



Purification and characterization of an alkali-organic solvent-stable laccase with dye decolorization capacity from newly isolated *Lysinibacillus fusiformis* W11

Xifeng Wang¹ · Pengxiao Chen² · Zhi Liu¹ · Zhihua Liu¹ · Liping Chen¹ · Haifeng Li¹ · Jianhang Qu¹

Received: 1 March 2023 / Accepted: 28 June 2023 / Published online: 14 August 2023
© The Author(s) under exclusive licence to Sociedade Brasileira de Microbiologia 2023

Abstract

A new *Lysinibacillus fusiformis* strain with abundant laccase activity was isolated from soil under forest rotted leaf and identified as *L. fusiformis* W11 based on its 16S rRNA gene sequence and physiological characteristics. The laccase *Lfu*Lac was purified and characterized. The optimum temperature and pH of *Lfu*Lac on guaiacol were 45 °C and pH 9, respectively. *Lfu*Lac kept 78%, 88%, 92%, 74%, and 47% of activity at pH 7–11, respectively, suggesting the alkali resistance of the enzyme. The effects of various metal ions on *Lfu*Lac showed that Cu²⁺, Mg²⁺, and Na⁺ were beneficial to laccase activity and 10 mM Cu²⁺ increased the activity of *Lfu*Lac to 216%. *Lfu*Lac showed about 90% activity at 5% organic solvents and more than 60% activity at 20%, indicating its resistance to organic solvents. In addition, *Lfu*Lac decolorized different kinds of dyes. This study enriched our knowledge about laccase from *L. fusiformis* W11 and its potential industrial applications.

Keywords Laccase · Alkali · Organic solvent · *Lysinibacillus fusiformis*

Introduction

Laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) is a copper-containing polyphenol oxidase that is widely distributed in plants, insects, fungi, and bacteria [1]. Laccases are applied in food, pharmaceutical, and environmental industries because they can oxidize a wide variety of compounds and reduce oxygen into water without producing harmful by-products [2–4]. During the biotechnological applications of laccases, reactions are often carried out under extreme conditions, such as extremely acidic and alkaline pH and high concentration of organic solvents [5, 6]. Thus, searching for robust laccases that can tolerate harsh conditions is an ongoing effort to date.

Compared with plant and animal laccases, microbial laccases are abundant and classified into fungal and bacterial

laccases [7]. Fungal laccases have been found in many species, such as *Trametes trogii*, *Coriolopsis caperata*, and *Trametes versicolor* [8–10]. Fungal laccases have been studied in a variety of biotechnological applications, but their applications are usually hampered due to long fermentation periods, narrow pH range, and intolerance to extreme conditions [11]. Many bacterial laccases are available under extreme conditions and expected to replace fungal laccases for application to bioremediation, industrial wastewater treatment, and dye decolorization [1, 7, 12]. Laccases are discovered in a number of bacteria, including *Bacillus subtilis*, *Escherichia coli*, *Streptomyces coelicolor*, *Stenotrophomonas maltophilia*, and *Thermus thermophilus* [13, 14]. More bacterial laccases need to be found to meet the biotechnological applications.

Lysinibacillus fusiformis is a Gram-positive and rod-shaped bacterium, which has been isolated from multiple environments [15]. *L. fusiformis* has been reported for its capability for azo dye decolorization, petroleum degradation, and deproteinization [16, 17]. Meanwhile, some extracellular enzymes of *L. fusiformis*, such as L-asparaginase and protease, have been reported, suggesting *L. fusiformis* was a multifunctional bacterium. The laccase of *L. fusiformis* has also been reported with ability of removal sulfonamides and tetracyclines residues, but other characteristics of the laccase

Responsible Editor: Lucy Seldin

✉ Xifeng Wang
wangxifeng@haut.edu.cn

¹ College of Biological Engineering, Henan University of Technology, Zhengzhou 450001, China

² School of Food and Strategic Reserves, Henan University of Technology, Zhengzhou 450001, China

need to be further studied [18]. In this study, a new strain of *L. fusiformis* W11 was isolated from soil under forest rotted leaf in Wuling Mountain, Chongqing, China, and showed significant laccase activity. This study purified the laccase *Lfu*Lac and investigated its characteristics.

