Multicopper oxidase laccases with distinguished spectral properties: A new outlook

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ABSTRACT

Multicopper oxidases (MCOs) has a unique feature of having the presence of four Cu atoms arranged into three (Type I, II and III) spectral classification. MCOs laccase due to its broad range of substrate specificity has numerous biotechnological applications. The two types of laccases include the typical blue and the atypical white, yellow laccases which have been isolated from diverse geographical locations globally. In the present study laccases were identified using Liquid Chromatograph Mass Spectrometer Studies (LCMS) study where blue laccase exhibited homology with Trametes villosa Q99044 and Q99046 and white, yellow laccase exhibited homology with Myrothecium verrucaria OX = 1859699; Q12737 and Trametes versicolor Q12717 respectively. The spectral comparison between laccases were determined via spectroscopic analysis where UV-spectra of blue laccase from Trametes versicolor had a peak at 605 nm (Type I Cu atom) whereas in case of white and yellow laccase the peak was absent and in addition had an absorption peak at 400nm. It was followed by X-Ray Diffraction (XRD) analysis of proteins where α-helix (10%) and β-sheet (22%) structure were observed in case of all the three laccases. However, the intensity of α-helix in white and yellow laccase was stronger as compared to the blue laccase whereas the intensity of β-sheet was stronger in case of blue laccase as compared to other two laccases. Further, Fourier-transform infrared spectroscopy (FTIR) analysis was performed which enabled the analysis of proteins where α-helix (1650–1658 cm⁻¹), β-sheets (1620–1640 cm⁻¹), amide I (1700–1600 cm⁻¹) amide II (bands at under 1400 cm⁻¹) and amide A, B (bands above 3000 cm⁻¹).

1. Introduction

Multicopper oxidases (MCOs) laccase is ubiquitous in nature being found in plants, bacteria, fungi, soil and insects [1]. They have been regarded as a versatile enzyme which has the capability to “catalyze one basic reaction from which all its activities originate” [2]. Laccase are glycosylated monomer or homodimer protein with less monosaccharide compounds (10–25%) in fungal and bacterial laccase as compared to plant laccases [3]. Laccase generally are described as having a UV spectrum at 280 and 600 nm with a shouldering at 330 nm. Typical laccase consists of three Cu centres (Type I, II and III) consisting of four Cu ions. Type I Cu is responsible for intense blue colour and has electro absorption at 605 nm and detectable electro paramagnetic resonance (EPR) spectrum. Type II unlike type I is colourless and EPR detectable. Type III Cu on the other hand has a pair of Cu atom which have weak absorbance at UV spectrum and lacks EPR spectrum. Type II and III together forms the trinuclear cluster of laccases where dioxygen binds and four electron reduction to water takes place. It is due these features that laccase has its application in wide range of industries [4] e.g., clarification of beverage, paper and pulp, textile industry, biosensor, bioremediation and organic synthesis [51]. However, the catalytic ability of enzymes is often hindered in harsh environments like high-acid and high-alkaline solutions due to transformations of enzyme structure which results in low operational stability, difficulties in recovery and reuse [5]. Nonetheless screening of new strains are requisite which can produce various laccase to tolerate harsh industrial processes (e.g. alkaline tolerant and thermo-stable) and can give maximum enzyme production with minimum energy consumption is the requirement of various biotechnological and environmental sector [4].

Laccase have been studied globally and have been identified with unique characteristic features. Purahong et al [6] has stated that “fungal community structure was similar across different regions, but was nevertheless variable in all regions”. Laccase have been identified from various geographical regions e.g. Ganoderma lucidum KMK2 (India) [7], Scytalidium thermophilum (Tunisia) [8], P. sanguineus (Mexico) [9], Cerrena unicolor (Poland) [10], Pycnoporus cinnabarinus (Australia) [11],

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2.1 Chemicals and reagents

The blue laccase from *Trametes versicolor* (38429-1G) and ABTS were purchased from Sigma. All other chemicals were of analytical grade and purchased from Merck, Hi-media, India.

