Decolorization and degradation of synthetic dyes by Irpex lacteus KUC8958

Yong-Seok Choi [a] , Yan Long [a] , Min-Ji Kim [a] , Jae-Jin Kim [a] & Gyu-Hyeok Kim [a]

[a] Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, Seoul, Korea
Published online: 05 Feb 2013.

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Decolorization and degradation of synthetic dyes by *Irpex lacteus* KUC8958

YONG-SEOK CHOI, YAN LONG, MIN-JI KIM, JAE-JIN KIM and GYU-HYEOK KIM

Division of Environmental Science & Ecological Engineering, College of Life Sciences & Biotechnology, Korea University, Seoul, Korea

This study was carried out to evaluate the dye decolorizing and detoxifying abilities of *Irpex lacteus*. The decolorization abilities of 14 strains of *I. lacteus* were investigated in agar-plates containing 3 synthetic dyes: Congo Red (CR), Orange II (OII), and Reactive Blue 4 (RB4). In an agar plate test, *I. lacteus* KUC8958 showed the highest dye decolorizing rate with all 3 dyes. Subsequently, we investigated the decolorizing and detoxifying abilities of *I. lacteus* KUC8958 on synthetic dyes in liquid media under both shaking and static conditions. *I. lacteus* KUC8958 showed high decolorization rates for CR and BR4 (more than 95%) under shaking conditions, but only moderate decolorization of OII (up to 53%). The dye decolorizing rates of *I. lacteus* KUC8958 were approximately 20% to 60% higher under shaking conditions than under static conditions. In a detoxification assay, the toxicities of CR and OII solutions increased, whereas the toxicity of RB4 decreased after decolorization by *I. lacteus* KUC8958. Subsequently, high-performance liquid chromatography analysis detected 2 compounds in CR and 1 compound in OII that were newly formed during the decolorizing process, and which might be involved in the increased toxicities. Further studies are required to identify these newly formed compounds.

Keywords: Synthetic dyes, decolorization, degradation, *Irpex lacteus*, acute toxicity.

Introduction

Since mauve, the first synthetic dye, was synthesized by Perkin in 1856, a large number of synthetic dyes have been developed and used extensively in many industries including the textile dyeing industry. It is estimated that worldwide, over 15×10^5 tons of synthetic dyes were manufactured each year in the late 2000s, whereas approximately 8×10^5 tons were produced in the late 1970s.[1]

The discharge of an enormous volume of wastewater containing dyes is an inevitable consequence, because the textile industry consumes large quantities of water and all dyes cannot be completely combined with fibers during the dyeing process.[2,3] O'Neill[4] summarized previous reports relevant to dye concentrations in textile effluents, and revealed that 10–500 mg/L of dyestuff is included in the effluents. These high concentrations of dyes in effluents interfere with the penetration of visible light into water, resulting in a hindrance to photosynthesis and a decrease in gas solubility, since less than 1 mg/L of dyes is highly visible.[3] Furthermore, synthetic dyes, which include an aromatic ring in their basic structure, are regarded as toxic, carcinogenic, and xenobiotic compounds.

Therefore, decolorization and detoxification of dye-containing wastewater need to be conducted before discharging wastewater into natural water bodies. Certain physical, chemical, and biological treatments are currently being used for dye wastewater treatment.[5] Although physical and chemical methods usually show high dye-removal efficiencies, high operating costs became a main drawback to the large-scale application of these methods. Due to the high chemical stability of synthetic dyes, conventional biological treatment using bacteria cannot remove dyes efficiently.

White-rot fungi have been considered by far the most efficient synthetic dye decolorizing microorganisms.[6] White-rot fungi produce lignin-modifying enzymes such as lignin peroxidase, laccase, and manganese peroxidase during their metabolic processes. Because lignin-modifying enzymes non-specifically degrade phenolic compounds, various synthetic dyes that have phenolic compounds in their basic structure can be oxidized by lignin-modifying enzymes, resulting in the decolorization of synthetic dyes. Several white-rot fungi, including *Bjerkandera adusta*, *Irpex lacteus*, *Phanerochaete chrysosporium*, and *Trametes versicolor*, have frequently been reported as effective dye-degrading species.[7–11]

Among these, the white-rot fungus *I. lacteus* is attracting attention as a notable species for the decolorization of synthetic dyes. Numerous studies have reported that *I. lacteus*

effectively removes chemically different dyes (e.g., Poly R-478 and Remazol Brilliant Blue-R) by its enzymatic action.[12–17] Novotný et al.[10] demonstrated that among the various white-rot fungi, *I. lacteus* is the one of the most promising organisms for application to enzyme biotechnology and bioremediation, including dye decolorization processes.

However, the intra-specific variation among *I. lacteus* strains in the decolorization of synthetic dyes has not been thoroughly investigated to date. Most previous work has focused on the ability of *I. lacteus* to decolorize synthetic dyes, and on the relationship between decolorization and enzyme production, even though intra-specific variation is frequently observed in the fungal bioprocessing of organopollutants.[18] In addition, changes in the toxicity of synthetic dyes during fungal decolorization by *I. lacteus* remain to be elucidated. The objectives of the present study were to evaluate the intra-specific variation among *I. lacteus* strains

in the decolorization of synthetic dyes, and to investigate the detoxifying ability of selected *I. lacteus* strains during the decolorization process.

