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Improvement of lignin-degrading enzymes production from the white-rot fungus (*Lentinus strigosus*) and its application in synthetic dye removal

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Cultivation of *Lentinus strigosus*, a white-rot fungus producing a ligninolytic enzyme, in a simple medium indicated the production of Lac and MnP in the secondary growth phase. The aim of this study was to optimize the nutrient composition for Lac and MnP production as well as to investigate the removal of synthetic dyes by the strain. Preliminary study showed cellobiose and L-asparagine as the suitable nutrient sources for highest Lac and MnP production. To enhance the production of these enzymes, the L₉ Taguchi design was employed to optimize the culture medium composition. The optimized medium (1 L) containing 15 g cellobiose, 0.3125 g peptone, 1.2500 g L-asparagine and 0.002 g CuSO₄ gave Lac and MnP activities of 33,955.83 and 3,764.57 U L⁻¹, respectively. The data also suggested L-asparagine had the largest influence on enzymes production. Further study showed that 0.9375 g L⁻¹ L-asparagine was the best concentration, giving maximum Lac (35,977.76 U L⁻¹) and MnP (1,838.49 U L⁻¹) activities. Removal of different structural synthetic dyes by *L. strigosus* was investigated. Less than 6% RBBR remained following 48 h of fungal cultivation, whereas RB5 was more slowly removed, requiring 96 h for 98% removal. Maximum removal (82%) of Indigo 4B was achieved in 8 d which was the slowest among the tested dyes. Furthermore, it was found that the increasing percentage of dye removal was in accordance with the increase in enzymes activities. In summary, it was clear that *L. strigosus* efficiently removed these dyes, and Lac and MnP were considered as the major enzymes involved in this process.

Key words: *Lentinus strigosus*, white-rot fungus, laccase, manganese peroxidase, reactive dye.

INTRODUCTION

*Lentinus strigosus*, an edible mushroom mostly found clustered on the logs and stumps of deciduous trees, belongs in the group of white-rot basidiomycetes (Yamaç et al., 2008). The white-rot fungi, such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus*, *Ganoderma spp.*, *Irpex lacteus*, *Dichomitus squalens* and *Ischnoderma resinosum*, have been widely demonstrated to be able to degrade numerous hazardous components, including synthetic dyes (Jeffries et al., 1981; Eichlerová et al., 2005; Lopez et al., 2007; Svobodová et al., 2007). Young and Yu (1997) considered the mechanism to occur through the oxidation of the aromatic or phenolic groups in the dyes by the extracellular ligninolytic enzymes-namely, lignin
peroxidase (LiP), laccase (Lac) and manganese peroxidase (MnP) (Young and Yu, 1997). The fungi usually secrete one or more of these three enzymes that are essential for the degradation of their natural growth substrate—namely, lignin. The potential application of ligninolytic enzymes in biotechnology, such as textile dye bleaching (Bajpai, 1999), pulp delignification (Breen and Singleton, 1999) and wastewater detoxification (Baldrain and Snajdr, 2006), has stimulated the investigation of novel, promising enzyme producers as well as the optimization of their culture media in order to significantly improve the production of enzymes.

