



ORIGINAL ARTICLE

Open Access



# Comprehensive analysis of the isozyme composition of laccase derived from Japanese lacquer tree, *Toxicodendron vernicifluum*

Mariko Takano<sup>1\*</sup> , Masaya Nakamura<sup>1</sup> and Masanobu Tabata<sup>2</sup>

## Abstract

We performed an analysis using isoelectric focusing to comprehensively clarify the isozyme composition of laccase derived from Japanese lacquer tree, *Toxicodendron vernicifluum*. When water extracts of acetone powder obtained from lacquer were subjected to isoelectric focusing, five bands within pI 7.35–9.30 and nine bands within pI 3.50–5.25 were detected using Coomassie staining. Similarly, laccase activity staining using guaiacol showed five bands within pI 7.35–9.30 and three bands within pI 3.50–4.25. However, laccase activity staining using gallic acid showed remarkable staining within pI 3.50–5.85, whereas staining was very weak within pI 7.35–9.30. When the water extracts of acetone powder were fractionated into the fractions containing bands within pI 7.35–9.30 and pI 3.50–5.85 by SP-Sepharose column chromatography, the former had a blue color and the latter a yellow color. The laccase activity was measured for each of the fractions in buffer solution in the pH range of 2.5–8.0. When syringaldazine, guaiacol, and 2,6-dimethoxyphenol were used as substrates, the yellow fraction showed considerably higher activity than the blue fraction for pH 5.5–7.5. When 3-methylcatechol and 4-methylcatechol were used as substrates, the yellow fraction showed higher activity for pH 4.5–6.5, and the blue fraction showed higher activity for pH 7.0–8.0. When 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was used as the substrate, both fractions showed maximum activity at optimum pH of 3.0–4.0. Conventionally, in research on blue laccase derived from lacquer, the non-blue fraction corresponding to the yellow fraction lower than pI 6 has been removed during the purification process and thus has not been analyzed. Our results indicated that yellow laccase was present in the non-blue components of lacquer and that it may play a role in urushiol polymerization with previously reported blue laccase.

**Keywords:** Laccase, *Toxicodendron vernicifluum*, *Rhus vernicifera*, Yellow laccase, Urushiol

## Introduction

The Japanese lacquer tree (*Toxicodendron vernicifluum* (Stokes) F. A. Barkl.) is one of the family Anacardiaceae that is widely distributed in East Asia [1]. Lacquer is resin and sap extracted from lacquer trees and has been used since Jomon Period as paint for cultural assets and lacquer wares in Japan, making it a natural material of cultural and social significance [2, 3].

The components of lacquer include urushiol, water, plant gum, water-insoluble glycoproteins, and enzymes (laccase, peroxidase, and stellacyanin) [4, 5]. Urushiol is a generic term for catechol derivatives with an alkyl side-chain that has 15 or 17 carbon atoms at C-3 of the benzene ring; and more than ten types have been reported with different side-chain structures [4, 5]. Laccase (Lac) is a phenoloxidase and is found widely in plants such as lacquer trees, fungi such as white-rot fungi, and bacteria [6]. Lacquer hardens and dries to form a film, as a result of urushiol becoming oxidatively polymerized by Lac [4, 7], and functions as a coating material [8]. Because drying and hardening properties are used as indicators of lacquer quality, Lac has a direct effect on quality [9].

\*Correspondence: marin@ffpri.affrc.go.jp

<sup>1</sup> Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

Full list of author information is available at the end of the article

Lacquer Lac was the first multicopper oxidase discovered and has been studied in detail in regard to the catalytic mechanisms, functions, properties, and structures of the isolated enzyme [10]. In recent years, detailed analyses of the carbohydrate structure and gene structure of Lacs have been reported [11, 12]. These studies have focused on two to three Lacs with pI 8–10 purified using Reinhammer's method [13]. However, comprehensive isozyme composition and diversity of lacquer Lac remain to be clarified.

In contrast, a wide variety of Lacs have been reported in white-rot fungi [6]. In addition, in white-rot fungi, ligninolytic oxidizing enzymes, including Lac, are known to have many isoenzymes with the same molecular weight but different pIs and these have been analyzed using isoelectric focusing and two-dimensional electrophoresis [14, 15].

In this study, we performed a comprehensive analysis of Lac isozymes derived from Japanese lacquer tree using isoelectric focusing.

## Materials and methods

### Samples

Four samples were used, consisting of "Hatsu-urushi" lacquer collected between mid-June and mid-July 2018 and "Sakari-urushi" lacquer collected between late July and late August, in Ninohe, Iwate Prefecture and Iida, Nagano Prefecture.

### Preparation of crude Lac enzymes

Ten milliliters of each lacquer sample was collected in a 50-mL centrifuge tube, and to this was added 30 mL of cooled acetone and mixed well. After remaining standing for three days at  $-20^{\circ}\text{C}$ , the samples were then centrifuged in a refrigerated centrifuge (13,000 rpm  $\times$  10 min), and the precipitate was washed with acetone until the supernatant became colorless. The precipitates were then collected and dried at  $6^{\circ}\text{C}$  for 3 days to obtain acetone powder. To this was added 15 mL of distilled water, and extraction was performed in a shaker overnight. After centrifugation to remove insoluble matter, 500 g/L of ammonium sulfate was added to the solution, and the mixture was stirred. The solution was again centrifuged to collect blue supernatant, to which ammonium sulfate was added until saturation reached 100% (767 g/L). The sample was centrifuged, and the supernatant removed to collect blue precipitate. To the blue precipitate, 5 mL of distilled water was added and dissolved, and after being desalted with a PD-10 column, the sample was concentrated using VIVA SPIN.

### Fractionation of Lac

SP-Sepharose was packed in a column (1 cm  $\times$  10 cm) and equilibrated with phosphate buffer (pH 6.0, 10 mM). After crude Lac enzymes were added to the column, 30 mL of the same phosphate buffer was added, and the eluate was collected (yellow fraction). Then, 20 mL of phosphate buffer (pH 6.0, 300 mM) was added and the eluate was collected (blue fraction).

