ENHANCED PRODUCTION OF LACCASE BY FUNGI UNDER SOLID SUBSTRATE FERMENTATION CONDITION

Filiz Boran and Ozfer Yeşilada *

Funalia trogii and *Trametes versicolor* were grown on agro byproduct wheat bran moistened with various natural moistening agents, and their effects on laccase production under solid substrate condition were investigated. Laccase was the main enzyme detected under this condition. High levels of laccase activity were obtained with solid substrate cultures moistened with olive oil mill wastewater (OOMW) or alcohol factory wastewater (vinasse). Among the cultures without inducer, *T. versicolor* culture was detected as a more effective laccase producer than *F. trogii* culture. Copper and xylidine were used as laccase inducers, and copper induced laccase production more than xylidine. The maximum laccase activity was detected as 14.18 U/mL with *F. trogii* grown on wheat bran moistened with 5 mM $CuSO_4.5H_2O$ added 25% vinasse. Azo dye decolorization activity of the supernatants from solid substrate cultures was also tested. While the use of 0.063 U/mL *F. trogii* laccase in reaction solution gave 66% decolorization in a minute, it was 14% for *T. versicolor*. This method can be a possible alternative for valorization of lignocellulosic materials and industrial wastewaters during solid substrate fermentation and for obtaining enzyme source with very high decolorization activity.

Keywords: Laccase; Olive oil mill wastewater; Solid substrate fermentation; Vinasse; Wheat bran

*Contact information: Department of Biology, Arts and Science Faculty, Inonu University, 44280, Malatya, Turkey. *Corresponding Author. Tel./fax: +90 422 3773704 E-mail address: oyesilada@inonu.edu.tr*

INTRODUCTION

Laccase (benzenediol: oxygen oxidoreductase, EC. 1.10.3.2) is a multinuclear copper-containing enzyme that oxidizes diverse substrates. Because it can be used in numerous biotechnological applications, its production in high amount is important. Laccase production yield depends on species, strains, cultivation conditions, and nutrient sources (Stajic et al. 2004). Although white rot fungi are the best laccase producers, they produce this enzyme in small amounts. But, they can produce high amounts of laccase in the presence of inducers and under different cultivation conditions (Nyanhango et al. 2002; Moldes et al. 2003; Stajic et al. 2006).

Laccase can be produced by liquid substrate fermentation (LSF) and solid substrate fermentation (SSF). While the growth medium for LSF is a nutrient-rich liquid, for SSF it is typically a moistened solid substrate (Rodriguez-Couto and Toca-Herrera 2007). SSF, which is a completely different cultivation condition than LSF, has several advantages over LSF. In SSF, the solid substrate not only provides the microorganism with a similar environment to their natural habitats, but it also functions as an attachment

place and a source of nutrient (Rodriquez-Couto et al. 2002). Therefore, this method of fermentation can be a suitable process for production of enzymes by fungi.

Various lignocellulosic substrates could be used as solid substrate in SSF (Moldes et al. 2003; Rodriquez-Couto and Sanroman 2005; Shah et al. 2005; Stajic et al. 2006; Pant and Adholeya 2007). Wheat bran, which is a cheap and natural lignocellulosic solid substrate, contains phenolic compounds (Kim et al. 2006; Murugesan et al. 2007; Li et al. 2007). The solid substrate must contain enough moisture to support growth and metabolism of microorganisms (Pandey 2003). Wastewaters such as olive oil mill wastewater (OOMW), alcohol factory wastewater (vinasse), cheese factory wastewater (cheese whey), and molasses, which is byproduct of sugar manufacturing, have high polluting characteristics that cause serious environmental problems. Because these wastewaters contain sufficient nutrients for microbial growth, they can be used as a moistening agent in SSF, forming an alternative method. This can also be an alternative method for utilization of these wastewaters (Pant et al. 2006).

Because color affects the ecosystem by interfering with the photosynthetic activity and hence lowering the dissolved oxygen concentration, dye decolorization and removal of dyes from wastewater is a central problem for the textile industries. Azo dyes, which constitute the largest chemical class of synthetic dyes, are relatively resistant to biodegradation, especially to treatment with conventional biological wastewater systems. Decolorization of textile wastewaters by physical and chemical methods has some disadvantages. Biotechnological methods, such as the use of fungi or their enzymes for decolorization (Yesilada et al. 2010), represent an alternative approach. Laccase is known as an effective enzyme for dye decolorization. But, this decolorization activity depends on the source of enzyme and also on the chemical structure of the dye (Abadulla et al. 2000; Rodriquez-Couto 2007; Michnrewicz et al. 2008).

The aim of this study is to investigate laccase production ability of white rot fungi *F. trogii* and *T. versicolor* in SSF moistened with various wastewaters. The role of moistening agents on laccase production activity of these fungi is discussed. The effect of copper and xylidine as an inducer on laccase production of these fungi was also investigated. Most of the studies with these strains are based on their laccase production potential under liquid culture conditions. Until now, there have been limited studies on possible inducing effect of wastewaters on laccase production ability of fungi under SSF conditions. According to our literature knowledge, this is the first report on laccase production ability of these fungal strains on wheat bran moistened with OOMW, vinasse, cheese whey, and molasses. Therefore, azo dye decolorization activity of supernatants from solid substrate fermentation cultures was also determined.

