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ORIGINAL ARTICLE

Characterization and kinetic properties of the purified *Trematosphaeria mangrovei* laccase enzyme

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Characterization

Abstract The properties of *Trematosphaeria mangrovei* laccase enzyme purified on Sephadex G-100 column were investigated. SDS–PAGE of the purified laccase enzyme showed a single band at 48 kDa. The pure laccase reached its maximal activity at temperature 65 °C, pH 4.0 with K_m equal 1.4 mM and V_{max} equal 184.84 U/mg protein. The substrate specificity of the purified laccase was greatly influenced by the nature and position of the substituted groups in the phenolic ring. The pure laccase was tested with some metal ions and inhibitors, $FeSO_4$ completely inhibited laccase enzyme and also highly affected by (NaN_3) at a concentration of 1 mM. Amino acid composition of the pure enzyme was also determined. Carbohydrate content of purified laccase enzyme was 23% of the enzyme sample. The UV absorption spectra of the purified laccase enzyme showed a single peak at 260–280 nm.
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1. Introduction

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are multicopper blue oxidases widely distributed in higher plants, in some insects and in a few bacteria. However the best known laccases are of fungal origin. Laccase production occurs in various fungi over a wide range of taxa. Fungi from the deuteromycetes, ascomycetes as well as basidiomycetes are known producers of laccase (Sadhasivam et al., 2008).

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. The substrate specificity of laccases varies from one organism to another. The spectrum of laccase oxidizable substrates can be expanded considerably in the presence of appropriate redox mediators. Due to their interesting catalytic properties laccases have

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gained considerable interest in various industrial areas (Johannes and Majcherczjk, 2000).

In this study, the physicochemical characterization of purified laccase from the marine derived fungus *Trematosphaeria mangrovei* with respect to amino acid analysis, carbohydrate content, UV absorption spectra, kinetic and stability properties were reported.

2. Materials and methods

2.1. The fungal strain and culture condition

The filamentous marine-derived fungus *T. mangrovei* used in this study was isolated from decayed wood samples collected from Abou Keer, Alexandria, Egypt (Atalla et al., 2010). Identified in the National Research Centre, Chemistry of Natural and Microbial Products Dept. [Microbial Culture Collection Unit (MCCU)] according to (Kohlmeyer and Kohlmeyer, 1991). The final optimized medium composition was found to be as follows (g/l): 16 sucrose, 2 peptone, 1 yeast extract, 50% sea water and addition of copper sulfate (2.5 mM) on sixth day of incubation with 20 days incubation period. The optimum initial pH was 6 while incubation at a temperature of 25 °C under static conditions.

2.2. Assay of laccase activity

Laccase (EC 1.10.3.2) activity was measured by using the method described by Bourbonnais et al. (1995) based on the oxidation of the substrate 2,2′-azino–bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS). The rate of ABTS oxidation was determined spectrophotometrically at 420 nm.

The reaction mixture contained 600 μL sodium acetate buffer (0.1 M, pH 5.0 at 27 °C), 300 μL ABTS (5 mM), 300 μL culture filtrate and 1400 μL distilled water. The mixture was then incubated for 2 min at 30 °C and the absorbance was measured immediately in one-minute intervals. One unit of laccase activity was defined as activity of an enzyme that catalyzes the conversion of 1 mole of ABTS per minute.

2.3. Physicochemical properties of the pure T. mangrovei laccase enzyme

2.3.1. Molecular weight determination by SDS–PAGE

The molecular weight of the purified laccase enzyme was determined by using SDS-gel electrophoresis (Laemmli, 1970). Low molecular weight markers; phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and lactalbumin (14.4 kDa) were used for the determination of the molecular weight of the enzyme.

2.3.2. Effect of temperature and pH value

These were carried out as described for crude enzyme. Where the activity was tested at different temperatures (15–90 °C) by standard enzyme assay and the optimum pH of the purified enzyme was studied over the range of (3.5–7.0).

2.3.3. Thermal and pH stability

Thermal stability was determined after different pre-incubation times (15–60 min at 35, 40, 45, 50, 55, 60, 65, 70 and 80 °C) as the residual activity. To determine the pH stability, the enzyme incubated in different buffers (0.1 M) for different times and the residual laccase activity was determined under standard assay conditions.

2.3.3.1. Effect of enzyme concentration. Different amounts of the purified enzyme (0.04 to 2.04 mg protein) were added to the reaction mixture to study its effect on the enzyme activity.

2.3.4. Effect of substrate concentration

The following substrate (ABTS) concentrations 1, 3, 5 (blank), 7, 10, 20, 30, 40, 50, 60 and 70 mM were examined to study its effect on the activity of the purified enzyme. Control was prepared using the same substrate concentration and adding dead enzyme.

