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Research article

Substrate specificity of a new laccase from *Trametes polyzona* WRF03

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ABSTRACT

Various aromatic compounds that are structurally analogous to lignin were tested as possible/preferred substrates for purified laccase from newly isolated white rote fungi, *Trametes polyzona* WRF03. The pH optima were tested using different substrates and kinetic studies were conducted at these pH optima. The pH optima in the presence of ABTS, α-naphthol, o-dianisidine, and catechol were 4.5 but 5.0 and 5.5 in the presence of guaiacol and pyrogallol, respectively. The initial velocities obtained from the kinetic study were analyzed using Graph Pad Prism 7 and Lineweaver-Burk plot to obtain kinetic constants (k_m and V_{max}) which were used to calculate substrate specificity. Amongst all the substrates tested, ABTS had the highest specificity-constant (181.51 $M^{-1}s^{-1}$), and therefore, the most preferred substrate was followed by α-naphthol, *o*-dianisidine, guaiacol, pyrogallol, and catechol. Resorcinol, orcinol, and veratryl alcohol did not display any considerable chemical shift in the presence of *Trametes polyzona* WRF03 laccase. Also, oxidation of phenolic substrates appeared to be dependent on the nature of the substituent groups and their relative position on the aromatic nucleus. Since most of these substrates are structural analogs of lignin and many recalcitrant environmental pollutants, the enzyme may find application in delignification, treatment of wastewater containing dyes, and polycyclic aromatic hydrocarbons (PAHs).

1. Introduction

Laccase (benzediol: oxygen oxidoreductase, EC 1.10.3.2) has drawn scientific attention over the past few decades because of its ability to act on many substrates in the presence of molecular oxygen (abundant in nature) as the only requirement for its catalysis; thus making the enzyme applicable to many industrial processes [1]. The increasing demand for laccase has therefore intensified the search for laccase homologs with unique properties since laccases from different species/strains have different properties. Laccase is a member of the polyphenol oxidases, with four copper atoms (+2 oxidation state) coordinated within their active site [2]. The four cupric ions are distributed within three copper sites namely T1, T2, and T3 copper sites and they coordinated the amino acids responsible for the catalytic mechanism of the enzyme. Laccases are found in plants and many microorganisms where they play various physiological functions. However, reports indicated that white-rot fungi basidiomycetes are the most efficient laccase-producers and other lignolytic enzymes in response to lignin degradation [3].

Phenols such as ortho (position 2) and para (position 4) –diphenols, methoxy phenols, aromatic amines, phenolic acids, and several other lignin-related compounds are natural substrates of laccase [4]. Using molecular oxygen as the electron acceptor, laccase can efficiently oxidize these substrates, simultaneously forming phenoxy radicals, which spontaneously rearrange to open the aromatic rings or promote their polymerization. Due to their low redox potentials (0.5–1.0 V), these phenolic compounds are potential substrates for laccase [5]. In addition, the range of substrates oxidized by laccase can be extended greatly by the introduction of low molecular weight redox mediators.

One of the distinct advantages of laccase over peroxidases is that it only requires the presence of the substrates and molecular oxygen, which is abundant in nature for its catalysis. In addition, its apparent stability, broader substrate specificity, and lack of inhibition in the presence of hydrogen peroxide give added advantage over peroxidases [6, 7]. These properties make the enzyme most attractive and suitable for application in myriads of industrial oxidative processes such as pulp bleaching [8], dye decolorization [9], environmental clean-up protocols (bioremediation and detoxification of pollutants) [10], bioconversion of agricultural and forestry residues [11], organic synthesis [12], biosensor designing [13], enzymatic and immunochemical assays [14]. Production of laccase by several species of white-rot fungi, including *Pycnoporus sanguineus, Ganoderma lucidum, Fomes fomentarius, Pleurotus ostreatus, Pycnoporus cinnabarinus, Trametes trogii,* and *Trametes versicolor*

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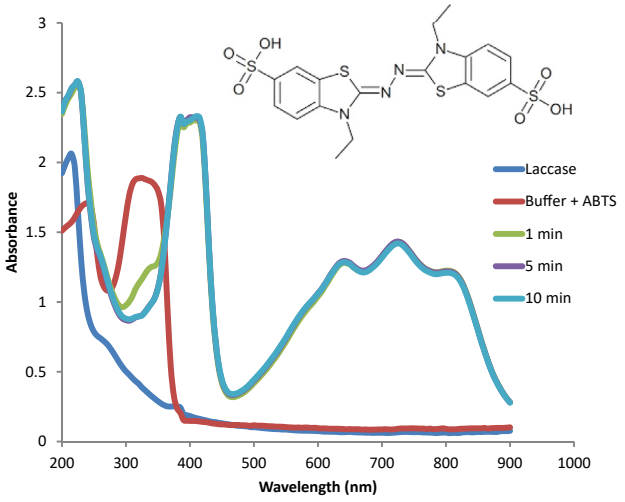


Figure 1. UV-Visible absorption spectrum of laccase catalyzed oxidation of ABTS with a characteristic peak at 420 nm.

