Mycobiology 37(1) : 53-61 (2009)

Screening of Biodegradable Function of Indigenous Ligno-degrading Mushroom Using Dyes

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(Received November 11, 2008. Accepted December 23, 2008)

The process of biodegradation in lingo-cellulosic materials is critically relevant to biospheric carbon. The study of this natural process has largely involved laboratory investigations, focused primarily on the biodegradation and recycling of agricultural by-products, generally using basidiomycetes species. In order to collect super white rot fungi and evaluate its ability to degrade lingo-cellulosic material, 35 fungal strains, collected from forests, humus soil, livestock manure, and dead trees, were screened for enzyme activities and their potential to decolorize the commercially used Poly-R 478 dye. In the laccase enzymatic analysis chemical test, 33 white rot fungi and 2 brown rot fungi were identified. The degradation ability of polycyclic aromatic hydrocarbons (PAHs) according to the utilized environmental conditions was higher in the mushrooms grown in dead trees and fallen leaves than in the mushrooms grown in humus soil and livestock manure. Using Poly-R 478 dye to assess the PAH-degradation activity of the identified strains, four strains, including *Agrocybe pediades,* were selected. The activities of laccase, MnP, and Lip of the four strains with PAH-degrading ability were highest in *Pleurotus incarnates*. 87 fungal strains, collected from forests, humus soil, livestock manure, and dead trees, were screened for enzyme activities and their potential to decolorize the commercially used Poly-R 478 dye on solid media. Using Poly-R 478 dye to assess the PAH-degrading activity of the identified strains, it was determined that MKACC 51632 and 52492 strains evidenced superior activity in static and shaken liquid cultures. Subsequent screening on plates containing the polymeric dye poly R-478, the decolorization of which is correlated with lignin degradation, resulted in the selection of a strain of *Coriolus versicolor*, MKACC52492, for further study, primarily due to its rapid growth rate and profound ability to decolorize poly R-478 on solid media. Considering our findings using Poly-R 478 dye to evaluate the PAH-degrading activity of the identified strains, *Coriolus versicolor*, MKACC 52492 was selected as a favorable strain. *Coriolus versicolor*, which was collected from Mt. Yeogi in Suwon, was studied for the production of the lignin-modifying enzymes laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP).

KEWWORDS : Biodegradation, Mushrooms

Mushrooms have the ability to degrade ligninocellulosic substrates, which are produced from natural materials used in the agriculture, woodland, animal husbandry, and manufacturing industries (Rinker, 2002), and are frequently put in landfills or burned in the field at great cost to the environment (Anoliefo *et al*., 1999). Mushrooms have, for many years, been eaten and appreciated for their flavor, and also prized for their economic and ecological benefits, as well as their medicinal properties. They feature a chemical composition which is very attractive from a nutritional standpoint. Various mushrooms have a long history of use in folk medicine, and the higher Basiomycetes species have been the subject of a great deal of interest, owing principally to their multifarious nutritional and pharmacological properties.

The ligninolytic system of *Pleurotus* spp. has been studied extensively in recent years. Three ligninolytic enzyme families have already been characterized: MnP, versatile peroxidase (VP), and laccase (Sannia *et al*., 1991; Hatakka, 1994; Asada *et al*., 1995; Tuor *et al*., 1995; Giardina *et al*., 1996; Camarero *et al*., 1999; Cohen *et al*., 2001). These enzymes can be employed in a variety of biotechnological and environmental applications. *Pleurotus* spp. and their enzymes may function as an efficient alternative for the bioremediation of resistant pollutants, as compared with non-ligninolytic micro-organisms. *P. ostreatus* shows great promise in its ability to degrade and mineralize toxic chemicals, including polycyclic aromatic hydrocarbons (PAHs), atrazine, organophosphorus, and wastewaters. Lignin is the second most abundant plant-based biopolymer in the biosphere after cellulose, and accounts for up to 35% of the dry weight of woody tissue. It is an aromatic polydispersed polymer that provides plant cell walls with rigidity, water impermeability, and

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resistance against microbial attack. The structural features of this heterogeneous polymer impose unusual restrictions on its biodegradability. The initial attack must be extracellular, non-specific, and oxidative (Kirk and Farrell, 1987). White-rot fungi, belonging primarily to the basidiomycetes, are characterized by their ability to degrade quickly and efficiently the lignin moiety of woody tissues (Eriksson *et al*., 1990). Lignin degradation by fungi or other specific enzymes has previously been the focus of a large number of biotechnological studies. Different fungal species can either modify or degrade completely all the major components of wood. The most significant rates of Poly R478 decolorization in liquid cultures have been observed with the following isolates: *Trametes cingulata, Trametes versicolor, Trametes pocas*, DSPM95 (a species to be identified), *Datronia concentrica* and *Pycnoporus sanguineus* (Tekere *et al*., 2001).