2.3.5. Kinetic constants of T. mangrovei laccase

Kinetic constants of laccase enzyme was investigated using 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as a substrate, in 0.1 M sodium acetate buffer, pH 4. K_m and V_{max} were calculated according to Lineweaver and Burk (1934).

2.3.6. Substrate specificity

To determine the substrate specificity of laccase, different substrates were added to the reaction mixture at a concentration of 5 mM. These included ABTS (control), phenol, Guaiacol (2-Methoxyphenol), catechol (1,2-Benzenediol), resorcinol (1,3-Benzenediol), *m*-cresol (3-Methylphenol), *p*-cresol (4-Methylphenol) and chloro-substituted phenol (4-Chlorophenol).

2.3.7. Effect of metal ions on enzyme activity

To determine the effect of different metal ions on enzyme activity, the enzyme was preincubated for 15 min with 1 mM of each of the listed metal ions ($MgSO_4$, $MnSO_4$, $FeSO_4$, KCl, NaCl, $CoCl_2$, $HgCl_2$, $CaCl_2$ and $BaCl_2$) prior to substrate addition, the remaining enzyme activity was assayed. The control was assayed without added metal ions.

2.3.8. Effect of inhibitors on enzyme activity

The effect of several inhibitors such as ethylene diamine tetra acetic acid (EDTA), sodium dodecyl sulfate (SDS), sodium cyanide, sodium azide and aniline on laccase activity was studied at a concentration of 1 mM. A control test was conducted in parallel in the absence of the inhibitor.

2.4. Amino acid analysis of the purified laccase enzyme

Pure enzyme sample was analyzed by LC 3000 Amino Acid Analyzer at the following conditions: Flow rate 0.2 ml/min, pressure of buffer from 0 to 50 bar, pressure of reagent from 0–150 bar, reaction temperature 123 °C.

Amino acid composition was carried out by acid hydrolysis with hydrochloric acid of proteins, Millipore (1987).

2.5. Carbohydrate content of purified laccase enzyme

The carbohydrate content of the purified enzyme was determined as glucose with phenol–H_2SO_4 according to the method of Dubois et al. (1956).

2.6. U.V absorption spectra of purified laccase enzyme

The laccase UV-absorbance spectrum was scanned from 200 to 1100 nm at room temperature on a Jasco V-630 Spectrophotometer.

3. Results

The present series of experiments were undertaken to investigate the properties of the purified laccase enzyme.

3.1. Molecular weight of pure laccase enzyme from T. mangrovei

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) of the purified laccase enzyme showed a single band at 48 kDa (Fig. 1).

3.2. Effect of temperature

The results in Fig. 2 showed a rapid increase in the laccase enzyme activity was detected up to 65 °C and above which the activity began to decrease.

3.3. Effect of pH value

The data in Fig. 3 indicated that the enzyme showed a maximum activity at pH 4.0, above or below the activity decreased. At pH 7.0 the enzyme became completely inactive.

3.4. Thermal stability of pure laccase enzyme at pH 4.0

The results in Fig. 4 showed that the stability of the enzyme activity depended on the temperature and time of heating.

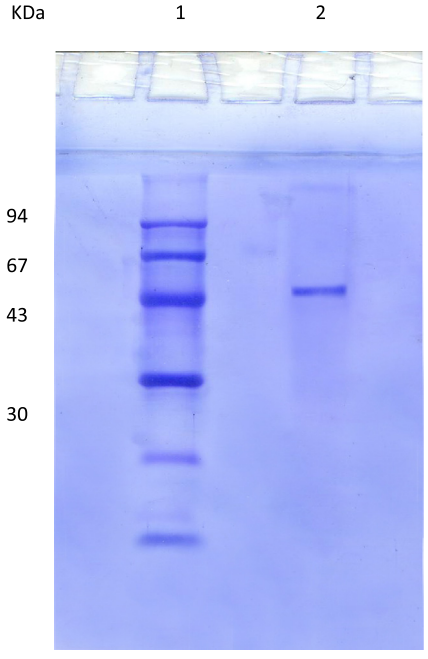

Figure 1 (SDS–PAGE) polyacrylamide disk electrophoresis of pure laccase from *T. mangrovei*. Lane 1: protein standard, lane 2: purified laccase enzyme.