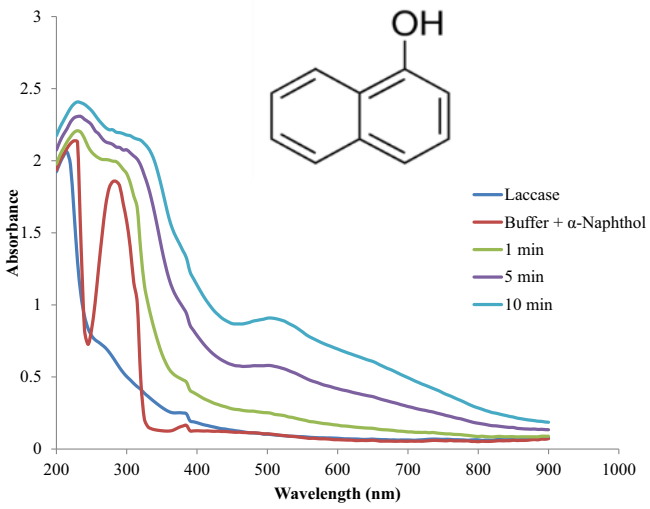


Figure 2. UV-Visible absorption spectrum of laccase catalyzed oxidation of α-naphthol with a broad peak around 510 nm.

is highlighted in many reports [15, 16, 17, 18, 19, 20, 21]. However, the substrate specificity of laccase differs markedly from one organism to another [22].

Factors that determine substrate specificity include the conformation and size of the substrate-binding pocket, the binding-site specific amino acid residues, and the redox potential difference between the T1 copper site and the substrate [23, 24]. Quantitatively, the Michaelis constant, k_m, and the catalytic efficiency, k_{cat}, describe the substrate specificity of an enzyme. Catalytic efficiency is the measure of the efficiency in the conversion of a given substrate by a given enzyme to a given product, hence the ratio, k_{cat}/k_m, is known as specificity constant [25]. There is available literature data reporting wide variances in the measured k_{cat}, k_m, and k_{cat}/k_m among some laccase homologs. The affinities of the purified laccase isoenzymes toward five selected substrates (2, 6-dimethoxyphenol, guaiacol, ferulic acid, veratric acid, and syringaldazine) were observed to be different [26]. However, the best substrate for all isoenzymes was 2,6-Dimethoxyphenol. Also, Wang et al. [27] determined the specificity constant of two purified laccases isoforms. The two laccase isoforms showed similar substrate specificity for the four investigated substrates, which were in the order of ABTS > Syringaldazine>2, 6-Dimethoxyphenol > guaiacol.

Since most of the laccases characterized so far exhibited huge differences in terms of their reactivity towards various phenolic and non-phenolic aromatic compounds, which invariably determine their applicability, the present study, therefore, sets to evaluate the kinetics and substrate specificity of laccase from a newly isolated white-rot fungus, *Trametes polyzona* WRF03.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals obtained from Sigma Aldrich, Germany constitute the major experimental chemicals in this study. They include ABTS (2, 2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), guaiacol, *o*-dianisidine, α-naphthol, pyrogallol, resorcinol, orcinol, bovine serum albumin (BSA) and Sephadex G-100, diethylaminoethyl cellulose (DEAE-cellulose) and folin-ciocalteau reagents.

2.2. Microorganism and lignocellulosic waste

A pure culture of a newly isolated and efficient laccase producing white-rot fungus, *Trametes polyzona* WRF03, was collected from the Microbial Culture Collection of Enzymology and Protein Chemistry Unit, Department of Biochemistry, University of Nigeria, Nsukka. The lignocellulosic waste (Wheat bran) used in solid-state fermentation for laccase production was purchased from a local market commonly known as Ogige main market in Nsukka Local Government Area of Enugu State, Nigeria.

Table 1. Spectral, kinetic properties and substrate specificity of laccase from *Trametes polyzona* WRF03.

Substrate	UV/Visible absorption maxima (nm)		v_{max} (μmol/min)[a]	k_m (μM)[b]	k_{cat} (s^{-1})[c]	Substrate Specificity (k_{cat}/k_m) (M^{-1} s^{-1})	Percentage Specificity (%)
ABTS	420	1429.00		8.66	1571.90	181.51	100
α-naphthol	510	941.00		16.66	1035.10	62.13	34.23
o-dianisidine	460	3858.00		89.26	4243.80	47.54	26.19
Pyrogallol	390	136.40		60.60	150.04	2.47	1.36
Guaiacol	460	366.90		191.00	403.59	2.11	1.16
Catechol	390	45.06		142.50	49.57	0.35	0.19
Resorcinol	-		-	-	-	-	-
Orcinol	-		-	-	-	-	-
Veratryl alcohol	-		-	-	-	-	-

[a] $_{Vmax}$ and [b]k_m values were determined by linear regression using GraphPad Prism 7 software/Lineweaver-Burk plot of the initial velocity. [c] k_{cat} values were calculated from V_{max} ($V_{max} = k_{cat}$ [Eo]) with the molecular weight (66 kDa) of *Tp*L. '-' = no measurable activity.