EXPERIMENTAL

Materials

Fungi

The white rot fungi *Funalia trogii* ATCC 200800 and *Trametes versicolor* 200801 were used. *F. trogii* growing on *Populus* sp. was originally collected from Malatya, Turkey. Alternatively, *T. versicolor* growing on *Cupressus* sp. was originally

collected from Adana, Turkey. These are stock cultures at Inonu University, Art and Science Faculty, Department of Biology, Malatya, Turkey. They were maintained on Sabouraud Dextrose Agar (SDA) plates at 4 °C, stored at 30 °C and subcultured every 2-3 weeks.

Methods

Solid substrate, moistening agents, and inducers

Wheat bran was used as a cheap and natural solid substrate. Four moistening agents were used to investigate their possible effect for enhancing enzyme production. The moistening agents were alcohol factory wastewater (vinasse) (25, 50, 100% concentrations), olive oil mill wastewater (OOMW) (25, 50, 100% concentrations), cheese factory wastewater (cheese whey) (25, 50, 100% concentrations), and byproduct of sugar factory (molasses) (1 and 5% concentrations). The influence of copper ($CuSO_4.5H_2O$; 1, 2, and 5 mM, final concentrations in moistening agents) and 2,5-xylidine (0.1, 1 and 2 mM final concentrations in moistening agents) on laccase production ability of these fungi was also investigated.

Preparation of inoculum

Fungi were incubated at 30 °C on slant Sabouraud Dextrose Agar (SDA). After one week, 10 mL of sterilized distilled water was added on slant SDA culture, and mycelial suspensions were prepared. Then, 5 mL of each suspension was used to inoculate 100 mL Sabouraud Dextrose Broth (SDB) in 250 mL Erlenmeyer flasks. The cultures were incubated at 150 rpm for five days and then, they were gently homogenized under sterile conditions. These homogenized mycelial suspensions were used as the inoculum.

Solid substrate fermentation

Erlenmeyer flasks (250 mL) containing 5 g wheat bran were moistened with 15 mL of moistening agent with and without inducers and autoclaved at 121 °C for 20 min. After autoclaving, the flasks were inoculated with 2 mL of inoculum under sterile conditions and incubated at 30 °C under static conditions for various time periods according to experimental design.

Sampling, extraction and assay

For the isolation of laccase, 40 mL of distilled water was added to solid cultures, and they were agitated on a rotary shaker at 200 rpm for 1h at 30 °C. After filtration, they were centrifuged at 4000 rpm for 15 min and the supernatants were used for the laccase assay.

Laccase (E.C. 1.10.3.2) activity was determined spectrophotometrically (Shimadzu-UV-1601, UV/Visible spectrophotometer) by monitoring the increase in absorbance at 420 nm. One unit was defined as the amount of enzyme that oxidized 1 μmol of ABTS [2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] per minute (Birhanli and Yesilada 2006).

Results are the mean of at least three different cultures.

In vitro dye decolorization

The decolorization of Reactive Blue 171 (diazo dye) by supernatants from solid substrate cultures of *F. trogii* and *T. versicolor* was investigated. The supernatant (10 µL, 25 µL, and 50 µL from cultures) containing mainly laccase enzyme (about 2.5 U/mL) was added into 0.1 mM sodium acetate buffer (pH 5.0), which contained 100 mg/L Reactive blue 171 dye. The total volume of reaction mixture was 3.0 mL. This was incubated at 30 °C for a minute or more. Decolorization was determined by monitoring the absorbance change at the maximum absorbance wavelength of the dye and expressed in terms of percentage. A control test was carried out in which the supernatant was replaced by the supernatant without crude laccase enzyme; the supernatant was separated by a membrane of 30 kDa molecular weight cut-off which gives a low molecular weight fraction containing the low MW metabolites (<30 kDa) without laccase. The dye structure is shown in Fig. 1.