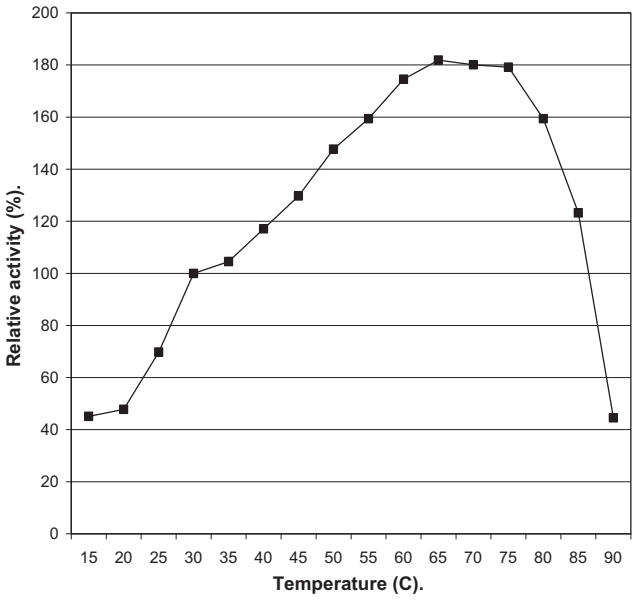

Figure 2 Effect of different temperatures of the reaction on pure laccase activity.

At 35 °C the enzyme was stable for 60 min, at 40 and 45 °C the enzyme retained about 56.77 and 55.71% of its activity after treatment for 60 min. Up to 45 °C the enzyme began to lose partially its activity and more adverse effect was found by heating at 65, 70 and 80 °C where the enzyme lose about 98% of its activity after treatment for 60 min.

3.5. pH stability of pure laccase enzyme at 65 °C

The results recorded in Fig. 5 showed that at pH 4.0 the enzyme activity possessed highest stability after 60 min exposure. At pH 4.5 the enzyme retained about 87.34% of its activity after 30 min exposure. At pH 3.5 the enzyme retained about 54.43% of its activity after 60 min exposure. Results also indicated that pH 5, 5.5 and 6.0 had adverse effects on the enzyme activity; about 80.17%, 88.61% and 91.14%, respectively of the enzyme activity was lost after 60 min. Despite pH 5 at

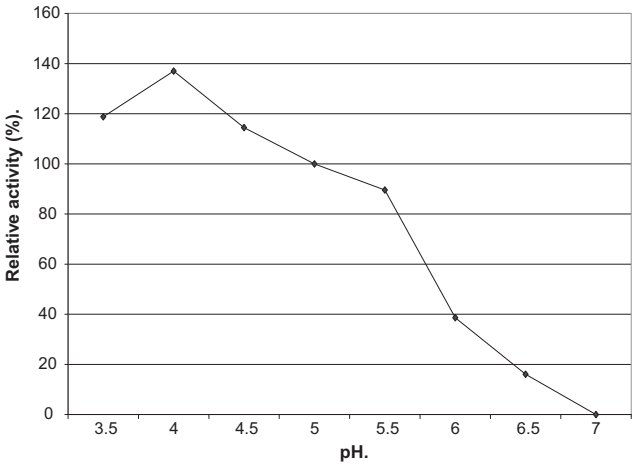

Figure 3 Effect of different pH values of the reaction on pure laccase enzyme activity.

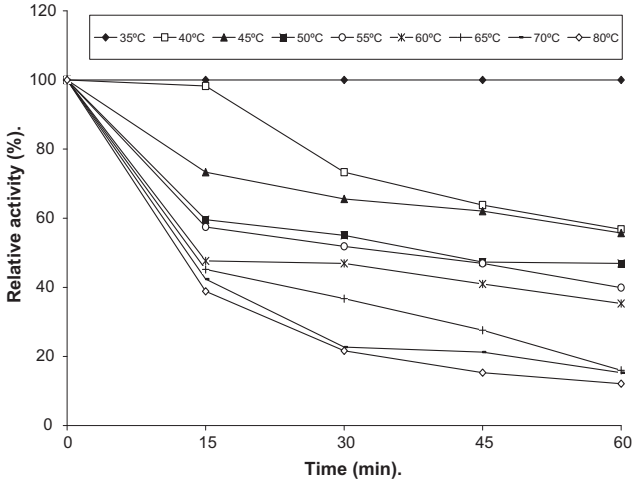

Figure 4 Thermal stability at pH 4.0 of the pure laccase enzyme from *T. mangrovei*.

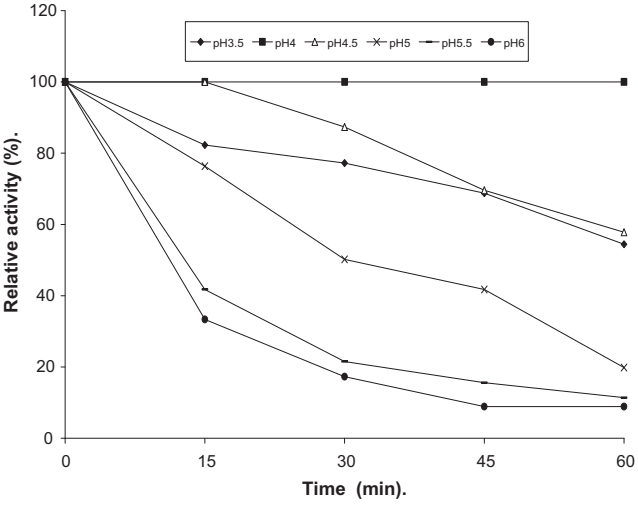

Figure 5 pH stability at 65 °C of the pure laccase enzyme from *T. mangrovei*.