### Electrophoresis

Isoelectric focusing was performed using CleanGel IEF and Pharmalyte, broad range pH 3.0–9.5, according to the manufacturer's instructions (Cytiva, Tokyo, Japan).

### Staining of the gel

Proteins separated in the gel were stained using Coomassie Blue R 250. Guaiacol (GU) and gallic acid were used for Lac activity staining.

### Measurement of enzymatic activities

The Lac activity was determined by preparing separate reaction solutions for the different substrates as described below, and measuring the changes in absorbance resulting from substrate oxidation within 1 min at  $25^{\circ}\text{C}$ . For GU, 100 mM solution (in 20% ethanol solution), and for syringaldazine (SA), 1 mM solution (in 100% ethanol), were prepared. For 3-methylcatechol (3MC), 4-methylcatechol (4MC), and 2,6-dimethoxyphenol (2,6-DMP), 10 mM aqueous solutions were prepared. For 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 40 mg/L aqueous solution was prepared. As a buffer solution, McIlvaine buffer (pH 2.5–8.0) was used [16]. The samples for which enzyme activity was measured contained 365  $\mu\text{L}$  of buffer solution, 25  $\mu\text{L}$  of the substrate solution, and 10  $\mu\text{L}$  of enzyme sample. Absorbance was measured at wavelengths of 414 nm for ABTS, 525 nm for SA, 460 nm for GU and 477 nm for 2,6-DMP [17]; absorbance for 3MC was measured at 430 nm and 4MC at 400 nm as described in "Results".

### Instruments

Multiphor II (Cytiva) was used for electrophoresis. An UV-VIS recording spectrophotometer UV-2400PC (Shimadzu, Kyoto, Japan) was used to measure the enzymatic activity and UV-VIS spectra of lacquer Lac.

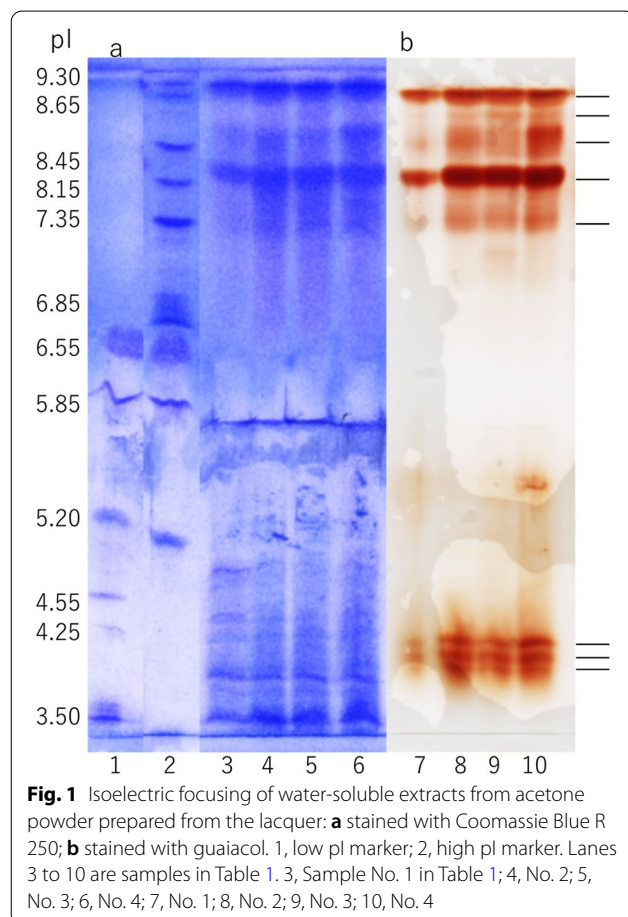
## Results

### Preparation of crude Lac enzymes

Acetone powder was prepared from each lacquer sample, which was mixed with distilled water to obtain a crude Lac enzyme solution. Table 1 shows the locations

**Table 1** Preparation of crude laccase from lacquer tree

Sample no.	Sampling locations	Timing of harvest	Amount of lacquer (g)	Acetone powder yield (g)	Total Lac activity (mAbs/min)	Lac activity per gram of acetone powder (mAbs/min)
1	Ninohe in Iwate	Late July–late August in 2018	34.9	0.81	568	706
2	Ninohe in Iwate	Mid-June–mid-July in 2018	38.0	2.58	1679	651
3	Iida in Nagano	Mid-June–mid-July in 2018	38.6	1.51	1541	1020
4	Iida in Nagano	Late July–late August in 2018	39.5	1.98	2972	1501



where the samples were collected, the timings of sample collection, the amounts of samples used, acetone powder yields, the total Lac activities, and the activities per gram of acetone powder. Acetone powder yield and Lac activity varied among the samples.

#### Isoelectric focusing

The results of isoelectric focusing on the four crude Lac enzyme samples are shown in Fig. 1. Using Coomassie staining, five bands were detected within pI 7.35–9.30 and nine bands within pI 3.50–5.20. Similarly,

when isoelectric focusing was performed, and Lac activity was determined using GU for 30 min, five bands were detected within pI 7.35–9.30 and three bands within pI 3.50–4.25. All five bands detected within pI 7.35–9.30 were found to be Lac. However, of the nine bands detected within pI 3.50–5.20, Lac activity was only found in three bands within pI 3.50–4.25.

Regarding the results of isoelectric focusing, the color development was weak in all bands of Sample 1, which had a low total Lac activity (Table 1); however, the positions and relative intensities of bands were the same in all four samples. These results suggest that the type and composition of isoforms and their compositional ratios were similar among all four samples.