Materials and methods

Isolation and identification

Sample was collected from soil under forest rotted leaf in Wuling Mountain, Chongqing, China. Ten grams of soil sample were properly mixed with 90 mL of distilled water in a 250-mL flask and serially diluted up to 10^{-6} . A 0.1-mL sample diluent was spread over solid lysogeny broth (LB) medium (10-g/L tryptone, 5-g/L yeast extract, 10 g/L NaCl, 10 g/L agar) containing 0.04% (v/w) guaiacol, which is a typical substrate of laccase [19]. The plates were incubated at 30 °C for 1–5 d. The colonies that showed red–brown circle on the plates were repeatedly transferred on LB solid plates until pure cultures were obtained.

Molecular characterization of the pure bacterial colony was conducted using 16S rRNA gene sequencing [20]. Genomic DNA was isolated with the TIANamp bacteria DNA Kit (Tiangen, Beijing, China). The 16S bacterial rRNA gene sequence was amplified by 27F with sequence 5'-AGA GTTTGATCATGGCTCAG-3' and 1492R with sequence 5'-CTACGGTTACCTTGTTAC GAC-3'. The gene sequence of 16S rRNA was verified by sequencing. A phylogenetic tree was constructed using neighbor-joining and maximum likelihood methods with MEGA 7 software, and bootstrap values were calculated from 1000 replications [21].

Purification of enzyme

LB medium was used for the production of laccase. Laccase was purified using the method described by Liu et al. [22]. The culture was centrifuged at 5000 rpm for 10 min. The supernatant was precipitated by ammonium sulfate, dialyzed in 0.1 M citrate-phosphate buffer (pH 7.0) for 48 h at 4 °C to remove ammonium sulfate and lyophilized. Then, the sample was loaded onto a DEAE-cellulose anion exchange column which was previously equilibrated with 0.1 M citrate-phosphate buffer (pH 7.0). After the unadsorbed proteins had been eluted, the adsorbed proteins were eluted with linear gradient concentration of NaCl from 0 to 1.0 M. All obtained fractions were monitored for laccase activity and the fractions containing laccase activity were pooled, concentrated, and dialyzed overnight. The sample was loaded onto a Sephadex G-100 column which was previously equilibrated with 0.1 M citrate-phosphate buffer (pH 7.0). Elution was collected with citrate-phosphate buffer and the eluted fractions with laccase activity were pooled and dialyzed. Protein content was then

determined by SDS-PAGE, and protein concentration was determined by Nanovue Plus (GE).

Biochemical characterization

The activity of *Lfu*Lac was determined using guaiacol as substrate [23, 24]. The reaction mixture containing 1 μ M *Lfu*Lac, 3 mM guaiacol, and 10 mM Cu^{2+} was incubated at set temperatures for 30 min. Absorbance of the reaction mixture at 465 nm was recorded to represent the production of tetraguaiacol. The optimum temperature was the temperature with the highest activity on guaiacol at the optimum pH during a 30-min reaction period. Optimum pH was determined by measuring the activity on guaiacol at 45 °C at different pH levels. Relative laccase activity was measured by setting the activity at the optimal temperature and pH as 100%. *Lfu*Lac was incubated at different temperatures at pH 9.0 for 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 h to determine its thermostability. *Lfu*Lac was incubated at different pH levels at room temperature for 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 h to determine pH stability. The amount of activity retained was detected. The activity of *Lfu*Lac without incubation was set as 100%. Cu^{2+} (CuCl_2), Mg^{2+} (MgCl_2), Zn^{2+} (ZnCl_2), Ni^{2+} (NiCl_2), Mn^{2+} (MnCl_2), Co^{2+} (CoCl_2), Na^+ (NaCl), and K^+ (KCl) were used to study the effects of metal ions on *Lfu*Lac. Five concentrations of metal ions (1, 2, 5, 10, and 20 mM) were tested. Five organic solvents (ethanol, acetone, methanol, chloroform, and DMSO) were tested at concentrations of 5%, 10%, and 20% (v/v) to study their effects on laccase activity.