Currently, a great deal of interest has been focused on the lignin-degrading fungi and ligninolytic enzymes, as their industrial potentials have been previously recognized in biomechanical pulping (Kashino *et al*., 1993), biobleaching (Paice *et al*., 1993; Katagiri *et al*., 1995; Ehara *et al*., 1997), and the degradation and detoxification of recalcitrant environmental pollutants including dioxins (Bumpus *et al*., 1985), chlorophenols (Joshi and Gold, 1993), nylon (Deguchi *et al*., 1997, 1998), polyethylene (Iiyoshi *et al*., 1998; Ehara *et al*., 2000), and dyes (Ollikka *et al*., 1993; Nishida *et al*., 1999). The principal objective of this study was to screen the lignin-degrading mushrooms indigenous to Korea, and to select the fungi that most potently degraded the lingo-cellulosic materials using a variety of chemical tests, enzyme assays, and physiological examinations.

Materials and Methods

Organisms. To select the super white rot fungi for our study of the degradation of ligno-cellulosic materials, 63 strains were collected from a variety of environmental samples obtained in Korea--forests with fallen leaves, humus soil, and dead tree stumps and logs--by the National Institute of Agricultural Scientific Technology, Rural Development Association. In addition, indigenous strains were immediately examined. The commercially available strain *Phanerochaete chrysosporium* ATCC64314 was utilized for comparison tests. A total of 81 strains were utilized for the screening of potent fungi (Table 1). *Phanerochaete chrysosporium*, a white rot basidiomycete, completely degrades wood (Kirk *et al.*, 1987). As cellulose constitutes 40 to 60% of all plant woody tissues, its degradation by *P. chrysosporium* is of great import. These organisms were maintained on potato dextrose agar (PDA)--at ambient temperature for those originating from tropical areas, and at 4°C for all others.

Table 1. Number of strains used in this study

Division	Total	Collected area			
		Forests	Humus soil	Dead tree stump and log	Etc.
Indigenous strains	83	24	8	21	10
Introduced strains	18	6	3	3	6
Total	81	30	11	24	16

Primary screening. Gum guaiac and syringaldazine are substrates for the enzyme system possessed by all lignin-degrading fungi. Different indicator compounds were added to the YMG agar plates in order to detect fungi that generated ligninolytic enzymes: 500 mg gum guaiac (Sigma) in 3 *ml* of 95% ethanol, 0.1% syringaldazine aqueous solution (Miller, 1988). A positive reaction of gum guaiac is a change in the reagent to blue-green, and syringaldazine is a pink to bright pink color. The positive reactions with different indicators were assessed by spot tests on fungal cultures.

Poly R-478 decolorization on solid cultures. YMG media were examined, all of which contained 0.02 percent (w/v) of the polymeric anthrapyridone chromophore poly R-478 (Sigma). A 2% (w/v) stock solution of poly R-478 was filter-sterilized and aseptically added at a final concentration of 0.2 g/liter to each of the previously autoclaved media. After fungal inoculation as described above, the diameters of growth and decolorization were measured daily.

Secondary screening. To assess the biodegradation ratio of fungi on liquid cultures, the primary screening was cultivated in a liquid YMG medium supplemented with 0.02% (w/v) poly R-478. Four mm-diameter agar plugs from 7-day-old cultures were utilized for the inoculation of 50 *ml* sterile medium in 125 *ml* Erlenmeyer flasks and incubated at 25°C in darkness. The medium was sterilized by 15 min of autoclaving at 121°C. The samples (1 *ml*) were collected on the 25th day, mycelial particles were pelleted at 12,000 rpm, and the supernatants were assayed for decolorization. On the selected days of incubation, extracellular culture fluid from three flasks was collected via filtration with Whatman filters (100-mm diameter) and assayed for decolorization.