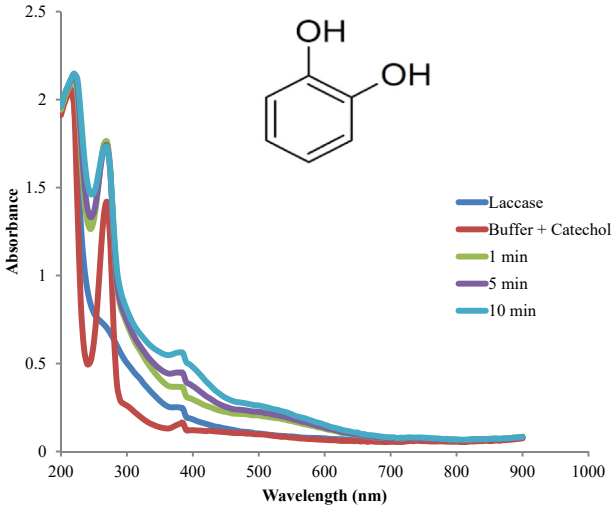


Figure 3. UV-Visible Absorbance spectrum of laccase catalyzed of oxidation of catechol with a characteristic peak at 390 nm.

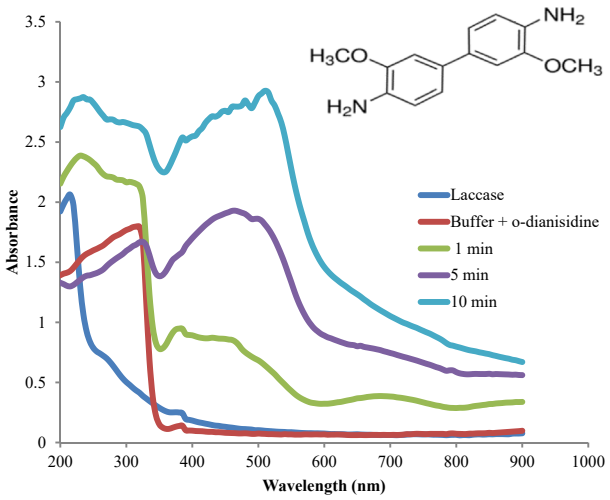


Figure 5. UV-Visible absorption spectrum of laccase catalyzed oxidation of *o*-dianisidine with a characteristic peak at 460 nm.

2.3. Solid-state fermentation for laccase production

Laccase was produced in 250 ml Erlenmeyer flasks containing 20 ml of fermentation medium composed of $(NH_4)_2SO_4$ (0.22 g/L), KH_2PO_4 (0.2 g/L), $MgSO_4.7H_2O$ (0.05 g/L), thiamine (0.001 g/L), $CuSO_4$ (0.08 g/L), $CaCl_2$ (0.01 g/L), $NaMO_4$ (0.05 g/L), $FeSO_4$ (0.03 g/L), $MnSO_4$ (0.07 g/L), $ZnSO_4$ (0.043 g/L), glucose (10 g/L) and solidified with wheat bran (g/L) [28]. Three PDA agar plugs cut from the growing edge of pure *Trametes polyzona* WRF03 cultures were inoculated into the media. The flasks were incubated for 9 days at room temperature (27 °C) after which enzyme was extracted with 50 ml of sodium acetate buffer (100 mM, pH 5.0) and filtered through cheesecloth. The filtrate was centrifuged at 10, 000 rpm for 10 min to remove mycelia and the supernatant was used as the crude enzyme extract.

2.4. Laccase activity assay

Laccase activity was assayed initially using ABTS by monitoring its oxidation in the presence of the enzyme at 420 nm ($_{420}$ nm 36,000 $mM^{-1}cm^{-1}$) according to the method of Shin and Lee [29]. Enzyme activity assay was made in 100 mM sodium acetate buffer (pH 4.5) at 30 °C. One unit of enzyme activity is the amount of enzyme that oxidizes 1.0 μmol of ABTS to product per minute.

2.5. Determination of protein content

According to the Lowry et al. [30] method, the estimation of the protein content was made using bovine serum albumin (BSA) standard.

2.6. Purification of crude enzyme extract

In line with the modified method of Sahay et al. [31], crude enzyme extract from *Trametes polyzona* WRF03 was purified by three protein purification steps – ammonium sulfate precipitation (90 % ammonium sulfate saturation)/dialysis, anion exchange chromatography (DEAE--cellulose column; 2 × 30 cm) and gel filtration (Sephadex G-100 column; (2 × 76 cm) as previously reported [28].