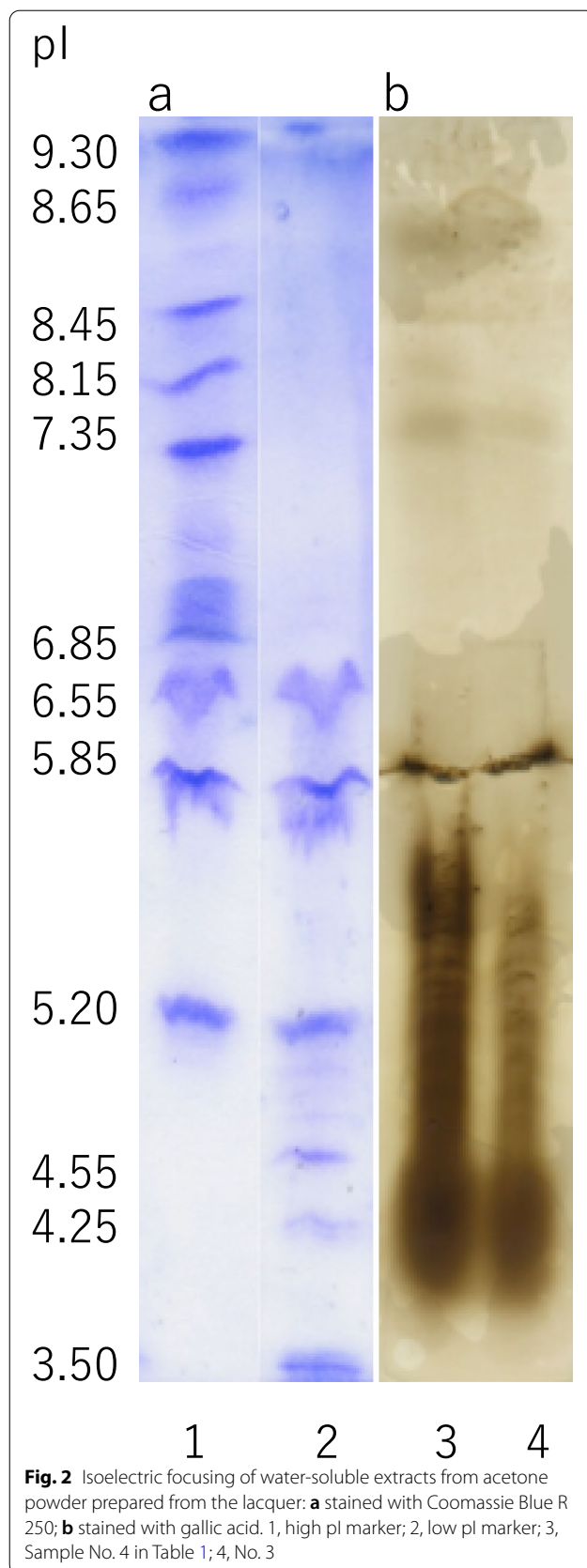
In addition, Lac activity staining using gallic acid, following isoelectric focusing of crude Lac enzymes, showed remarkable color development in the bands within pI 3.50–5.85, whereas color development was very weak in the five bands within pI 7.35–9.30 (Fig. 2). These results showed that Lac with pI 7.35–9.30 and pI 3.50–5.85 had different character on their enzymatic activity.

#### Spectral changes of 3MC and 4MC oxidized by Lac

Various aromatic compounds, such as SA, 2,6-DMP, and GU, have been used for measuring Lac activity. In addition to these compounds, two others were used as substrates in this study: 3MC, which has a similar chemical structure to urushiol; and 4MC, which has a similar chemical structure to thitsiol. First, 3MC and 4MC were oxidized with Lac to measure their spectral changes during the color development process. Color development by oxidation of 3MC showed a spectrum with a maximum value at 430 nm, and of 4MC a maximum at 400 nm (Fig. 3). Accordingly, Lac activity was assayed by measuring absorbance at 430 and 400 nm for 3MC and 4MC, respectively.

#### Activity of crude Lac enzymes

The activity of crude Lac enzymes extracted from acetone powder was measured in McIlvaine buffer of pH 2.5–8.0 [16]. The results for using ABTS, SA, and 2,6-DMP as substrates are shown in Fig. 4a, and for 3MC, 4MC, and



GU in Fig. 4b. The optimum pHs for Lac activity were pH 3.0–4.0 for ABTS, pH 6.0–8.0 for SA, and pH 6.0–7.0 for 2,6-DMP. When 3MC, 4MC, and GU were used as substrates, Lac activity was high at pH 5.0–8.0. ABTS showed high Lac activity in acidic conditions, whereas the other substrates showed high Lac activity in the neutral to weakly alkaline conditions.

#### Fractionation of crude Lac enzymes

As shown in the results described above (Fig. 2), Lac with pI 7.35–9.30 and pI 3.50–5.85 had different character on their enzymatic activity. For analysis of the difference between these two enzymes, crude Lac was fractionated as follows. The Lac samples were separated by SP-Sepharose column chromatography into two fractions: one containing Lac with pI 7.35–9.30 (exhibiting a blue color) and one with pI 3.50–5.85 (exhibiting a yellow color) (Fig. 5). The blue fraction showed maximum absorption at around 600 nm—a characteristic of blue Lac; whereas no maximum absorption was observed around 600 nm for the yellow fraction (Fig. 6).