Poly-R 478 decolorization on liquid media under culture conditions. The chemical structures and visible spectra of Poly R are provided in Fig. 1-③ (Gold, 1988). Fungi selected in the primary screening were cultivated in a liquid YMG medium supplemented with 0.02% (w/v) poly R-478, under static and agitated conditions (rotary shaker-150 rpm, 2.5 cm diameter circle). The composition of the culture medium was identical to YMG but without

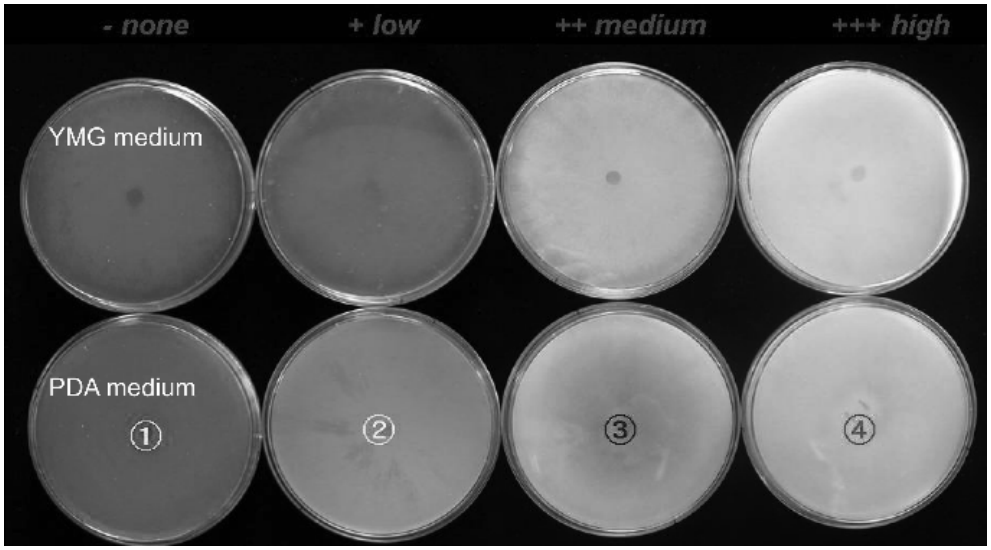

Fig. 1. Degradation activity of polycyclic aromatic hydrocarbon (PAH) with Poly-R 478 dye by the selected strains. ① *Mamasmius androsaceus*, MKACC51196; ② *Collybia susaquosa*, MKACC50146; ③ *Crinipellis stypitaria*, MKACC51272; ④ *Coriolus versicolor*, MKACC52492.

the addition agar. In order to prepare the fungal inocula, the mycelia obtained from a 40 *ml*, 7-day-old agitated culture (150 rpm) on YMG was washed twice in distilled water; 10 *ml* of distilled water was then added to the washed material and the mycelia were fragmented with 2 g of homogenizer (Junsei, Japan). Static cultures (25 *ml* in 250 *ml* flasks) and agitated cultures (50 *ml* in 250 *ml* flasks) were inoculated with 2 *ml* of the suspension. The flasks were closed with silicone caps and incubated for seven days at 25°C. The results were expressed by reference to controls (non-inoculated flasks) treated and incubated under identical conditions. Samples of the extracellular fluids (1 *ml*) were taken and centrifuged (5 min, 12,000 rpm). Poly R-478 decolorization was assessed by decreases in the absorbance ratio (OD_{520}/OD_{350}) after a 10-fold dilution in water (Jaouani, 2003). The decolorization ratio (D.R.) was defined as the absorbance ratio of the sample divided by the absorbance ratio of the control. Decolorization was measured by the decrease in absorbance at different wavelengths of the visible spectrum after a 50-fold dilution, on the 3th, 6th, 12th, and 15th day.