The expression of ligninolytic enzymes is known to be influenced by the culture conditions including the types and concentrations of carbon and nitrogen sources, the pH of culture medium and the presence of inducers. Identifying the medium composition to maximize the enzyme yield in minimally controlled batch fermentation is therefore important. Traditionally, fermentation processes are optimized by changing one independent variable or factor at a time while keeping the others at some fixed values. This single dimensional search is slow and laborious, especially if a large number of independent variables are involved. Consequently, statistical methods are increasingly preferred for fermentation optimization because they reduce the total number of experiments needed and provide a better understanding of the interactions among factors on the outcome of the fermentation (Revankar and Lele, 2006a). Statistical techniques such as the Taguchi method have gained broad acceptance in fermentation optimization (discussed below). The Taguchi method of orthogonal array experimental design involves the study of a given system by changing the values (or levels) of a set of independent variables (or factors) over the range of interest. For a given number of independent variables tested at a given number of levels, the Taguchi method specifies orthogonal arrays for combining the various variables and levels in the minimum acceptable number of experimental trials. This method determines the optimal levels of the important controllable factors based on the concept of robustness and “signal-to-noise” (S/N) ratio (Roy, 1990). The desired design is sought by selecting the best performance under conditions that produce a consistent performance. The conclusions drawn from the experiments are valid over the entire experimental space spanned by the levels of the controlled factors (Phadke and Dehndad, 1988). Whereas the traditional experiment design focuses on the average process performance characteristics, the Taguchi method concentrates on the effect of variation on the process characteristics. In addition, the Taguchi method approach facilitates the identification of the influence of individual factors and interactive effects of factors on performance with a few well-defined experimental sets (Prasad et al., 2005). Many previous studies have proved that the Taguchi method allows the rapid construction of accurate models for optimization. Prasad et al. (2005) applied the Taguchi method for the production of Lac by *P. ostreatus* 1804. Eight factors (pH, glucose, wheat bran, urea, inoculum, yeast extract, KH$_2$PO$_4$ and inducer concentrations) with three levels were assigned for the culture condition optimization. The optimized conditions showed an enhanced Lac production of 32.3% (from 538.8 to 803.3 U). In 2009, the Taguchi method was used for the optimization of growth media for Lac production by *Cryptococcus albides* (Singhal et al., 2009). Five factors (pH, CuSO$_4$, glucose, meat peptone and inducer) at four levels were designed. After optimization, Lac production increased seven times from 32 to 219 IUmg$^{-1}$. Periasamy and Palvanann (2010) optimized eight factors (pH, glucose, yeast extract, malt extract, mineral solution, inoculum, inducer and amino acid concentrations) influencing Lac production by *P. ostreatus* IMI395545 by the Taguchi method. An improved production of Lac by 86.8% (from 485.0 to 906.3 U) resulted from the optimized conditions (Periasamy and Palvanann, 2010).

The present study aimed to apply the Taguchi method for the optimization of culture medium for the production of ligninolytic enzymes by the white-rot fungus *L. strigosus*. The direct influence of these ligninolytic enzymes on the decolorization of reactive dye solution was also assessed.

**MATERIALS AND METHODS**

**Microorganism and culture conditions**

A culture of white-rot fungus, *L. strigosus*, obtained from the Biotechnology Research and Development Office, Department of Agriculture, Thailand, was used in this study. The fungal stock culture was maintained through a periodic transfer on potato dextrose agar (PDA) at 4°C until use. To prepare the inoculum, the fungus was transferred onto a fresh PDA plate and incubated at 30°C for 5 d. The agar disks (with a diameter of 7 mm and taken from the growing edge of a 5-day-old agar culture) were inoculated into 200 mL minimal medium (MM; composed of the following in gL$^{-1}$: glucose 10.0; K$_2$HPO$_4$ 1.0; MgSO$_4$$\cdot$7H$_2$O 0.5; KCl 0.5; FeSO$_4$$\cdot$7H$_2$O 0.1; MnSO$_4$$\cdot$4H$_2$O 0.008; Zn (CH$_3$COO)$_2$ 0.003; Ca(NO$_3$)$_2$$\cdot$4H$_2$O 0.006; CuSO$_4$$\cdot$5H$_2$O 0.003; at pH 5.5) and incubated at 30°C with shaking at 150 rpm. Samples were periodically taken for the analysis of cell dry weight and the activities of laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP). All experiments were performed in triplicates.

**Enzymes assay**

The enzyme assay was carried out using the culture filtrate sampled at intervals from each experiment. The culture liquids were centrifuged at 10,000 x g for 20 min at 4°C. The assay was performed in triplicate. Lac activity was measured by monitoring the oxidation of 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 420 nm (molar extinction coefficient = 36,000 M$^{-1}$cm$^{-1}$) according to (Eggert et al., 1996). One unit of laccase activity was defined as the amount of enzyme that oxidizes 1 μmol ABTS in 1 min. LiP activity was measured by monitoring the oxidation of veratryl alcohol in the presence of H$_2$O$_2$ at 310 nm (molar extinction coefficient = 9,300 M$^{-1}$cm$^{-1}$) according to (Tien and Kirk, 1984). One unit of LiP activity was defined as the amount of enzyme catalyzing...
The production of fungal enzymes is influenced by many culturing parameters such as medium composition. The first experiment focused on the types of carbon and nitrogen sources. The second experiment optimized the medium composition using the Taguchi methodology and the third experiment optimized the concentration of L-asparagine, in order to improve the production yield of Lac and MnP by L. strigosus. All experiments were performed in triplicate.

