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Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment

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Abstract Laccases are enzymes belonging to the group of oxidases. Laccases catalyze the oxidation of a variety of phenolic compounds, diamines and aromatic amines. Twenty-four fungal isolates were isolated from biodeteriorated ancient paper and parchment. These isolates were identified and found to belong to six genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus* and *Trichoderma*, and were tested for producing laccase enzyme. *Trichoderma harzianum* have the ability of secreted laccase enzyme. The maximum production of laccase enzyme by *T. harzianum* was observed at 35 °C and pH 5 after 6 days. The highest activity of laccase achieved at 35 °C and pH 5 during the reaction. FTIR analysis revealed that the structure of extracted fungal pigments has aromatic ring and phenols group. Crude laccase was capable to decolorize different pigment structures. The enzyme showed great decolorization efficiency toward the extracted yellow pigment produced from *Asp. terrus* and *Asp. ochareceous* treated by 200 µl of partially purified enzyme. Laccase enzyme was used to decolorization pigment secreted from deteriorated pigmented fungi on paper and parchment during 30 days by using a pieces of paper and parchment inoculated by spore suspension. The results indicated that a high removal effect of fungal pigment on paper (71.21%) was recorded comparing to parchment samples (32.39%).

Introduction

The use of enzyme in the diverse field of industrial application is of greater importance in recent years. Many of such potential enzymes are widely distributed in nature; laccase is one among them which is oldest and most studied enzymatic system. Laccase is currently the focus of much attention because of its diverse applications such as dye decolorization, waste detoxifications and bioremediation applications. Laccases catalyze the oxidation of a broad range of substrates such as ortho and para-diphenols, methoxy-substituted phenols, aromatic amines, phenolic acids and several other compounds

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coupled to the reduction of molecular oxygen to water with one electron oxidation mechanism (Atallah et al., 2013).

Laccase is most widely distributed in a wide range of higher plants, fungi and bacteria (Benfiled et al., 1964; Diamantidis et al., 2000). Laccases are secreted out in the medium extracellulary by several fungi during the secondary metabolism but not all fungal species produce laccase such as Zygomycetes and Chytridiomycetes (Morozova et al., 2007). Fungi belong to Deuteromycetes, Ascomycetes as well as Basidiomycetes are known producers of laccase (Gochev and Krastanov, 2007; Sadhasivam et al., 2008).

Archives, museums and libraries worldwide conserve many historical document collections of important cultural value for all humankind. These documents may be composed of paper but oldest may be of parchment (Kraková et al., 2012). Many microorganisms that cause deterioration to ancient paper and parchment have been exploited for the production of color, because the microbial colors have the advantage of being climate independent, do not require large area for their growth and can be produced in any quantity in shorter period (Sanjay et al., 2007). Fungal degradation of library materials and paintings causes different kinds of damage depending on the species of organism responsible for the attack and the characteristics of the substratum. Damage can occur because of mechanical stress, production of staining compounds or enzymatic action (Blyskal, 2009; López-Miras et al., 2013; Pinzari et al., 2010; Santos et al., 2009; Sterflinger, 2010).

Microbial pigments are typically composed of many complex chemical substances that are formed during metabolic process (Szczepanowska and Lovett, 1992). The fungal species are found on paper and produce characteristic stains: *Alternaria solani*, *Pencillium notatum*, *Fusarium oxysporium* and *Chaetomium globosum* (Szczepanowska and Lovett, 1992). Among the bacteria, *Bacillus*, *Micrococcus* and *Pseudomonas* were commonly found on paper and leather and *Actinomycetes* were also present such as *Streptomyces*. Yeasts, such as *Candida*, were isolated (Valentin, 2010(.

Chemical compounds were effective in removing fungal stains present varying degree of toxicity and should be handled with extreme care (Tavzes et al., 2013; Szczepanowska and Lovett, 1992). The enzyme laccase has recently been employed in bioremediation, biofuel, biosensor, and organic synthesis applications (Dubé et al., 2008) as well as in textile industries to decolorize a variety of pigments and dyes (Ramsay and Goode, 2004) and may have cultural heritage applications (Konkol et al., 2009). The use of laccase in decolorization of unwanted pigment on ancient paper or parchment instead of using chemicals is considered a safety solution. Ramsay and Nguyen (2002) found that after decolorization by laccase enzyme, toxicity of few dyes remained the same while some became nontoxic.

The current work was designed to optimize the conditions of laccase enzyme activity and its use for decolorizing the fungal pigment on ancient paper and parchment.

Materials and methods

Isolation and identification of fungal isolates

All fungi were isolated from biodeteriorated paper from Kasr Abdin, Cairo, Egypt, and biodeteriorated parchment from Othman's manuscript, Cairo, Egypt, on Czapek's agar medium (Difco, 1984).

Identification of fungal isolates was accomplished depending on colonial characters of the pure culture, microscopic characters and dimensions of informative character of each fungal isolate using a specific program (Axio Vision 4.7) for measurement (with help of computerized Carl Zeiss microscope Axioplane 2) and comparing it with that are present in the identification references (Gilman, 1969; Traute et al., 1980; Alexopoulos et al., 1985).

