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The Role of Enzymes Produced by White-Rot Fungus *Irpex lacteus* in the Decolorization of the Textile Industry Effluent

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The textile industry wastewater has been decolorized efficiently by the white rot fungus, *Irpex lacteus*, without adding any chemicals. The degree of the decolorization of the dye effluent by shaking or stationary cultures is 59 and 93%, respectively, on the 8th day. The higher level of manganese-dependent peroxidase (MnP) and non-specific peroxidase (NsP) was detected in stationary cultures than in the cultures shaken. Laccase activities were equivalent in both cultures and its level was not affected significantly by the culture duration. Neither lignin peroxidase (LiP) nor Remazol Brilliant Blue R oxidase (RBBR ox) was detected in both cultures. The absorbance of the dye effluent was significantly decreased by the stationary culture filtrate of 7 days in the absence of Mn (II) and veratryl alcohol. In the stationary culture filtrate, three or more additional peroxidase bands were detected by the zymogram analysis.

***Key words*:** decolorization, peroxidases, textile industry wastewater, *Irpex lacteus*

The extracellular ligninolytic enzyme system of white-rot fungi can degrade a wide variety of recalcitrant compounds, such as xenobiotics, lignin, and various types of dyes (Paszczynski and Crawford, 1995). The advantages of the ligninolytic peroxidases are that they are substrate non-specific, extracellular, and tolerant to pollutants at high concentrations. They thus can degrade various recalcitrant compounds, even the complex mixture of pollutants. Three extracellular enzymes that can biodegrade dyes have been identified: lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and manganese independent peroxidases (MiP) (Field *et al.*, 1993). Laccase is another extracellular enzyme that can biodegrade dyes (Field *et al.*, 1993; Reddy, 1995). White-rot fungus, *Irpex lacteus*, has been reported to degrade lignin, polycyclic aromatic hydrocarbons (PAHs), and numerous synthetic dyes in shallow stationary cultures (Capelari and Zadražil, 1997; Novotný *et al.*, 2000; Novotný *et al.*, 2001; Kasinath *et al.*, 2003). In addition, the fungus is able to degrade the anthraquinone dye in the contaminated soil (Bhatt *et al.*, 2000) as well as decolorizes various types of synthetic dyes in aqueous cultures and packed-bed bioreactors (Kasinath *et al.*, 2003).

The dye effluent from the textile industry contains the mixture of various dyes that are usually aromatic and difficult to degrade. Such dye effluent was treated conventionally by the activated sludge process with mixed bacteria. The efficacy of such process is low. Some anaerobic microorganisms can degrade dyes by their azoreductase activity but the effluent could be toxic (Brown and DeVito, 1993; Chung and Stevens, 1993). These problems limit the application of bacteria in the decolorization of dyes.

In this study, the efficacy of *I. lacteus* on the decolorization of the textile industry effluent was investigated by measuring the ligninolytic enzyme activity.

Materials and Methods

Fungus strain and culture conditions

The *I. lacteus* strain KR 35W was provided by Prof. Kyu-Jung Kim, from Kangnung National University. The strain was maintained on MGPY agar slants (1% malt extract, 1% glucose, 0.5% peptone, and 0.5% yeast extract) at 4°C. 20 ml textile industry wastewater in 250 ml Erlenmeyer flasks was incubated in the state of stationary or shaking at 28°C. The wastewater sample was obtained from the Busan Dyeing Industry Complex in March 2003. The fungus was grown, without shaking, for 1 week in the MGPY medium, preinoculated with four 0.9 cm mycelium-covered discs that had been obtained from a fresh MGPY agar culture. The liquid inoculum was gently homogenized, and used at 10% (v/v) dilution.