Effect of carbon and nitrogen sources on Lac and MnP production

To investigate the effect of the carbon source on the production of Lac and MnP, the production of enzymes was carried out in 200 mL MM. Eight sources of carbon were used: glucose, fructose, sucrose, maltose, lactose, cellobiose, soluble starch and glycerol. Yeast extract was fixed as the nitrogen source. The reactions were incubated at 30°C with shaking at 150 rpm. Samples were periodically taken for the analysis of cell dry weight and the activities of Lac and MnP.

Similarly, the effect of the nitrogen source on enzyme production was investigated in 200 mL MM. Yeast extract, beef extract, peptone, L-asparagine, NH₄NO₃, (NH₄)₂SO₄ and urea were applied as the nitrogen sources. The reactions were incubated at 30°C with shaking at 150 rpm. Samples were periodically taken for the analysis of cell dry weight and the activities of Lac and MnP.

Optimization of Lac and MnP production by Taguchi methodology

In this study, four factors at three levels of variations (Table 1) were used in the experiments. The factors optimized included the concentrations of cellobiose, peptone and L-asparagine. The concentration of copper sulfate was included in the optimization since the Cu²⁺ ion has been reported to affect the production of white-rot fungi Lac and MnP (Baldrian and Gabriel, 2002; Chen et al., 2003; Galhaup and Haltrich, 2001). The various combinations of factors and levels were in accordance with Taguchi’s L₉ orthogonal array. The factor level combinations for all the experiments are shown in Table 2. Submerged batch fermentations were conducted using various media formulations (5, 10 and 15 g/L cellobiose; 0.3125, 0.6250 and 1.25 g/L peptone; 0.3125, 0.6250 and 1.25 g/L L-asparagine and 0.004, 0.002 and 0.001 g/L CuSO₄ in 200 mL MM. The reactions were incubated at 30°C with shaking at 150 rpm. Samples were periodically taken for the analysis of Lac and MnP activities.
Optimization of L-asparagine concentration

The effect of L-asparagine concentration on the production of Lac and MnP was further investigated. The experiment was carried out in 200 mL MM containing 15.0 gL\(^{-1}\) cellulose, 0.3125 gL\(^{-1}\) peptone and 0.002 gL\(^{-1}\) CuSO\(_4\). The concentrations of L-asparagine were varied at 0.3125, 0.6250, 0.9375, 1.2500, 1.5625, 1.8750, 2.1875 and 2.500 gL\(^{-1}\). The reactions were incubated at 30°C with shaking at 150 rpm. Samples were periodically taken for the analysis of cell dry weight and the activities of Lac and MnP.

Decolorization of synthetic dye solution

The reactive dyes used in this study-namely, Remazol Brilliant Blue R (RBBR), Reactive Black 5 (RB5) and Indigo 4B, were obtained from Dy Star Thai Com., Ltd., Thailand. RBBR, RB5 and Indigo 4B are representatives of the synthetic dyes based on anthraquinone-, tetrasulphonated diazo- and indigoid-structures, respectively. Decolorization experiments were carried out in 500 mL flasks. The dye solutions of RBBR, RB5 and Indigo 4B (500 mgL\(^{-1}\) final concentration) were prepared in 200 mL of the optimized MM. Then, 20 agar disks of L. strigosus growing mycelia were inoculated followed by incubation at 30°C with 150 rpm shaking. Samples were periodically taken for the analysis of the percentage of decolorization and the activities of Lac and MnP.

RESULTS AND DISCUSSION

Fungal growth and activity of enzymes

The production of Lac, MnP and LiP by L. strigosus was confirmed as a liquid cultivation in MM as mentioned in the methodology. Figures 1a and b demonstrate the Lac and MnP activities, respectively, in relation to the fungal cell dry weights when L. strigosus was grown in MM at 30°C for 14 d. The fungal cell dry weight sharply increased during the primary growth phase from days 1 to 4, reached the secondary growth phase from days 4 to 6, and then became stationary and decreased on day 8 of cultivation. The activity of Lac was detectable in the primary growth phase, reached a maximum (669.81 UL\(^{-1}\)) after 6 d of cultivation, and tended to decrease on day 7 of cultivation. Similarly, MnP activity was clearly detected (34.04 UL\(^{-1}\)) in the secondary growth phase on day 7, even though this MnP level was far lower than that of Lac. High production levels of ligninolytic enzymes in the secondary growth phase of white-rot fungi was also reported by