2.2 Liquid chromatograph mass spectrometer studies of various laccases

The sequence of blue laccase and isolated white and yellow laccase were determined using LCMS studies. The white and yellow laccase were produced as per previous study Agrawal et al. [21] and Agrawal and Verma [19, 20]. The extracellular proteins were concentrated and considered for further analysis. The concentrated yellow and blue laccase were alkylated using urea (6M) and tris buffer (100 mM, pH 7.8) and DTT (200 mM in tris buffer) and vortex for 60 min at 28 ± 2 °C followed by adding iodoacetamide (200 mM in tris buffer) as an alkylating agent and again vortex for 60 min at 28 ± 2 °C. After incubation reducing agent was added to remove the alkylating agent and incubated for 60 min. The sample was diluted to reduce concentration of urea to 0.6 M and then 20 μg chymotrypsin was added and samples were digested overnight at 37 °C. Then the pH was reduced to less than 6 and analyzed using Advance Biopetide Column 2.1 × 100 mm, 27 micron using QTOF-LCMS at Agilent Technologies, Centre of Excellence Manesar, Gurugram India.

The concentrated white laccase was dissolved in tris buffer (pH 7), digested, desalted followed by analysis using HR-LCMS Orbitrap at IIT Bombay SAIF by analytical column: PepMap RSLC C18 2 um, 100 A x 50 cm, Pre-column: Acclaim PepMap 100, 100um x 2cm nanoviper, with mobile phase as solvent A: 0.1% FA in milliq water, solvent B: 80:20 (ACN: milliq water) + 0.1% FA. The data was analyzed using Thermo Proteome Discoverer 2.2.

2.3 3D ribbon representation of various laccases

The LCMS study enabled the identification of the sequence similarity of laccase from existing database. The sequence obtained (https://www.uniprot.org/) was used for the prediction of the 3D ribbon representation of multicopper oxidases from SWISS-MODEL Server or Repository (https://swissmodel.expasy.org/) [39, 40, 41, 42, 43].

2.4 Purification and the spectral studies of various laccases

The production and extraction of laccases were performed from isolated strains as per previous reports [19, 20, 21] and unpublished data for spectral studies. The cell free supernatant was concentrated using ammonium sulphate (80 %) precipitation and dialyzed using dialysis membrane-110. Further the dialysed sample was purified using ion exchange chromatography, DEAE-Sephadex column and equilibrated with acetate buffer (0.1 M pH 5). The sodium chloride gradient (0–1 M) was applied at a flow rate of 0.5 mL/min. The collected fractions were analyzed for laccase activity and protein content [21]. The purified white and yellow laccase along with the blue laccase was considered for further spectral studies.

2.4.1 UV-absorption of various laccases for spectral analysis

The blue laccase are typical laccases and have been studied extensively for the past few years. On the other hand, white/yellow laccase also known as atypical laccase are less studied and scattered information is available. Atypical laccase differs from typical laccase in UV-absorption spectra at 605 nm. Except for the lack of absorbance all other features of atypical laccase are similar to the typical laccase [22]. The UV visible spectra of all three laccases were determined using UV-visible spectro-photometer (Halo- DB, Dynamica Asia Limited Hong Kong) at a wavelength range of 200–800 nm followed by a comparative spectral analysis [21].

2.4.2 Spectral assessment of various laccases using XRD

The XRD studies were performed for detecting the presence of α helix and β sheets. Purified white and yellow laccase as mentioned under section 2.4 were lyophilized using lyophilizer (Allied Freezer, New Delhi, India). The blue laccase and lyophilized proteins of white and yellow laccases were scanned at 2θ range of 10–25° [21] by PANalytical EMPYREAN, Netherlands using Cu as the anode material at Department of Physics, Central University of Rajasthan.