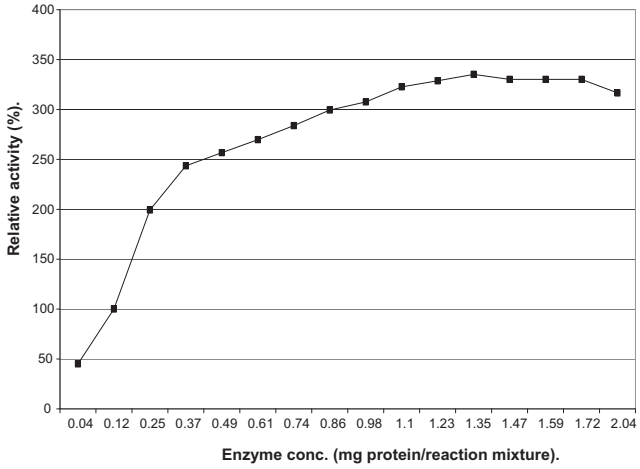

Figure 6 Effect of different enzyme concentrations on pure enzyme activity.

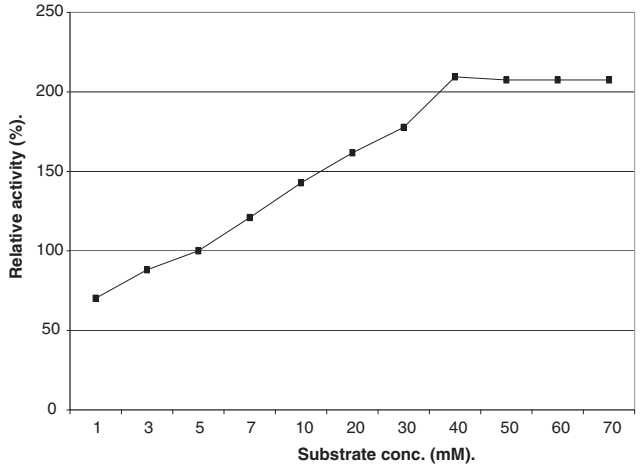

Figure 7 Effect of substrate concentrations on pure enzyme activity.

which 50% of the enzyme activity was retained after 30 min incubation.

3.6. Effect of enzyme concentration on enzyme activity of purified laccase

The results in Fig. 6 showed successive increase of laccase enzyme activity with increasing enzyme concentration from 0.04 to 1.35 mg per reaction mixture where the maximum enzyme activity was obtained. The laccase activity increased 235.27% as compared to the control experiment 100% at 0.12 mg protein. With further increase in the enzyme concentration, the activity remained nearly constant.

3.7. Effect of substrate concentration on pure enzyme activity

Results in Fig. 7 indicated that the activity increased linearly up on increasing the ABTS concentration until reaching maximum at 40 mM. Higher substrate concentrations resulted in a constant enzyme activity. It was a loss in enzyme quantity.

3.8. Kinetic parameters (K_m and V_{max} values) of T. mangrovei laccase

The kinetic parameters of the purified *T. mangrovei* laccase enzyme were determined by using ABTS as a substrate at the following concentrations (1, 3, 5, 7, 10, 20, 30, 40 mM) and calculated from Lineweaver and Burk plots. The results indicate that the K_m was 1.42 mM and V_{max} was 184.84 U/mg protein.

3.9. Substrate specificity

A series of phenolic and non phenolic aromatic compounds were examined as possible enzyme substrates at a concentra-

Table 1 Substrate specificity of *T. mangrovei* laccase enzyme.

Substrate (5 mM)	Relative activity (%)
ABTS	100
Phenol	–
Guaiacol	10.66
Catechol	5.71
Resorcinol	–
p-Cresol	15.60
m-Cresol	–
4-Chlorophenol	–

The rate of oxidation of each substrate is expressed relative to the rate of ABTS oxidation (100%).

tion of 5 mM, in 0.1 M Na–acetate buffer (pH 4.0) and the reaction mixture containing the same amount of enzyme for all tested substrates.

As shown in Table 1 the nature and substitution on the phenolic ring affected the oxidation rate of laccase enzyme. The purified enzyme strongly oxidized ABTS. The enzyme has also slight activity with guaiacol (2-Methoxyphenol), *p*-cresol (4-Methylphenol) and catechol (1,2-Benzenediol). No activity was observed on phenol, resorcinol, *m*-cresol (3-Methylphenol) and 4-Chlorophenol.