Screening of laccase producing fungi

All collected fungal isolates were cultured on Petri plates containing sterilized potato dextrose agar (PDA) (ATCC, 1982) supplemented with 0.04% guaiacol (Sigma, USA) and 0.01% (w/v) chloramphenicol (to avoid bacterial growth) adjusted at pH 5.5. These Petri plates were incubated at 28–30 °C for 72 h and then screened for the formation of reddish brown zones around the fungal colonies (Kalra et al., 2013).

Fermentation process for growth and enzyme production

Standard inoculum (disk of fungal growth 2 mm) of the most potent fungus was cultivated into 250 ml flasks containing 100 ml of productive liquid medium (Kalra et al., 2013) which contained the following: 3 g peptone, 10 g glucose, 0.6 g KH_2PO_4, 0.001 g $ZnSO_4$, 0.4 g K_2HPO_4, 0.0005 g $FeSO_4$, 0.05 g $MnSO_4$ and 0.5 g $MgSO_4$ per L, pH 5.5, and then incubated at 30 °C for 12 days on rotary shaker (150 rpm). Fungal growth and enzyme activity were assayed periodically.

Guaiacol assay method for laccase assay

Oxidation of guaiacol has been reported for laccase assay by Kalra et al. (2013). The reddish brown color developed due to oxidation of guaiacol by laccase is used to measure enzyme activity at 450 nm. The reaction mixture can be prepared as follows:

(a) Guaiacol (2 mM) 1 ml.
(b) Sodium acetate buffer (10 mM) 3 ml.
(c) Enzyme source 1 ml (fungal supernatant).

A blank was also prepared that contains 1 ml of distilled water instead of enzyme. The mixture was incubated at 30 °C for 15 min and the absorbance was read at 450 nm using UV spectrophotometer. Enzyme activity was expressed as International Units (IU), where 1 IU is the amount of enzyme required to oxidize 1 μmol of guaiacol per min. The laccase activity in U/ml is calculated by this formula:

$$\text{E.A} = A \times V/t \times e \times v$$

where
E.A = Enzyme activity
A = Absorbance
V = Total mixture volume (ml)
v = enzyme volume (ml)
t = incubation time
e = extinction coefficient for guaiacol (0.6740 μM/ cm).

Effect of temperature, pH and incubation period on enzyme production

In order to record the optimum temperature, pH value and incubation period for laccase enzyme production, the productive medium was prepared and inoculated by standard inoculum of tested fungus as mentioned before. Five different temperatures, i.e., 20, 25, 30, 35 and 40 °C were investigated. Also, different levels of initial pH values ranged from 4:7 were applied. The proper time for the maximum laccase production was detected during 264 h fermentation period on productive medium. One flask containing 100 ml medium was taken as a sample periodically every 6–18 h, and filtrated using filter paper No. 1 to determine the growth dry weight (g/100 ml) and laccase enzyme activity in filtrate.

Characterization of enzyme activity

Effect of temperature and pH of buffer during the oxidation of guaiacol reaction was studied. Temperature was studied by incubating the enzyme mixture containing enzyme, guaiacol and sodium-acetate buffer at different temperatures (25 °C, 30 °C, 35 °C, 40 °C and 45 °C) for 15 min. pH buffer of enzyme mixture was adjusted at different values (3, 5, 7, 9 and 11) to record the optimum pH. After incubation for 15 min, the absorbance of enzyme catalyzed reaction was recorded. Then the optimum temperature and pH of the enzyme activity were detected (Kalra et al., 2013).

Pigment decolorization assay by laccase enzyme

Extraction of crude laccase enzyme

The supernatant of cultivated culture was saturated by 80% ammonium sulfate to recover the extracellular protein enzyme and then centrifugate at 10,000 rpm for 15 min. The pellet was dissolved in 10 mM phosphate buffer (pH 6.5). The sample was dialyzed by a large volume of 10 mM phosphate buffer (pH 6.5) using dialysis membrane -60. Dialyzed product was kept in the refrigerator at 4 °C (Majolagbe et al., 2012). The extraction process of pigment was preformed according to Chiu and Poon (1993).

Extraction of fungal pigment

The fungal pigments were extracted from fungal supernatant according to Chiu and Poon (1993).

Pigment decolorization assay

Different amounts of partially purified laccase enzyme (100, 150, 200, 250, 300 and 350 µl) were added to 1 ml of extracted pigment of each fungi and then the content was incubated at room temperature for 40 min. Control sample was prepared in parallel by adding acetate buffer instead of laccase under the same conditions. All measurements were done in triplicate. The absorption spectrum of pigment was measured by Spectrophotometer at the specific wavelength for each pigment. The effect of pigment decolorization was determined by the decrease in absorbance under the maximum wavelength of the dye. The efficiency of decolorization was expressed in terms of decolorization percentage (%) (Zhao et al., 2011).

$$\text{Decolorization } (\%) = (\text{initial absorbance} - \text{observed absorbance}) \times 100/\text{Initial absorbance}$$

Application of laccase enzyme to decolorize fungal pigment

Pieces of aging paper and parchment (10 × 15 cm) were inoculated by 5 ml spore suspension of isolated pigmented fungi containing 4.5×10^4 spore/ml and were sprayed onto each piece individually. After one month, the paper and parchment were over grown with the fungal hyphae and secreted pigment, and then treated with laccase at the optimum concentrations obtained from previous experiment for each pigment were added to samples and incubated at room temperature. The change of color was measured by using colorimeter during 370 min reaction period at room temperature. The percentage of decolorization pigment (%) was calculated as mentioned before.