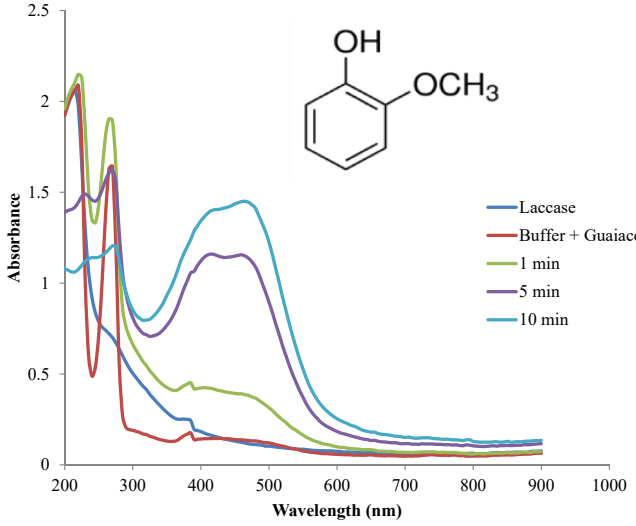


Figure 4. UV-Visible absorption spectrum of laccase catalyzed oxidation of guaiacol with a characteristic peak at 460 nm.

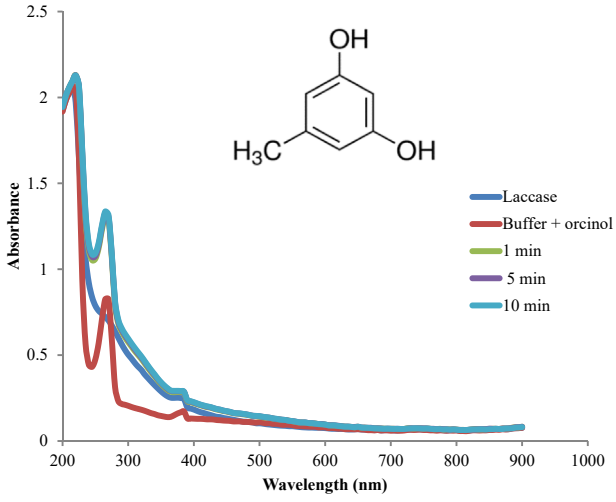


Figure 6. UV-Visible absorption spectrum of laccase catalyzed oxidation of orcinol with no characteristic peak.

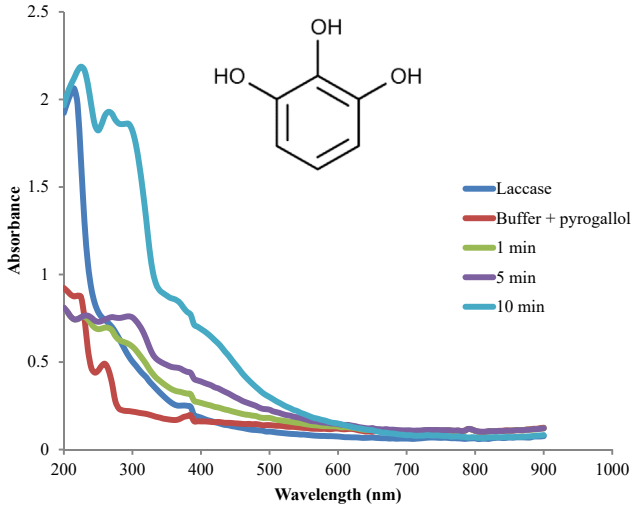


Figure 7. UV-Visible absorption spectrum of laccase catalyzed oxidation of pyrogallol with a characteristic peak at 390 nm.

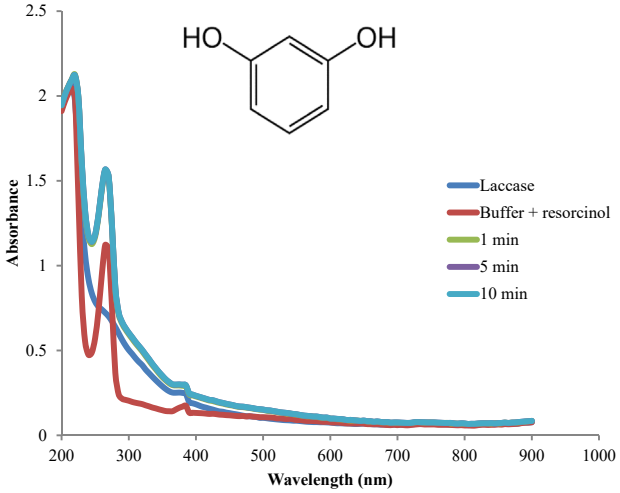


Figure 8. UV-Visible absorption spectrum of laccase catalyzed oxidation of resorcinol with no characteristic peak.

2.7. Substrate specificity studies of Trametes polyzona WRF03 laccase

2.7.1. Spectra scan of laccase-catalyzed reaction

Spectral scans of laccase-catalyzed oxidation of the different phenolic compounds (guaiacol, pyrogallol, catechol, orcinol, resorcinol, α-naphthol, veratryl alcohol and non-phenolic compounds (ABTS and *o*-dianisidine) were monitored at 200–900 nm using UV-Visible spectrophotometer (Jenway 6405) to determine their wavelengths of maximum absorbance (WMA). The reaction mixture contained 0.1 ml of each potential substrate (final conc. 100 μM) in 2.8 ml of sodium acetate buffer (0.1 M, pH 4.5). The oxidation was monitored for 10 min for a possible spectral shift in the presence and absence of enzyme. It was monitored for 10 min using a UV-Visible spectrophotometer (Jenway 6405) and the WMA was identified.