Figure 1. Effect of time on the activities of (a) laccase (Lac) and (b) manganese peroxidase (MnP) with cell dry weight when L. strigosus was grown in minimal media at 30°C for 14 d. The vertical bars represent the standard deviation range for mean values. (laccase — , manganese peroxidase — , cell dry weight — )
Effect of carbon sources on the activities of Lac and MnP production

The effect of the carbon source on Lac and MnP production by L. strigosus is shown in Figures 2a and b, respectively. Among all tested carbon sources, the cultivation of L. strigosus in MM containing cellobiose yielded the highest activities of Lac and MnP (6,374.27 and 285.72 UL⁻¹, respectively) on day 4 of cultivation and they reached maximum activities on day 10 (6,947.26 and 409.49 UL⁻¹, respectively). The advantage of cellobiose for the production of fungal ligninolytic enzymes was supported by (Galhaup et al., 2002), who reported that the maximum Lac and MnP activities were achieved from the cultivation of Trametes pubescens MB89 in a basal medium containing cellobiose. Soluble starch, glucose and glycerol were also reported as optimum carbon sources for high production of Lac and MnP from Ganoderma sp. (Revankar and Lele, 2006a), Coriolus versicolor MTCC138 (Revankar and Lele, 2006b), Ganoderma sp. KU-Alk4 (Teerapatsakul et al., 2007), and Fomes fomentarius (Songulashvili et al., 2007). In some cases, a sequential addition of different carbon sources, such as fructose followed by glycerol, led to an increase in Lac and MnP production in Trametes hirsuta relative to glucose alone (Rodriguez et al., 2006). These findings imply that the carbon source appears to regulate Lac and MnP expression in white-rot fungi, and the activity of Lac and MnP can be increased by the choice of carbon source.

The nitrogen source also had a marked effect on Lac and MnP production as shown in Figures 3a and b, respectively. Supplementation with organic nitrogen sources, like L-asparagine, peptone and yeast extract, clearly showed higher production of Lac and MnP by L. strigosus than from inorganic nitrogen sources such as NH₄NO₃ and (NH₄)₂SO₄. Although, most studies reported that yeast extract, peptone and tryptone were often preferred nitrogen sources over L-asparagine, in the present study, L-asparagine was the best among all tested nitrogen sources for Lac and MnP production by L. strigosus. Replacement of L-asparagine with other nitrogen sources failed to enhance the production of Lac and MnP. Hou et al. (2004) showed the advantage of peptone and yeast extract for Lac production by P. ostreatus strain 32 (Hou et al., 2004). Revankar and Lele (2006a) reported that an organic nitrogen source was essential for Lac production by the white-rot fungus WR-1 and could not be substituted with an inorganic nitrogen source. Further, they found that yeast extract and peptone were the most appropriate nitrogen sources for Lac production by the white-rot fungus WR-1 and by C. versicolor MTCC 138, respectively (Revankar and Lele, 2006a; Revankar and Lele, 2006b). Lac production by Ganoderma sp. KU-Alk4 was also supported by yeast extract supplementation (Teerapatsakul et al., 2007). In some cases, higher production of Lac and MnP could be obtained from the cultivation of white-rot fungi in the presence of inorganic nitrogen sources. The highest Lac
production by *T. versicolor* 145 was obtained from a medium containing (NH₄)₂PO₄ compared with those containing casein hydrolysate or peptone (Mikiashvili et al., 2005). Elisashvili et al. (2001) showed that Lac activity in *Cerrena unicolor* IBB62 depended on the nitrogen source in the culture medium, and the highest Lac activity was observed in the medium with (NH₄)₂SO₄ (Elisashvili et al., 2001).

**Optimization of Lac and MnP production by Taguchi methodology**

From previous study, cellulose and L-asparagine were shown to be the best carbon and nitrogen sources, respectively, for Lac and MnP production from *L. strigosus*. They were further varied to determine the optimal concentration by the Taguchi method. Apart from the carbon and nitrogen sources, the concentration of CuSO₄ was also varied since there were reports that Cu²⁺ was able to induce the production of Lac and MnP by white-rot fungi (Galhaup and Haltrich, 2001; Baldrian and Gabriel, 2002; Chen et al., 2003).