Materials and methods

Fungal isolates

A total of 14 strains of *I. lacteus* (KUC8075, KUC8411, KUC8508, KUC8604, KUC8605, KUC8813, KUC8817, KUC8842, KUC8843, KUC8853, KUC8958, KUC9014, KUC9124, and KUC9126), obtained from the Korea University Culture collection, were used in this study. Four strains of *I. hydnoides* (KUC8501, KUC8502, KUC8504, and KUC8505) and 2 strains of *T. versicolor* (KUC8413 and KUC8867) were also used as reference isolates. The fungi were transferred from stock cultures to malt extract glucose agar (MEGA) media containing 5 g/L malt extract,
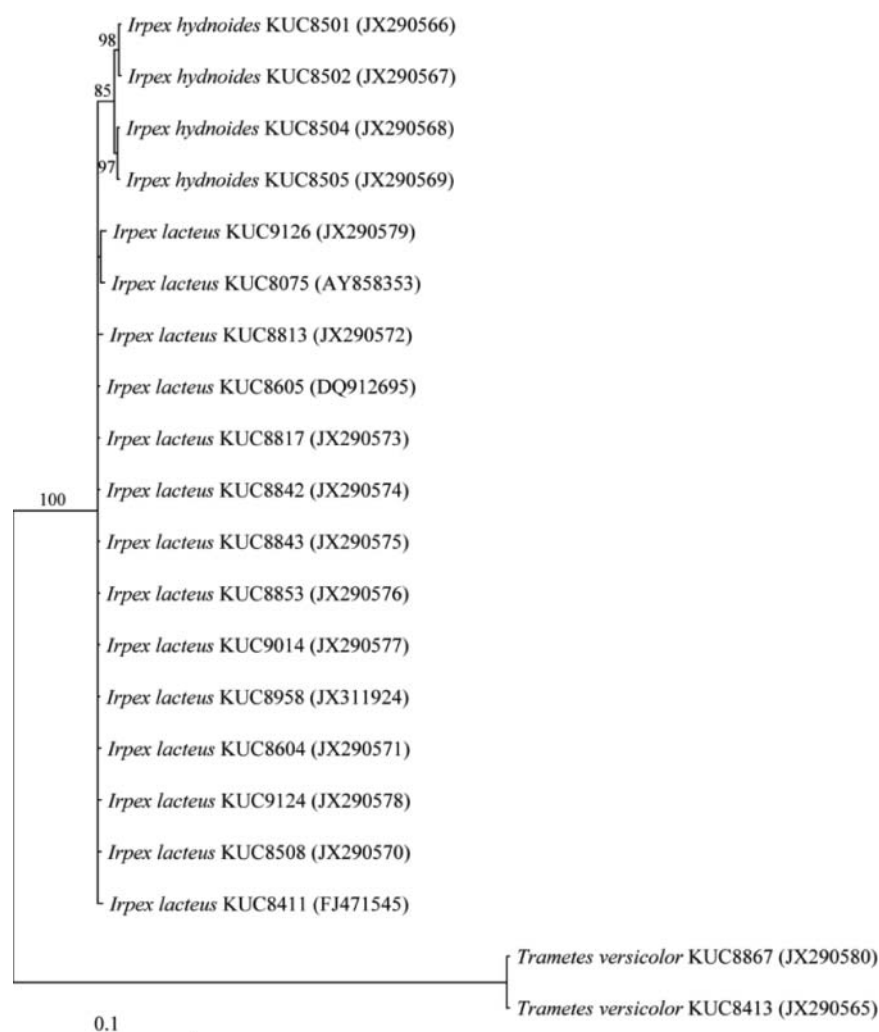

Fig. 1. Phylogenetic tree of *Irpex lacteus* resulting from a Bayesian analysis in a combined ITS and LSU rDNA sequence. Two strains of *Trametes versicolor* were used as an outgroup. Bootstrap values above 60% based on 1,000 replicates are shown on the branches. The GenBank accession numbers are given in parentheses with the species names.

Table 1. Mobile phase program for the HPLC gradient.

Mobile phase	% A	% B	Curve
0 min	100	0	Linear
40 min	20	80	Linear
45 min	20	80	Linear
50 min	100	0	Linear
65 min	100	0	Linear

1 g/L glucose, and 20 g/L agar, and incubated at 27°C in the dark until the mycelium covered approximately 60% of the area of the plate.

The identity of all fungi used in this study was confirmed using molecular methods. Briefly, whole internal transcribed spacer and partial large subunits of rDNA were amplified by polymerase chain reaction (PCR) using 2 primer sets ITS4/ITS5 and LR0R/LR3.[19,20] Subsequently, the sequences of the PCR products were compared with data sets from GenBank and deposited into the Gen-Bank DNA sequence database (Fig. 1).

Decolorization of synthetic dyes in solid media

The decolorization abilities of the fungal isolates for synthetic dyes in solid media were determined using the *in vitro* bioassay described by Novotný et al.[21] Briefly, a fungal disk (5-mm diameter) was removed from a precultured plate and inoculated into the center of a MEGA media plate containing 100 mg/L Congo Red (CR), Reactive Blue 4 (RB4), or Orange II (OII). The plates were then incubated at 27°C in the dark for 6 days, and all tests were performed

in triplicate. After incubation, the diameter of the decolorized area was determined.

Decolorization of synthetic dyes in liquid media

The KUC8958 strain of *I. lacteus*, which was identified as an outstanding fungus for dye decolorization on solid media (see later), was further investigated for its ability to decolorize and detoxify synthetic dyes in liquid media.

To determine the decolorizing ability of *I. lacteus* KUC8958 for synthetic dyes in liquid media, 100-mL aliquots of malt extract glucose (MEG) medium containing each dye (5 g/L malt extract, 1 g/L glucose, and 0.1 g/L of dye) were prepared in 250-mL flasks, and autoclaved at 121°C for 15 min. Ten inocula (5-mm diameter) of the fungus were then inoculated into the flasks. Incubation was carried out at 27°C under 2 conditions, shaking (150 rpm) and static. Dye-containing MEG media without inoculation and inoculated MEG media without dye were used as controls.