Assay of lignin-modifying enzymes. Enzymes were produced in shallow stationary cultures using the liquid YMG medium. 4 mm-diameter agar plugs from 7-day-old cultures were used to inoculate 50 *ml* sterile medium in 125 *ml* Erlenmeyer flasks, followed by incubation at 25°C in darkness. The medium was then sterilized by 15 min of autoclaving at 121°C. Samples (100 *ml*) were collected on the 15th day, the mycelial particles were pelleted at 12,000 rpm, and the supernatants were assayed for LMEs. On the selected days of incubation, extracellular culture fluid from three flasks was collected via filtration with Whatman filters (100-mm diameter) and assayed for LME activity. Laccase activity was determined by the method developed by Niku-Paavola *et al.* (1988). All enzymes were spectrophotometrically determined at room temperature with a Beckman DU-650. The laccase reaction mixture contained 14 mM 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) in 0.1 M glycine-HCl buffer at pH 3.0 in a final volume of 1.0 *ml*. The reaction was monitored by measuring the change in absorbance at 405 nm (D'souza, 1996). The enzyme units were expressed in nanokatals per liter. One katal is defined as one mole of product formed per second. MNP activity was determined by the method developed by Paszczynski *et al.* (1988) by monitoring the oxidation of Mn^{2+} to Mn^{3+}, whereas the LIP activity was determined by measuring the rate of H_2O_2-dependent oxidation of veratryl alcohol to veratraldehyde (Dass, 1990; Tien, 1988), and the activities were expressed as units per liter (shown as U/1 in the figures). One unit of enzyme is defined as 1 mmol of veratryl alcohol oxidized per min and 1 μmol of Mn(II) oxidized per min for LIP and MNP, respectively.

Determination of mycelial dry weight. Mycelial dry weights were determined by vacuum filtering the cultures with preweighed (110-mm-diameter) filter papers (Whatman, Inc., NO. 2). The filters containing the mycelial mass were placed in preweighed 50-mm-diameter aluminum pans and dried to constant weight at 80°C.

Results and Discussion

Primary screening. Reactions with the two different indicators, gum guaiacol and syringaldazine, were assessed

Table 2. Comparison of reaction of the fungal strains with different indicators on YMG agar plate

No.	Strains	Scientific name	Chemical test	
			Gum guaiac	Syringaldazin
1	MKACC 50028	*Coriolus versicolor*	+++	–
2	MKACC 50046	*Agaricus campestris*	+	+++
3	MKACC 50051	*Agrocybe arvalis*	+++	+++
5	MKACC 50104	*Collybia subaquosa*	++	–
6	MKACC 50118	*Daedalea dickinsii*	+	+
7	MKACC 50126	*Coprinus pilcatilis*	+	–
8	MKACC 50143	*Naematoloma sublateritium*	++	++
9	MKACC 50146	*Clitocybe nebularis*	+++	+
10	MKACC 50148	*Coriolus hirsutus*	+++	+++
11	MKACC 50149	*Psilocybe xeroderma*	+	+
12	MKACC 50164	*Gymnopilus spectabilis*	+	–
13	MKACC 50169	*Cordyceps nutans*	+++	+++
14	MKACC 50181	*Agaricus britorquis*	++	++
15	MKACC 50206	*Grifola frondosa*	+	–
16	MKACC 50215	*Ganoderma lucidum*	++	–
17	MKACC 50231	*Agrocybe erebia*	+	+
18	MKACC 50286	*Lycoperdon perlatum*	+	++
19	MKACC 50300	*Agrocybe semiorbicularis*	+	–
20	MKACC 50354	*Agaricus praeclaresquamosus*	+++	+
21	MKACC 50378	*Calvatia craniiformis*	++	++
22	MKACC 50522	*Cryptoporus volvatus*	+	–
23	MKACC 50544	*Lysurus mokusin f.sinensis*	–	–
24	MKACC 50551	*Oudemansiella mucida*	+++	+++
25	MKACC 50552	*Gymnopilus* sp.	+	+++
26	MKACC 50560	*Coriolus hirsutum*	+	+
27	MKACC 50568	*Lycoperdon perlatum*	+	–
28	MKACC 50619	*Coriolus brevis*	+++	+++
29	MKACC 50634	*Collybia confluens*	+++	+++
30	MKACC 50635	*Collybia dryophilla*	+	+++
31	MKACC 50651	*Clitocybe irina*	+	–
32	MKACC 50653	*Agrocybe aegerita*	–	++
34	MKACC 50733	*Collybia butylacea*	++	+
35	MKACC 50873	*Clitocybe hydrogramma* var. *alba*	+++	+++
36	MKACC 50878	*Naematoloma fascicurale*	+	+
37	MKACC 50881	*Collybia peronata*	+++	+++
38	MKACC 50894	*Coprinus domesticus*	+	+
39	MKACC 50896	*Coprinus stercoreus*	+++	–
40	MKACC 50902	*Coprinus sterquilinus*	+	++
41	MKACC 50911	*Coprinus gonophyllus*	+	+
42	MKACC 50951	*Coriolus versicolor*	+	+
43	MKACC 50976	*Armillaria mellea*	–	–
44	MKACC 51032	*Oudemansiella platyphylla*	+	–
45	MKACC 51042	*Cordyceps longissima*	–	–
46	MKACC 51043	*Agaricus subrutilescens*	+	+
47	MKACC 51047	*Hohenbuehelia myxotricha*	–	+
48	MKACC 51060	*Lycoperdon hiemail*	–	–
49	MKACC 51063	*Coriolus versicolor*	+++	+++
51	MKACC 51124	*Clitocybe nebularius*	+	++
52	MKACC 51141	*Daedaleopsis confrogosa*	++	+++
53	MKACC 51160	*Pleurotus incarnates*	+	–
55	MKACC 51170	*Agrocybe pediades*	+	–
56	MKACC 51173	*Agrocybe erebia*	+	+
57	MKACC 51180	*Agrocybe salicacola*	+	–
58	MKACC 51181	*Agrocybe dura*	+	++
59	MKACC 51182	*Coprinus rhizophorus*	+	++
60	MKACC 51183	*Coprinus picaceus*	+	+
61	MKACC 51184	*Coprinus radians*	+	+