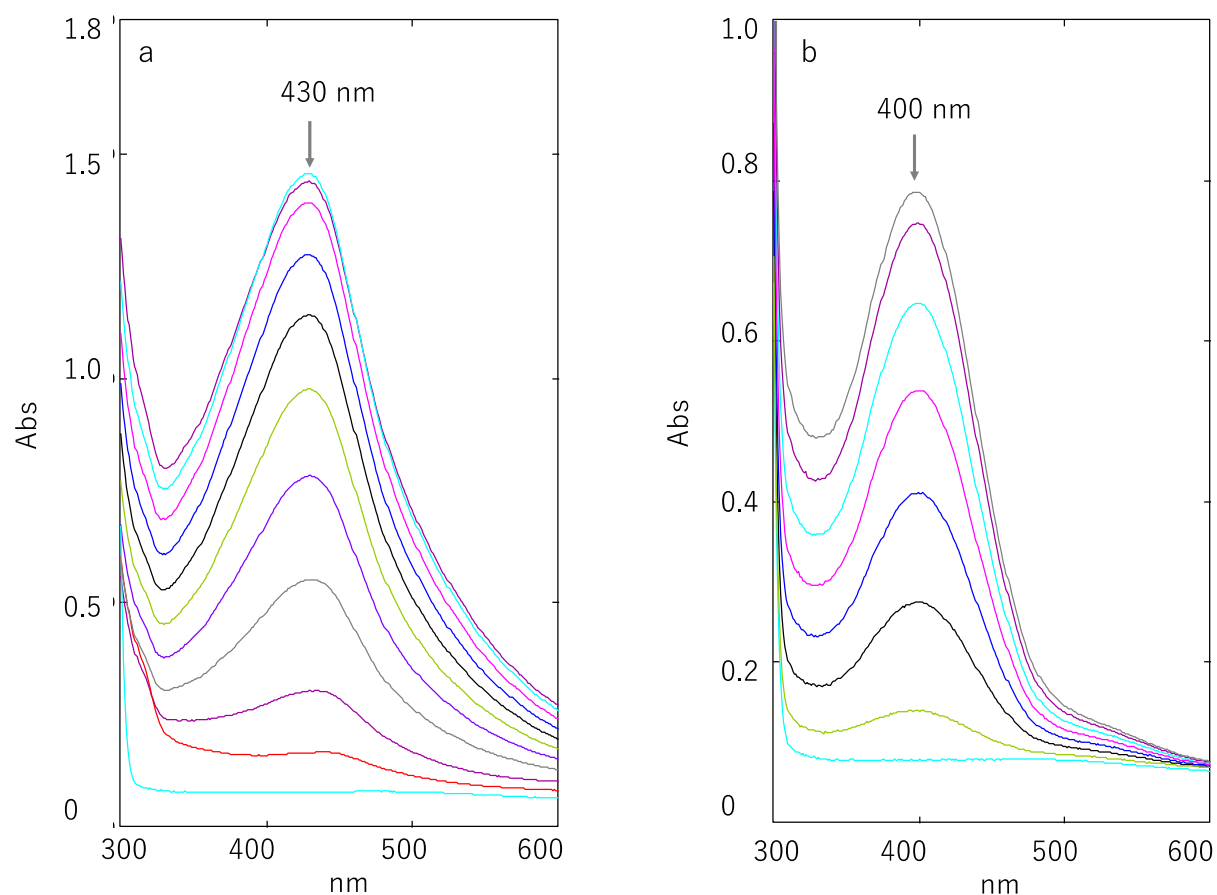
#### Isoelectric focusing of the fractionated samples

The blue and yellow fractions were subjected to isoelectric focusing, and the results of Coomassie staining and Lac activity staining by GU are shown in Fig. 5. Coomassie staining showed that the blue fraction contained bands within pI 7.35–9.30, and the yellow fraction contained bands within pI 3.50–5.85. When the duration of GU staining was extended to over an hour, the Lac activity was confirmed widely, corresponding to multiple bands within pI 4.25–5.85 in addition to three bands near pI 4 (Fig. 1b). These results suggested that characteristics of Lac activity differed between the blue and yellow fractions.

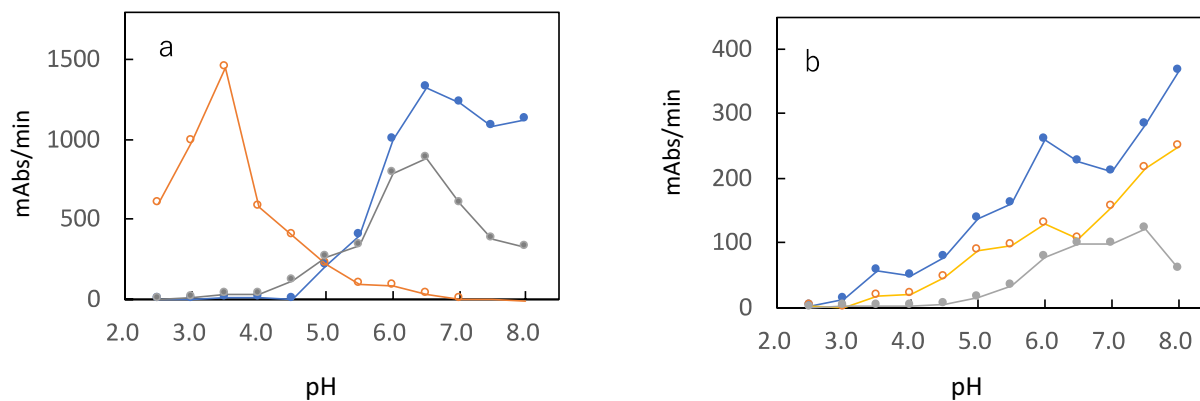
#### Lac activity of the fractionated samples

To compare characteristics of Lac activity between the blue and yellow fractions, Lac activity was measured for each of the substrates within the range of pH 2.5–8.0 (Fig. 7). For comparison of Lac activity of these 2 fractions working in lacquer, Lac activity contained in the same amount of lacquer was assayed.