3.10. Effect of metal ions on the activity of purified enzyme

Results in Fig. 8 showed that, $FeSO_4$ completely inhibited laccase enzyme, while in the presence of $CoCl_2$, $MgSO_4$ and $HgCl_2$ the enzyme retained about 66.01, 64.38 and 57.58% of its initial activity, respectively. The metal ions KCl and NaCl have similar effects on the enzyme activity where the enzyme retained about 71% of its initial activity, the other metal ions $BaCl_2$, $CaCl_2$ and $MnSO_4$ have little effect on the enzyme activity.

3.11. Effect of inhibitors on the activity of the purified enzyme

The oxidase inhibitors sodium azide and sodium cyanide caused about 77.77 and 51.48% inhibition of laccase activity,

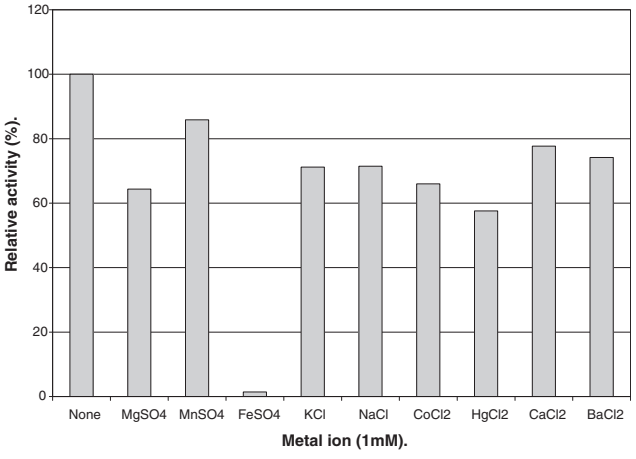

Figure 8 Effect of metal ions on *T. mangrovei* laccase activity. The enzyme activity without added metal ions was taken as 100% activity.

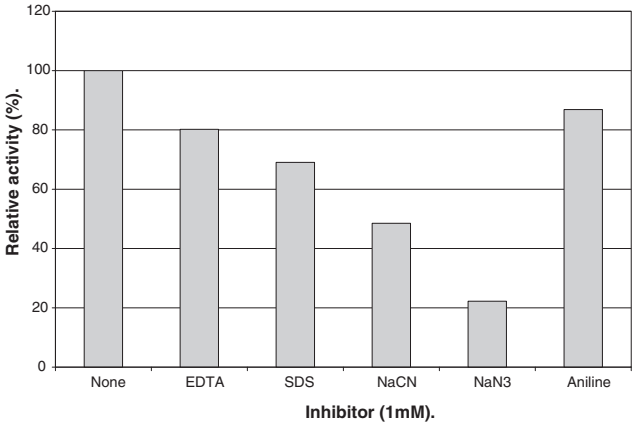

Figure 9 Effect of inhibitors on *T. mangrovei* laccase activity. Activity without inhibitors added was taken as 0% inhibition.

respectively. However the enzyme retained 69.04% of its activity in the presence of 1 mM sodium dodecylsulfate (SDS). Other inhibitors, EDTA and aniline have low effect on laccase activity (Fig. 9).

3.12. Amino acid analysis of the purified laccase enzyme

The amino acid analysis of the purified laccase enzyme indicated that the enzyme contained 13 amino acids (Fig. 10). The enzyme rich in alanine 15.5% and serine 13.2% of the total amino acids. Also contained high amounts of glutamic acid, threonine and aspartic acid with 11.4%, 11.1% and 10.4% of the total amino acids, respectively. Valine, proline, leucine comprised considerable proportions which amounted to 22.8% of the total amino acids.

On the other hand the enzyme was poor in arginine, histidine, glycine, isoleucine and lysine which all comprised only about 15.6% of the total amino acids.

3.13. Carbohydrate content of purified laccase enzyme

The results showed that the total carbohydrate content of the pure laccase enzyme from *T. mangrovei* contributed 23% of the enzyme sample and the protein content was 77%.

3.14. U.V absorption spectra of purified laccase enzyme

The absorption spectra of the purified laccase enzyme was recorded between 200 and 300 nm. The spectrophotometric curve of the purified enzyme Fig. 11 exhibited a maximum absorption at 260–280 nm.

4. Discussion

The characterization and purification of the crude laccase enzyme obtained from the filamentous marine-derived fungus *T. mangrovei* was described in the previous study (Atalla et al., 2010).

SDS-gel electrophoresis of the purified laccase enzyme showed a single band at 48 kDa. This result agreed with that reported about the molecular masses of *Ganoderma lucidum* laccases (40 and 66 kDa) (D'Souza et al., 1999).