Table 2 demonstrates the variation in Lac and MnP activities according to the experiments conducted based on the Taguchi method. The experimental data was analyzed using the Qualitek-4 software (Nutek, Inc., Bloomfield Hills, MI, USA), with the larger-the-better attribute selected for establishing the optimum fermentation medium composition and identifying the individual factors influencing Lac and MnP production. The average effect of the factors at the designed levels on Lac and MnP production by *L. strigosus* is shown in Table 3. The difference between levels 2 and 1 (L₂ - L₁) of each factor indicates the relative influence of the effects. The larger the difference, the stronger is the influence (Prasad et al., 2005). Among the studied factors influencing Lac and MnP production, L-asparagine showed the strongest influence (14,760.3 and 1,789.4, respectively) compared to other factors, followed by cellulose (11,246.6 and 1,227.3, respectively), CuSO₄ (9,163.7 and 1,064.6, respectively) and peptone (3,727.0 and 363.3, respectively). Figures 4a and b show the influence of each individual factor on Lac and MnP activities. An increase in the concentrations of cellulose and L-asparagine resulted in an increase in enzyme production. Interestingly, an increase in CuSO₄ concentration led to higher Lac and MnP activities up to level 2, but subsequent increases resulted in a decrease in enzymes activities. It has been reported that production of ligninolytic enzymes in fungal cultures is influenced by heavy metals that in turn affect the levels of

**Table 3. Main effects of selected factors on laccase (Lac) and manganese peroxidase (MnP) activities.**

| Factor       | Level 1 Lac | Level 1 MnP | Level 2 Lac | Level 2 MnP | Level 3 Lac | Level 3 MnP | L₂ - L₁
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</thead>
<tbody>
<tr>
<td>A: cellulose</td>
<td>7,390.4</td>
<td>882.6</td>
<td>10,693.7</td>
<td>1,292.8</td>
<td>18,636.9</td>
<td>21,099.9</td>
<td>11,246.6</td>
</tr>
<tr>
<td>B: peptone</td>
<td>14,252.3</td>
<td>1,578.0</td>
<td>10,525.3</td>
<td>1,214.7</td>
<td>11,943.4</td>
<td>1,492.5</td>
<td>3,727.0</td>
</tr>
<tr>
<td>C: L-asparagine</td>
<td>5,688.3</td>
<td>606.3</td>
<td>10,584.2</td>
<td>1,283.3</td>
<td>20,448.6</td>
<td>2,395.7</td>
<td>14,760.3</td>
</tr>
<tr>
<td>D: CuSO₄</td>
<td>11,206.7</td>
<td>1,417.3</td>
<td>17,338.9</td>
<td>1,966.3</td>
<td>8,175.3</td>
<td>901.7</td>
<td>9,163.7</td>
</tr>
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**Figure 3.** Effect of nitrogen sources on the activities of (a) laccase (Lac) and (b) manganese peroxidase (MnP) when *L. strigosus* was grown in minimal media at 30°C for 14 d. The vertical bars represent the standard deviation range for mean values. ( ■ yeast extract; ■ L-asparagine; ▲ peptone; ◇ beef extract; ▼ urea; ● NH₄NO₃; ○ (NH₄)₂SO₄)
transcriptional and translational regulation. Cu^{2+} serves as cofactor in the catalytic core of Lac, thus a minimum concentration (in the milli mole range) of Cu^{2+} is necessary for the production of active Lac (Majeau et al., 2010). The strong positive effect of the addition of Cu^{2+} on the production of Lac and MnP was observed in several species such as Ceriporiopsis subvermispora (Salas et al., 1995), T. versicolor (Collins and Dobson, 1997), P. cryosporium (Dittmer et al., 1997), and Trametes hirsute (Soden and Dobson, 2001). However, a decrease in the fungal growth rate accompanied with a prolonged lag phase was sometimes observed in the presence of Cu^{2+}. For example, Cu^{2+} inhibited the growth of Ganoderma lucidum at concentrations of less than 1 mM (Baldrian, 2003), while only 1.6 mM of Cu^{2+} decreased the growth rate of Trametes trogii (Levin et al., 2002). Excess Cu^{2+} may have a toxic effect on the fungal biomass and thus decrease Lac production (Majeau et al., 2010). The peptone concentration showed high Lac and MnP activities up to level 1, then the activities decreased when the peptone concentration increased up to level 2, and subsequent peptone concentrations up to level 3 led to an increase in activities. This might have been due to the other constitutive effects of the culture media (Prasad et al., 2005).