2.4.3 Spectral evaluation of various laccases using FTIR

The spectral studied of typical and a typical laccase were further extended to FTIR studies. It has been stated that the FTIR has advantages over other techniques as they can be used for obtaining spectrum of protein at a wide range of environments, requiring less time as well as sample. Thus, FTIR analysis was performed for the blue laccase and purified white and yellow laccase at a wavenumber range of 500–4000 cm⁻¹ [21] at IIT Bombay, Sophisticated Analytical Instrument Facility (SAIF), India.

2.5 SDS-PAGE and silver staining of various laccases

The blue laccase from *Trametes versicolor* and white laccase from *M. verrucaria* ITCC 8447 was determined using SDS-PAGE as per Laemmli [46]. The bands were confirmed by Commassie Brilliant Blue R-250

*Pycnoporus sanguineus* (Brazil) [12], *Peniophora* species (Netherland) [13], *M. verrucaria* (China) [14] and *Stropharia aeruginosa* (Netherland) [15]. Among them atypical white/yellow laccases have been identified which exhibits advantages over typical blue laccase. The blue laccases require the presence of mediator (shuttles in between the substrate and laccase) as compared to yellow/white laccases which function effectively in the absence of mediator [52]. These atypical laccases have also been reported to be stable at acidic pH and high temperature as well (55–60 °C) [14, 17]. White laccase from *M. verrucaria* 24G-4, MD-R-16 and NF-05 were reported by Sidhu et al. [18], Sun et al [17] and Zhao et al [16]. *M. verrucaria* 24G-4 was obtained from Japan, *M. verrucaria* MD-R-16 was isolated from roots of pigeon pea from China and *M. verrucaria* NF-05 was isolated from soil of China. Similarly, the strain *M. verrucaria* ITCC 8447 was isolated from Shree nagar, Rajasthan India during rainy season. Thus, it can be inferred that the strains have been isolated from wide range of climatic and geographical habitats. On the other hand, yellow laccase from *S. aeruginosa* CBS 839.87 was from Netherlands [15] and *Stropharia* sp. ITCC 8422 was isolated from Rajasthan, India during rainy season [19, 20].

The systematic spectral studies have not been reported till date to understand and examine the differences among the spectral properties of various laccases considering the diverse geographical distribution of laccases. Thus, in order to allow better understanding of the spectral characteristic features and differences among the typical and atypical nature of laccases the present study was designed where the sequence homology using LCMS and detailed spectral analysis was performed using two multicopper oxidase laccases producing strains which were isolated from western province of Rajasthan, India. These strains *M. verrucaria* ITCC 8447 and *Stropharia* sp. ITCC 8422 were deposited at Indian Type Culture Collection [19, 20, 21]. In order to examine distinguished spectral properties, blue laccase from *Trametes versicolor* was used as standard (control) along with two isolated strains (*M. verrucaria* ITCC 8447 and *Stropharia* sp. ITCC 8422) which are efficient producers of white and yellow laccase. The sequence homology of white, yellow and blue laccase was performed using LCMS studies. Further all the three laccases were primarily analyzed using UV visible spectra, followed by detailed spectroscopic analysis consisting of X-Ray Diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) for determining the typical and atypical nature of laccase.
staining method. The yellow laccase from Stropharia sp. ITCC 8422 was determined using silver staining protocol, where the gel was fixed using fixing solution (methanol 50%, acetic acid 10%, formaldehyde 50 μL in 100 mL) for 60 min, washed with 50% ethanol (3 times) for 20 min each, followed by sensitizing the gel with sodium thiosulfate (20 mg/100 mL) for 60 s. The gel was washed followed by treatment with silver nitrite solution (200 mg/100 mL) for 30 min and washing. Later developing solution (sodium carbonate 6g, hypo solution 2mL, formaldehyde 50 μL) was added and incubated till the bands appeared followed by the addition of 5% acetic acid to stop the reaction.