Table 2. Continued

No.	Strains	Scientific name	Chemical test	
			Gum guaiac	Syringaldazin
62	MKACC 51192	*Psilocybe merdaria*	+	++
63	MKACC 51193	*Psilocybe coprophila*	+	–
65	MKACC 51195	*Psilocybe stuntzii*	+	++
66	MKACC 51196	*Mamasmius androsaceus*	+	–
69	MKACC 51208	*Stropharia rugosoannulata*	+	+
70	MKACC 51212	*Hohenbuehelia grisea*	+	+
71	MKACC 51213	*Phaeolus schweinitzii*	+	–
72	MKACC 51215	*fustulina hepatica*	+	++
73	MKACC 51267	*Inonotus mikadoi*	+++	+++
74	MKACC 51272	*Crinipellis stypitaria*	+++	+++
76	MKACC 51281	*Coprinus atramentarius*	+	+
77	MKACC 51290	*Collybia erythropus*	–	–
78	MKACC 51595	*Pleurotus eryngii*	++	++
79	MKACC 51606	*Pleurotus eryngii*	+++	+++
80	MKACC 51632	*Pleurotus ostreatus*	+++	+++
81	MKACC 52492	*coriolus versicolor*	+++	+++
82	MKACC 52494	*Coriolus versicolor*	+++	+++
83	MKACC 52495	*Coriolus versicolor*	+++	+++
84	MKACC 52496	*Coriolus versicolor*	+++	+++
85	MKACC 52497	*Coriolus versicolor*	+++	+++
86	MKACC 52499	*Coriolus versicolor*	+++	+++
88	MKACC 53300	*Bjerkandera adusta*	+++	+++
89	MKACC 53301	*Bjerkandera adusta*	+++	+++

Table 3. Decolourization of Poly R 478 by white rot fungi in YMG media and PDA on day 7