The influence of the factors on the production of Lac and MnP is shown in Tables 4 and 5, respectively. Analysis of variance (ANOVA) was used to analyze the results and to determine the percentage contributions of each factor. The nitrogen source (that is L-asparagine, factor C) and the carbon source (that is cellobiose, factor A) were significant factors for the production of Lac and MnP. The confidence levels further confirmed that L-asparagine, cellobiose and CuSO_4 had a highly significant effect on the production of Lac and MnP (P < 0.01), whereas peptone was less effective but still significant (P < 0.05). To this end, maximum activities of Lac (33,955.83 UL^{-1}) and MnP (3,764.57 UL^{-1}) were obtained from the same condition-namely, 15 g L^{-1} cellobiose, 0.3125 g L^{-1} peptone, 1.2500 g L^{-1} L-asparagine and 0.002 g L^{-1} CuSO_4.

A confirmation test was conducted for the production of Lac and MnP using the above identified optimum process parameters. The confirmation revealed comparable predicted values with confirmation of the enzymes’ activity levels. The confirmation data showed the highest
activities of Lac (32,140.95 UL\(^{-1}\)) and MnP (3,491.36 UL\(^{-1}\)) within 12 d of incubation. This condition favored the preferential production of Lac and MnP and therefore maximized the production of both enzymes.

Optimization of L-asparagine concentration

In the present study, L-asparagine contributed the highest impact factor in the production of Lac and MnP (Tables 4 and 5, respectively). The effect of L-asparagine concentrations on Lac and MnP production was further studied and this is shown in Figures 5a and b, respectively. Increasing the L-asparagine concentration from 0.3125 to 0.9375 gL\(^{-1}\) led to a sevenfold increase (from 5,850.59 to 39,777.76 UL\(^{-1}\)) in Lac activity and a ninefold increase (from 283.50 to 1,838.49 UL\(^{-1}\)) in MnP activity. A further increase to 2.5000 gL\(^{-1}\) L-asparagine, however, did not enhance Lac and MnP activities, but had a negative effect on Lac and MnP activities. This experiment confirmed the important role of nitrogen sources, as well as their concentrations, in Lac and MnP production by *L. strigosus*. Galhaup et al. (2002) reported the effect of nitrogen concentration on Lac production by *T. pubescens* MB 89. Peptone concentrations higher than 10 gL\(^{-1}\) could not raise the Lac production by *T. pubescens* MB 89; however, a decrease in the peptone concentration to lower than 10 gL\(^{-1}\) did decrease the Lac production. Wu et al. (2005) also reported that an increase in the concentration of ammonium tartrate led to a decrease in ligninolytic enzyme production by *P. ostreatus* (Wu et al., 2005). This was consistent with the study by (Stajic et al., 2006) who reported that the cultivation of *Pleurotus eryngii* in higher concentrations of (NH\(_4\))\(_2\)SO\(_4\) resulted in a decrease in Lac production.

Decolorization of synthetic dye solution

Figures 6 to 8 show the relationship between Lac and MnP activities and %color removal of RBBR, RB5 and Indigo 4B, respectively, when *L. strigosus* was grown in
the optimized medium containing these color solutions at 30°C for 8 d. The removal of RBBR was observed after 24 h of incubation, whereas for RB5 and Indigo 4B, removal was observed after 42 and 48 h of incubation, respectively. After these incubation times, all three colors were continuously and sharply reduced until they reached maximum color removal of 94.45, 98.19 and 81.92% for RBBR, RB5 and Indigo 4B, respectively. Robinson et al. (2001) suggested that the removal of color by white-rot fungi depended on two mechanisms - namely, cell biosorption and enzymatic biodegradation (Robinson et al., 2001).

