelial growth inhibition (<10%). And other six isolates were selected via gallic acid reaction test (Table 1). Most of fungal isolates selected from gallic acid reaction test efficiently degraded four PAHs in liquid medium than the isolates selected from tolerance test (Fig. 1). And they demonstrated degradation of PAHs higher than *Phanerochaete chrysosporium*, which is a well-known and widely used degrader of PAHs (Brodkorb and Legge, 1992). *P. incarnata* KUC8836 completely decolorized RBBR within ten days and oxidized gallic acid to a dark brown color. Additionally, the species showed the highest tolerance to both four and mixed PAHs. This species simultaneously degraded four PAHs and produced three ligninolytic enzymes with high efficiency during degradation of PAHs (Fig. 2). It considered that the species can be useful for bioremediation of xenobiotics in contaminated environments. As we compared degradation rates of PAHs excluding *P. incarnata* KUC8836, the means of degradation rate of individual PAH by five isolates via gallic acid were 68.3% (PHE), 55.5% (ANT), 60.3% (FLT), 56.3% (PYR) and showed higher than other five isolates via tolerance test (i.e., 54.6%, PHE; 40.3%, ANT; 35.9%, FLT; 38.6%; PYR). And these isolates via gallic acid produced three ligninolytic enzymes with higher efficiency than the isolates via tolerance (Fig. 2). To select a fungal isolate for degradation of xenobiotics, we found that gallic acid reaction test might be a better method than tolerance test. Even though the selected isolates via gallic acid did not show high tolerance in mixed four PAHs, they demonstrated their capability for biodegradation of PAHs producing ligninolytic enzymes. Thus, we suggest that oxidation test of gallic acid should be primarily performed than other test for bioremediation procedure. Most of all, it can be a great opportunity to achieve remediation of various contaminated environments with xenobiotics if the species is obtained, such as *P. incarnata* KUC8836 which showed the greatest efficiency in all characteristics. The capability of the best species, *P. incarnata* KUC8836, to survive and grow in the presence of a high concentration of toxic molecules (e.g., dyes and various PAHs) is an adaptive feature that, together with their xenobiotic degradation capacity, makes them very promising candidates for biotechnological applications and bioremediation. These selected species may potentially enhance the biodegradation of PAH-contaminated environments. Other fungi not examined in this study may also be effectively used for bioremediation if they demonstrate a high tolerance to PAHs. Such fungi have a high potential for effective use in multiple biotechnological applications.

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