Enzyme assays

The laccase activity was determined by the oxidation of 2,2-

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azino-bis(3-ethylbenzthiazoline)-6-sulfonate (ABTS) (Wolfenden and Willson, 1982). The MnP activity was determined by the method of Wariishi *et al.* (1992). The LiP activity was assayed as described by Tien and Kirk (1984), using veratryl alcohol as the substrate. The RBBR oxidase (RBBR Ox) was assayed by the method of Shin *et al.* (1997). The non-specific peroxidase (NsP) activity was measured using 0.5 mM *o*-dianisidine as the substrate, in the presence of 4 mM H_2O_2 in 50 mM of sodium acetate buffer, pH 5.5 (Claiborne and Fridovich, 1979). One enzyme unit was defined as the activity producing 1 μmol of the products per min (laccase, MnP, LiP, NsP) or the consumption of 1 μmol substrate per min (RBBR Ox) under the assay conditions.

Colorimetric analyses

The decolorization rate of wastewater was determined by measuring the optical density at 600 nm. The spectrum change of the filtered dyes effluent due to the generation of MnP was recorded after 10 min with 7-day-old culture filtrate from the stationary cultures, in the presence of 0.5 mM $MnSO_4$, 0.4 mM H_2O_2 and 50 mM sodium malonate (pH 4.5). The spectrum change caused by the production of NsP was recorded after 10 min, in the presence of 4 mM H_2O_2 and 50 mM sodium acetate buffer (pH 5.5), with the 7-day-old culture filtrate from the stationary cultures.

Peroxidase activity

The discontinuous non-denaturing electrophoresis in a slab gel was carried out as described previously (Davis, 1964). The gel was soaked in the solution of 50 mg *o*-dianisidine dissolved in 100 ml of 50 mM sodium acetate buffer (pH 5.5) with 0.75 ml of 3% H_2O_2. The gel was incubated at room temperature until a brown band appeared (Claiborne and Fridovich, 1979).

Results and Discussion

Characteristics of the textile industry wastewater

The textile industry wastewater contains various recalcitrants: dyes (reactive, acid, metal complex, disperse), detergents (non-ionic, anionic), sizing agents (polyvinyl alcohol, polyester, starch, carboxyl cellulose), bleaching agents, oxidizing and reducing agents, thickening agent, and auxiliaries (complexing agents, dispersing agents, levelling agents, emulsifiers, wetting agents). The characteristic of the textile industry wastewaters varies widely. The physicochemical property of the wastewater used in this experiment was as follows: BOD; 330±8 ppm, COD; 370 ±10 ppm, SS (Suspended solid); 360±4 ppm, pH; 9.9±0.6.

Decolorization of textile industry wastewater

In the initial period of culture, the decolorization rate in the stationary culture was not different from that in the culture shaken. After three days, the decolorization was more rapid in the stationary culture than in the shaken ones. The stationary culture removed 90% of color and the shaken culture removed 40% (Fig. 1). The anthraquinone based RBBR was decolorized efficiently in both cultures by *I. lacteus* (Kasinath *et al.*, 2003). Malachite Green was degraded equally efficiently in the stationary and the shaken cultures

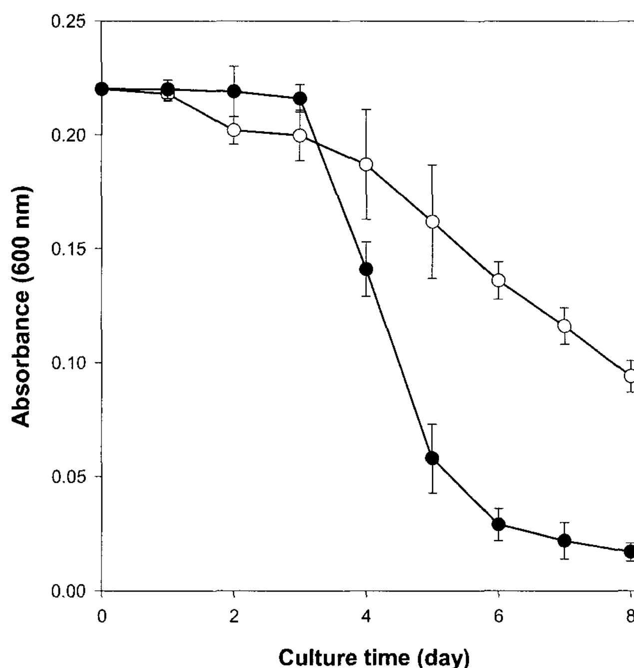

Fig. 1. Decolorization of dye effluents in shaken (open circle) and stationary cultures (closed circle) of *I. lacteus*.