Sampling to determine dye decolorization was carried out every 2 days, for 2 weeks after inoculation. Two-milliliter samples were drawn in a presterilized syringe and filtered using a 0.45-μm syringe filter to remove mycelia from the sample. Filtered samples were used immediately in an absorbance assay. The decolorizing rates of samples were estimated using a spectrophotometer, by measuring the absorbance at 495, 595, and 485 nm for CR, RB4, and OII, respectively.

Detoxification assay

A detoxification assay was conducted according to the standard procedures of the Organization for Economic

Table 2. Decolorization of synthetic dyes by *Irpex lacteus* and other white rot fungi in solid media.

Fungal isolate	Isolate no.	% Dye removal								
		Congo red			Reactive blue 4			Orange II		
Irpex lacteus	KUC8075	61.4	(0.9)[a]	C[b]	69.5	(1.3)	FG	59.6	(4.7)	CD
Irpex lacteus	KUC8411	73.6	(1.3)	B	73.7	(1.3)	F	42.7	(2.5)	E
Irpex lacteus	KUC8508	78.3	(1.8)	AB	90.0	(1.5)	B	70.5	(2.7)	B
Irpex lacteus	KUC8604	78.0	(1.0)	AB	85.0	(4.9)	CD	29.0	(4.2)	F
Irpex lacteus	KUC8605	80.7	(2.0)	A	88.7	(1.4)	BC	0.0	(0.0)	G
Irpex lacteus	KUC8813	50.5	(2.0)	D	59.1	(2.2)	H	0.0	(0.0)	G
Irpex lacteus	KUC8817	77.8	(1.2)	AB	90.0	(1.0)	B	0.0	(0.0)	G
Irpex lacteus	KUC8842	54.2	(2.2)	D	62.7	(1.3)	H	61.8	(1.6)	C
Irpex lacteus	KUC8843	32.4	(2.1)	G	0.0	(0.0)	J	0.0	(0.0)	G
Irpex lacteus	KUC8853	39.2	(1.8)	F	0.0	(0.0)	J	0.0	(0.0)	G
Irpex lacteus	KUC8958	82.1	(1.1)	A	100.0	(0.0)	A	76.4	(2.8)	A
Irpex lacteus	KUC9014	80.5	(1.3)	A	79.9	(1.8)	E	68.8	(2.7)	B
Irpex lacteus	KUC9124	23.1	(0.4)	H	29.3	(3.2)	I	0.0	(0.0)	G
Irpex lacteus	KUC9126	28.1	(4.6)	G	86.9	(0.6)	BCD	76.0	(1.9)	A
Irpex hydnoides	KUC8501	30.2	(4.7)	G	84.6	(1.3)	CD	0.0	(0.0)	G
Irpex hydnoides	KUC8502	39.2	(3.8)	F	67.6	(1.8)	G	0.0	(0.0)	G
Irpex hydnoides	KUC8504	20.5	(0.6)	H	68.4	(4.7)	G	0.0	(0.0)	G
Irpex hydnoides	KUC8505	32.3	(3.2)	G	84.1	(2.3)	DE	0.0	(0.0)	G
Trametes versicolor	KUC8413	44.0	(0.9)	E	68.3	(0.8)	G	57.5	(0.8)	D
Trametes versicolor	KUC8867	50.1	(0.3)	D	84.0	(1.6)	DE	71.2	(1.0)	B

[a]Values represent the average of 10 replicates; numbers in parentheses indicate the standard deviation.
[b]Numbers followed by the same letter in each column are not significantly different ($\alpha = 0.05$) according to Tukey's multiple comparison test.
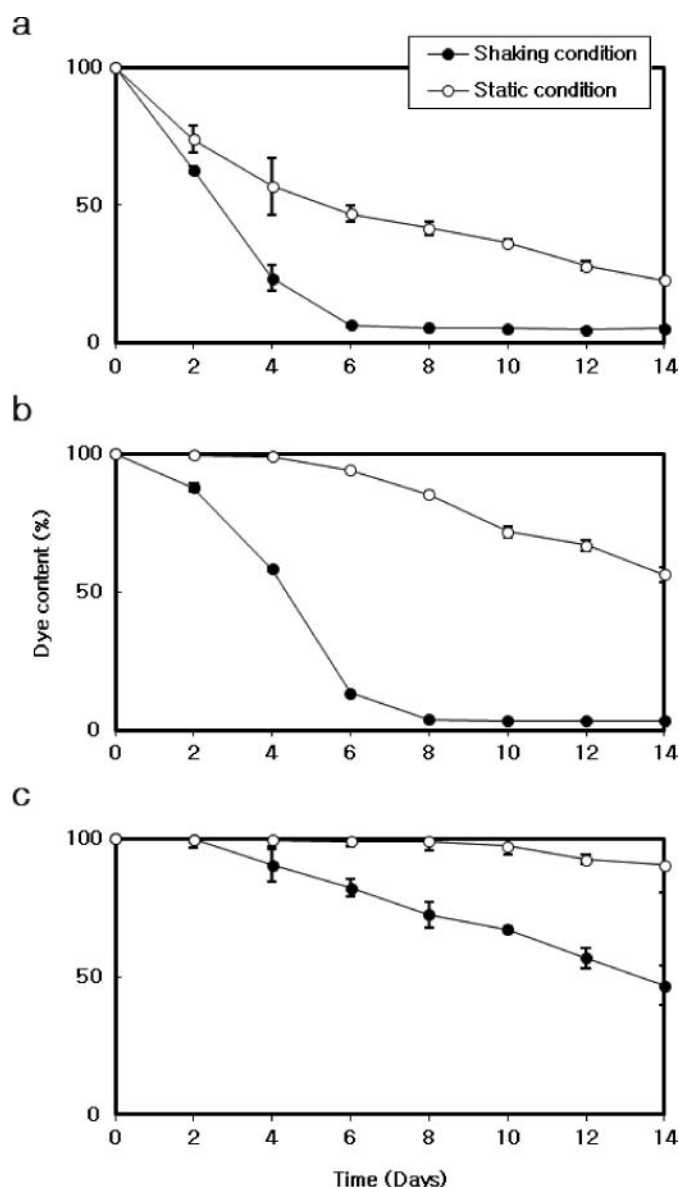
Fig. 2. Decolorization of liquid media containing Congo red (a), Reactive blue 4 (b) and Orange II (c) by *Irpex lacteus* KUC8958 under shaking and static conditions.