FTIR analysis

The fungal pigments were analyzed using Fourier Transformer Infrared (FTIR) spectrometer. Each pigment was air-dried on a glass slide in the form of a thin film for the evaluation of chemical structures using a potassium bromide pellet technique (Stuart, 2004). The analysis measurement was carried out in National Research Center, physics division, spectroscopy department at infrared spectrum ranging from 340 to 4000 cm^{-1}.

Results and discussion

Screening for laccase producing fungi

Twenty-four fungal isolates were obtained from deteriorated ancient paper and parchment on Czapek's medium. All fungal isolates were identified depending on their morphological characters according to Gilman (1969), Traute et al. (1980) and Alexopoulos et al. (1985). They belonged to six main genera namely *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus* and *Trichoderma*. Among of them produced pigment and the other non produced pigment. Data in Table 1 show the incidence of fungal strains isolated from the deteriorated samples and their state of producing pigment. Five fungal strains produced pigments and the other strains non produced.

All collected fungal isolates were examined for producing laccase enzyme using guaiacol assay method. From all isolates only *Trichoderma harzianum* isolated from ancient paper produced laccase enzyme as shown in Fig. 1. It has been detected by formation of reddish brown zone around its colony due to the oxidation–reduction reaction.

These results are in line with this obtained by Gochev and Krastanov (2007) who found that many of *Trichoderma* sp. extensively studied as sources of cellulases also have been reported as sources of laccases. Moreover, *T. harzianum* and *Trichoderma longibrachiatum* are the sources of laccases production as reported by Holker et al. (2002) and Veľazquez-Cedęno et al. (2004) respectively.

Table 1 Incidence of fungal strains isolated from the deteriorated paper and parchment.

Ancient paper		Ancient parchment	
Fungal strains	Pigment production	Fungal strains	Pigment production
Alternaria alternata	Non-pigmented	*Aspergillus niger*	Non-pigmented
Alternaria geophila	Non-pigmented	*Aspergillus flavus*	Non-pigmented
Aspergillus niger	Non-pigmented	*Aspergillus versicolor*	Non-pigmented
Aspergillus flavus	Non-pigmented	*Aspergillus terrus*	Yellow pigment
Aspergillus terrus	Yellow pigment	*Aspergillus ochareceous*	Yellow pigment
Aspergillus fumigates	Non-pigmented	*Penicillium expansum*	Non-pigmented
Cladosporium cladosporidis	Non-pigmented	*Penicillium regulosm*	Non-pigmented
Penicillium expansum	Non-pigmented	*Penicillium purpurogenum*	*Red pigment*
Penicillium regulosm	Non-pigmented	*Penicillium lanosum*	*Non-pigmented*
Penicillium purpurogenum	Red pigment		
Penicillium islandicum	Brown pigment		
Penicillium raistrickii	Red pigment		
Rhizopus sp.	Non-pigmented		
Trichoderma viride	Non-pigmented		
Trichoderma harzianum	Non-pigmented		

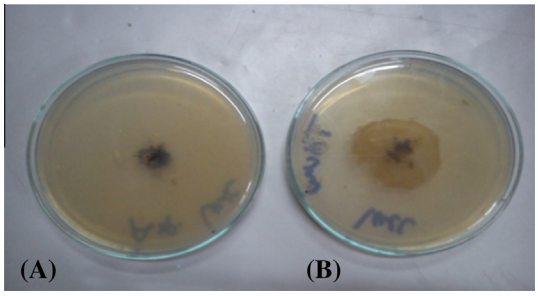

Fig. 1 Reddish-brown colonies zone apparently due to laccase produced by *Trichoderma harzianum* as a positive result (B) compared with *Asp. niger* as a negative result (A). Under the same condition.

Laccase production as affected by culture conditions

T. harzianum was cultivated on productive medium to study the effect of incubation period, pH and temperature on laccase production and the samples were taken periodically. The incubation time plays an important role in the growth and enzyme secretion. Fig. 2 shows that the laccase production by *T. harzianum* occurred during 3rd day (66 h) and the enzyme production reached its maximum at 6th day (138 h) being 1.286 U/ml and biomass production reached its maximum at 8th day (186 h) being 2.70 g/100 ml and then the rate of enzyme and mass production is declined gradually. No enzyme production was observed after 9th day. The enzyme production decreased may be due to depletion of macro and micronutrients in production medium. These results are similar with Kalra et al. (2013) and Desai and Nityanand (2011) who found that the highest enzyme activity was observed at 6th day. Sadhasivam et al. (2008) reported that the onset of laccase activity in *T. harzianum* occurred on 2nd day and reached its maximum on 4th day and the rate of enzyme production declines gradually and no enzyme production was observed after 8th days.