2.7.2. Determination of pH optima for laccase activity

At the WMA of each of the substrates, the optimum pH of laccase activity was assayed for each of the substrates at different pH value

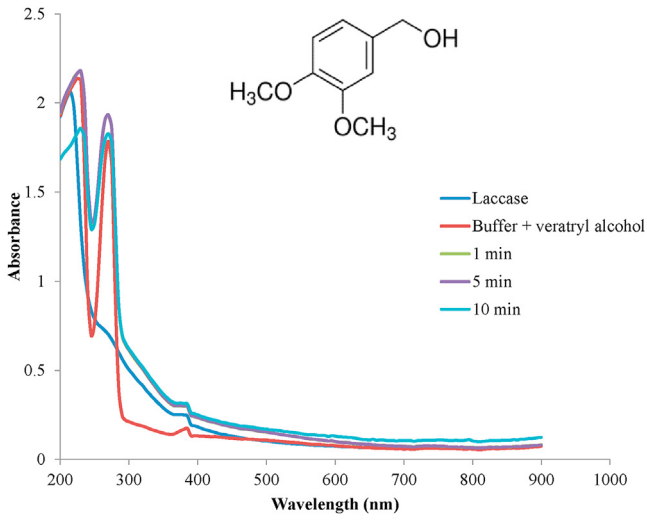


Figure 9. UV-Visible absorption spectrum of laccase catalyzed oxidation veratryl alcohol with no characteristic peak.

ranging from 3.0-10.0 using sodium acetate buffer (0.1 M, pH 3.5–5.5), phosphate buffer (0.1 M, pH 6.0–7.5) and Tris-HCl buffer (0.1 M, pH 8.0–9.5). The reaction mixture contained 0.1 ml of each of the substrate (final conc. 100 μM) in 2.8 ml of respective pH and 0.1 ml of purified enzyme. One unit of enzyme activity was defined as the amount of enzyme required to convert 1 μmol of the substrate to the product in 1 min under the assay conditions employed.

2.7.3. Determination of substrate specificity constant

Kinetic studies of laccase-catalyzed oxidation of these substrates were carried out at their respective pH optima for laccase activity. All laccase activity assays were done at the same temperature of 30 °C. The initial velocity data were analyzed using Graph Pad Prism 7 Software and Lineweaver-Burk plot to determine the kinetic constants, k_m, and $_{Vmax}$. If Michaelis-Menten model fits, $k_2 = {}_{Vmax} = k_{cat}$ [E_{total}]. The specificity constant was calculated from the ratio of k_{cat}/k_m value for each substrate catalyzed to product and was used to predict the preferred substrates for the enzyme.

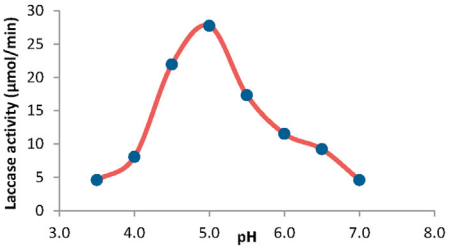
3. Results

3.1. Purification of laccase

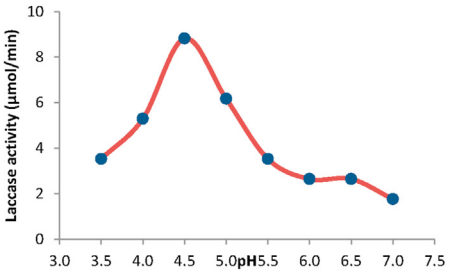
The crude enzyme extract from *Trametes polyzona* WRF03, cultured with wheat bran (carbon source), and lignocellulosic support under solid-state fermentation system showed a specific activity of 1637 U/mg protein. After ammonium sulfate purification, ion-exchange chromatography, and gel filtration, laccase from *Trametes polyzona* WRF03 (TpL) was purified approximately 13-fold with a specific activity of 21523 U/mg protein and percentage yield of 2.8.

3.2. Spectra scan of laccase oxidation of substrates

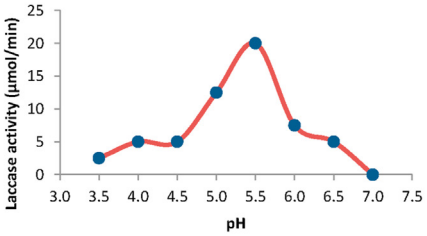
The wavelengths of maximum absorbance (WMA) of *Tp*L catalyzed oxidation of substrates are shown in Table 1. *Tp*L oxidized a broad range of phenolic substrates (guaiacol, pyrogallol, catechol, and α-naphthol) and non-phenolic substrates (ABTS and o-dianisidine). Resorcinol, orcinol, and veratryl alcohol did not display any considerable chemical shift in the presence of laccase as shown in Figures 1, 2, 3, 4, 5, 6, 7, 8, and 9.