Table 3. Toxicity of liquid media containing synthetic dyes before and after decolorization by *Irpex lacteus* KUC8958.

Decolorization procedure	Toxicity units ($100/EC_{50}$)		
	Congo red	Reactive blue 4	Orange II
Before decolorization	4.60	5.10	3.03
After decolorization	11.71	3.14	24.69

ues were transformed into toxic units (TU $= 100/EC_{50}$) for comparison of toxicities before and after decolorization.

HPLC analysis

Three milliliters of each sample were removed from the dye-containing MEG media before and after decolorization using a pre-sterilized syringe, and filtered using a 0.45-μm syringe filter. A high-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA), consisting of 2 pumps (Waters 515), an auto sampler (Waters 717 plus), a photo diode array detector (Waters 2996, wavelength range: 190–800 nm), and an RP-C18 column (250 mm × 4.6 mm; LOT: 10659; Thermo Scientific, Waltham, MA, USA) were used to perform the analysis. Water-acetonitrile solutions, in the ratios 95:5 (A) and 20:80 (B), were prepared as the mobile phase, filtered using a 0.45-μm filter, and degassed by ultrasonic waves prior to use. The flow rate was 1.0 mL/min, and the injection volume was 20 μL. A gradient was used to ensure the clear separation of different components in the samples (Table 1). The analysis was performed at room temperature.

Results and discussion

Decolorization of synthetic dyes in solid media

The decolorization rates (expressed as percentages) of *I. lacteus* on synthetic dyes in solid media are shown in Table 2. There were obvious differences in decolorizing rates among the *I. lacteus* isolates. Among the 14 isolates of *I. lacteus*, 8 isolates (KUC8075, KUC8411, KUC8508, KUC8604, KUC8842, KUC8958, KUC9014, and KUC9126) were able to form decolorization zones on dye-containing plates regardless of the type of dye.

Although 4 isolates of *I. lacteus* (KUC8605, KUC8813, KUC8817, and KUC9124) showed moderate decolorization rates for CR and RB4, they had no decolorization effect on OII. Two isolates of *I. lacteus* (KUC8843 and KUC8853) only decolorized a small zone of the medium containing CR (32.4–39.2%). These data provide evidence of individual variations among the strains, although the accurate reason is not yet fully understood. On the basis of the intra-specific variation of *I. lacteus* observed in this study, we suggest that in order to select appropriate *I. lacteus* isolates for use in dye decolorization processes, their

Co-operation and Development (OECD) with neonates of *Daphnia magna* (less than 24 h old).[22] Sample solutions of MEG media before and after decolorization were filtered using a 0.45-μm syringe filter. Five dilutions (50%, 25%, 12.5%, 6.25%, and 3.125%) of each sample and 1 control were prepared in 4 replicates, with 10 mL of test solution and 5 individual test organisms placed in each vessel. Test was conducted at 20 $\pm$ 2°C with 16-h light and 8-h dark photoperiods for 48 h. During the test, organisms were not fed. Immobilization (defined as no response to gentle agitation) of the test species was used to calculate the EC_{50} value by the trimmed Spearman-Karber method. EC_{50} val-
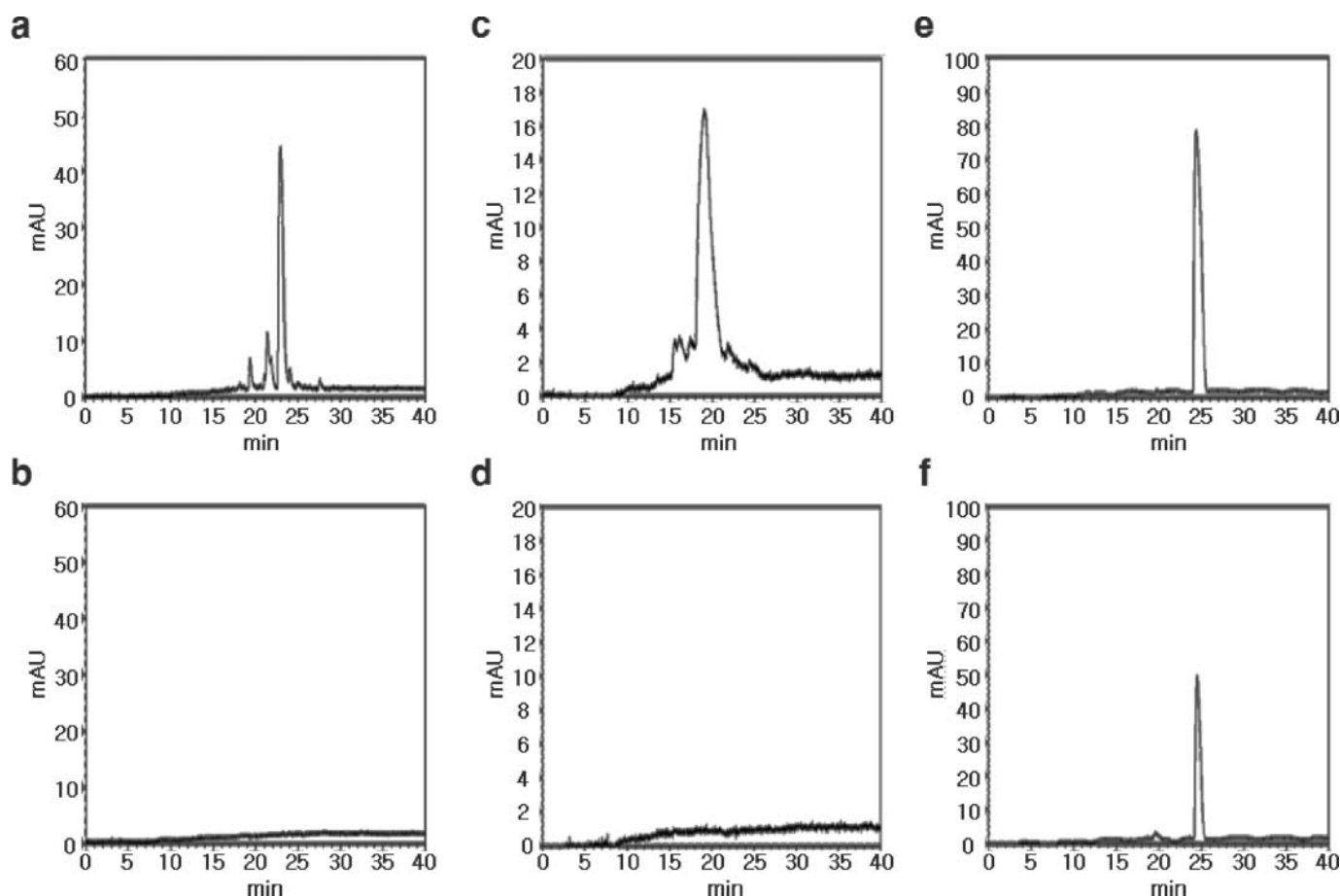
Fig. 3. HPLC analysis of Congo red at 495 nm (a, before decolorization; b, after decolorization by *Irpex lacteus* KUC8958), Reactive blue 4 at 595 nm (c, before decolorization; d, after decolorization by *Irpex lacteus* KUC8958) and Orange II at 485 nm (e, before decolorization; f, after decolorization by *Irpex lacteus* KUC8958).