Fig. 3 shows that, 35 °C was the optimum incubation temperature for the highest growth of *T. harzianum* and enzyme production was obtained after 8 and 6 days being 2.923 g/100 ml and 1.479 U/ml respectively. Shraddha et al. (2011) reported that the effect of temperature is limited in case of laccase production. The optimal temperature of laccase differs greatly from one strain to another. The optimum temperature range for laccase production by *Pycnoporus sanguineus* is between 25 °C and 30 °C (Pointing et al., 2000).

Fig. 4 shows that the optimum pH for growth and enzyme production by *T. harzianum* was observed at pH 5 after 8 and 6 days respectively. The corresponding values were 3.113 g/100 ml and 1.896 U/ml in respective order. This result is similar with Thurston (1994) who found that when fungi are grown in the medium at pH 5.0, the laccase will produce in excess but most studies showed that pH between 4.5 and 6.0 is suitable for enzyme production. The results are in agreement with those obtained by Kalra et al.(2013) who found that the optimum pH value for enzyme activity was 4.5–5.5. Shraddha et al. (2011) stated that the optimum value of pH varies according to the substrate because different substrate causes different reactions for laccases.

Characterization of laccase enzyme

The effect of temperature and pH of buffer on the reaction of laccase enzyme was shown in Fig. 5. The enzyme activity was increased with increasing the temperature from 20 to 35 °C with the maximum activity at 35 °C recorded 3.81 U/ml and then, rapidly decreased at 40 °C. Similar study and results were obtained by Sadhasivam et al. (2008) who found that the maximum of laccase enzyme activity was found at 35 °C. Palonen et al. (2003) and Xu et al. (1996) indicated that in general, laccases are stable at 30–50 °C and rapidly lose activity at temperatures above 60 °C. This result is in disagreement with those obtained by Kalra et al. (2013) who found that the optimum temperature for enzyme activity was 45–50 °C. In this study, the optimum pH for maximum laccase activity was observed at pH 5 to record 4.21 U/ml enzyme activity when guaiacol was used as substrate. Above pH 5 enzyme activity decreased gradually (Fig. 5b), and this result is in line with Holker et al. (2002) and Robles et al. (2002) who revealed that the optimal pH range for fungal laccase was ranged from 4.0 to 6.0.

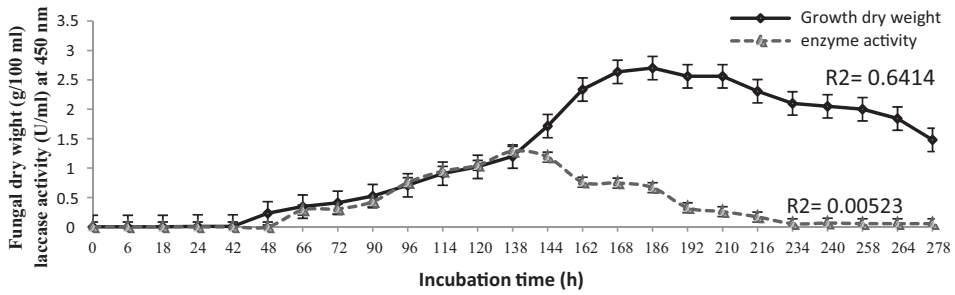

Fig. 2 Effect of incubation period on growth and laccase production producing by *Trichoderma harzianum* with pH 7 under shaking conditions during 278 h at 30 °C. The bar at the point indicates ±SE.