When 3MC was used as the substrate, the yellow and the blue fractions exhibited the highest Lac activity at pH 6.0 and 8.0, respectively. At pH 4.5–6.0, the yellow fraction showed higher activity than the blue fraction, and the blue fraction showed higher activity than the yellow at pH 7.0–8.0. For SA, the yellow fraction showed maximum Lac activity at pH 6.5 and the blue fraction at pH 8.0. In the range of pH 5.0–7.5, the yellow fraction showed higher activity than the blue fraction. For 2,6-DMP, the yellow fraction showed

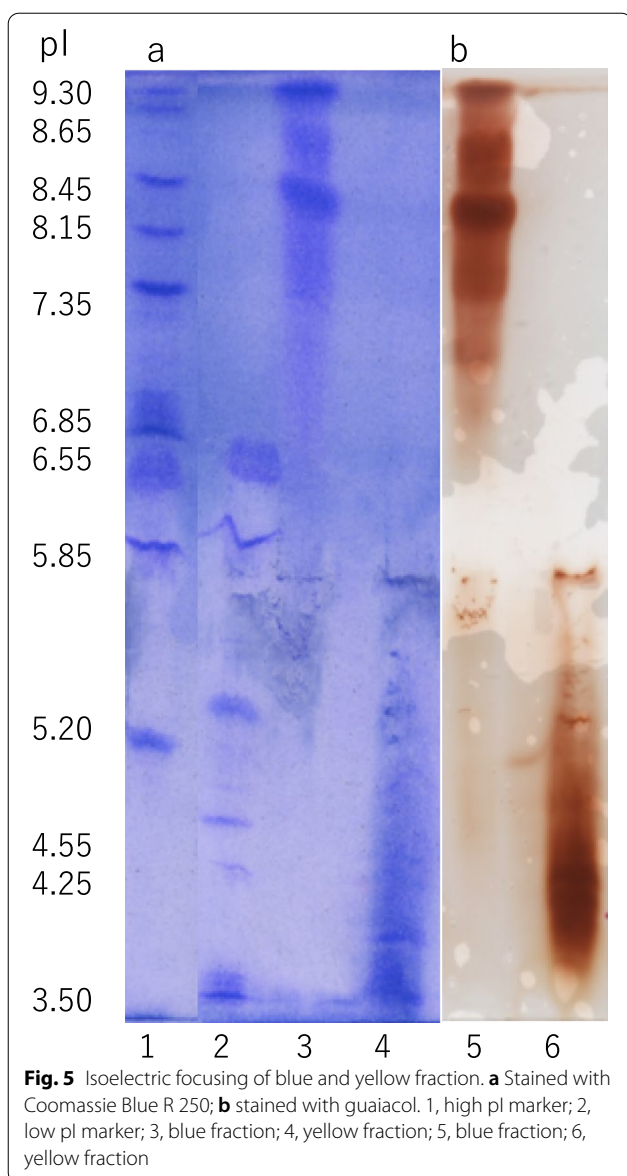


**Fig. 3** Spectra change of 3-methylcatechol and 4-methylcatechol oxidized by laccase. Spectral changes were recorded at intervals of 2 min. **a** 3MC; **b** 4MC

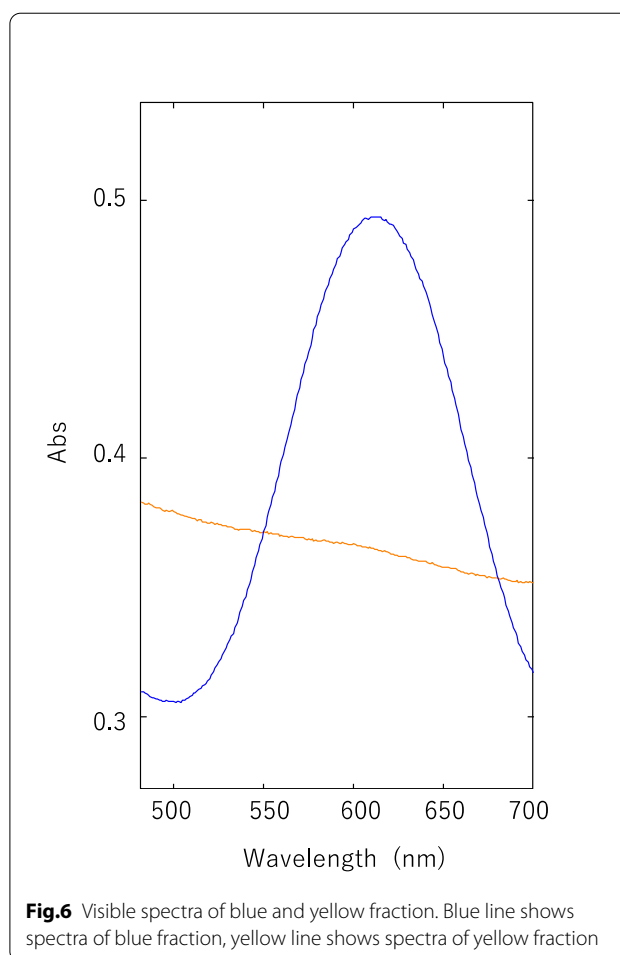


**Fig. 4** Laccase activity of water-soluble extracts from acetone powder prepared from the lacquer. **a** Yellow line shows laccase activity measured with ABTS; blue line, SA; gray line, 2,6-DMP. **b** yellow line, 4MC; blue line, 3MC; gray line, GU