Dye decolorization assays

Dye decolorization assays were performed as previously described [25, 26]. The reaction mixture of 1 μ M *Lfu*Lac, 15 mg/L dyes, and 10 mM Cu^{2+} was incubated at 30 °C for 1 h. Dye decolorization was determined by recording changes in absorbance at the following wavelengths for different dyes: crystal violet (590 nm), malachite green (624 nm), Coomassie brilliant blue R-250 (556 nm), Congo red (497 nm), Acid red 27 (520 nm), methyl orange (465 nm), Acid blue 25 (595 nm), and Disperse blue 60 (670 nm). Samples without the enzyme were used as control. Dye decolorization was calculated using the following equation:

$$\% \text{Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100.$$

Results

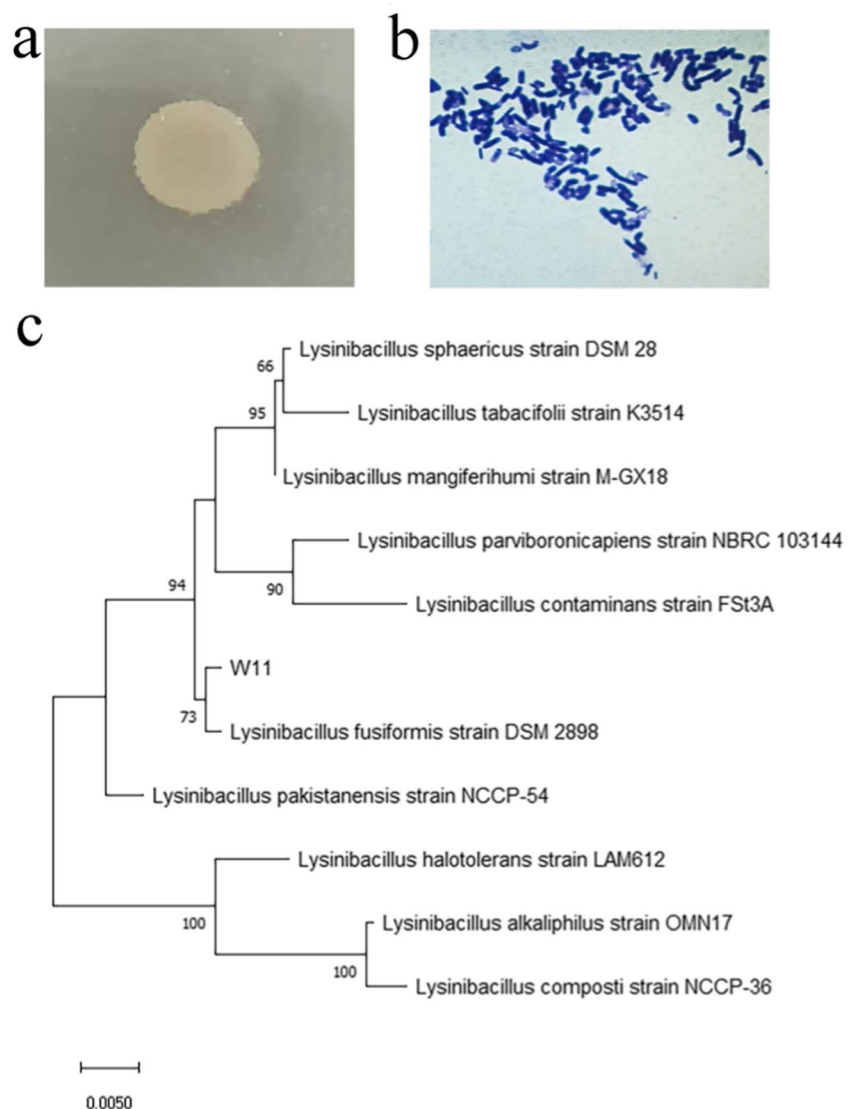
Screening and identification of laccase-positive strain

The soil sample was cultured in LB–guaiacol medium to screen for laccase-positive strains. Eight strains showed a red–brown oxidized zone on the plates, indicating that they

were laccase active. Strain W11 was screened as the best laccase-secreting strain because of the biggest red–brown oxidized zone on the LB–guaiacol plate (Fig. S1). The colony of W11 was irregular, moist, flat, and slightly convex (Fig. 1a). The strain was Gram-positive and had rod-shaped cells (Fig. 1b).

The 16S rRNA gene sequence (1403 nt) of the strain was amplified, purified, and sequenced to identify W11. The 16S rRNA sequence of W11 (accession no: OQ418029) showed the 99.67% similarity to the sequences of *L. fusiformis* DSM2898. A phylogenetic tree was constructed by neighbor-joining and maximum likelihood methods, and the results revealed that W11 clustered with the members of *L. fusiformis* (Fig. 1c). According to the analysis of 16S rRNA sequence and morphological characteristics, strain L-11 was identified as *L. fusiformis* and designated as *L. fusiformis* W11. The laccase secreted by *L. fusiformis* W11 was labeled as *LfuLac*.