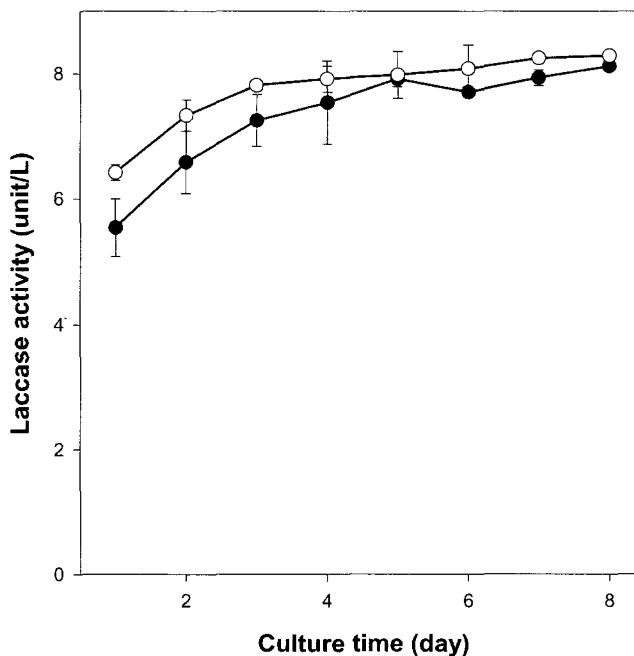

Fig. 2. Laccase activities of shaken (open circle) and stationary cultures (closed circle) of *I. lacteus* grown in textile industry wastewater.

of *Phanerochaete chrysosporium* (Sani *et al.*, 1998). However, the degradation of the structurally similar dye Crystal Violet by *P. chrysosporium* was five times more efficient in the shaken culture than in the stationary culture (Sani *et al.*, 1998). It was speculated that the decolorization mechanism of the dyes effluent by *I. lacteus* is different from the mechanism mentioned above because of the complexity of the textile industry wastewater.

Enzyme production

The LiP and RBBR Ox activities were negligible in the sample, suggesting that these enzymes play only a negligible role in the decolorization of the dyes effluent. Although fungal LiP has been repeatedly implicated in the bleach of a diverse range of synthetic dyes (Ollikka *et al.*, 1993; Sayadi and Ellouz, 1995; Young and Yu, 1997), its activity was not detected in any of the *I. lacteus* cultures in the presence of various synthetic dyes (Novotný *et al.*, 2001). Our results are in agreement with these observations. The laccase activities are shown in Fig. 2. The difference in the two culture systems was not different significantly. This indicates that laccase in not involved in the decolorization of the dye effluent. In contrast, laccase was detected when *Trametes modesta* was used for the decolorization of the textile dyes (Nyanhongo *et al.*, 2002).