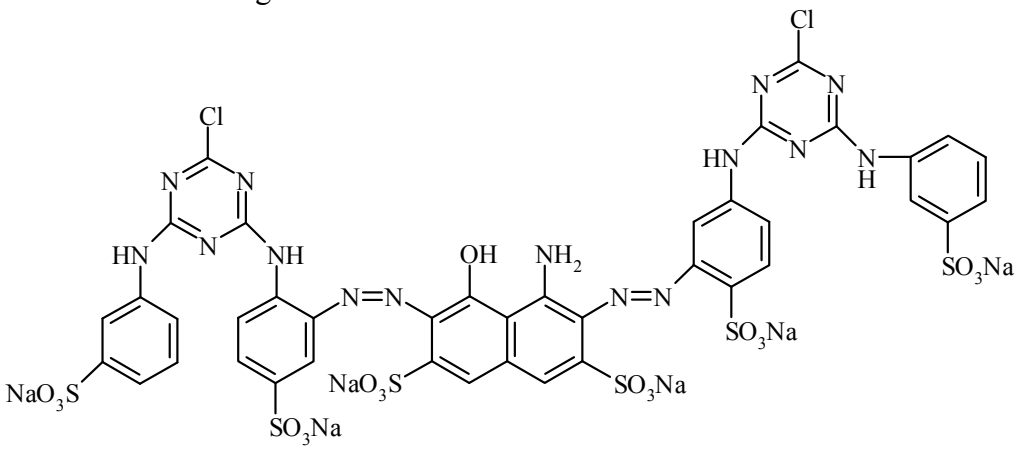

Fig. 1. Chemical structure of reactive blue 171

RESULTS AND DISCUSSION

Effect of Various Moistening Agent Concentrations on Laccase Production of *F. trogii* and *T. versicolor*

Wheat bran is a cheap lignin-carbon source that provides fungi with a similar environment to their natural habitats (Hernandez Fernaud et al. 2006). It is a good substrate for laccase production (Revenkar et al. 2007). Souza et al. (2006) reported that the inductive capability of wheat bran depends on its phenolic compound content. In SSF, solid substrate must be moistened with moistening agents for supporting the growth of fungi. Here, different moistening agents such as cheese whey, molasses, OOMW, and vinasse were used as cheap moistening agents. No extra commercial source was used in the current study. This gives significant economic advantage. Molasses, which is a by-product of sugar factory, is used as a carbon and energy source in various fermentations. Cheese whey, a liquid waste that is generated during the process of cheese making, is a good source of protein, vitamins, minerals and lactose that may be used to stimulate cell growth and product formation (Aksu and Eren 2007). Because of its high levels of

organic compounds, cheese whey causes pollution problems when discharged directly into the environment. OOMW contains phenolics, and it has high chemical oxygen demand and black color (Yesilada et al. 1998). Therefore, it causes environmental problems. It includes includes sugar, tannins, polyphenols, polyalcohols, pectins, and lipids (Hamdi et al. 2003). Yesilada et al. (1998) and Fenice et al. (2003) reported this effluent as a low-cost growth medium. It induces laccase production of white rot fungi (Massadeh and Modallal 2008). The environmental impact of vinasse is also very high due to its organic and inorganic matter content and its dark color (Yesilada and Fiskin 1995). Hence, all these wastewaters must be treated or utilized. One way to utilize and reduce these wastewaters is to use them as a nutrient source for enzyme production in fungal fermentations. Therefore, *F. trogii* and *T. versicolor* were grown on wheat bran moistened with these wastewaters as moistening agents. Laccase was the main enzyme detected under this condition. Ligninase or Mn-peroxidase could not be detected under SSF condition. Fungal mycelia completely colonized the solid substrate within 5 d. Table 1 shows the laccase activities obtained on the 5th and 10th days by these fungi cultivated on solid substrate moistened with different concentrations of these wastewaters. Type and concentration of wastewaters greatly affected the laccase production ability of these fungi under SSF conditions.

Table 1. Effect of Various Wastewaters and their Concentrations on Laccase Production

Laccase Activity (U/mL)				
	F. trogii		*T. versicolor*	
Wastewater Concentrations (%)	5d	10d	5d	10d
Cheese whey (25)	2.70 ± 0.59	3.40 ± 0.62	0.81 ± 0.11	1.02 ± 0.20
Cheese whey (50)	0.10 ± 0,01	0.01± 0.01	0.05 ± 0.01	0.09 ± 0.01
Molasses (1)	2.77 ± 0.38	1.85 ± 0.74	2.98 ± 0.49	3.22 ± 0.23
Molasses (5)	2.35 ± 1.73	1.07 ± 0.77	2.06 ± 0.37	3.35 ± 1.44
OOMW (25)	3.12 ± 0.44	3.46 ± 0.62	4.97 ± 0.31	6.36 ± 1.58
OOMW (50)	3.19 ± 0.87	1.11 ± 0.44	2.03 ± 0.13	1.56 ± 1.32
OOMW (100)	3.76 ± 0.36	2.29 ± 0.92	1.76 ± 0.44	1.75 ± 0.85
Vinasse (25)	3.31 ± 0.39	4.30 ± 0.90	4.93 ± 0.74	6.08 ± 0.70
Vinasse (50)	3.32 ± 0.88	2.40 ± 0.21	1.74 ± 0.16	8.51 ± 5.17

Among the moistening agents tested, OOMW and vinasse were detected as the most suitable and effective agents. The best OOMW concentration for laccase production was detected as 25%. This concentration gave the highest activity as 3.46 ± 0.62 U/mL and 6.36 ± 1.58 U/mL on 10th day for *F. trogii* and *T. versicolor*, respectively. While the highest laccase activity obtained with *F. trogii* cultivated on wheat bran moistened with 25% vinasse was 4.30 ± 0.90 U/mL on 10th day, it was 6.08 ± 0.70 U/mL for *T. versicolor* on the same day.