Poly R 478 decolourization			
None (–)	Low (+)	Medium (++)	High (+++)
Agrocybe aegerita	*Agrocybe dura*	*Agrocybe semiorbicularis*	*Daedalea dickinsii*
Agaricus campestris	*Psilocybe xeroderma*	*Agrocybe pediades*	*Pleurotus incarnates*
Coprinus cinereus	*Coriolus hirsutum*	*Agrocybe salicacola*	*Coriolus hirsutus*
Gymnopilus spectabilis	*Coprinus sterquilinus*	*Agrocybe arvalis*	*Cordyceps nutans*
Lysurus mokusin f. *sinensis*	*Agrocybe dura*	*Collybia confluens*	*Agaricus praeclaresquamosus*
Lycoperdon hiemail	*Stropharia rugosoannulata*	*Naematoloma fascicurale*	*Coriolus brevis*
Coprinus lagopus	*Hohenbuehelia grisea*	*Daedaleopsis confrogosa*	*Collybia dryophilla*
Pleurotus mutilus	*Fustulina hepatica*	*Crinipellis stypitaria*	*Coriolus versicolor*
Coprinus rhizophorus	*Collybia subaquosa*		*Bjerkandera adusta*
Coprinus picaceus	*Naematoloma sublateritium*		*Pleurotus ostreatus*
Coprinus radians	*Grifola frondosa*		*Pleurotus eryngii*
Psilocybe merdaria	*Oudemansiella mucida*		
Psilocybe coprophila	*Gymnopilus* sp.		
Psilocybe subensis			
Psilocybe stuntzii			
Mamasmius androsaceus			
Asroeareachnoidea fisch			
Panaeolus separatus			
Phaeolus schweinitzii			
Coprinus pilcatilis			
Agaricus britorquis			
Ganoderma lucidum			
Calvatia craniiformis			
Cryptoporus volvatus			
Clitocybe irina			
Collybia butylacea			
Clitocybe hydrogramma			
Collybia peronata			

with the fungal strains on YMG agar plates. The positive reactions with gum guaiacol and syringaldazine correlated well among the very positive strains (+++), respectively. But MKACC50028, MKACC50146, MKACC 50354, and MKACC50896 evidenced a very positive reaction (+++) only with gum guaiac and not with syringaldazine (Table 2) and MKACC50046, MKACC50635, and MKACC51141 showed a very positive position only with syringaldazine. The MKACC50051, MKACC50148, MKACC50169, MKACC50551, MKACC50619, MKACC 50634, MKACC 50873, MKACC50881, MKACC51267, MKACC51272, and MKACC51606 strains evidenced strong positive reactions with all of the tested indicators. However, MKACC50544, MKACC50976, MKACC51042, MKACC51060, and MKACC51290 evidenced no reaction with any of the tested indicators.

Fungal strains evidencing positive reactions on gum guaiac indicator plates were identified as 73 strains from the collected samples, and 58 of the strains evidenced a positive reaction on syringaldazine.

Poly R-478 decolorization on solid culture. 81 fungi, including 18 introduced strains, were screened for their potential to degrade poly R-478. A total of 53 fungal strains decolorized the polymeric dye after 7 days of growth on the YMG and PDA medium supplemented with 0.02% poly R-478. 11 strains degraded poly R-478 very positively (+++), but 29 strains, including *Agrocybe aegerita*, appeared negative as compared to other strains. *Coriolus hirsutus*, *Cordyceps nutans*, *Coriolus brevis*, *Coriolus versicolor*, and *Pleurotus eryngii* evidenced profoundly positive reactions with all the tested indicators and poly R-478 degradation (Table 3, Fig. 1).

Secondary screening. When using Poly-R 478 dye to assay the PAH-degrading activity of selected strains, the tested fungi were classified into four groups according to their decolorization ability. 11 strains, including MKACC 50169, were classified in group A. However, only two strains, MKACC50635 and 50354, were classified in group D (Table 4). The control strain, ASI89003, was included in group B. Most of the fungi, including groups B, C, and D yielded positive decolorization results on liquid culture. Fig. 2 shows that the visible spectra of fluids of the fungi grown in the liquid medium contained Poly-R. The curve of the non-inoculated control was similar to the

Table 4. Grouping of selected fungal strains based on the patterns of poly R-478 decolorization shown by the spectral analysis of their fluids from the dye-containing cultures

Group A	Group B	Group C	Group D
MKACC 50169, 50118, 50300, 51193, 51181, 51208, 51212, 50286, 51173, 50149, 51215	MKACC 51170, 51272, 51160, 51180, 51632, 52492, ASI89003	MKACC 50560, 50148, 50619, 50028, 51192, 51194	MKACC 50635, 50354