The MnP activity was shown to correlate to the dyes

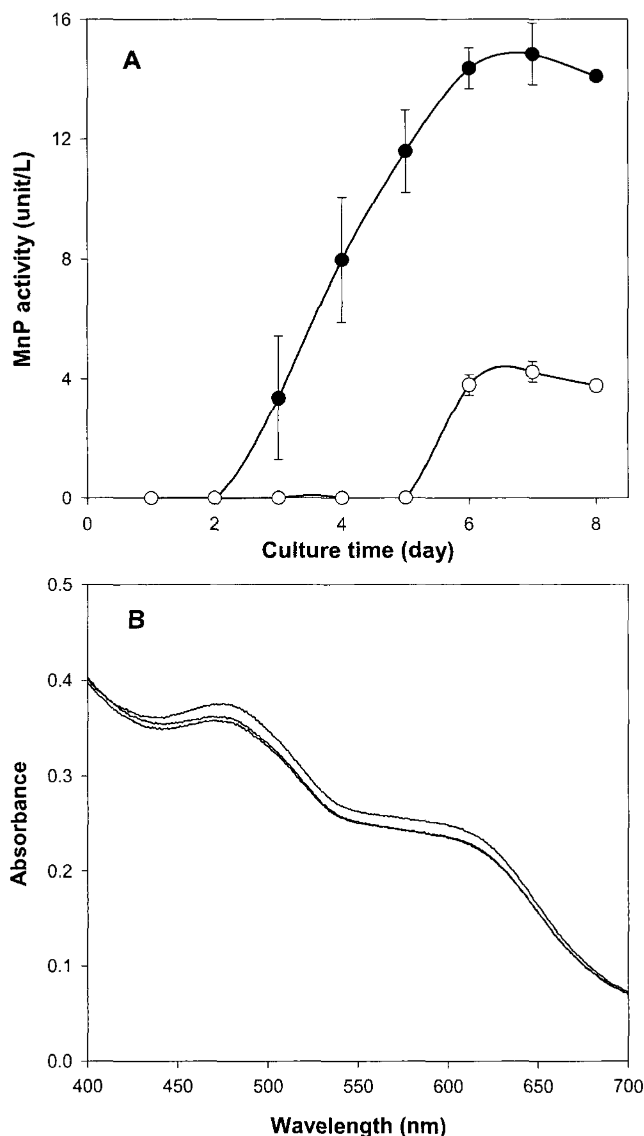

Fig. 3. MnP activities of shaken (open circle) and stationary cultures (closed circle) of *I. lacteus* (A), and spectra of textile industry wastewater (B). The spectra were recorded with 7-day old culture filtrate of stationary cultures in the presence of $MnSO_4$, H_2O_2 and sodium malonate (pH 4.5) after 10 min. Without enzyme (upper), without H_2O_2 (middle), with H_2O_2 (lower).