In vinasse media, the highest activities for *F. trogii* were determined in 25% vinasse media on 10th day, but, the highest value for *T. versicolor* was obtained in 50% vinasse media. However, *T. versicolor* also produced high a amount of enzyme in 25% vinasse media. Therefore, it was preferred to use the same vinasse concentration (25%) throughout the study. Based on these results, 25% OOMW and 25% vinasse were selected and used as the moistening agents for further studies.

Time Course of Laccase Production by *F. trogii* and *T. versicolor* during Incubation on Wheat Bran Moistened with 25% OOMW and 25% Vinasse

Laccase production of these fungi on wheat bran moistened with 25% OOMW and also 25% vinasse was monitored for 20 days. The mixtures produced high amounts of laccase during growth.

Increase in laccase activity of *F. trogii* from 5th to 15th day and decrease in its activity on 20th day were observed on wheat bran moistened with OOMW. The maximum laccase activity was 4.10 U/mL. On the other hand, laccase activity of *T. versicolor* on the 5th and 10th day were 4.97 and 6.36 U/mL, respectively, and the maximum was 8.36 U/mL on 20th day (Fig. 2). Fenice et al. (2003) reported that maize stalks moistened with OOMW stimulates the laccase production of *P. tigrinus*, for which the highest level of laccase activity was 1.31 U/mL. Many phenolic compounds have been detected in OOMW (Ramos-Cormenzana et al. 1996). The inducing effect of phenolic and other organic compounds present in OOMW on laccase production under LF conditions, which is a different cultivation condition than SSF, was reported (Perez et al. 1998; Yesilada et al. 1998; Dias et al. 2004). de la Rubia et al. (2008) reported also that addition of OOMW to liquid cultures of white rot fungi increases fungal growth and laccase activity.

The time course of laccase production for *F. trogii* incubated on wheat bran moistened with vinasse was similar to wheat bran moistened with OOMW. Its maximum laccase activity was detected as 4.30 U/mL on the 10th day, and then the activity decreased.

On the other hand, the maximum laccase activity value for *T. versicolor* was 12.22 U/mL on the 20th day (Fig. 2). This value is about five-fold higher than the maximum value obtained with *F. trogii*. Hence, *T. versicolor* cultures were detected as more effective laccase producers than *F. trogii* cultures. Enhanced production of laccase by *A. flavus* (0.35 U/mL) and *P. ostreatus* (0.11 U/mL) on lignocellulosic solid substrates moistened with distillery effluent was also reported by Pant and Adholya (2007). But, these reported activities were very low.

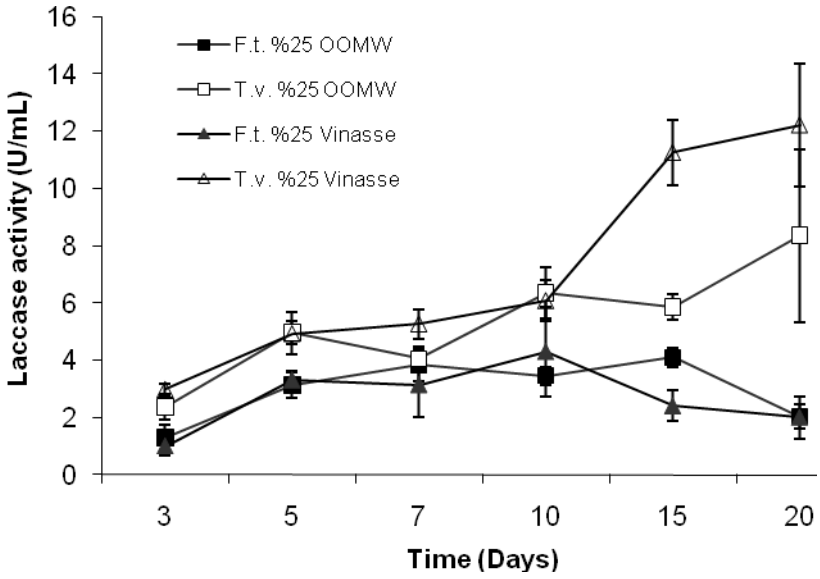

Fig. 2. Time course of laccase production by *F. trogii* and *T. versicolor* cultures incubated on wheat bran moistened with 25% OOMW and also 25% vinasse

Effect of Copper on Laccase Production of *F. trogii* and *T. versicolor* during Incubation on Wheat Bran Moistened with 25% OOMW and 25% Vinasse

Copper is an effective laccase inducer (Galhaup and Haltrich 2001). Therefore, the effect of copper on laccase production of these fungi under SSF condition was studied. To test this effect, copper was added before inoculation, and the cultures were incubated for 10 days. As can be seen from Fig. 3 and 4, the presence of copper greatly induced the laccase production ability of these fungi.