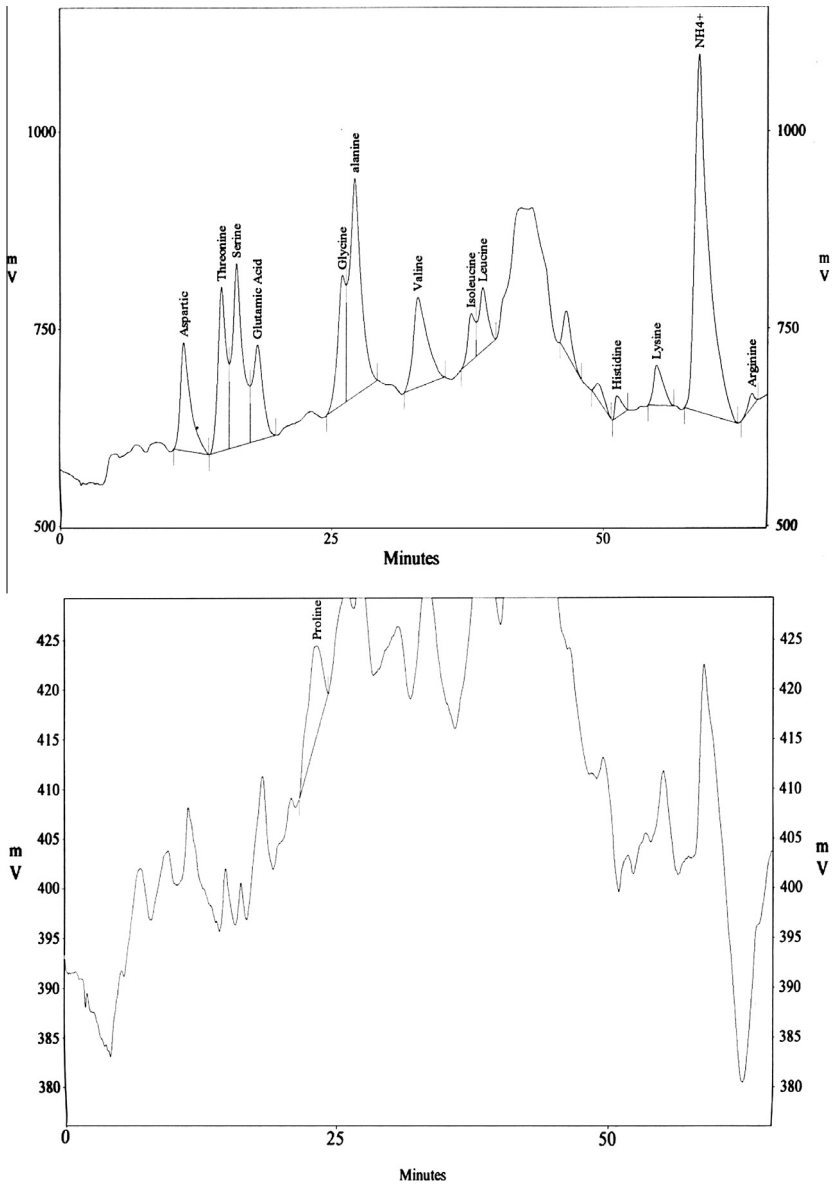

Figure 10 Amino acid analysis of the purified laccase enzyme. Total of amino acids = 100%.

The general properties of the purified laccase enzyme were studied. Optimum temperature of the reaction of the purified enzyme was found to be 65 °C. These results do not differ from other laccases with optima temperature between 50 and 70 °C (Baldrian, 2006). This result to some extent was similar to that reported by Liers et al. (2007) who found that the highest activity of *Xylaria polymorpha* laccase enzyme was observed between 55 and 60 °C. Laccase from *Galerina sp*. exhibited optimal activity on substrate 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) at 60 °C (Ibrahim et al., 2011).

Dias et al. (2003) reported that *Phanerochaete chrysosporium* ATCC 24725 laccase enzyme displayed maximum activity at 50–60 °C with the standard ABTS assay. The enzyme proved to be almost fully active between 45 and 70 °C but only 37% of maximum activity was achieved at 25 °C. The results obtained in the present study, concurred with these observations.

The optimum pH of the purified enzyme with ABTS as the substrate was found to be 4.0. This result is similar to that obtained by Šušla et al. (2007) who found that the optimum pH of *Dichomitus squalens* purified laccase enzyme was 4.0. Studies with laccases from *Coriolus hirsutus*, *Trichoderma atroviride*, *Chalara (syn. Thielaviopsis) paradoxa CH 32* and *Cerrena unicolor* 059 showed that the optimal pH range for fungal laccase was from 4.0 to 6.0 (Holker et al., 2002 and Robles et al., 2002).