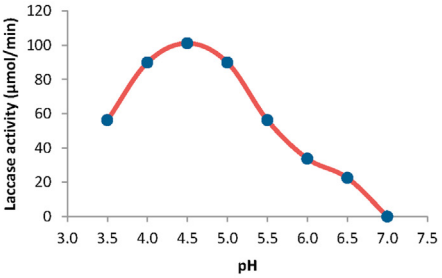
(a)Optimum pH of *Tp*L with guaiacol as substrate



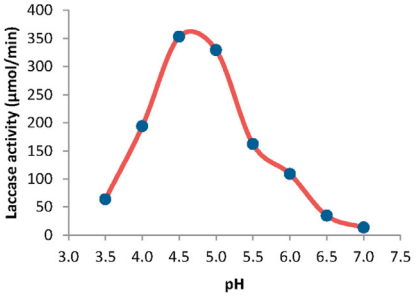
(b) Optimum pH of *Tp*L with catechol as a substrate



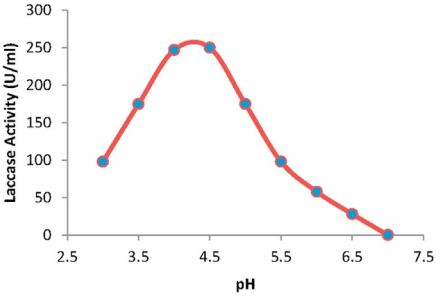
(c) Optimum pH of *Tp*L with pyrogallol as substrate



(d) Optimum pH of *Tp*L with α-naphthol as a substrate



(e) Optimum pH of *Tp*L with *o*-dianisidine as substrate



(e) Optimum pH of *Tp*L with as substrate

Figure 10. Optimum pH of *Tp*L on various substrates using 0.1 M sodium acetate buffer (pH 3.0–5.5), 0.1 M sodium phosphate buffer (pH 6.0–7.5) and 0.1 M Tris-HCl buffer (pH 8.0–10.0).

3.3. pH optima for laccase activity

Oxidation of all the substrates described a characteristic bell-shaped pH-activity profile with pH optima in the acidic range. *Tp*L showed optimum pH of 4.5 for ABTS, α-naphthol, o-dianisidine, catechol whereas for guaiacol and pyrogallol 5.0 and 5.5 were observed, respectively (Figure 10).

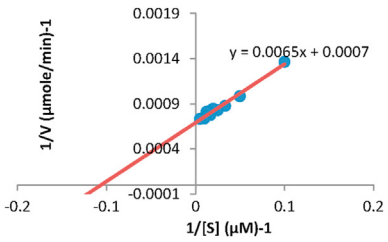
3.4. Substrate specificity constant

Kinetic parameters ($_{Vmax}$ and k_m) of *Tp*L catalyzed oxidation of the various substrates obtained were presented in Figure 11. The substrate specificity of TpL was computed as the ratio of k_{cat} to k_m for the various oxidized substrates. The enzyme showed a higher substrate specificity value (181.51 M-1s-1) with ABTS than other substrates. ABTS was then used as a standard to evaluate the percentage specificity of the enzyme with other substrates. The *Tp*L showed a percentage specificity of 34.24, 25.80, 1.66, 1.36, and 6 % for α-naphthol, *o*-dianisidine, guaiacol, pyrogallol, and catechol, respectively (Table 1).

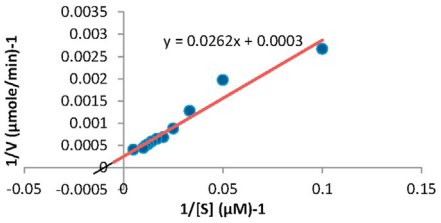
4. Discussion

Laccase has been reported to have broad substrate specificity because of its ability to oxidize a wide range of substrates. This catalytic ability towards aromatic substrates (predominantly phenols) is of great importance in delignification [32, 33], decolourization, and detoxification of dyes from textile effluents or removal of stains from biomaterials [34] and the treatment of contaminated environments [35].

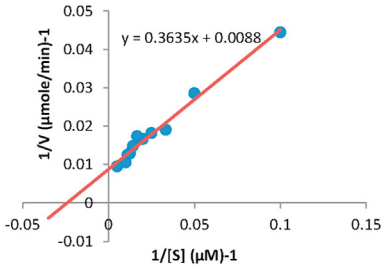
In the present study, *Tp*L oxidized phenolic compounds such as guaiacol, pyrogallol, catechol, α-naphthol as well as non-phenolic compounds (ABTS and *o*-dianisidine) although at differing oxidation rate. Spectral scans of laccase-catalyzed oxidation of various aromatic substrates revealed different maximum absorption wavelengths in the Ultraviolet-Visible region of the spectrum. Pyrogallol and catechol showed absorption maxima at 390 nm (UV) nm while ABTS, α-naphthol, *o*-dianisidine, and guaiacol showed absorption maxima of 420 nm, 510 nm, 460 nm, and 460 nm, respectively. However, there were no significant chemical shifts in the absorption spectra of resorcinol, orcinol, and veratryl alcohol indicating little or no oxidation.