3. Result and discussion

3.1. Liquid chromatograph mass spectrometer studies of various laccases

M verrucaria ITCC 8447 has been reported to produce 1549.7 U/L of laccase after the optimization of various nutritional and physiological parameters [21]. On the other hand, yellow laccase by Stropharia sp. ITCC 8422 has been reported to produce 164.4 U/L on 18th day [19, 20]. The extracellular white/yellow laccases were concentrated and analyzed using LCMS for the confirmation of the production of laccase by these two strains as well as to identify its sequence homology with the existing database.

The LCMS analysis of blue laccase from Trametes versicolor exhibited homology with laccase-1, laccase-2 from Trametes villosa (White-rot fungus) with the accession number Q99044 and Q99046. The white laccase from M. verrucaria ITCC 8447 exhibited identity with MCOs bilirubin oxidase from Myrothecium verrucaria OX¼1859699 with accession Q12737. In the study by Sulistyaningdyah et al [18] NH2-terminal amino acid sequence of laccase from Myrothecium verrucaria 24G-4 exhibited homology with bilirubin oxidase from M. verrucaria MT-1 which was further validated using experimental analysis. Similarly, M verrucaria ITCC 8447 via experimental analysis proved the production of laccase [21]. The laccase of Stropharia sp. ITCC 8422 exhibited homology with laccase-5 from Trametes versicolor (White-rot fungus; Coriolus versicolor) with accession number Q12717. This confirms the production of laccase by Stropharia sp. ITCC 8422.

3.2. 3D ribbon representation of various laccases

The sequence of MCOs were identified after LCMS for blue laccase and the sequence obtained were ADGPAFINQCPISSGHSF and AEDVDADVKAANPVPKAW for laccase 1 and 2 from Trametes villosa and Trametes versicolor respectively. On the other hand, for white and yellow laccases sequences were AAFDGWAEDITEPGSFK and RLVSISCDPNFTF from Myrothecium verrucaria OX¼1859699; Q12737 and Trametes versicolor (White-rot fungus; Coriolus versicolor) Q12717 respectively. The LCMS studies enabled the prediction of the 3D ribbon representation which has been represented in Figure 1 (a–d). It was also observed that all the laccase has the presence of 4 x Copper (II) ion and ranged from 519-572 amino acid residues.

3.2.1. UV-absorption of various laccases for spectral analysis

Blue laccase from Trametes versicolor had the presence of peak at 605 nm (Figure 2b) which represents the presence Type I Cu atom, responsible for the intense blue colour of typical blue laccase [11]. The purified white laccase is different from blue laccase, where peak at 605 nm was absent which is typically present in type I of blue laccase (Figure 2c) and has an absorption peak at 400 nm (Palmieri et al 1997). Additionally, shouldering at 330 nm was absent which corresponds to T3 binuclear copper. The absence of peak at 605 nm can be due to the incomplete oxidation of copper which is typical feature of white laccase and it is in

Figure 1. 3D ribbon representation of various laccases as obtained after LCMS study using the sequence homology (a–b) blue; (c) white; (d) yellow.
accordance with the study reported by Plameiri et al. [23]; Zhou et al. T versicolor Figure 2.

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production of yellow laccase by

absorption maximum at 605 nm (Figure 2a) thereby, con

sents its atypical nature. Similar data has been reported by Schliephake et al [11] where at 605 nm an increase in absorption was observed and for white laccase the study was similar to Plameiri et al. [23]; Zhou et al. [25]; Zhao et al. [14] and for yellow laccase the study was similar to Daroch et al [15].

The XRD analysis was performed to analyze the structure of blue, white/yellow laccases. The XRD analysis of proteins at an angle of 2θ and approximately 10° and 22° represents the α-helix and β-sheet structure [26, 27]. The XRD of blue laccase had the presence of α-helix at 10° along with β-sheet at 22°. Similar observation was observed in case of white [21] and yellow laccase (Table 1). However, the intensity of white and yellow laccase α-helix was stronger as compared to the blue laccase. On the other hand, β-sheet structure was stronger in case of blue laccase as compared to white and yellow laccase (Figure 3).