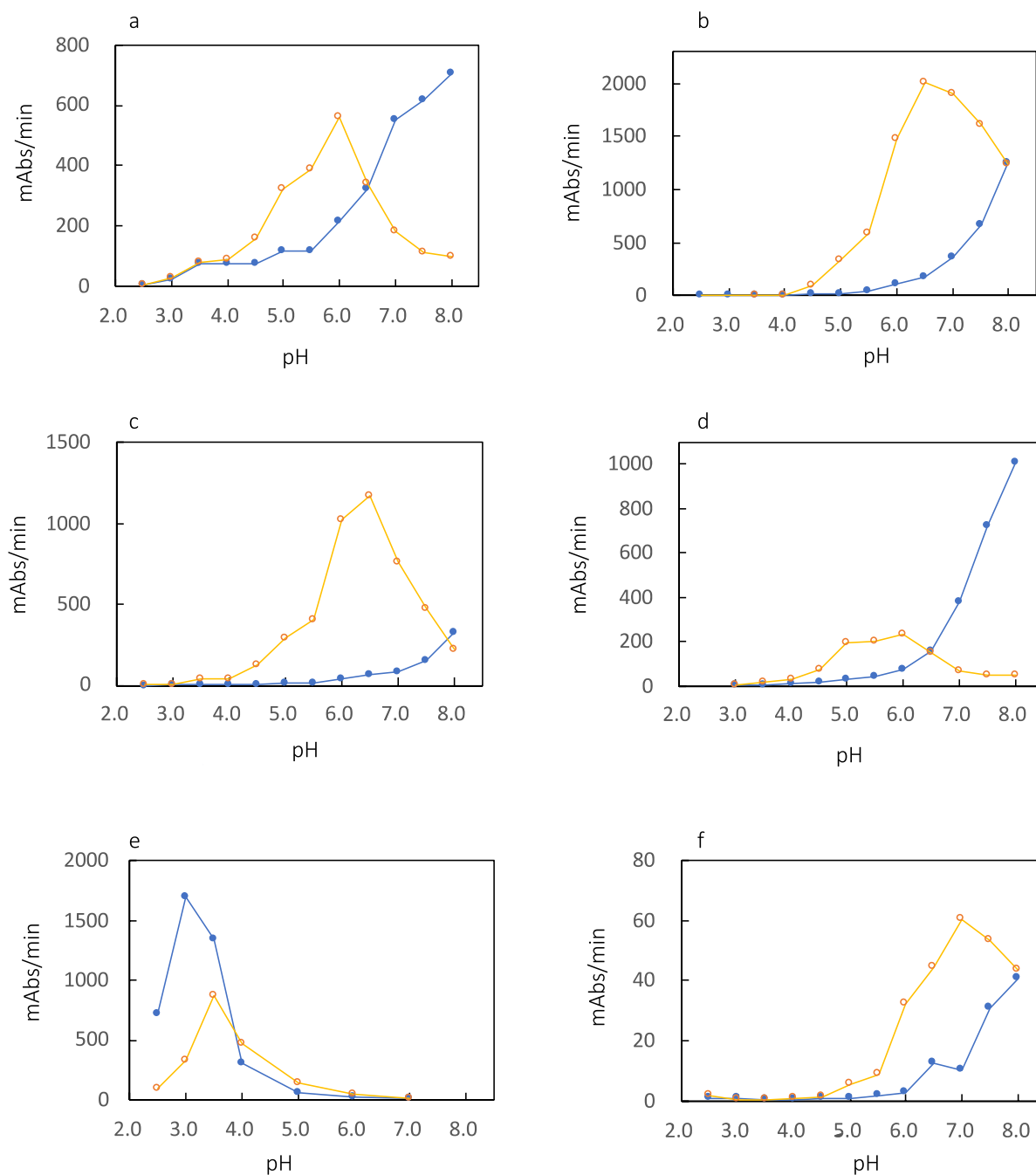
maximum Lac activity at pH 7.0 and the blue fraction at pH 8.0. The yellow fraction showed higher activity at pH 4.5–7.5 than the blue fraction. For 4MC, the yellow fraction showed maximum Lac activity at pH 5.0–6.0, and the blue fraction at pH 8.0. The yellow fraction had higher activity than the blue fraction at pH 4.5–6.0, and the blue fraction had higher activity than the yellow at pH 7.0–8.0. For ABTS, the yellow fraction showed maximum Lac activity at pH 3.5, and the blue fraction at pH 3.0. At pH 2.5–3.5, the blue fraction had higher activity than the yellow. For GU, the yellow and blue fractions showed maximum Lac activities at pH 7.0 and 8.0, respectively. At pH 5.0–7.5, the yellow fraction showed higher activity than the blue.



Although the results shown were for Sample No. 4, which had the highest total Lac activity among all samples (Table 1), similar results were obtained for the remaining three samples.

## Discussion

In this study, we performed an analysis using isoelectric focusing to clarify the comprehensive composition of the isozymes of Lac derived from lacquer. Isoelectric focusing performed on crude Lac enzymes that had been extracted from acetone powder showed multiple bands indicating Lac activity detected by GU in the ranges of pI 3.50–5.20 and 7.35–9.30 (Fig. 1). However, laccase activity detected by gallic acid showed remarkable staining within pI 3.50–5.85, whereas staining was very weak within pI 7.35–9.30 (Fig. 2). For elucidating the difference between Lac with pI 3.50–5.85 and 7.35–9.30, crude Lac was fractionated and analyzed. When the crude Lac enzymes were separated into the fractions containing the bands within pI 3.50–5.85 and 7.35–9.30, the latter samples were blue in color and the former were yellow



**Fig. 7** Laccase activity of blue and yellow fraction. **a** 3MC; **b** SA; **c** 2,6-DMP; **d** 4MC; **e** ABTS; **f** GU. Blue line shows laccase activity of blue fraction; yellow line shows laccase activity of yellow fraction. The amount of blue and yellow fraction used corresponded to 1  $\mu$ L of crude Lac (2.6 mL) prepared from Sample 4

(Fig. 6). The yellow fraction showed Lac activity equal to or higher than the blue fraction (Fig. 7). The yellow fraction is conventionally removed during the purification process of lacquer Lac and has thus seldom been

analyzed. This paper is the first to report the importance of the yellow fraction as a component of lacquer Lac.

Lacquer Lac was the first multicopper oxidase found and has long been studied in detail to understand the

enzyme properties, including structure and functions [6, 10]. Isoelectric points around pI 8–10 have been reported for purified lacquer Lac, and the enzymes are known to exhibit blue color with a maximum absorbance at around 600 nm in the UV–VIS spectra [13].

The blue fraction analyzed in this study had a maximum absorbance at around 600 nm and contained bands within pI 7.35–9.30. These characteristics were consistent with previously reported characteristics of the blue fraction of lacquer Lac. In contrast, the crude Lac enzymes of the yellow fraction had isoelectric points within pI 3.50–5.85 and showed no distinct maximum absorption at around 600 nm in the visible spectrum—characteristics not consistent with those of conventional blue Lac (Figs. 5, 6).