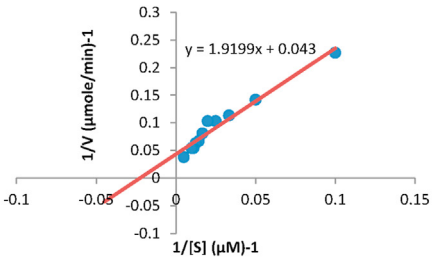
(a) Effect of substrate (ABTS) concentration



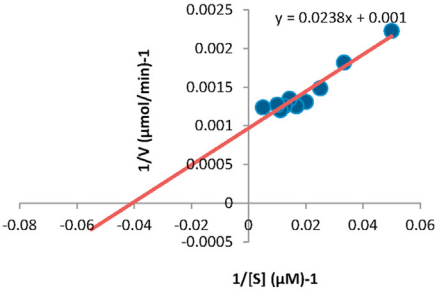
(b) Effect of Substrate (o-dianisidine) concentration



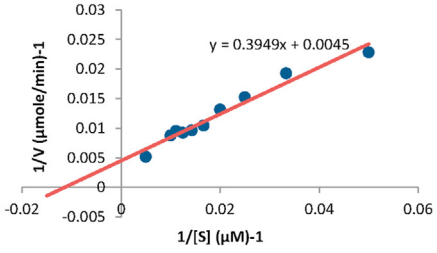
(c) Effect of substrate (pyrogallol) concentration



(d) Effect of Substrate (catechol) concentration



(e) Effect of substrate (α-naphthol) concentration



(f) Effect of Substrate (guaiacol) concentration

Figure 11. Lineweaver-Burk plot of the initial velocity of the effect of different substrate concentrations on *Tp*L.

Although, the actual oxidation products of some of these substrates have not been elucidated, the oxidation of ABTS (a non-phenolic heterocyclic compound) according to Johannes and Majcherezyk [36], resulted in the formation of a cation radical, ABTS azine ($ABTS^{\cdot+}$) and subsequently to ABTS dication ($ABTS^{2+}$). During the enzymatic oxidation, ABTS turned from colourless to blue green and was responsible for the characteristic peak observed at 420 nm. The oxidation of α-naphthol with a hydroxyl group on naphthalene ring resulted in the formation of insoluble purple products, which absorbs at 510 nm [37]. Conversely, the oxidation of various phenolic substrates appeared to follow a particular pattern as reported by Viikari [38]. The phenolic substrates first lose one electron abstracted by laccase to produce aryloxy radicals These radicals are highly unstable and may undergo further non-enzymatic oxidation or reduction reactions, radical coupling to other phenolic structures, or polymerize to form deeply coloured products. For example, Laccase-catalysed oxidation of catechol produces o-benzoquinone intermediates [39]. Due to the high reactivity of this quinine intermediates, they could react spontaneously to form dimers, oligomers and polymers [40, 41]. Thus, the dark brown colour formed from the oxidation of catechol or pyrogallol by laccase are usually as a result of their oxidation and responsible for the characteristic peak observed around 390 nm.

The activity of laccase in the presence of selected phenolic compounds was investigated under different pH conditions. All the phenolic substrates showed a dumb-bell shaped curve typical of an enzyme-catalyzed reaction. The optimum pH of *Tp*L catalyzed oxidation of the substrates varied between 4.5 and 5.5 depending on the substrates. The optimum pH of laccase activity with ABTS, catechol, *o*-dianisidine, and α-naphthol was 4.5 while that of guaiacol and pyrogallol were 5.0 and 5.5, respectively. This is in agreement with many reports on literature. However, the observed variations in pH might be due to the nature of substrates with respect to the different roles of substrate protonation in the reaction mechanism [2, 42].

Fungal laccases are known generally to possess a greater affinity for ABTS than for other substrates [43]. The laccase from *Trametes polyzona* WRF03 is consistent with this generalization. Hence, in the present study, the k_m value showed that *Tp*L has a higher affinity towards ABTS than other substrates tested. Interestingly, the k_m value (8.66 µM) of *Tp*L towards ABTS was much lower than the k_m values 12.5, 47, 66.9, 69, 250, 302.7, and 333.3 µM for laccase from *Trametes versicolor* [44], *Ganoderma lucidium* [45], *Trametes sp*. LS-10C [46], *Trametes trogii* S0301 [47], *Pleurotus sp*. [48], *Cerrena unicolor* GSM-01 [27] and *Trametes orientalis* [1] respectively. This indicated that *Tp*L had a higher affinity for ABTS

when compared with laccase from these organisms. However, this may be greatly dependent on the reaction conditions including pH, temperature, substrate concentration, and ionic strength.