actual abilities to decolorize different synthetic dyes need to be confirmed prior to application.

Another species of the genus *Irpex*, *I. hydnoides*, showed no ability to decolorize OII, whereas approximately 30% of CR and 75% of RB4 was decolorized, with little intra-specific variation (Table 2). Two strains of *T. versicolor* were able to form decolorizing zones on plates containing synthetic dyes, but the efficiency was somewhat lower than some strains of *I. lacteus*. Among all the white-rot fungi tested, *I. lacteus* KUC8958 significantly removed the origin color of each dye tested in solid media, exhibiting decolorization rates of 82.1% for CR, 100% for RB4, and 76.4% for OII. Therefore, *I. lacteus* KUC8958 was selected for further investigation in liquid media.

Decolorization of synthetic dyes in liquid media

The synthetic dye decolorizing ability of *I. lacteus* KUC8958, which emerged as the most effective dye-degrader in the agar-plate screening, was tested in liquid media under both shaking and static conditions. Over 95% of CR was decolorized within 8 days under shaking con-

ditions, whereas 42% of CR remained under static conditions (Fig. 2a). After 14 days' incubation, 77% of CR was removed under static conditions. Similar results were obtained in the RB4 decolorization test (Fig. 2b); 56% of RB4 remained after 14 days' incubation under static conditions, whereas over 95% of RB4 was removed under shaking conditions. In the case of OII, the decolorization rate under shaking conditions (53%) was higher than that under static conditions (9%), even though the decolorization rates under both shaking and static conditions were considerably lower than those for CR and RB4.