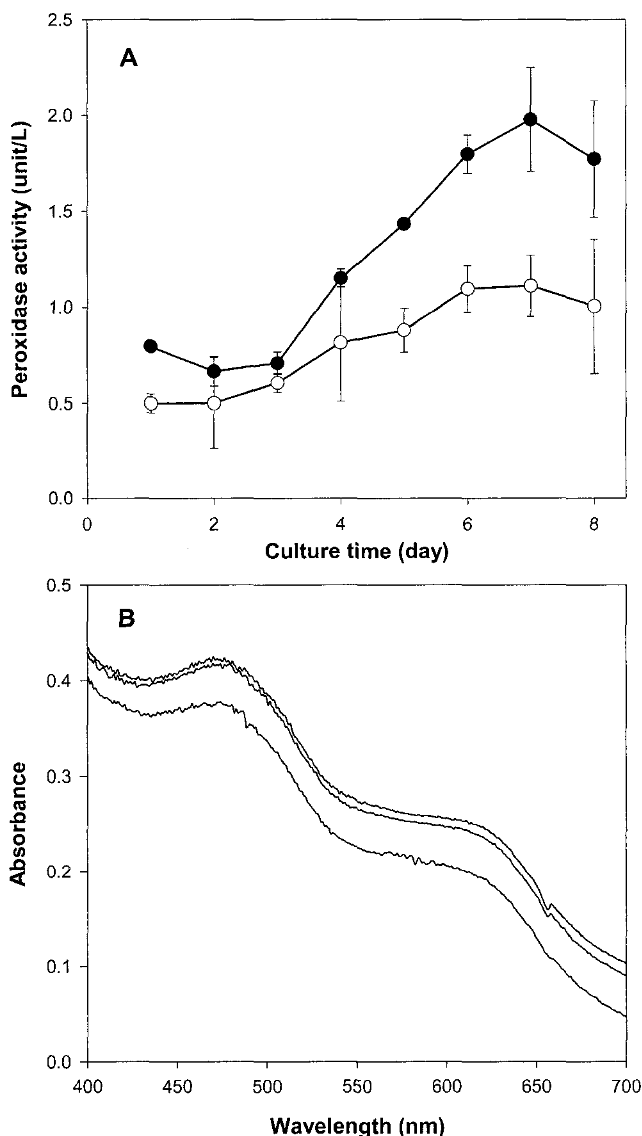

Fig. 4. NsP activities of shaken (open circle) and stationary cultures (closed circle) of *I. lacteus* (A), and spectra of textile industry wastewater (B). The spectra were recorded with 7-day old culture filtrate of stationary cultures in the presence of 4 mM of H_2O_2 and 50 mM of sodium acetate (pH 5.5) after 10 min. Without enzyme (upper), without H_2O_2 (middle), with H_2O_2 (lower).

decolorization. The MnP activity in the stationary cultures was about 4-fold higher than in the shaken cultures. The MnP activity reached the maximum after in 7 days in both cultures (Fig. 3A). With the 7 day old culture filtrate, however, in the presence of 0.5 mM $MnSO_4$, 0.4 mM H_2O_2 and 50 mM sodium malonate (pH 4.5), the decrease in the absorbance spectrum at the visible wavelength of the dye effluent was minimal (Fig. 3B). The result indicates that MnP may be involved in the degradation of textile dyes. Other enzymes may be also involved in the degradation as postulated by Swamy and Ramsay (1999). Presently, the exact role of MnP from *I. lacteus* in the decolorization of dyes is controversial. The MnP level in the *I. lacteus* was reduced in the presence of various chemically different dyes (Novotný *et al.*, 2001) while the decolorization rate of the RBBR decreased by two-fold in the presence of MnP inhibitors in the same fungus (Kasinath *et al.*, 2003).

Our data show the correlation between the decolorization of the dye effluent and the production of NsP (Fig. 4A). Even in the absence of Mn (II) or veratryl alcohol, the absorbance of the effluent was significantly decreased by 7-day old stationary culture filtrate (Fig. 4B), suggesting that NsP plays an important role in the decolorization of the dye effluents.

Zymogram analysis

To identify the amount and the number of peroxidases produced in cultures, the culture filtrate was applied to the non-denaturing polyacrylamide gel electrophoresis and stained with *o*-dianisidine in the presence of H_2O_2, at pH 5.5. As shown in Fig. 5, only one peroxidase band appeared in the shaken culture filtrate while three or more bands appeared in the stationary culture filtrate. The role of the peroxidases in the decolorization of the dye effluents is not clear yet. We, however, speculated that in the stationary culture, NsP might be involved in the decolorization of the textile industry wastewater.

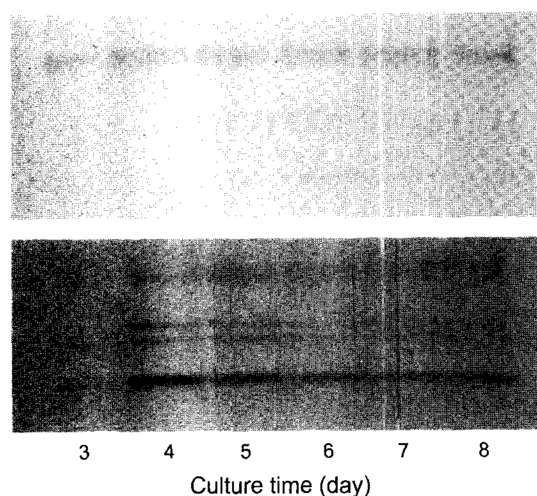

Fig. 5. Zymograms of NsP, from the culture filtrate of shaken (upper) and stationary (lower) cultures, in textile industry wastewater. Activity staining was performed with *o*-dianisidine.

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