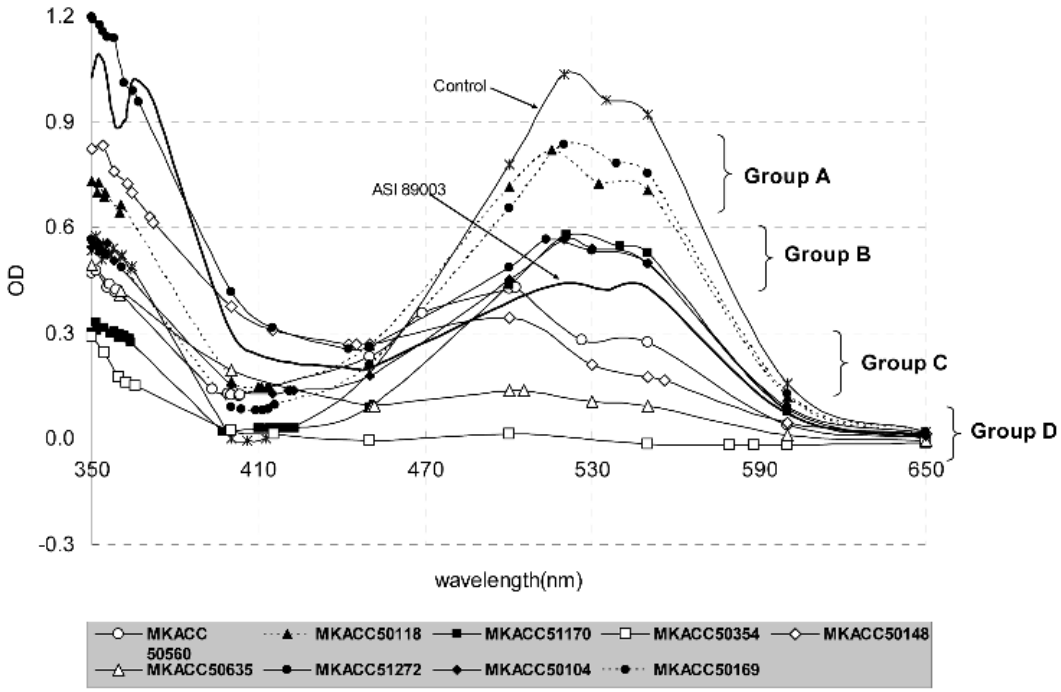

Fig. 2. Visible spectra of fluids from the fungal strains grown under the liquid medium containing Poly-R.

Table 5. Poly-R 478 decolorization by selected fungi on liquid media under shaken and static conditions

(Incubation periods: 15 days)

Strains	Static incubation		Shaking incubation	
	Decolourisation	Mycelial growth (mg/50 *ml*)	Decolorization	Mycelial growth (mg/50 *ml*)
MKACC50028	0.768	537	0.393	549
MKACC50118	0.757	497	0.441	579
MKACC52492	0.752	578	0.417	772
MKACC51595	0.751	531	0.685	460
MKACC51170	0.748	495	0.218	767
MKACC50635	0.725	420	0.518	422
MKACC50619	0.711	460	0.839	564
MKACC50148	0.709	607	0.808	312
MKACC51632	0.693	578	0.238	857
MKACC51160	0.660	292	0.339	551
MKACC50560	0.613	601	0.392	558
MKACC52499	0.600	589	0.380	429
MKACC51272	0.542	705	0.837	409
MKACC50104	0.400	305	0.502	297

*Decolorization ratio was defined as the absorbance ratio (OD_{520}/OD_{350}) of the sample/ the absorbance ratio of the control.

results of Gold (1988).

Poly-R 478 decolorization on liquid media under culture conditions. Using the Poly-R 478 dye to assay the PAH-degrading activity of the identified strains, decolorization by MKACC50104 proved most effective under static conditions and MKACC51170 proved most effective in shaking liquid cultures. On the other hand, MKACC 50028 evidenced the poorest results under static conditions and MKACC50619 evidenced the poorest results in shaking liquid cultures. The degradation of the majority of the selected fungi was more prominent in shaking liquid cultures than in static cultures (Table 5). However, MKACC 50104, MKACC 50148, MKACC50619, and MKACC 51272 evidenced better results in the static cultures. The mycelial growth of fungi was also evaluated under different culture conditions. The mycelial growth of MKACC 52492 was the best on liquid culture media under shaken conditions, and the following strain was MKACC51170. In the static liquid cultures, the MKACC51272 evidenced good mycelial growth. Particularly, MKACC51170 generated good results for both decolorization and mycelial growth. Fig. 3 evidenced poly-R 478 decolorization on liquid media under shaken and static conditions by the selected fungi.