A comparison of decolorization rates under shaking and static conditions has also been performed by other researchers. Rigas and Dritsa[23] reported that the efficiency of some basidiomycetes strains in decolorizing Poly R-478 was higher under shaking conditions than under static conditions. Better oxygen transfer and nutrient distribution with agitation may be responsible for the higher decolorization rate. It has reported that dissolved oxygen (DO) is an important factor that enhances the rate of dye decolorization. Decolorization of dyes by *P. chrysosporium* was strongly dependent on the DO concentration of the culture. [16,24]
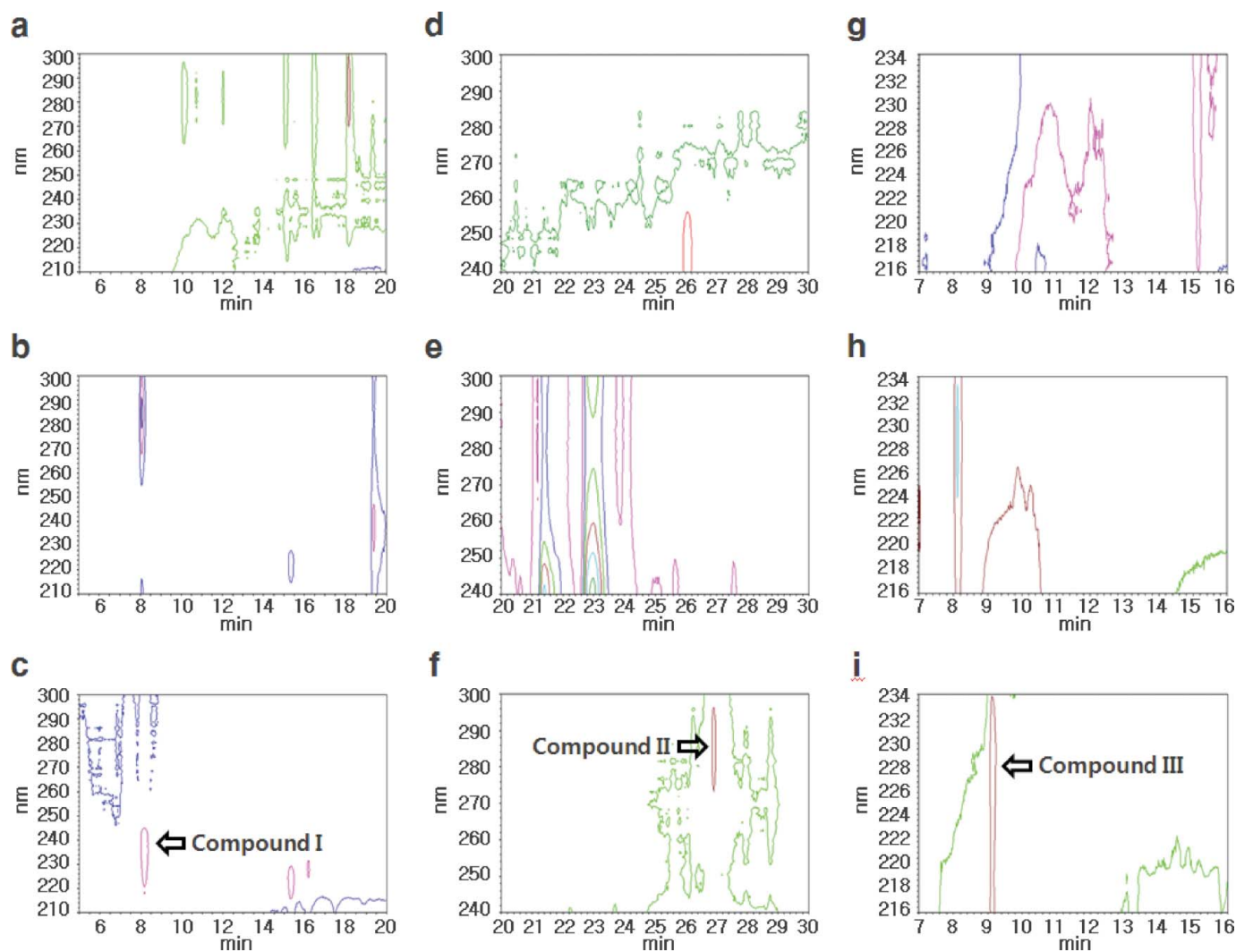
Fig. 4. Diode array detection results of Congo red (Compound I and II) and Orange II (Compound III) (a, d and g, *Irpex lacteus* KUC8958 incubated in MEG media without dye; b, e and h, dye-containing MEG media without fungal inoculation; c, f and i, dye-containing MEG media with fungal inoculation after 14 days of incubation) (color figure available online).

Therefore, the higher DO concentration could be responsible for the higher dye decolorization rate under shaking conditions.

Detoxification assay

TU values of samples before and after decolorization are shown in Table 3. After decolorizing treatment by *I. lacteus* KUC8958, the toxicity of RB4 decreased, whereas the toxicities of the 2 azo dyes (CR and OII) increased. This increased toxicity may be affected by several factors such as pH value and metabolites. The pH value may affect the TU values of samples before and after decolorization because the TU value is calculated based on the immobilization rate of the water flea.[25] Nevertheless, no significant pH value changes were detected in the samples before and after decolorization in this study. Therefore, there is no relationship between pH value changes and the increased toxicities

of both dyes (CR and OII) in this study. Consequently, metabolites, which have frequently been reported to be the main factor causing increased toxicity, were investigated using HPLC analysis. [26, 27]