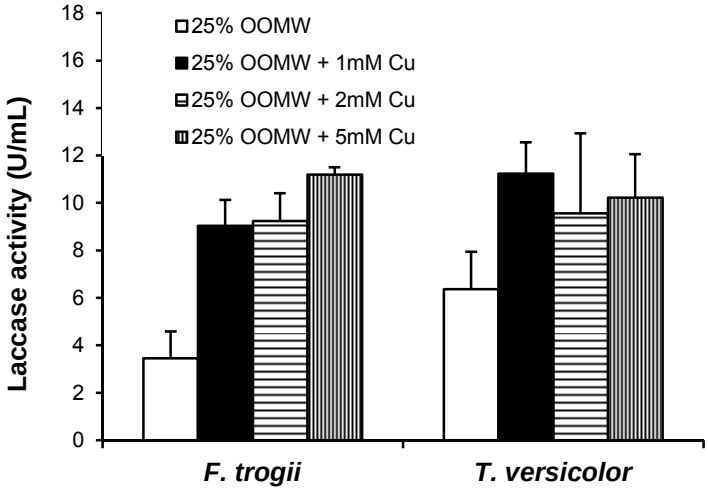

Fig. 3. Laccase production on wheat bran moistened with copper-added 25% OOMW

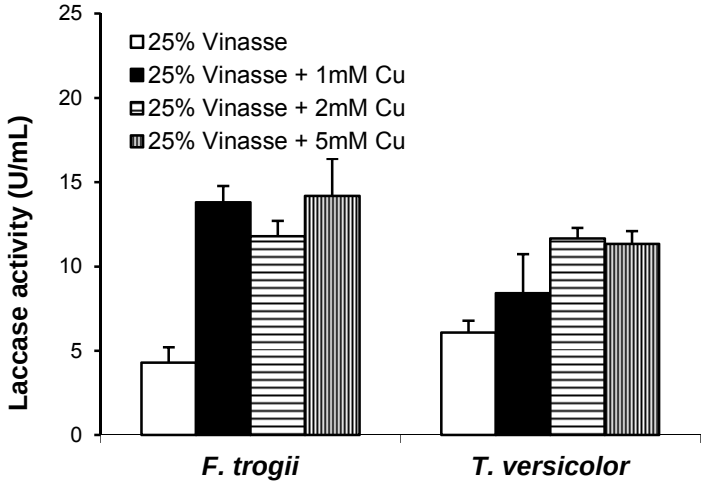

Fig. 4. Laccase production on wheat bran moistened with copper-added 25% vinasse

On wheat bran moistened with 25% OOMW media, for *F. trogii,* the laccase obtained for control (OOMW without copper) was 3.46 U/mL, whereas the values were 9.03 U/mL, 9.24 U/mL, and 11.19 U/mL for 1 mM, 2 mM, and 5 mM copper-added cultures, respectively. Similarly, copper induced the laccase production ability of *T. versicolor*, and laccase activity of the control ranged from 6.36 U/mL to 11.24 U/mL for 1mM copper-added culture. When the concentration of copper in the medium was increased to 5 mM, the laccase activity detected was higher than the control but lower than the cultures with 1 mM copper. The highest activity values for *F. trogii* and *T. versicolor* on wheat bran moistened with 25% OOMW media were detected in the presence of 5 and 1 mM copper, respectively.

While the presence of copper improved the laccase activity of *F. trogii* about three-fold, this increase was about two-fold for *T. versicolor* (Fig. 3). Similarly, the presence of copper in wheat bran moistened with 25% vinasse media induced the laccase production capacity of fungi (Fig. 4).

The maximum values obtained in this medium were 14.18 U/mL and 11.65 U/mL for *F. trogii* and *T. versicolor*, respectively. These values were about three-fold and two-fold higher than their controls. These results showed the positive inducing effect of copper on laccase activity of fungi incubated under SSF conditions, and 1.0 mM was sufficient for this inducing effect.

Because the composition of these wastewaters was different, copper could probably affect the laccase production differently. Moldes et al. (2003) reported that laccase production of *Trametes hirsuta* on barley bran was strongly stimulated by the addition of copper.

Effect of Xylidine on Laccase Production of *F. trogii* and *T. versicolor* during Incubation on Wheat Bran with 25% OOMW and 25% Vinasse

The effect of inducers on laccase production differs from fungus to fungus (Rodriquez-Couto et al. 2004). Xylidine has also been reported as an important laccase inducer under semi-solid-state condition (Rodriquez-Couto et al. 2002). Therefore, the

effect of 2,5-xylidine on laccase production activity of these fungi under the SSF conditions used was examined.

As shown in Fig. 5, addition of xylidine to cultures moistened with OOMW slightly improved the laccase activity levels, but no significant differences were detected between the laccase levels of cultures with and without xylidine and also between the cultures with different concentrations of xylidine. On the other hand, addition of xylidine to cultures moistened with vinasse affected the laccase production of two fungi differently. While xylidine did not induce the laccase production of *F. trogii*, the presence of 1 or 2 mM xylidine induced the laccase production of *T. versicolor* (Fig. 6).