Fig. 1 Characterization of strain W11. **a** Colony characteristic of W11, **b** gram staining of W11, and **c** phylogenetic analysis of the 16S rRNA gene sequence of strain W11 Bar, 0.005 substitutions per nucleotide position



Purification of *LfuLac* from *L. fusiformis* W11

The laccase activities in the liquid medium were 0.11, 0.35, 0.87, and 1.01 U/mL in 1 to 4 days, respectively, and it reached the maximum level of 1.28 U/mL on the 5th day. The extracellular laccase from *L. fusiformis* W11 was purified, and a summary of purification data is shown in Table 1. *LfuLac* was purified by ammonium sulfate precipitation, DEAE-cellulose anion exchange, and Sephadex G-100 gel filtration chromatography, generating a 19.11-fold increase in purity and 44.31% yield. The purified *LfuLac* was examined by SDS-PAGE (Fig. 2).

Effect of pH and temperature on the activity and stability of *LfuLac*

Assays were performed using guaiacol as substrate to determine the laccase activity of *LfuLac*. As shown in

Table 1 Summary of purification steps of laccase *LfuLac* from *L. fusiformis* W11

Purification step	Total activity (U/mL)	Total protein (mg/mL)	Purification fold	Yield(%)
Crude extract	1.28	1.212	1	100
Salt precipitation	1.09	0.78	1.43	85.11
DEAE-cellulose anionexchange	0.76	0.28	2.71	60.23
Sephadex G-100 gel filtration chromatography	0.61	0.03	19.11	44.31

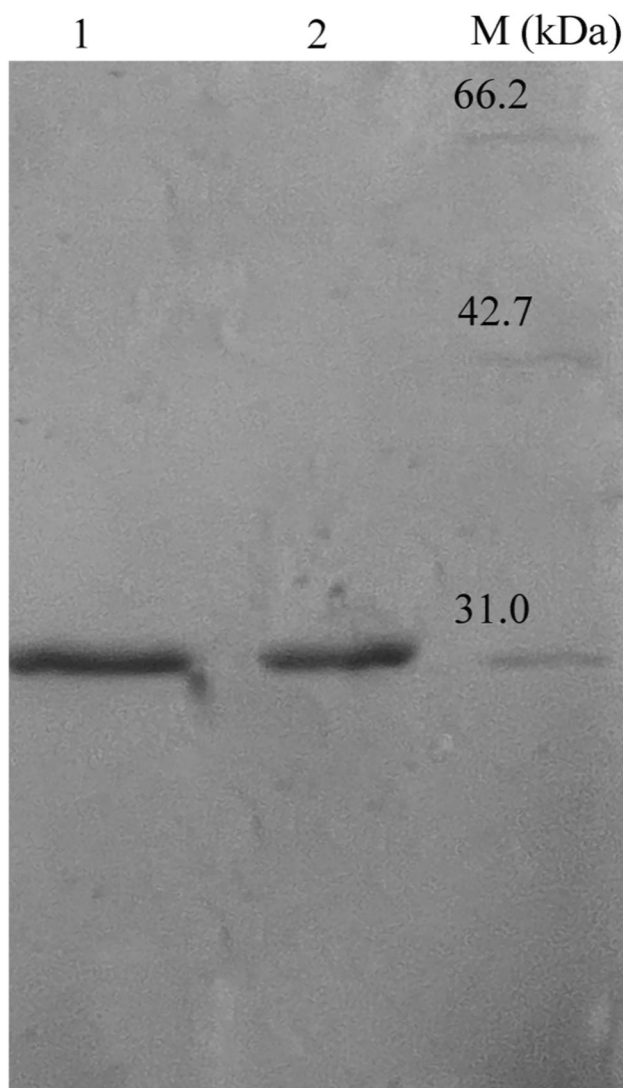
**Fig. 2** SDS-PAGE analysis of purified *LfuLac*. Purified *LfuLac* (lane 1 and lane 2) and protein molecular mass markers (kDa) (lane M)

Fig. 3a, the laccase activity of *LfuLac* increased gradually from 25 °C to 45 °C. *LfuLac* reached its maximum activity of 76 U/mL at 45 °C and decreased at higher temperatures. The effect of pH on the laccase activity of *LfuLac* was

examined at 45 °C. *LfuLac* kept more than 70% activity over a wide range of pH 7.0–11.0 and reached its highest activity at pH 9.0 (optimum pH) (Fig. 3b).