The kinetic parameters (v_{max} and k_m) influence the substrate specificity value which means that the lower the k_m value or higher k_m (rapid turnover), the more the affinity of that substrate to the enzyme and the higher the substrate specificity [44]. The specificity constant (k_{cat}/k_m) is expressed as a measure of an enzyme's efficiency in converting substrates into products per active site per unit time when the enzyme is saturated with the substrate. A comparison of different specificity constant of various substrates obtained from kinetic data would help to determine the best substrate for the enzyme [49]. Hence, there is a positive correlation between an enzyme's specificity constant and its preference for a given substrate. The purified *Tp*L showed notable differences in its substrate specificity when different substrates were used. Interestingly, ABTS (a non-phenolic aromatic substrate) was the most preferred substrate than other tested substrates judging by its higher specificity constant value. This is in agreement with the report of Saito et al. [50], Park and Park [51], and Olajuyigbe et al. [52]. The non-phenolic substrates such as ABTS, α-naphthol, *o*-odianisidine had higher specificity constant than the phenolic substrates such as guaiacol, pyrogallol, catechol, resorcinol, orcinol and veratryl alcohol. The percentage of specificity with ABTS was about 3 times greater than α-naphthol and about 385 times greater than catechol. The high value of k_{cat}/k_m for ABTS indicated a very high frequency with which enzyme and substrate molecules collide [49]. The lower value of specificity constant observed for the phenolic substrates was related to irreversible enzyme inactivation by the reaction products [37].

The preference of laccase towards various phenolic substrates depended on the type and relative position of the substituent groups attached to their respective benzene ring [53]. In general, laccase activity towards substituted-phenols decreases from ortho-, through para-to meta-substituted phenols [2]. In the present study, the ortho-substituted compounds (guaiacol, pyrogallol and catechol) were better substrates while the meta-substituted compounds such as resorcinol, orcinol and veratryl alcohol showed the no detectable oxidation. The number and position of the hydroxyl groups on the aromatic ring was observed to affect the oxidation of the ortho-substituted phenols. For example, *Tp*L had a higher affinity towards pyrogallol, which has three hydroxyl groups and thus, was oxidized better than catechol with two adjacent hydroxyl groups. Catechol (-OH in ortho-position) was more readily oxidized by laccase than its meta-substituted isomer (resorcinol), indicative of meta-substituted phenols as poor substrates for laccase.

Substitution of methoxy- (-OCH_3) or hydroxyl- (-OH) group at the ortho-position of the aromatic ring seemed to enhance the oxidation of an adjacent hydroxyl-group by the enzyme. In contrast, ortho-methylation (-CH_3) of the phenol ring seems to confer lowers its oxidation, which may be attributable to the absence of a lone-pair of electrons [54]. Min et al. [55] highlighted that substitution of –OH, –OCH_3, or –CH_3 group on the aromatic ring confers easy oxidation by laccase. However, *Tp*L did not oxidize substrates such as resorcinol and orcinol (with –OH) and veratryl alcohol (with –OCH_3) containing those groups at various positions.

The lack of reaction towards meta-substituted phenols may be due to the absence/or limited formation of aryloxy radicals unlike the *ortho*- and *para*-substituted phenols [56]. Laccase catalyzed oxidation of the *ortho*- and *para*-substituted phenols leads to the generation of aryloxy radicals formed by the abstraction of one electron and one proton from the substituent group. In the process, aryloxy radicals generate resonance structures that are stabilized in *para* and *ortho* positions. Meta-substituents do not form resonance-stabilized structures, thus, leading to no/low reactivity of laccases toward meta-substituted phenols [56].

Apart from the formation resonance-stabilized structures, the oxidation of phenolic substrates, which invariably affect the substrate specificity of laccase, was related to the redox potential of the substrates [57]. Since the rate-limiting step of laccase catalyzed reaction is in fact the transfer of the first electron from the substituent group of the phenolic substrate to the type-1 copper atom at the active site of the enzyme, the efficiency of oxidation of substituted phenols would therefore depend on redox-potential, which is a function of the substituent groups present [57]. As with orcinol in the present investigation, there was limited oxidation in the presence of laccase due to the diminished electron density on the phenoxy group arising from the presence of an electron-withdrawing substituent group. Furthermore, steric effect may contribute to the oxidative efficiency of the enzyme on a given substrate. However, electronic contributions may predominate because of the relatively small sizes of the substituent groups involved in the present study [58].

5. Conclusion

Laccase from *Trametes polyzona* WRF03 (*Tp*L) was able to oxidize various phenolic and non-phenolic aromatic substrates although at varying degrees. ABTS was identified as the most sensitive substrates for the enzyme. Laccase catalyzed oxidation of phenolic substrates is dependent on the number, relative position, and type of substituent groups on the aromatic ring of the compounds. Since the enzyme showed relatively high catalytic efficiency towards aromatic substrates such as ABTS, guaiacol, α-naphthol (a derivative of naphthalene), and *o*-dianisidine that are structural analogues of lignin and many recalcitrant environmental pollutants, the enzyme may be useful in many industries where radical oxidative process is required.

Declarations

Author contribution statement

Tobechukwu C. Ezike: Performed the experiments; Analyzed and interpreted the data.

Jerry O. Udeh: Analyzed and interpreted the data.

Parker E. Joshua, Sabinus O. O. Eze: Conceived and designed the experiments.

Arinze L. Ezugwu: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chukwurobe V. Isiwu: Performed the experiments.

Ferdinand C. Chilaka: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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