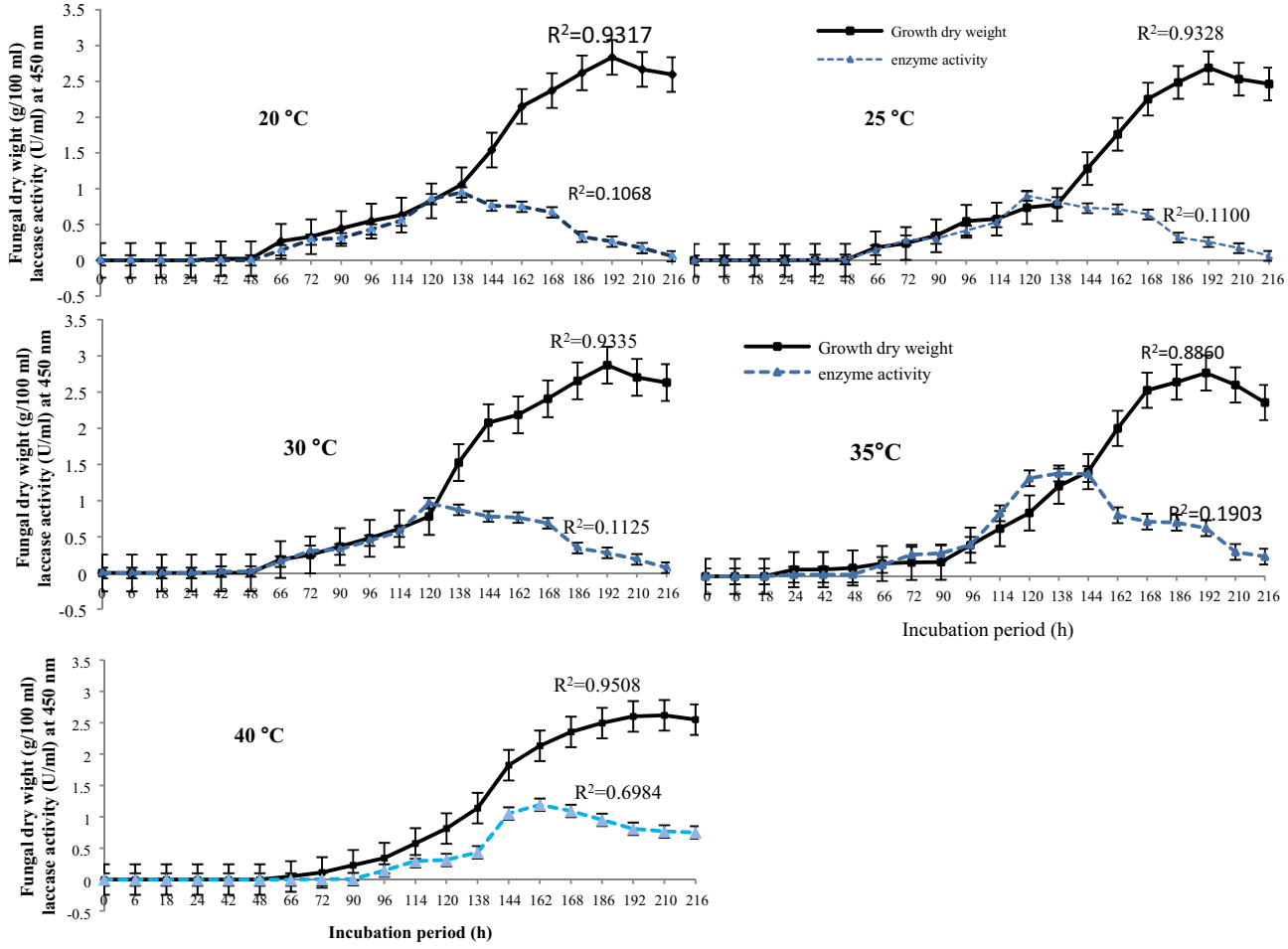

Fig. 3 Effect of incubation temperature on growth and laccase production producing by *Trichoderma harzianum* at pH 7 under shaking condition during 216 h. The bar at the point indicates ±SE.

FTIR of fungal pigments

Five fungal strains, *Asp. ochareceous* and *Asp. terrus* (yellow pigment), *Pen. purpurogenum* and *Pen. raistrickii* (red pigment) and *Pen. islandicum* (brown pigment), were isolated from deteriorated paper and parchment. They found to have the ability to secrete extracellular pigments, and their pigments were extracted and analyzed by FTIR. There was an aromatic ring and phenols group in the structure of pigments as shown in Fig. 6a–e, where the absorption ranges of aromatic rings were at 300–3100 cm^{-1} and phenols at 3100–3600 cm^{-1}. Laccases catalyze the oxidation of a broad range of substrates such as ortho and para-diphenols, methoxy-substituted phenols, aromatic amines, phenolic acids and several other compounds coupled to the reduction of molecular oxygen to water with one electron oxidation mechanism (Atallah et al., 2013). This analysis interprets the potentiality of laccase to decolorize the fungal pigment. The substrate specificity of laccases varied from one organism to other.