Stability of the enzyme with respect to temperature was also studied. At 35 °C the enzyme was stable for 60 min., at 40 and 45 °C the enzyme retained about 56.77 and 55.71% of its activity after treatment for 60 min. Up to 45 °C the enzyme began to lose partially its activity and more adverse effect was found by heating at 65, 70 and 80 °C where the enzyme lose about 98% of its activity after treatment for 60 min. These results are to some extent similar to that reported by Forootanfar

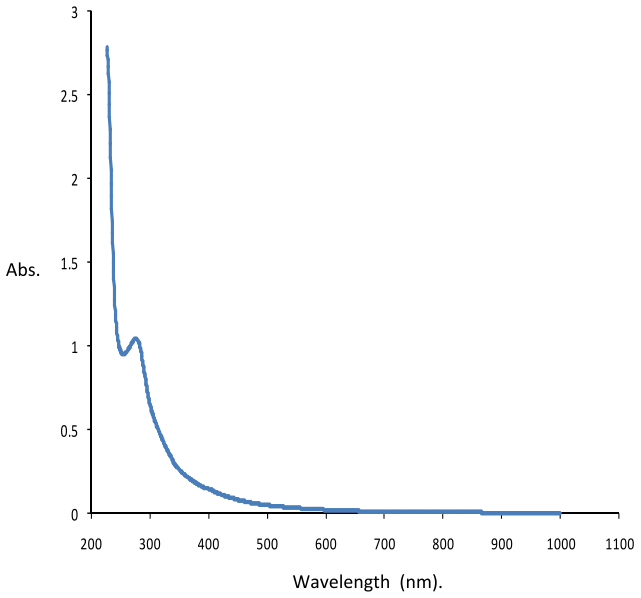

Figure 11 UV absorption spectra of purified laccase enzyme.

et al. (2011) who indicated that at 50 °C the purified enzyme retained 50% of its initial activity after 1 h. However, it was almost completely inactive at higher temperatures.

Depending on the source of the microorganism, thermal stability varied at different temperatures. For example, the laccase from *T. harzianum* retained 70% of its initial activity after 1 h incubation at 55 °C (Sadhasivam et al., 2008), whereas the laccase from *Daedalea quercina* was very stable below 45 °C (Baldrian, 2004).

Our study showed that the enzyme below pH 4.0 and above pH 4.5 was partially unstable. This result is similar to that reported about the good stability of *T. atroviride* laccase in slightly acidic pH values (Chakroun et al., 2010). This stability is rare among fungal laccases, for example laccases from *Melanocarpus albomyces* (Kiiskinen et al., 2002), *Pleurotus ostreatus* (Palmieri et al., 2003) and *Perenniporia tephropora* (Ben Younes et al., 2007) have good stability at neutral and slightly alkaline pH values.

The effect of enzyme concentration on enzyme activity was carried out under standard conditions with varying amounts of enzyme (mg protein) added. The results showed that, the maximum laccase activity was obtained with 1.35 mg protein of the purified enzyme per reaction mixture. On the other hand, 5.429 mg protein of the crude enzyme per reaction mixture was the best concentration for the maximum enzyme activity. This result indicated that the crude sample contains high amount of protein with low laccase activity but the pure sample contains low protein with high enzyme activity.

The effect of substrate (ABTS) concentration on the activity of the purified laccase enzyme was studied. Results indicated that the activity increased linearly up on increasing the ABTS concentration to reach maximum at 40 mM and higher substrate concentrations than 40 mM resulted in a constant enzyme activity.

The kinetic parameters (K_m and V_{max} values) of the purified *T. mangrovei* laccase enzyme was determined by using ABTS as a substrate and calculated from Lineweaver and Burk plots (1934). The results indicated that the K_m value was 1.42 mM and V_{max} value was 184.84 U/mg protein. This result is lower than that reported by Chakroun et al. (2010) who found that the K_m value of *T. atroviride* laccase enzyme was 2.5 mM towards ABTS.

Robles et al. (2002) reported that the K_m of laccase from *Chalara (syn. Thielaviopsis) paradoxa CH 32* by using ABTS as a substrate at pH 4.5 was 0.77 mM. While the K_m 1.6 mM was found with *Ceriporiopsis subvermispora* at pH 3.5 using Guaiacol (Fukushima and Kirk, 1995). This observation of the different K_m values confirms the suggestion of Banerjee and Vohra (1991) who attributed these differences to be due to alteration of culture conditions, pH and substrate used.

Substrate specificity of *T. mangrovei* laccase enzyme was studied on different phenolic and non phenolic aromatic compounds. The results indicated that, purified enzyme strongly oxidized ABTS. The enzyme has also slight activity with Guaiacol (2-Methoxyphenol), *p*-cresol (4- Methylphenol) and catechol (1,2-Benzenediol). No activity was observed on phenol, resorcinol, 3-Methylphenol (*m*-cresol) and 4-Chlorophenol. The enzyme showed higher affinity and activity toward ABTS than other substrates. These results are similar to that reported by Zouari-Mechichi et al. (2006) about the substrate specificity of both laccases obtained from *Trametes trogii* and Laccase from *Galerina sp.* showed the highest activity toward ABTS, a non-phenolic heterocyclic compound, while the activity with the phenolic substrates was much lower (Ibrahim et al., 2011).