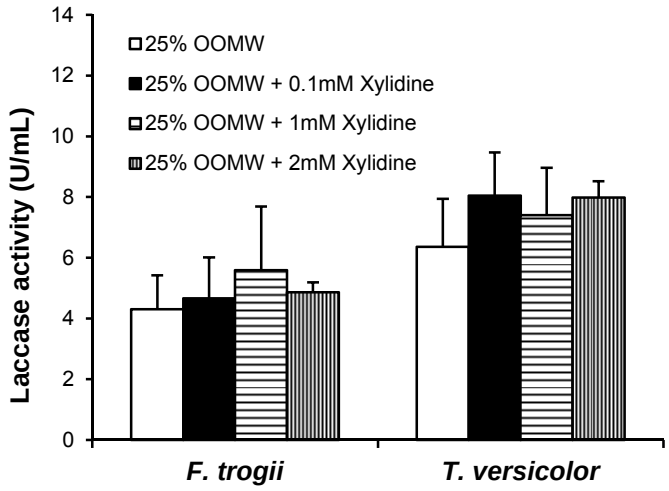

Fig. 5. Laccase production on wheat bran moistened with xylidine-added 25% OOMW

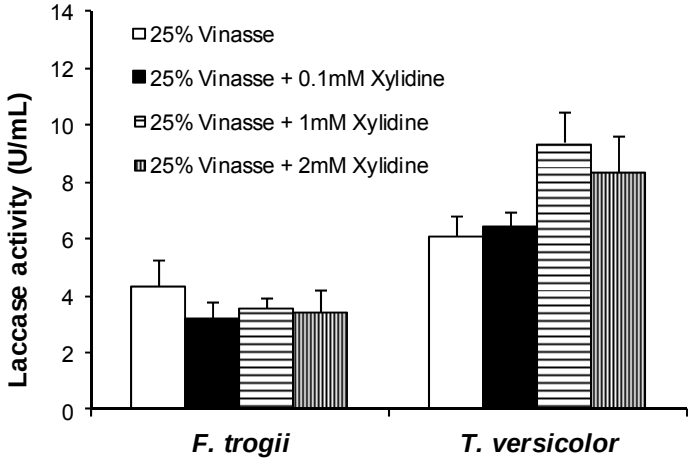

Fig. 6. Laccase production on wheat bran moistened with xylidine-added 25% vinasse

Our results showed that copper was a more effective laccase inducer on solid substrate fermentation cultures than xylidine. Laccase activity values obtained with copper-added cultures were higher than xylidine-added cultures. Similar result was also reported by Rodriquez-Couto et al. (2004), who reported an acute effect of copper sulphate on laccase activity of solid state cultures of *T. hirsuta*.

Dye Decolorization

The fungi used in our study produced laccase as the only extracellular enzyme. Wheat bran contains phenolic compounds, which may act as mediators for dye decolorization by laccase enzyme (Murugesan et al. 2009). Therefore, the dye decolorization activity of the supernatants (crude laccase sources) from solid substrate fermentation cultures was tested. Reactive Blue 171, which is a naphtol type diazo dye, was used as a model dye. The crude laccase sources could decolorize the dye to a different extent. The crude laccase from *F. trogii* culture was determined to have a higher and faster decolorization capacity than the crude laccase from *T. versicolor* culture. The crude laccase source of 0.027 U/mL from *F. trogii* culture showed 45% and 66% decolorization activity after one and two minutes of incubations, respectively. Increase in the amount of laccase in reaction solution gave fast decolorization. The use of 0.063 U/mL gave 66% in a minute, whereas the use of 0.133 U/mL reached the same value in half a minute. Fig. 7 shows the absorption spectra of untreated Reactive Blue 171 (100 mg/L) dye solution and Reactive blue 171 (100 mg/L) dye solution treated with 0.063 U/mL laccase enzyme from *F. trogii*.

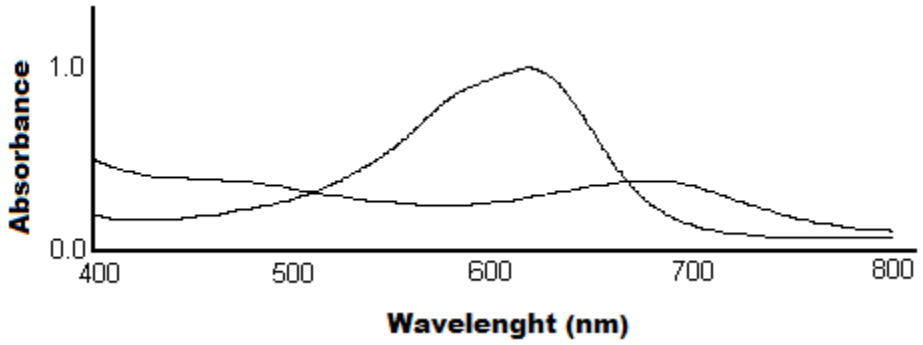

Fig. 7. Spectra of untreated (top) and treated dye

As shown in this figure, the spectrum of this azo dye was greatly diminished after one minute of incubation. On the other hand, decolorization activity of the crude laccase source of 0.026 U/mL from *T. versicolor* culture was as low as 6% and 11% decolorization values after one and two minutes of incubations, respectively. When the enzyme amount in the reaction medium was increased from 0.026 U/mL to 0.063 U/mL, dye decolorization percentages were detected as 14% and 24% for one and two minutes, respectively. The enzyme amount of 0.133 U/mL gave 27% and 43% decolorization values for the same incubation periods. This result showed that the organism, from which the enzyme source was obtained, is an important factor for effective dye decolorization. It has been reported that Methyl Orange (monoazo dye) could be decolorized by *T. hirsuta* laccase (about 65% decolorization in 24h).