Temperature and pH stability were examined. The activity of *LfuLac* remained almost 100% after incubation at 10 °C and 0 °C for 1 h and then decreased gradually with increasing incubation temperatures (Fig. 3c). *LfuLac* retained about 74% and 40% of its activity after incubation at 30 °C and 40 °C for 1 h, respectively. The activity of *LfuLac* almost reduced to 0 after incubation at 60 °C for 1 h. As shown in Fig. 3d, *LfuLac* kept 78%, 88%, 92%, 74%, and 47% of its activity at pH 7–11, suggesting the alkali resistance of the enzyme under these conditions. However, the activity of *LfuLac* decreased to 50% at pH 5 and was 9% at pH 4, indicating that it was unstable against strong acid conditions.

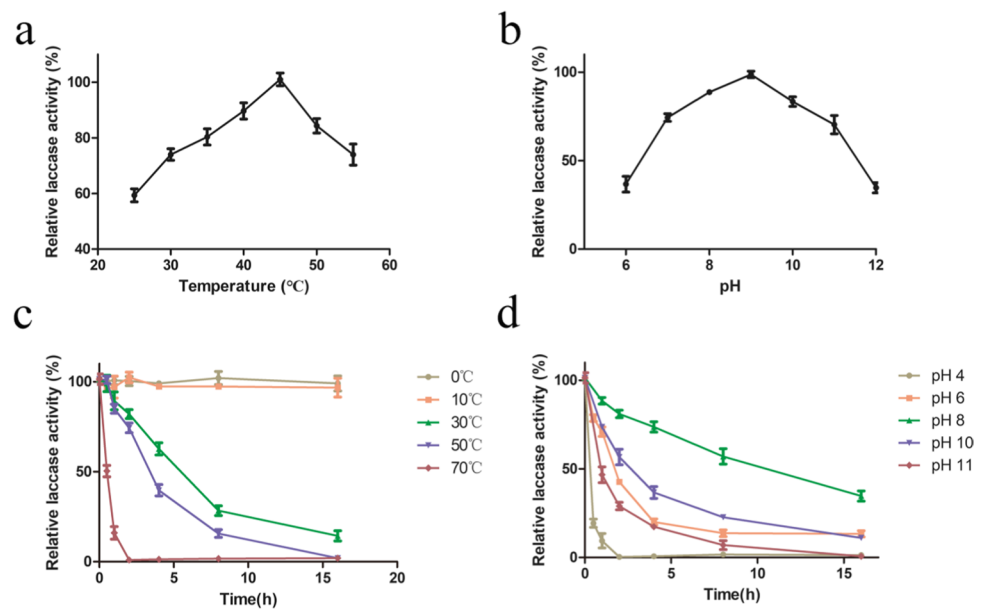
Effect of metal ions on the activity of *LfuLac*

The reaction mixtures were added with Cu^{2+} , Mg^{2+} , Zn^{2+} , Ni^{2+} , Mn^{2+} , Co^{2+} , Na^+ , and K^+ to study the effects of metal ions on *LfuLac*. Five concentrations of metal ions (1, 2, 5, 10, and 20 mM) were tested. As shown in Fig. 4, Cu^{2+} , Mg^{2+} , and Na^+ were beneficial to laccase activity. To be specific, 10 mM Cu^{2+} increased the activity of *LfuLac* to 216%, suggesting that *LfuLac* was a Cu^{2+} -activated laccase. Moreover, 2 mM Mg^{2+} and Na^+ 10 mM increased the laccase activity to 152% and 125%, respectively. The addition of K^+ , Mn^{2+} , and Co^{2+} did not show obvious effects on *LfuLac*. Zn^{2+} and Ni^{2+} suppressed the activity of *LfuLac* and exhibited dose-dependent inhibition effects at high concentrations.