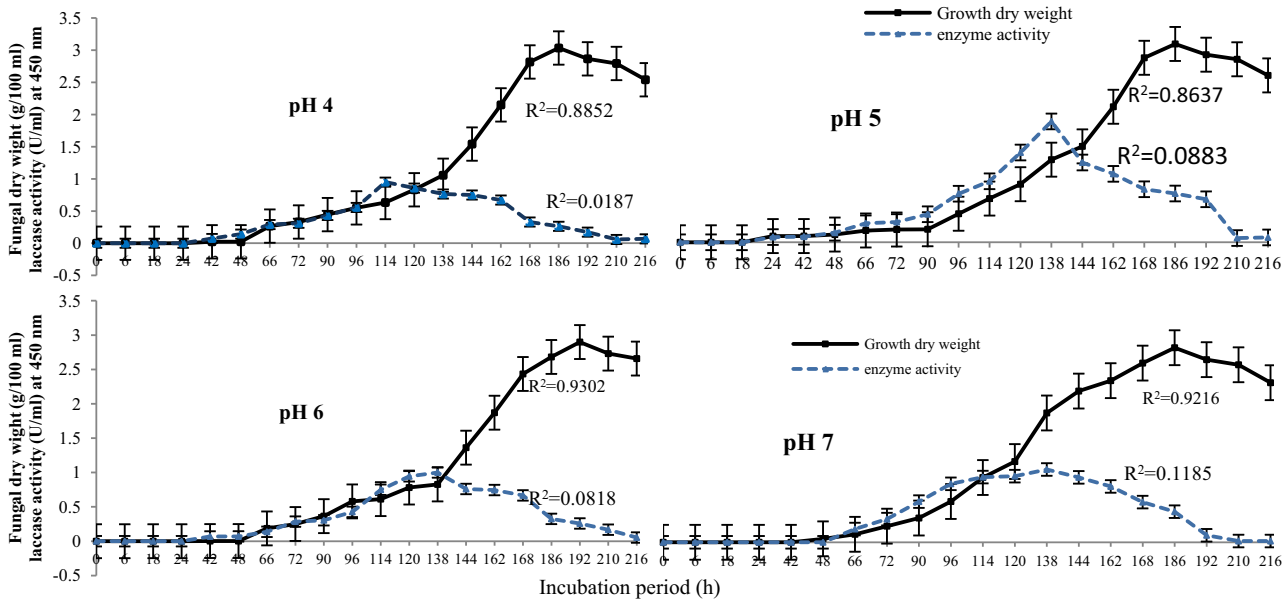

Fig. 4 Effect of different pH value on the growth and laccase production producing by *Trichoderma harzianum* at 35 °C under shaking condition. The bar at the point indicates ±SE.

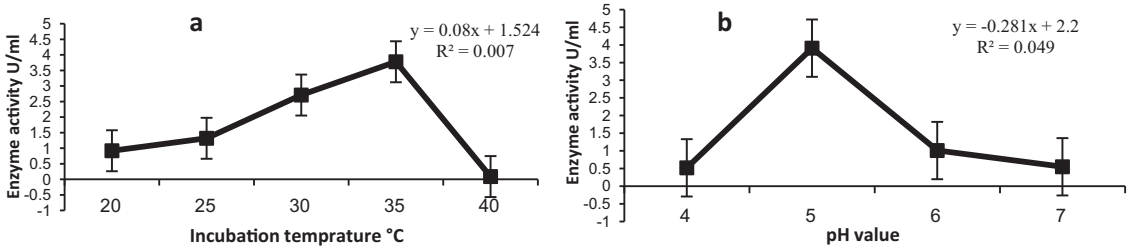

Fig. 5 Effect of temperature and buffer pH on laccase activity during the reaction period (15 min). The bar at the point indicates ±SE.

Decolorization of fungal pigments by laccase enzyme

In vitro on extracted pigment

The decolorization of fungi pigments was investigated by partially purified laccase. Different concentrations of laccase were added to 1 ml extracted pigments and then the content was incubated at room temperature for 40 min. The optimized concentration of enzymes was 200 µl for *Asp. terrus*, 250 µl for *Asp. ochareceous*, *Pen. islandicum* and 300 µl for both *Pen. purpurogenum* and *Pen. raistrickii* pigment. The corresponding values of decolorization percentage were 90.71%, 84.62%, 79.71%, 69.25% and 38.60% in respective order (Table 2).

Konkol et al. (2009) observed that use of purified laccase appears to provide a rapid, easy to use, and environmentally sound method of decolorizing microbially produced pigments on historic marble. Long recognized for its ability to decolorize chemical pulps and textiles, laccase from white-rot fungi may now have applications in cultural heritage where conventional cleaning techniques may cause more damage or are simply cost prohibitive.

On deteriorated paper and parchment

In this experiment, the decolorization of fungal pigment on deteriorated paper and parchment by laccase enzyme produced by *T. harzianum* was recorded at interval from 0 to 360 min. The decolorization percentage of treated sample was determined by colorimeter and tabulated in Table 3 and Figs. 7 and 8.

The data in Table 3 showed that laccase enzyme had highly effect on fungal pigment on biodeteriorated paper. The highest percentage of decolorization was obtained after 180 min with red pigment of *Pen. purpurogenum* (71.21%), whereas the decolorization percentage of red pigment produced by *Pen. raistrickii* recorded 21.42%. Brown pigment of *Pen. islandicum* and yellow pigment of *Asp. terrus* gave 47.53% and 43.75% after 210 and 150 min, respectively. The lowest effect of enzyme was observed with yellow pigment of *Asp. ochareceous*.

The pigmented parchment by tested fungi was measured for color before and after enzyme treatment. Laccase enzyme had less effect on fungal pigment of biodeteriorated parchment compared with the effect of laccase on paper. Bleaching of pigments appeared after 120 min to record the maximum value of decolorization percentage with yellow pigment of *Asp. ochareceous* (32.39%) after 210 min followed by yellow pigment of *Asp. terrus* after 270 min (21.79%), whereas the laccase enzyme gave low values of decolorization percentage with other tested fungi such as the lowest effect with red pigment produced by *Pen. Raistrickii* (10.12%) after 240 min.