HPLC analysis

Dye degradation was confirmed by detecting peaks at the λ_{max} of each dye by HPLC (Fig. 3). In the case of CR and RB4, several peaks were identified as impurities by extracting the absorption curves of these peaks (Figs. 3a and 3c). No impurity peak was detected in the sample of OII (Fig. 3e). The peaks of CR and RB4 completely disappeared after 14 days of incubation, confirming the complete degradation of these 2 dyes by *I. lacteus* KUC8958 (Figs. 3b and 3d). The area of the peak in the case of OII decreased moderately after 14 days of incubation, indicating that OII was partly degraded by *I. lacteus* KUC8958 (Fig. 3f).
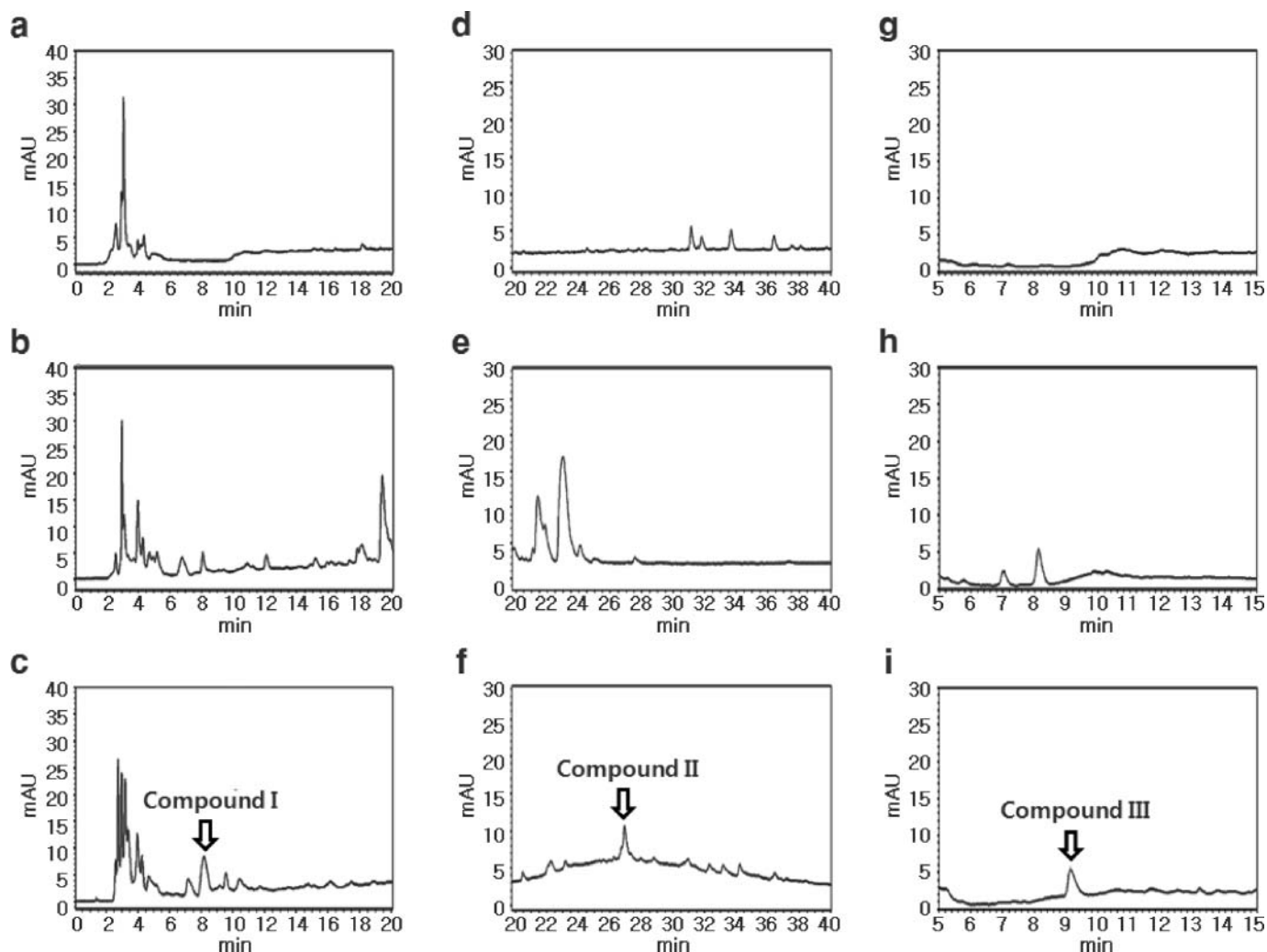
Fig. 5. Results from diode array detection showing Compound I and Compound II in Congo Red solution at 238 nm and at 285 nm, respectively, and Compound III in Orange II solution at 228 nm (a, d and g, *Irpex lacteus* KUC8958 incubated in MEG media without dye; b, e and h, dye-containing MEG media without fungal inoculation; c, f and i, dye-containing MEG media with fungal inoculation after 14 days of incubation).

Due to the different absorption properties of metabolites produced during dye degradation, diode array detection (DAD) was performed to detect all peaks in the samples. The samples, which were obtained after 2-week incubations of 3 types of liquid media (dye-containing MEG medium with fungal inoculation, fungi-inoculated MEG medium without dye, and the dye-containing MEG medium without fungal inoculation), were analyzed using DAD (Fig. 4). From the DAD results, 3 new peaks were extracted at the λ_{max} of each compound, and are shown in 2-dimensional graphs in Figure 5.

Interestingly, a new peak at 8.10 min (Compound I) was found in MEG medium containing the dye after 14-day incubation (Fig. 5c). This peak did not occur in inoculated MEG medium incubated without the dye (Fig. 5a), or in the MEG sample with dye before incubation (Fig. 5b). Therefore, the new peak, Compound I, was identified as a metabolite or intermediate produced during the

degradation of CR. Another compound (Compound II) was revealed using the same method (Fig. 5f). In the case of OII, one peak (Compound III) was identified in the OII sample incubated with *I. lacteus* KUC8958 for 14 days (Fig. 5i). These compounds might be responsible for the increased toxicity of CR and OII after incubation. Further studies are necessary to separate and identify these compounds using mass spectrometry and NMR analysis. In addition, a long-term decolorization test (more than 2 weeks) should be performed to confirm whether these compounds are metabolites or intermediates produced during the decolorizing process.

Conclusion

In the present study, significant differences in the rates of dye decolorization of 14 isolates of *I. lacteus* were confirmed

by the agar-plate decolorization test. Among the strains of *I. lacteus* used in this study, *I. lacteus* KUC8958 showed a remarkable potential to decolorize all 3 synthetic dyes tested. In the liquid media test, the decolorization rates exhibited by this strain for the 3 dyes under shaking conditions were higher than those seen under static conditions. After 14 days of incubation, 95% of both CR and RB4 in liquid media were removed under shaking conditions, whereas only 77% of CR and 44% of RB4 were removed under static conditions. In the case of OII, although 47% of OII remained under shaking conditions, only 10% of OII was removed from the media under static conditions.

The detoxification test confirmed an increase in the toxicity of the 2 dyes (CR and OII). HPLC analysis revealed that 3 compounds were newly formed during the decolorization of CR and OII by *I. lacteus* KUC8958, suggesting that these are toxic metabolites or intermediates. The identification of the chemical structures of these compounds is necessary to determine whether these compounds can be biodegraded easily, as these compounds will be released into natural water bodies.

Acknowledgments

References

[1] Jiang, H.; Murmann, J. P. Regional institutions, ownership transformation, and migration of industrial leadership in China: the case of the Chinese synthetic dye industry, 1978–2008. Australian School of Business Research Paper No. 2011 STRE 01, Australian School of Business, The University of New South Wales, **2011**.

[2] Brown, D. Effects of colorants in the aquatic environment. Ecotox. Env. Saf. **1987**, *13*, 139–147.

[3] Banat, I.M.; Nigam, P.; Singh, D.; Marchant, R. Microbial decolorization of textile-dye-containing effluents: a review. Biores. Technol. **1996**, *58*, 217–227.