The biodegradation was confirmed by the presence of ligninolytic enzymes - namely, Lac, MnP and LiP peroxidase. Only Lac and MnP were detected in L. strigosus; thus both enzymes were quantitatively investigated at various time intervals during incubation as shown in Figures 6 to 8. The activities of Lac and MnP (both less than 10 UL⁻¹) were slightly detected during the first 48 h of incubation which corresponded to the low removal levels of all tested colors. After that, the activities of Lac and MnP sharply increased and reached their maximum levels at 1,051.25 and 63.58 UL⁻¹, respectively, for RBBR removal, at 16,298.36 and 1284.96 UL⁻¹, respectively, for RB5 removal and at 14,655.31 and 1,200.00 UL⁻¹, respectively, for Indigo 4B removal. The increases in activity of Lac and MnP were relative with the increases in color removal, as it has also been reported that Lac and MnP were the main ligninolytic enzymes for the removal of color and organic matter in effluents containing synthetic dyes (Kim et al., 2004; Vaithanomsat et al., 2010).

The removal of RBBR, RB5 and Indigo 4B by L. strigosus in the present study was comparable with previous extensive studies. It was shown that L. strigosus could tolerate higher concentrations of synthetic dyes (initial RBBR, RB5 and Indigo 4B concentrations of 1,000, 500 and 500 mg L⁻¹, respectively), as well as being more efficient in the removal of those synthetic dyes. Swamy and Ramsay (1999) reported the removal of 40 mg L⁻¹ RBBR and 60 mg L⁻¹ RB5 by P. chrysosporium and T. versicolor at a maximum level of 83% (for RBBR) and 100% (for RB5) within 11 and 15 d of incubation, respectively. Balan and Monteiro (2001) demonstrated that Phellinus gilvus, Pleurotus sajor-caju, Pycnoporus sanguineus and P. chrysosporium removed 0.02% Vat Blue I (Indigo-blue dye) at maximum amounts of 100, 94, 90 and 70%, respectively, within 4 d of incubation (Balan and Monteiro, 2001). I. lacteus was reported to have removed 100 mg L⁻¹ RBBR and RB5 at maximum amounts of 90% (for RBBR and RB5) within 2 and 10 d of incubation, respectively (Maximo and Ferreira, 2004). Kim et al. (2004) reported T. versicolor KCTC16781 capability of 100 mg L⁻¹ RB5 at a maximum amount of 99% within 40 h of incubation. Dastronina sp. KAPI0039 removed 1,000 mg L⁻¹ RBBR and 600 mg L⁻¹ RB5 at maximum amount of 96.05 and 88.01%, respectively, within 48 h of incubation (Vaithanomsat et al., 2010).

Takken all together, L. strigosus is one of the most interesting white-rot fungi, in terms of ligninolytic enzyme
production, for applications on an industrial scale.

When dye structures were considered, the best removal efficiency (94% within 48 h of incubation) by *L. strigosus* was observed for RBBR, an anthraquinone-based structure. The same result, which showed the best color removal from anthraquinone-based synthetic dyes, was also obtained from the studies by (Swamy and Ramsay, 1999; Robinson et al., 2001; Ozsoy et al., 2005; Palmieri et al., 2005; Murugesan et al. 2007). RB5 (an azo-based synthetic dye) and Indigo 4B (an indigoid-based synthetic dye) were shown to be the second best (92% within 96 h of incubation) and third best (81.92% within 192 h of incubation), respectively, for removal efficiency. This corresponded with the study by (Zhao...
and Zhang, 2005) who suggested that the azo- and indigoid-based structures were rather complicated and, therefore, more difficult to be degraded by fungal enzymes. Moreover, it was suggested that high concentrations of the azo- and indigoid-based synthetic dyes were toxic to \textit{C. versicolor} and \textit{P. ostreatus} (Ramsay and Nguyen, 2002; Erkurt et al., 2007).

**Conclusion**

There has been growing interest in studying the ligninolytic enzymes from fungi with the expectation of finding more effective systems for their application in various biotechnological approaches. It can be concluded that one of the key factors to increase the yield of ligninolytic enzymes is the optimization of the production medium. In the present study, Lac and MnP production by \textit{L. strigosus} were successfully optimized using the Taguchi methodology. High production levels of Lac and MnP of this strain, as well as its ability to degrade dye, could be advantageous with respect to synergistic reactions in bioremediation and biotransformation processes. This strain, therefore, could be an attractive source for an industrial application.

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