Effects of organic solvents on the activity of *LfuLac*

Five organic solvents (ethanol, acetone, methanol, chloroform, and DMSO) were tested at concentrations of 5%, 10%, and 20% (v/v). The laccase activity without any chemicals of 76 U/mL was set as 100%. As shown in Fig. 5, *LfuLac* showed more than 90% activity at 5% organic solvents and more than 60% activity at 20%, indicating that the enzyme displayed decent resistance to the tested chemicals. In 10% ethanol, acetone,

Fig. 3 Biochemical characterization of *LfuLac*. **a** and **b** Optimum temperature and pH of *LfuLac* determined with guaiacol as the substrate. Relative laccase activity was measured by setting the activity at the optimal temperature and pH as 100%. **c** and **d** Thermostability and pH stability of *LfuLac*. The activity of *PthLac* without incubation was set as 100%. The error bars represent the standard error of the mean of triplicate measurements



methanol, chloroform, and DMSO, *LfuLac* exhibited 87.13%, 80.32%, 82.19%, 80.19%, 76.87%, 92.52%, and 89.89% activity, respectively. Compared with other organic solvents, *LfuLac* displayed stronger resistance to ethanol with more than 65% activity at 20% concentrations. Hence, *LfuLac* remained active at high concentrations of the chemicals and might have potential for industrial applications.

Dye decolorization capacity of *LfuLac*

As shown in Table 2, nine dyes from three categories were used to test the dye decolorization capacity of *LfuLac*. The results showed that *LfuLac* partly decolorized all the tested dyes. *LfuLac* showed the strongest decolorization capacity to azo dye than the two other dyes and the least decolorization rate to anthraquinone. The decolorization capacities of *LfuLac* to Congo red, Acid Red 27, and methyl orange were 45.18%, 45.11%, and 36.53%, respectively.

Discussion

L. fusiformis is one of the most recognized species in the genus *Lysinibacillus*. It was first isolated in 1901 from the surface of *Beta vulgaris* [15]. Since then, many strains of *L. fusiformis* have been isolated from various environmental samples, such as soil, wastewater, and potato phyllosphere [27, 28]. In this study, a new strain was isolated from soil under forest rotted leaf, which is habitable and rich in lignocellulose, and was identified as *L. fusiformis* W11 by its 16S

rRNA sequence and morphological characteristics. Laccase was able to oxidize non-phenolic lignin, which might make *L. fusiformis* W11 secrete laccase to adapt to its habitat. The laccase activity of *LfuLac* was 44 U/mL when incubation with guaiacol as the substrate under the optimal reaction conditions. The value is higher than that of laccase from *Agaricus blazei* U2-4 (25.4 U/mL) and *Colletotrichum gloeosporioides* (38 U/mL) [29, 30]. Hence, *L. fusiformis* W11 could be a laccase-secreting microorganism. However, the activity is lower than that of laccase from *Bacillus subtilis* MTCC 2414 (100 U/mL), suggesting that the conditions need to be further optimized to increase the production of *LfuLac*.

Several extracellular laccases were purified with the three consecutive procedures, such as laccase from *Marasmius scorodoni*, laccase from *Marasmius* sp. BBKAV79, and ThLacc-S from *Trametes hirsute* [31, 32]. Compared with laccase activities of 432.8 U/mg from *Marasmius scorodoni* and 0.226 U/mg laccase from *Marasmius* sp. BBKAV79, the laccase activity of *LfuLac* was moderate 20 U/mg. The molecular weight of *LfuLac* was about 31 kDa, which was consistent with SLAC (PDB ID: 3CG8) from *Streptomyces coelicolor*, suggesting *LfuLac* might be a small bacterial laccase [13].

Alkali-stable laccases have been studied in many reports. ThLacc-S from *Trametes hirsute* retained about 70% of its activity after incubation at pH 10 for 72 h, but the activity decreased to about 10% at pH 11 [24]. Laccase from *Bacillus tequilensis* SN4 kept more than 75% activity at pH 9.0 for 24 h, but higher pH was not tested [33]. The