The effect of different metal ions on the activity of laccase enzyme was investigated. Results showed that all the metal ions used exhibited inhibition effects to the enzyme activity but with different degrees. $FeSO_4$ showed complete inhibition effect on laccase enzyme while in the presence of $CoCl_2$, $MgSO_4$ and $HgCl_2$ the enzyme retained about 66.01, 64.38 and 57.58% of its initial activity, respectively. The metal ions KCl and NaCl have less effect on the enzyme activity where the enzyme retained about 71% of its initial activity, the other metal ions $BaCl_2$, $CaCl_2$ and $MnSO_4$ have little effect on the enzyme activity.

Sadhasivam et al. (2008) showed that the metals such as Co, Hg, Fe, K, Mg, Mn, Na, Ba and Ca at a concentration of 1 mM had no significant effect over *Trichoderma harzianum* WL1 laccase activity except Hg, which caused 17.2% inhibition. On the other hand the purified laccase from the edible mushroom *Lentinula edodes* was inhibited in the presence of 1 mM Sn^{2+} (99%), Ca^{2+} (70%), Zn^{2+} (64%), Hg^{2+} (55%), K^{+} (54%) and Cd^{2+} (45%) (Nagai et al., 2002). The observations indicated that the effect of metal ions on laccase activity was highly dependent on its source and the type of metals used, which had a great influence on the catalytic activity of the enzyme.

Effect of a range of potent laccase inhibitors on the enzyme activity was tested. The oxidase inhibitors sodium azide and sodium cyanide caused about 77.77 and 51.48% inhibition of laccase activity, respectively. However the enzyme retained 69.04% of its activity in the presence of 1 mM sodium dodecylsulfate (SDS). Other inhibitors, EDTA and aniline have low effect on laccase activity, similar to *T. harzianum* laccase which was mildly inhibited by the metal chelator EDTA 1 mM concentration (16.8% inhibition) Sadhasivam et al. (2008). *T. atroviride* laccase was strongly inhibited by the typical laccase inhibitor sodium azide, but it was not sensitive to EDTA and SDS (Chakroun et al., 2010).

Robles et al. (2002) stated that sodium azide and potassium cyanide caused complete inhibition of *Chalara (syn. Thielaviopsis) paradoxa CH 32* laccase activity at 2 mM final concen-

tration. Also inhibited by the chelating agent EDTA and sodium dodecylsulfate caused complete inhibition of laccase activity at a final concentration of 0.1%.

Laccase activity was completely inhibited by the common metalloenzyme inhibitor, sodium azide (NaN_3) at the concentration of 20 μM. It was reported that the binding of sodium azide to the types 2 and 3 copper sites affects internal electron transfer, thus inhibiting the activity of *Sclerotium rolfsii* laccase Ryan et al. (2003).

The amino acid analysis of the purified laccase enzyme indicated that the enzyme contained 13 amino acids. Alanine 15.5% and serine 13.2% were the major amino acids. Glutamic acid, threonine and aspartic acid comprised considerable proportions which amounted to 11.4%, 11.1% and 10.4% of the total amino acids, respectively. On the other hand the enzyme was poor in arginine, histidine, glycine, isoleucine and lysine which all comprised only about 15.6% of the total amino acids. These results were similar to that reported about the amino acid composition of *Trametes sp*. AH28–2 laccase (Xiao et al., 2004).

Carbohydrate content of purified laccase enzyme was estimated to be 23% of the enzyme sample, while it contained 77% protein. This result is similar to that showed by Xiao et al. (2004) who indicated that *Trametes sp*. AH28–2 laccase B was a monomeric glycoprotein with 25% carbohydrate content. This agree with that is known about fungal laccases where laccase typically contains 15–30% carbohydrate, which may contribute to the high stability of the enzyme.

U.V absorption spectra of purified laccase enzyme was recorded between a range of 200–300 nm. The spectrophotometric curve of the purified enzyme exhibited a maximum absorption at 260–280 nm. This result is similar to the absorption spectra of the purified laccase enzymes (LA and LB) obtained from *P. ostreatus* NRRL (0366) strain where LA exhibited a maximum absorption at 260–280 nm and 260–275 nm for LB enzyme (Hala, 1996). Also Liers et al. (2007) found that UV absorption spectra of *X. polymorpha* showed characteristic absorption maxima at 280 nm.

It can be concluded that, the marine–derived fungus *T. mangrovei* laccase enzyme exhibited interesting enzymatic properties, such as thermal stability at 35, 40 and 45 °C (1 h), has broad substrate specificity, showed very low inhibition with Mn, Ca, Ba, K, Na, Mg, aniline, EDTA and SDS.

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