Lacquer Lac has been previously studied for the purpose of analyzing the blue proteins contained in lacquer. Therefore, the non-blue fractions have been removed during the purification of the blue Lac. In many such studies, a purification process that used an ion-exchange resin was set up, and proteins or other components of pI 6.0 or lower were removed [12, 13]. The Lacs from the bands within pI 3.50–5.85 detected in this study should correspond to the fractions that were previously removed.

There was a large difference in Lac activity for the blue and yellow fractions depending on the type of substrate and pH (Fig. 7). When SA, 2,6-DMP, and GU were used as substrates, the yellow fraction showed higher activity than the blue fraction in the pH range of 5.5–7.5. At pH 6–7, especially, Lac activity was mostly derived from the yellow fraction and not the blue fraction. The SA, 2,6-DMP, and GU have been used as versatile substrates to measure the activity of lacquer Lac. The previously reported Lac activity of lacquer acetone powder may have originated from the yellow rather than the blue fraction.

For 3MC and 4MC, the yellow fraction showed higher activity than the blue fraction at pH 4.5–6.5, and the blue fraction showed higher activity than the yellow at pH 7.0–8.0. Compared with SA, 2,6-DMP, and GU, 3MC has a higher structural similarity to urushiol (Fig. 8). The results suggest that in the polymerization of urushiol, the blue and yellow fractions acted complementarily over a wide pH range.

When ABTS was used as the substrate, significant differences in Lac activity were observed compared to other substrates. The optimum pH of lacquer Lac has been reported to be pH 5.0–8.0 when SA is the substrate [6, 18]. In this study, when SA, 2,6-DMP, 3MC, 4MC, and GU were used as substrates, the optimum pH was 5.0–8.0. However, when ABTS was the substrate, both the blue and yellow fractions had optimum pH of 3.0–4.0.

ABTS is widely used as a substrate to measure activity of Lac and peroxidases. The other substrates used had a skeleton similar to the structure of wood components; however, ABTS had a different chemical structure. The Lac of white-rot fungi is considered to have an acidic optimum pH [6]; however, in recent years, studies on yellow and white Lac derived from white-rot fungi have reported that optimum pH of SA is pH 6–7, whereas optimum pH of ABTS is in the range of pH 3–4 [19, 20].

The color development test following isoelectric focusing that used gallic acid confirmed strong color development in multiple bands within pI 3.5–5.85 of the yellow fraction, whereas color development was weak in the five bands within pI 7.35–9.30 of the blue fraction.

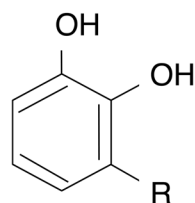
The GU color development test performed on the yellow fraction confirmed Lac activity in multiple bands within pI 4.25–5.85, in addition to three bands within pI 3.50–4.25, by extending the reaction time. However, as the reaction time for the Lac activity measurement was shorter than 5 min, it was likely that the three bands that developed color in this short time contributed to the reaction.

All these results indicated that the yellow fraction was at least as important as the blue fraction as a Lac component of lacquer.

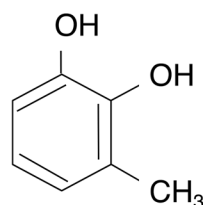
Lac is a multicopper oxidase, and the active center responsible for its catalytic function is composed of four copper atoms, which are classified into types I, II, and III [10]. Among them, it has become clear that type I contributes to development of the blue color. An earlier analysis of lacquer Lac reported that removing copper from Lac resulted in decreased enzyme activity and loss of the blue color [21].

In this study, although the bands within pI 3.5–5.2 contained in the yellow fraction were very low in amount and intensity compared to the bands within pI 7.35–9.30, the bands were very clear and were not smeared as often occurs due to degeneration of the protein (Fig. 1). In addition, as discussed earlier, the yellow fraction showed a Lac activity equal to or greater than the blue fraction, with no results indicating deactivation of the enzyme (Fig. 7). Therefore, it is unlikely that the deterioration or degradation of the blue fraction resulted in the deterioration or loss of type I copper and that the loss of blue color gave rise to the yellow fraction. These results indicated that the yellow fraction represented a completely different enzymatic protein from the blue fraction. In white-rot fungi, multiple isozymes of Lac have been reported, including yellow and white Lac, which do not exhibit blue color [17, 22, 23]. Our results indicated that yellow Lac was also present in lacquer.

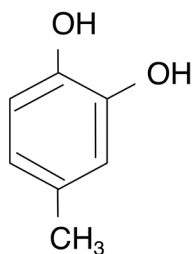




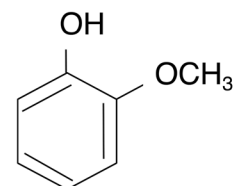
Urushiol  
R=15, 17 carbon chains with 0-3 olefins



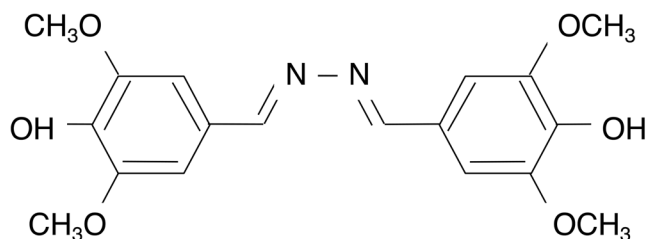
3-Methylcatechol (3MC)



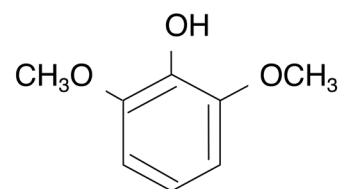
4-Methylcatechol (4MC)



Guaiacol (GU)



Syringaldazine (SA)



2,6-Dimethoxyphenol  
(2,6-DMP)

**Fig. 8** Chemical structures of urushiol and substrates used for laccase activity measurement in this study

## Conclusions

In this study, we comprehensively analyzed lacquer Lac using isoelectric focusing and found multiple Lacs not previously described. Among them, more than one Lac enzyme with an isoelectric point within pI 3.5–5.85 was suggested to be yellow Lac, which did not exhibit a blue color. Enzymatic activity was measured for the isolated blue and yellow fractions at pH 2.5–8.0 using several substrates, showing that the yellow fraction had an activity equal to or greater than the blue fraction. These results indicated that there were multiple

Lac enzymes in lacquer that could be involved in the polymerization and drying of urushiol, in addition to the blue Lacs that have been commonly studied.