[4] O'Neill, C.; Hawkes, F.R.; Hawkes, D.L.; Lourenco, N.D.; Pinheiro, H.M.; Delee, W. Colour in textile effluents—Sources, measurement, discharge consents and simulation: a review. J. Chem. Technol. Biotechnol. **1999**, *74*, 1009–1018.

[5] Robinson, T.; Mcmullan, G.; Marchant, R.; Nigamn, P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Biores. Technol. **2001**, *77*, 247–255.

[6] Wesenberg, D.; Kyriakides, I.; Agathos, S.N. White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol. Adv. **2003**, *22*, 161–187.

[7] Guo, M.; Lu, F.P.; Liu, M.Y.; Li, T.P.; Pu, J.; Wang, N.; Liang, P.; Zhang, C.Y. Purification of recombinant laccase from *Trametes versicolor* in *Pichia methanolica* and its use for the decolorization of anthraquinone dye. Biotechnol. Lett. **2008**, *30*, 2091–2096.

[8] Heinfling, A.; Martinez, M.J.; Martinez, A.T.; Bergbauer, M.; Szewzyk, U. Purification and characterization of peroxidases from the dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol. Letts. **1998**, *165*, 43–50.

[9] Keharia, H.; Madamwar, D. Transformation of textile dyes by white-rot fungus *Trametes versicolor*. Appl. Biochem. Biotechnol. **2002**, *102*, 99–108.

[10] Novotný, Č.; Cajthaml, T.; Svobodová, K.; Šušla, M.; Šašek, V. *Irpex lacteus*, a white-rot fungus with biotechnological potential - review. Folia Microbiol. **2009**, *54*, 375–390.

[11] Paszczynski, A.; Pastigrigsby, M.B.; Goszczynski, S.; Crawford, R.L.; Crawford, D.L.; Mineralization of sulfonated azo dyes and sulfanilic acid by *Phanerochaete chrysosporium* and *Streptomyces chromofuscus*. Appl. Environ. Microbiol. **1992**, *58*, 3598–3604.

[12] Novotný, Č.; Rawal, B.; Bhatt, M.; Patel, M.; Šašek, V.; Molitoris, H.P. Capacity of *Irpex lacteus* and *Pleurotus ostreatus* for decolorization of chemically different dyes. J. Biotechnol. **2001**, *89*, 113–122.

[13] Kasinath, A.; Novotný, Č.; Svobodová, K.; Patel, K.C.; Šašek, V. Decolorization of synthetic dyes by *Irpex lacteus* in liquid cultures and packed-bed bioreactor. Enz. Microb. Technol. **2003**, *32*, 167–173.

[14] Pocedič, J.; Hasal, P.; Novotný, Č. Decolorization of organic dyes by *Irpex lacteus* in a laboratory trickle-bed biofilter using various mycelium supports. J. Chem. Technol. Biotechnol. **2009**, *84*, 1031–1042.

[15] Svobodová, K.; Erbanova, P.; Sklenar, J.; Novotný, Č. The role of Mn-dependent peroxidase in dye decolorization by static and agitated cultures of *Irpex lacteus*. Folia Microbiologica **2006**, *51*, 573–578.

[16] Svobodová, K.; Senholdt, M.; Novotný, Č.; Rehorek, A. Mechanism of reactive orange 16 degradation with the white rot fungus *Irpex lacteus*. Proc. Biochem. **2007**, *42*, 1279–1284.

[17] Svobodová, K.; Majcherczyk, A.; Novotný, Č.; Kües, U. Implication of mycelium-associated laccase from *Irpex lacteus* in the decolorization of synthetic dyes. Biores. Technol. **2008**, *99*, 463–471.

[18] Lee, H.; Choi, Y.-S.; Kim, M.-J.; Huh, N.Y.; Kim, G.-H.; Lim, Y.W.; Kang, S.M.; Cho, S.-T.; Kim, J.-J. Degrading ability of oligocyclic aromates by *Phanerochaete sordida* selected via screening of white rot fungi. Folia Microbiologica **2010**, *55*, 447–453.

[19] Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bact. **1990**, *172*, 4238–4246.

[20] White, T.J.; Bruns, T.D.; Lee, S.; Taylor, J.W.A. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to methods and applications*; Innis, M.A.; Gelfand, D.H.; Sninsky, J.J.; White, T.J., Eds.; Academic Press: New York, USA, 1990; 315–322.

[21] Novotný, Č.; Svobodová, K.; Kasinath, A.; Erbanová, P. Biodegradation of synthetic dyes by *Irpex lacteus* under various growth conditions. Int. Biodet. Biodeg. **2004**, *54*, 215–223.

[22] OECD. *Daphinia* sp., Acute immobilization test. Guideline for testing of chemicals, No. 202; 2004.

[23] Rigas, F.; Dritsa, V. Decolourisation of a polymeric dye by selected fungal strains in liquid cultures. Enzym. Microb. Technol. **2006**, *39*, 120–124.

[24] Glenn, J.K.; Gold, M.H. Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **1983**, *45*, 1741–1747.

[25] Zou, E.; Bu, S. Acute toxicity of copper, cadmium, and zinc to the water flea, Moina-Irrasa (Cladocera). Bull. Environ. Contam. Toxicol. **1994**, *52*, 742–748.

[26] Ekici, P.; Leupold, G.; Parlar, H. Degradability of selected azo dye metabolites in activated sludge systems. Chemosphere **2001**, *44*, 721–728.

[27] Olukanni, O.D.; Osuntoki, A.A.; Kalyani, D.C.; Gbenle, G.O.; Govindwar, S.P. Decolorization and biodegradation of Reactive Blue 13 by *Proteus mirabilis* LAG. J. Hazard. Mater. **2010**, *184*, 290–298.