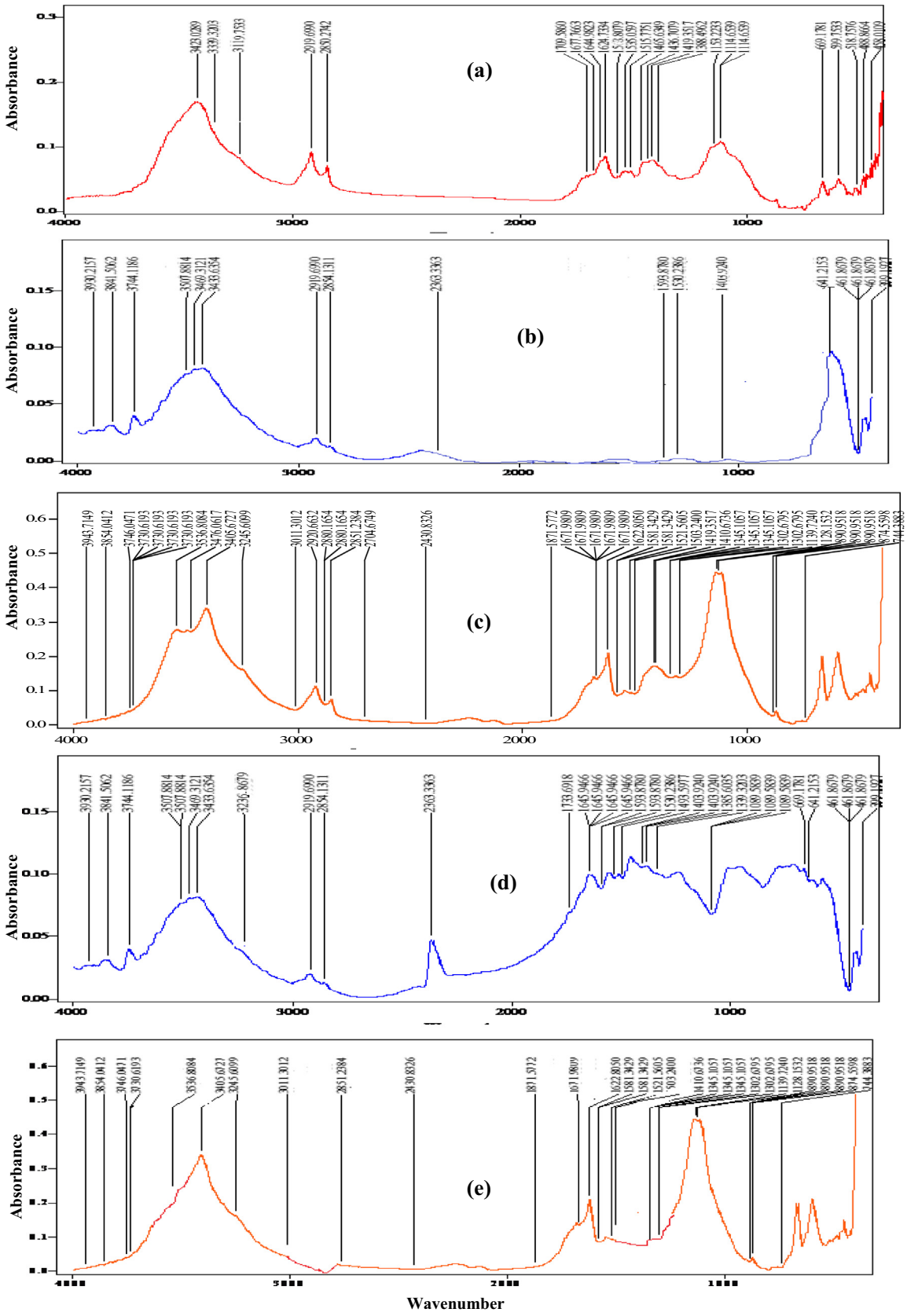

Fig. 6 FTIR analysis of fungal pigments: (a) *Asp. ochareceous*, (b) *Asp. terrus*, (c) *Pen. raistrickii*, (d) *Pen. purpurogenum* and (e) *Pen. islandicum*.

Table 2 Pigment decolorization assay by partially purified laccase enzyme.

Pigment of fungi					
Enzyme concentrations (µl)	*Asp. ochareceous* [yellow pigment (at 400 nm)]	*Asp. terrus* [yellow pigment (at 400 nm)]	*Pen. islandicum* [brown pigment (at 620 nm)]	*Pen. purpurogenum* [red pigment (at 530 nm)]	*Pen. raistrickii* [red pigment (at 530 nm)]
		% Decolorization			
100	22.67 ± 0.02	43.70 ± 0.14	29.98 ± 0.03	32.03 ± 0.06	27.36 ± 0.01
150	64.83 ± 0.09	74.69 ± 0.05	30.83 ± 0.10	33.75 ± 0.02	27.67 ± 0.03
200	84.62 ± 0.04	90.71 ± 0.02	32.47 ± 0.05	63.45 ± 0.04	27.58 ± 0.01
250	84.76 ± 0.02	0.00	79.71 ± 0.02	69.00 ± 0.13	31.02 ± 0.07
300	0.00	0.00	0.00	69.25 ± 0.05	38.60 ± 0.06
350	0.00	0.00	0.00	0.00	0.00

±: Standard error of three replicates values.

Table 3 Decolorization percentage (%) of deteriorated paper and parchment treated by laccase enzyme at room temperature.