3.2.2. Spectral assessment of various laccases using XRD

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3.2.3. Spectral evaluation of various laccases using FTIR

FTIR analysis enables the analysis of proteins and polypeptides with nine specific IR absorption bands i.e., amide A, B and I to VII of which the amide bands I and II exhibits very distinctive vibrational bands of the protein backbone [28, 29, 30]. The amide I band of protein is the most sensitive region to the spectra 1700–1600 cm⁻¹ which is contributed due to the C–O stretch vibrations in the peptide linkages which is approximately 80%. Amide I band is related to the secondary structure of protein whereas amide II is due to the in-plane NH bending which constitutes 40–60% of the potential energy and CN stretching vibrations as well which constitutes 18–40%. It is due to these factors that amide I is more sensitive than amide II bands in the FTIR spectra of proteins [28, 31]. While obtaining the FTIR spectra of protein it has to be taken into consideration that in case water is present in the sample three peaks can be detected at 3400 cm⁻¹, 2125 cm⁻¹ and 1645 cm⁻¹ representing O–H stretching, H₂O association and H–O–H association. In all the cases absorbance at 1645 cm⁻¹ was observed which could be due to the amide I band overlapping with H₂O band which thereby is also responsible for higher absorbance [31].

The blue laccase had the presence of α-helix and β-sheets at 1650–1658 cm⁻¹ and 1620–1640 cm⁻¹ and the band detecting the presence of amides III (bands under 1400 cm⁻¹) and amides A, B (bands above 3000 cm⁻¹) were also visible distinctly in the FTIR spectrum (Figure 4). White laccase had peaks at 1650–1658 cm⁻¹ and 1620–1640 cm⁻¹ representing α-helix and β-sheets respectively whereas the bands at under 1400 cm⁻¹ and above 3000 cm⁻¹ signifies the presence of amides II and amides A, B [32] which are very clearly present in case of white laccase. The band present in 1600–1700 cm⁻¹ represents the amide I and II. The amide I band due to the presence of overlap broad underlying components i.e., α helix, β sheet and random structures are featureless (Figure 4). In case of yellow laccase, the FTIR spectra was similar to that of white laccase where the peaks at 1650–1658 cm⁻¹ and 1620–1640 cm⁻¹ where present however it was slightly more intense as compared to white laccase. The bands below 1400 cm⁻¹ (amides II) and the band below 3000 cm⁻¹ (amides A and B) were similar to that of white laccase. The secondary structure band assignment also consists of random and coil structure at 1642-1657 cm⁻¹ representing α-helix, β-sheet and random structure [33]. While obtaining the FTIR spectra of protein it has to be taken into consideration that in case water is present in the sample three peaks can be detected at 3400 cm⁻¹, 2125 cm⁻¹ and 1645 cm⁻¹ representing O–H stretching, H₂O association and H–O–H association. In all the cases absorbance at 1645 cm⁻¹ was observed which could be due to the amide I band overlapping with H₂O band which thereby is also responsible for higher absorbance [31].

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3.3. Comparative spectral assessment and application of various laccases

The UV visible spectra of white laccase exhibited an absorption peak at 400nm and absence of peak at 605 nm which represents Type I Cu [23]. White laccase lacks typical blue colour which can be due to the change in the valence state of Cu²⁺ and lack of the EPR signal due to the Fe²⁺ which has low spin electronic configuration [14]. The replacement of Cu²⁺ can be inferred but the lack of absorption can also be due to the incomplete oxidation of the copper which has an occupied electron configuration of d10 and no d-d transition [14]. It has to be noted that white laccase has the preference of neural pH as compared to other laccase where the optimal pH ranges from 3-5 [21]. The other characteristic feature is the presence of anomalous metal content which contributes towards its unique feature. White laccase was identified from Pleurotus ostreatus where blue spectra were lacking and consisted of one Cu, one Zn and two Fe atoms. On the other hand, yellow laccase was...
produced as a result of solid-state fermentation where yellow brown laccase lacks the typical blue spectra and had an atypical EPR spectrum [1].