## Abbreviations

Lac: Laccase; GU: Guaiacol; SA: Syringaldazine; 3MC: 3-Methylcatechol; 4MC: 4-Methylcatechol; 2,6-DMP: 2,6-Dimethoxyphenol; ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

## Acknowledgements

The authors thank Mr. Masayuki Seko and Mr. Yoshihiro Takeuchi for collecting the lacquer.

**Authors' contributions**

MT, MN, MT conceived, planned the work leading to the report. MT performed most of the experiments and interpreted the evidence. All authors read and approved the final manuscript.

**Funding**

This work was supported by Japan Society for the Promotion of Science, Grant Number 19H00551.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

<sup>1</sup> Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan. <sup>2</sup> Tohoku Research Center, Forestry and Forest Products Research Institute, Morioka, Iwate 020-0123, Japan.

Received: 15 October 2020 Accepted: 18 January 2021

Published online: 26 January 2021

**References**

- Suzuki M, Yonekura K, Noshiro S (2007) Distribution and habitat of *Toxicodendron vernicifluum* (Stokes) F. A. Barkl. (Anacardiaceae) in China. *Jpn J Histor Bot* 15:58–62
- Miyamoto M, Kakuda A (2008) An exhibition of Shisui Rokkaku, who established Japanese 'National Treasure'. Executive committee on an exhibition of Shisui Rokkaku. pp. 82–111
- Snyder DM (1989) An overview of oriental lacquer. *J Chem Educ* 66:977–980
- Kumanotani J (1995) Urushi (oriental lacquer)—a natural aesthetic durable and future-promising coating. *Prog Org Coat* 26:163–195
- Kamiya Y, Miyakoshi T (2001) Synthesis of urushiol components and analysis of urushi sap from *Rhus vernicifera*. *J Oleo Sci* 50:865–874
- Madhavi V, Lele SS (2009) Laccase: properties and applications. *BioResources* 4:1694–1717
- Lu R, Harigaya S, Ishimura T, Nagase K, Miyakoshi T (2004) Development of a fast drying lacquer based on raw lacquer sap. *Prog Org Coat* 51:238–243
- Kamiya Y, Niimura Y, Miyakoshi T (2000) Evaluation of synthesized lacquer films using pyrolysis-gas chromatography/mass spectrometry. *Bull Chem Soc Jpn* 73:2621–2626
- Japanese Standards Association (1979) JIS K5950–1979 refined rhus lacquer. Japanese Standards Association, Tokyo
- Solomon EI, Sundaram UM, Machonkin TE (1996) Multicopper oxidases and oxygenases. *Chem Rev* 96:2563–2605
- Tumurbaatar O (2015) Enzymatic digestion and mass spectroscopies of N-linked glycans in lacquer stellacyanin and laccase from *Rhus vernicifera*. Dissertation, Kitami institute of Technology. <https://doi.org/10.19000/00008428>
- Nitta K, Kataoka K, Sakurai T (2002) Primary structure of a Japanese lacquer tree laccase as a prototype enzyme of multicopper oxidases. *J Inorg Biochem* 91:125–131
- Reinhammar B (1970) Purification and properties of laccase and stellacyanin from *Rhus vernicifera*. *Biochim Biophys Acta* 205:35–47
- Leisola MSA, Kozulic B, Meussdoerffer F, Fiechter A (1987) Homology among multiple extracellular peroxidases from *Phanerochaete chrysosporium*. *J Biol Chem* 262:419–424
- Lobos S, Larraín J, Salas L, Cullen D, Vicuña R (1994) Isoenzymes of manganese-dependent peroxidase and laccase produced by the lignin-degrading basidiomycete *Ceriporiopsis subvermispora*. *Microbiology* 140:2691–2698
- McIlvaine TC (1921) A buffer solution for colorimetric comparison. *J Biol Chem* 49:183–186
- Palmieri G, Giardina P, Bianco C, Scaloni A, Capasso A, Sanna G (1997) A novel white laccase from *Pleurotus ostreatus*. *J Biol Chem* 272:31301–31307
- Lu R, Honda T, Sung M, Jung J, Miyakoshi T (2017) Analysis of fresh sap collected from Ryukyu lacquer tree. *Anal Sci* 33:1253–1257
- Ike PTL, Moreira AC, Almeida FG, Ferreira D, Birolli WG, Porto ALM, Souza DHF (2015) Functional characterization of a yellow laccase from *Leucoagaricus gongylophorus*. *Springerplus* 4:654. <https://doi.org/10.1186/s40064-015-1464-y>
- Pozdnyakova NN, Turkovskaya OV, Yudina EN, Rodakiewicz-Nowak Y (2006) Yellow laccase from the fungus *Pleurotus ostreatus* D1: purification and characterization. *Appl Biochem Microbiol* 42:56–61
- Omura T (1961) Studies on laccases of lacquer trees. III Reconstruction of laccase from its protein and copper. *J Biochem* 50:389–393
- Leontievsky AA, Vares T, Lankinen P, Shergill JK, Pozdnyakova NN, Myasodova NM, Kalkkinen N, Golovleva LA, Cammack R, Thurston CF, Hatakka A (1997) Blue and yellow laccases of ligninolytic fungi. *FEMS Microbiol Lett* 156:9–14
- Daroch M, Houghton CA, Moore JK, Wilkinson MC, Carnell AJ, Bates AD, Iwanejko LA (2014) Glycosylated yellow laccases of the basidiomycete *Stropharia aeruginosa*. *Enzyme Microb Technol* 58–59:1–7

**Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:**

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)