Time (min)	*Asp. ochareceous* [yellow pigment (at 400 nm)]	*Asp. terrus* [yellow pigment (at 400 nm)]	*Pen. islandicum* [brown pigment (at 620 nm)]	*Pen. purpurogenum* [red pigment (at 530 nm)]	*Pen. raistrickii* [red pigment (at 530 nm)]
On paper sample					
0	0.00	0.00	0.00	0.00	0.00
30	0.00	1.79 ± 0.02	0.00	5.88 ± 0.54	0.00
60	0.00	20.92 ± 0.73	1.46 ± 0.08	13.08 ± 0.87	0.00
90	0.00	25.09 ± 1.90	12.10 ± 1.92	35.94 ± 1.26	10.37 ± 0.22
120	0.00	34.79 ± 1.22	29.61 ± 2.57	45.73 ± 0.05	16.85 ± 0.03
150	7.86 ± 0.15	43.75 ± 2.51	43.75 ± 1.45	52.16 ± 0.92	17.38 ± 0.02
180	15.73 ± 1.02	43.75 ± 2.09	46.55 ± 0.71	71.21 ± 1.31	21.42 ± 0.04
210	25.56 ± 1.16	43.75 ± 2.09	47.53 ± 0.26	71.21 ± 1.31	21.42 ± 0.04
240	25.56 ± 1.16	43.75 ± 2.09	47.53 ± 0.26	71.21 ± 1.31	21.42 ± 0.04
On parchment sample					
0	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00
150	7.91 ± 0.01	2.86 ± 0.02	0.00	4.47 ± 0.05	1.35 ± 0.11
180	18.66 ± 0.01	6.49 ± 0.31	4.05 ± 0.15	10.90 ± 0.02	4.59 ± 0.05
210	32.39 ± 0.02	14.24 ± 0.01	10.28 ± 0.01	14.60 ± 0.33	7.88 ± 0.46
240	32.39 ± 0.02	20.26 ± 0.01	13.86 ± 0.14	14.60 ± 0.33	10.12 ± 0.09
270	32.39 ± 0.02	21.79 ± 0.01	18.89 ± 0.12	14.60 ± 0.33	10.12 ± 0.09
300	32.39 ± 0.02	21.79 ± 0.01	18.89 ± 0.12	14.60 ± 0.33	10.12 ± 0.09
330	32.39 ± 0.02	21.79 ± 0.01	18.89 ± 0.12	14.60 ± 0.33	10.12 ± 0.09
360	32.39 ± 0.02	21.79 ± 0.01	18.89 ± 0.12	14.60 ± 0.33	10.12 ± 0.09

±: Standard error of three replicates values.

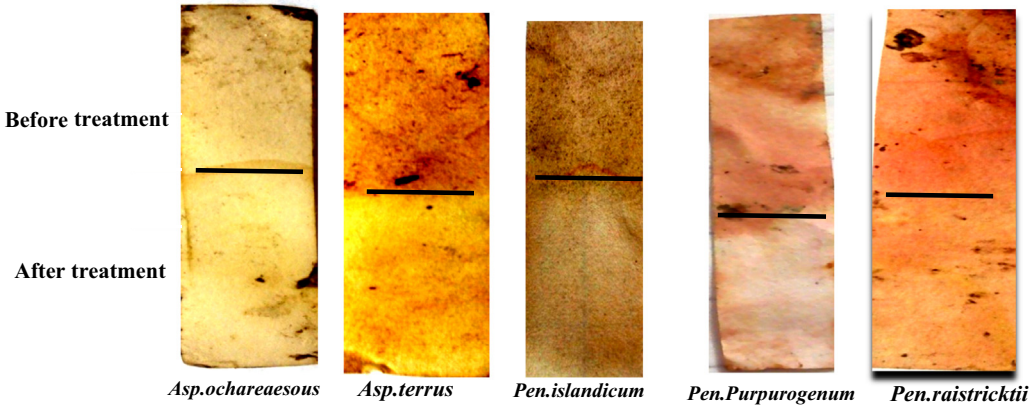

Fig. 7 Application of laccase on pigmented deteriorated paper.

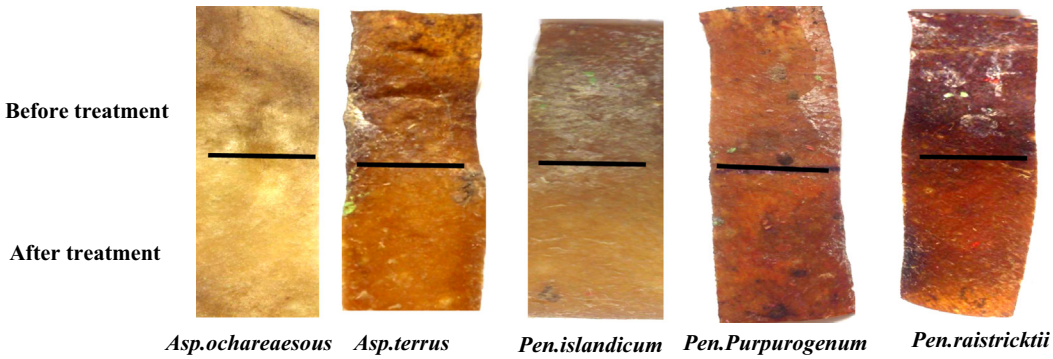

Fig. 8 Application of laccase on pigmented deteriorated parchment.

Conclusion

T. harzianum could be considered one of the most important sources for laccase production at 35 °C and pH 5. Using laccase enzyme showed potentials to decolorize different pigment structures by various degrees and can be exploited for handling the fungal pigments on documentary heritage.

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