The supernatants were also separated by a membrane of 30 kDa molecular weight cut-off, obtaining low molecular weight fractions and testing their decolorization activity. The low molecular weight fraction containing the low MW metabolites (<30 kDa) without laccase showed no decolorization activity. This shows that Reactive Blue 171 is a

good substrate especially for crude laccase enzyme of *F. trogii* from wheat bran medium, and this enzyme needs no low MW metabolites for its dye decolorization activity.

CONCLUSIONS

1. Lignocellulosic material moistened with wastewaters could be used as a solid substrate for fungal growth and laccase enzyme production. Because this enzyme was the only enzyme detected in these media, it can easily be obtained.
2. Copper was also detected as an effective inducer for laccase production under the conditions selected here.
3. These solid substrate cultures which contain high amounts of crude laccase can also be used for dye decolorization.
4. This method could also be an alternative method for valorization and bioremediation of the industrial wastewaters such as OOMW and vinasse.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Inonu University research fund. We also thank Dr. Mustafa Sahiner for English corrections.

REFERENCES CITED

Abadulla, E., Robra, K. H., Gubitz, G. M., Silva, L. M., and Cavaco-Paulo, A. (2000). "Enzymatic decolorization of textile dyeing effluents," *Text. Res. J.* 70, 409-414.

Aksu, Z., and Eren, A. T. (2007). "Production of carotenoids by the isolated yeast of *Rhodotorula glutinis*," *Biochem. Eng. J.* 35, 107-113.

Birhanli, E., and Yesilada, O. (2006). "Increased production of laccase by pellets of *Funalia trogii* ATCC 200800 and *Trametes versicolor* ATCC 200801 in repeated-batch mode," *Enzyme Microb. Technol.* 39, 1286-293.

Dias, A. A., Bezerra, R. M., and Pereira, A. N. (2004). "Activity and elution profile of laccase during biological decolorization and dephenolization of olive mill wastewater," *Bioresour. Technol.* 92, 7-13.

de la Rubia, T., Lucas, M., and Martinez, J. (2008). "Controversial role of fungal laccases in decreasing the antibacterial effect of olive mill waste-water," *Bioresour. Technol.* 99, 1018-1025.

Fenice, M., Sermanni, G. G., Federici, F., and D'Annibale, A. (2003). "Submerged and solid-state production of laccase and Mn-peroxidase by *Panus tigrinus* on olive mill wastewater-based media," *J. Biotechnol.* 100, 77-85.

Galhaup, C., and Haltrich, C. (2001). "Enhanced formation of laccase activity by the white-rot fungus *Trametes pubescens* in the presence of copper," *Appl. Microbiol. Biotechnol*. 56, 1-2.

Hamdi, M. (2003). "Future prospect and constraints of olive millwastewater use and treatment: A Review." *Bioprocess Eng*. 8, 209-214.

Hernandez Fernaud, J. R., Marina, A., Gonzalez, K., Vazquez, J., and Falcon, M. A. (2006). "Production, partial characterization and mass spectrometric studies of the extracellular laccase activity from *Fusarium proliferatum*," *Appl. Microbiol. Biotechnol.* 70, 212-221.

Kim, K. H., Tsao, R., Yang, R., and Cui, W. S. (2006). "Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions," *Food Chem*. 95, 466-473.

Li, W., Pickard, D. M., Beta, T. (2007). "Effect of thermal processing on antioxidant properties of purple wheat bran," *Food Chem*. 104, 1080-1086.

Massadeh, M. I., and Modallal, N. (2008). "Ethanol production from Olive Mill Wastewater (OMW) pretreated with *Pleurotus sajor-caju*," *Energy Fuels* 22,150-154.

Michnrewicz, A., Ledakowicz, S., Ullrich, R., and Hofrichter, M. (2008). "Kinetics of the enzymatic decolorization of textile dyes by laccase from *Cerrena unicolor*," *Dyes Pigm*. 77, 295-302.

Moldes, D., Gallego, P. P., Couto S. R, and Sanroman, A. (2003). "Grape seeds: The best lignocellulosic waste to produce laccase by solid state cultures of *Trametes hirsuta*," *Biotechnol. Lett*. 25, 491-495.

Murugesan, K., Nam, I., Kim, Y., and Chang, Y. (2007). "Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture" *Enzyme Microb. Technol.* 40, 1662-1672.

Murugesan, K., Yang, I. H., Kim, Y.M., Jeon, J. R., and Chang, Y. S. (2009). "Enhanced transformation of malachite green by laccase of *Ganoderma lucidum* in the presence of natural phenolic compounds," *Appl. Microbiol. Biotechnol.* 82, 341-350.

Nyanhongo, G. S., Gomes, J., Gübitz, G., Zvauya, R., Read, J. S., and Steiner, W. (2002). "Production of laccase by newly isolated strain of *Trametes modesta*," *Bioresour. Technol*. 84, 259-263.