In order to assess the degradation of poly-R 478 on liq-

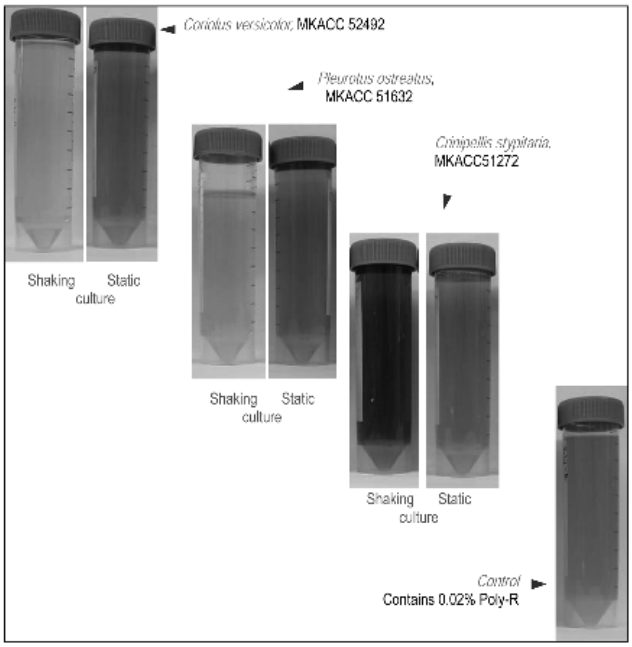

Fig. 3. Poly-R 478 decolorization by selected fungi on liquid media under shaken and static conditions.

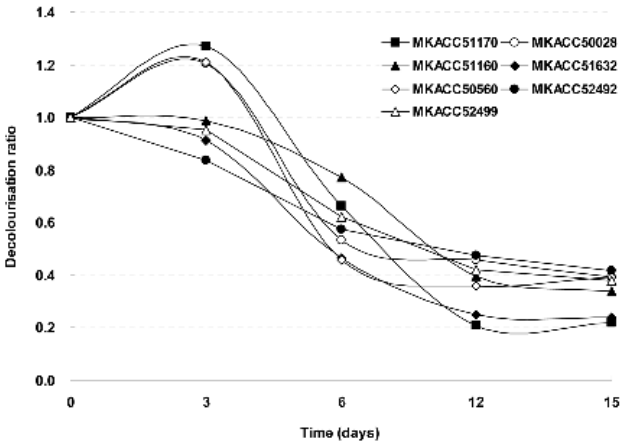

Fig. 4. Poly-R 478 decolorization on liquid media under shaken conditions by selected fungi. The decolorization ratio was defined as the absorbance ratio (OD_{520}/OD_{350}) of the sample/ the absorbance ratio of the control.

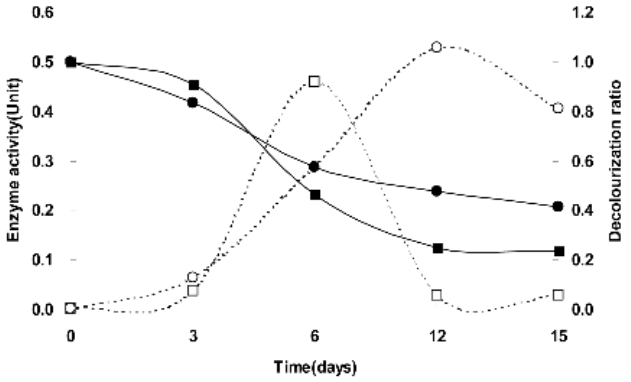

Fig. 5. Typical profile of the Poly-R 478 decolorization correlated with laccase activity by *Coriolus versicolor* (MKACC 52492) and *Pleurotus ostreatus* (MKACC 51632). (■), decolorization ratio of *P. ostreatus*; (□), laccase activity of *P. ostreatus*; (●), decolorization ratio of *C. versicolor*; (○), laccase activity of *C. versicolor*.

uid media by the selected fungi over a given course of time, the decolorization ratios were assessed. As a result, the degradation of the majority of strains steeply increased until the sixth day, and slightly increased after 12 days (Fig. 3). In general, decolorization coincided with the synthesis of oxidative enzymes (Jaouani, 2003) as shown in Fig. 4. The laccase activity of the MKACC 51635 strain reached a maximum on the sixth day and the laccase activity of the MKACC 52492 strain reached a maximum on the 12th day.

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