The yellow laccase on the other hand are artificially reduced blue laccase which also lacks absorption spectra at 605 nm and an EPR spectrum [33, 34]. The yellow laccase occurs by the reduction of Type I Cu site by aromatic product of lignin degradation or the binding of specific amino acid of the polypeptide of enzyme to a molecule of the modified product produced by lignin degradation. The other factor which is responsible for the production of yellow laccase could be due to heterogeneity induced by glycosylation [15]. A “yellow” laccase with “blue” spectroscopic features was identified from Sclerotinia sclerotiorum (Moț et al 2012). The modified molecule of the apoenzyme (yellow laccase) performs electron transfer mediator similar to the role of mediator e.g., ABTS as in case of blue laccase [33, 34]. Thus, enabling yellow laccase to act effectively in the absence of mediator to oxidise non-phenolic compounds in contrast to blue laccase where mediator is required, thereby increasing its biotechnological and industrial potential [19, 20, 35]. Yellow laccases and blue laccase have four copper atoms like the blue laccase whereas white laccases contain one copper, two zinc ions and one iron ion per protein molecule [23].

The substrate affinity of laccase has been reported and it was observed that ABTS was the most effective substrate for blue, white (M. verrucaria ITCC 8447) [47,53] and yellow laccase (data not shown). The

| Table 1. The peaks detected in the XRD analysis of the blue, white and yellow laccases. |
|---------------------------------|-------------------------------------------------|---------------------------------|-------------------------------------------------|-------------------------------------------------|
| **Blue Laccase**                | **α helix**                                     | **White Laccase**               | **α helix**                                     | **Yellow Laccase**                              |
|                                 | 10.03, 10.14, 10.15, 10.23, 10.24, 10.26, 10.32, 10.44, 10.54, 10.60, 10.66, 10.70, 10.72, 10.73, 10.75, 10.86, 10.87, 10.90, 10.95 and 10.97 | 10.02, 10.71', 11.40', 11.43' and 11.48' | 10.02, 10.06, 10.08, 10.16, 10.19, 10.31 and 10.35', 10.54', 10.61', 10.84', 10.98' | 10.02, 10.06, 10.08, 10.16, 10.19, 10.31 and 10.35', 10.54', 10.61', 10.84', 10.98' |
|                                 | β sheet                                        |                                 | β sheet                                        |                                 |
|                                 | 22.01, 22.02, 22.05 to 22.10, 22.24, 22.31 to 22.34, 22.38, 22.40, 22.60, 22.76, 22.81 and 22.94 | 22.55' and 22.69'                  | 22.07, 22.10, 22.22, 22.91, 22.92, 22.98 and 22.99 |

![Figure 3. The XRD of various laccases from T versicolor, M verrucaria ITCC 8447 and Stropharia sp. ITCC 8442. (The XRD of white laccase has been reproduced/adapted from Agrawal et al [21] with permission from Elsevier).](image-url)
Km and Vmax values of the purified white laccase has been reported as 2.5 mM and 1818.2 μmol/min/L. Similarly, for yellow laccase from *Pleurotus ostreatus* D1 it was observed that the Km and Vmax for syringaldazine, 2,6-dimethoxyphenol, and ABTS were higher than the corresponding values for yellow laccase from *Panus tigrinus* 8/18, and the values were close to the blue laccase from *Pleurotus ostreatus* strain. Thus, it has been observed that the Km and Vmax varies from strain to strain however maximum affinity for laccase has been reported towards ABTS [33, 34, 35, 48]. Laccases have also been used for its effective bioderection potential and they have effectively removed various structural different dyes e.g. azo, anthraquinone [14, 19, 20, 50]. In addition, blue laccase from *Trametes versicolor* [49] white laccase from *M. verrucaria* ITCC 8447 [21] and yellow laccase from *Stropharia* sp. ITCC 8422 also has high potential in the delignification of agricultural biomass. The white and yellow laccase has more advantage over blue laccase they can oxidizes non-phenolic compounds in the absence of mediators [33, 34], thereby further broadening its application in various biotechnological sectors.