Pandey, A. (2003). "Solid-state fermentation," *Biochem. Eng. J.* 13, 81-84.

Pant, D., Gangi, R. U., and Adholeya, A. (2006). "Cultivation of oyster mushrooms on wheat straw and bagasse substrate amended with distillery effluents," *World J. Microbiol. Biotechnol.* 22, 267-275.

Pant, D., and Adholeya, A. (2007). "Enhanced production of ligninolytic enzymes and decolorization of molasses distillery wastewater by fungi under solid state fermentation," *Biodegradation* 18, 747-659.

Perez, J., de la Rubia, T., Hamman, O. B., and Martinez, J. (1998). "*Phanerochaete flavido-alba* laccase induction and modification of manganese peroxidase isoenzyme pattern in decolorized Olive Oil Mill Wastewaters," *Appl. Environ. Microbiol.* 64, 2726-2729.

Ramos-Cormenzana, A., Juarez- Jimenez, B., and Garvia-Pareja, M. P. (1996). "Antimicrobial activity of olive mill wastewaters (alpechin) and biotransformed olive oil mill wastewater," *Int. Biodeter. Biodegr.* 38, 283-290.

Revankar, M. S., Desai, K. M., and Lele, S. S. (2007). "Solid-state fermentation for enhanced production of laccase using indigenously isolated *Ganoderma* sp.," *Appl. Biochem. Biotechnol.* 143, 16-26.

Rodriquez-Couto, S., Dominguez, A., and Sanroman, M. A. (2002). "Production of manganese-dependent peroxidase in a new solid-state bioreactor by *Phanerochcete chrysosporiurn* grown on wood shavings. Application to the decolorization of synthetic dyes," *Folia Microbiol*. 47, 417-421.

Rodriquez-Couto, S., Gundin, M., Lorenzo, M., and Sanroman, M. A. (2002). "Screening of supports and inducers for laccase production by *Trametes versicolor* in semi-solid-state conditions," *Process Biochem*. 38, 249-255.

Rodriquez-Couto, S., Rosales, E., Gundin, M., and Sanroman, M. A. (2004). "Exploitation of a waste from the brewing industry for laccase production by two *Trametes* species," *J. Food Eng.* 64, 423-428.

Rodriquez-Couto, S., and Sanroman, M. A. (2005). "Cocunut flesh: A novel raw material for laccase production by *Trametes hirsuta* under solid-state conditions. Application to Lissamine Green B decolourization," *J. Food Eng.* 71, 208-213.

Rodriquez-Couto, S. (2007). "Decolorization of industrial azo dyes by crude laccase from *Trametes hirsute*," *J. Hazard. Mater.* 148, 768-770.

Rodriquez-Couto, S., and Toca-Herrera, J. L. (2007). "Laccase production at reactor scale by filamentous fungi," *Biotechnol. Adv*. 25, 558-569.

Shah, M. P., Reddy, G. V., Banerjee, R., Babu, P. R., and Kothari, I. L. (2005). "Microbial degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi (*Phylosticta* spp. MPS-001 and *Aspergillus* spp. MPS-002)," *Process Biochem*. 40, 45-451.

Souza, D. F., Tychanowich, G.K., Souza, C. G. M., and Peralta, R. M. (2006). "Co-production of ligninolytic enzymes by *Pleurotus pulmonarius* on wheat bran solid state cultures," *J. Basic Microbiol.* 46, 126-134.

Stajic, M., Persky, L., Cohen, E., Hadar, Y., Brceski, I., Wasser, P. S., and Nevo, E. (2004). "Screening of laccase, manganese peroxidase, and versatile peroxidase activities of the genus *Pleurotus* in media with some raw plant materials as carbon sources," *Appl. Biochem. Biotechnol.* 117, 155-164.

Stajic, M., Persky, L., Friesem, D., Hadar, Y., Wasser, P. S., Nevo, E., and Vukojevic, J. (2006). "Effect of different carbon and nitrogen sources on laccase and peroxidase production by selected *Pleurotus* species," *Enzyme Microb Technol.* 38, 65-73.

Yesilada, O., and Fiskin, K. (1995). "The use of white rot fungi for evaluation and degradation of vinasse," *Turk J Biol*. 19, 181-189.

Yesilada, O., Sik, S., and Sam, M. (1998). "Biodegradation of olive oil mill wastewater by *Coriolus versicolor* and *Funalia trogii*: effects of agitation, initial COD concentration, inoculum size and immobilization," *World J. Microbiol. Biotechnol*. 14, 37-42.

Yesilada, O., Yildirim, C. S., Birhanli, E., Apohan, E., Asma, D., and Kuru, F. (2010). "The evaluation of pre-grown mycelial pellets in decolorization of textile dyes during repeated batch process," *World J. Microbiol. Biotechnol.* 26, 33-39.

Article submitted: April 11, 2011; Peer review completed: August 10, 2011; Revised version accepted: September 9, 2011; Published: September 13, 2011.