3.4. Distinguish features of atypical laccases

The white laccases from *M. verrucaria* 24G-4, MD-R-16, NF-05 and ITCC 8447 were stable at a pH 8–11.5, 4.6–6.5, 2–7 and 7–9 [16-18,21]. The stability of the enzyme over a wide range of acidic to alkaline pH (2–11) and the thermal stability ranged from 20-60 °C. The molecular weight of white laccase from *M. verrucaria* ITCC 8447 ranged from 63-75 kDa (Figure 5d). On the other hand, yellow laccase from *Stropharia aeruginosa* is stable over 4–12 pH range for Yel 3p (5–9) and Yel 1p (4–12) with the optimal temperature being 40 °C with molecular weight being reported as 55 kDa due to its monomeric nature Daroch et al [15]. In the present study the molecular weight of blue and glycosylated yellow laccase from *Trametes versicolor*, *Stropharia* sp. ITCC 8422 was 63–75 and 100–135 kDa respectively (Figure 5b, f). The molecular weight of laccase ranges from 63 to 100 kDa which is contributed due to glycosylation (10–50 %) (Table 2). However, the molecular weight can be as high as 320 kDa [36, 37, 38]. Glycosylation (is a reaction where a glycosyl donor attaches to hydroxyl or other functional group of another molecule which acts as a glycosyl acceptor) in laccases is responsible for secretion, thermal stability, proteolytic susceptibility, activity and copper [1, 44].

| White Laccase | pH | Temperature (°C) | Molecular weight (kDa) | Reference |
|---------------|----|-----------------|------------------------|-----------|
| *M. verrucaria* 24G-4 | 8–11.5 | 30–50 | 62 | [18] |
| *M. verrucaria* MD-R-16 | 4.6–6.5 | 35–55 | - | [17] |
| *M. verrucaria* NF-05 | 2–7 | 20–60 | 66 | [16] |
| *M. verrucaria* ITCC 8447 | 7–9 | 30–40 | –63–75 | [21] |

| Yellow Laccase | | | |
|----------------|-----------------|-----------------|-----------|
| *Stropharia aeruginosa* | | | |
| Yel 1p | 4–12 | Up to 40 | 55 | [15] |
| Yel 3p | 5–9 | | |

| Blue Laccase | | | |
|--------------|-----------------|-----------------|-----------|
| *Trametes versicolor* IBL-04 | 5–8 | 25–40 | 63 | [45] |
| *Trametes versicolor* | 2.5–4 | 0.50 | 97 | [16] |
4. Conclusion

The sequence of white/yellow and blue laccase exhibited homology with *Trametes villosa, Myrothecium verrucaria* OX = 1859699, *Trametes versicolor* after LCMS study. The present study enabled better understanding of the typical and atypical laccases. Various spectral analysis i.e., UV-spectra, XRD and FTIR, of blue laccase exhibited different features as compared to other two atypical laccases. The presence of peak at 605 nm in the UV visible spectra, was observed in case of blue laccase whereas it was absent in other two laccases. The XRD data showed that the α-helix intensity was stronger in white/yellow laccase as compared to the blue laccase, whereas the β-sheet structure was stronger in case of blue laccase. Similarly, the FTIR data showed that the β-sheets were more intense with blue laccase as compared to white and yellow laccase. The spectral analysis help infer that the blue laccases are different as compared to white and yellow laccase which is in correlation with the previous study [15, 19, 20, 23].

Declarations

Author contribution statement

K. Agrawal: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

P. Verma: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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