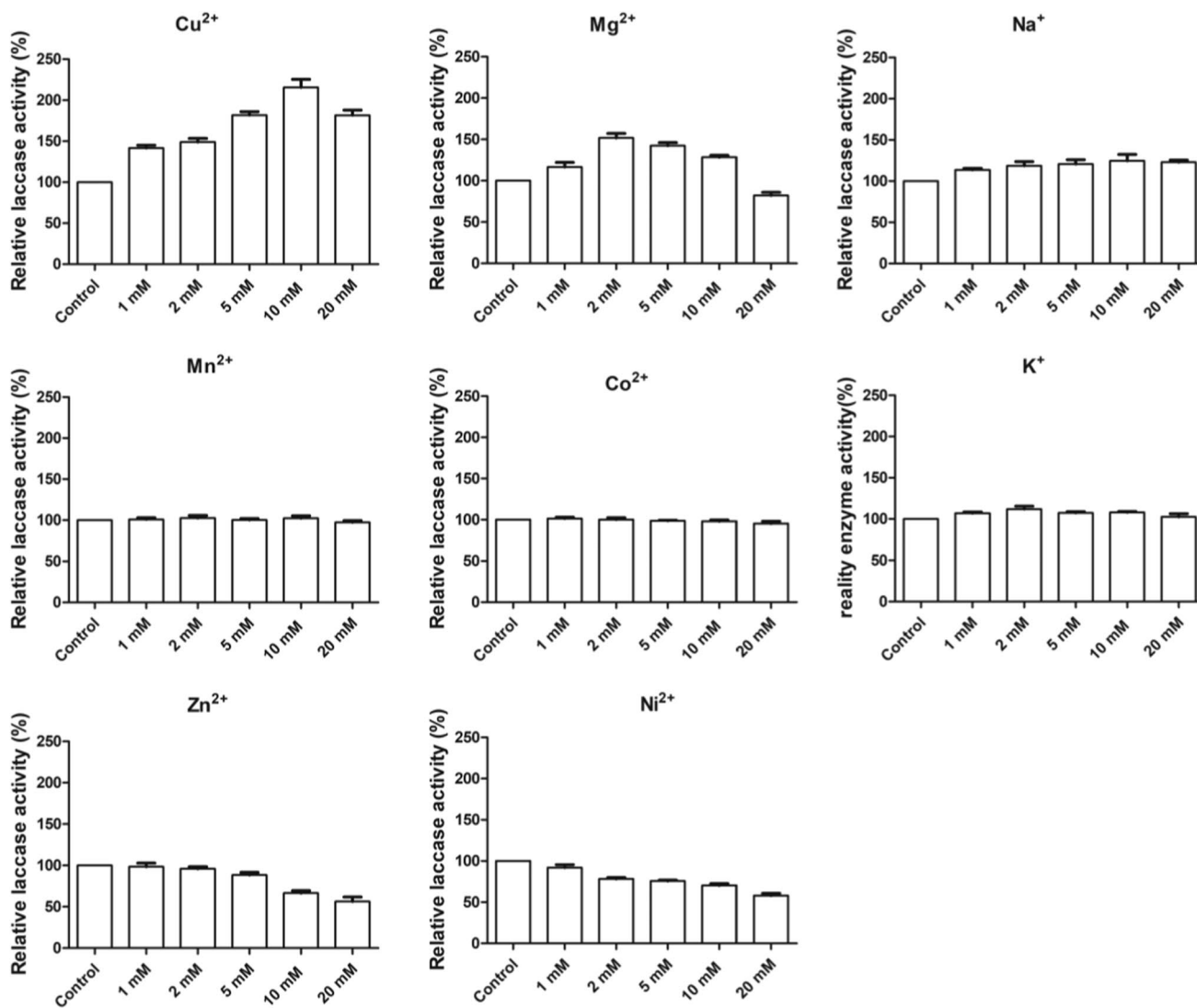


Fig. 4 Activity of *LfuLac* in the presence of different metal ions at the concentrations of 1–20 mM. The enzymatic activity in the absence of each metal ion was set as 100%. The error bars represent the standard error of the mean of triplicate measurements

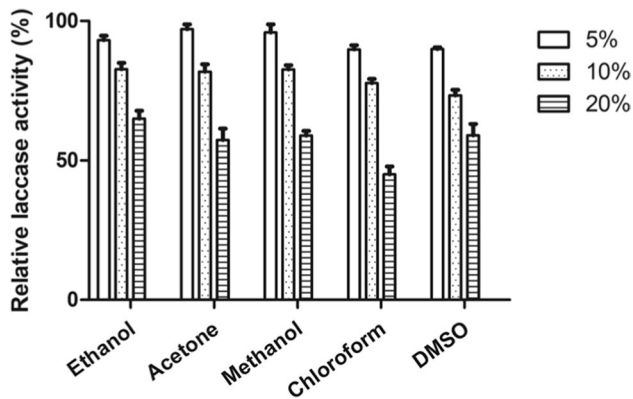


Fig. 5 Inhibitory effect of different organic solvents on *LfuLac* activity. The laccase activity without any chemicals was set as 100%

Table 2 Ability of *PthLac* to decolorize different dyes

Dyes	%Decolorization
Crystal violet	32.67
Malachite green	25.76
Coomassie brilliant blue R-250	31.35
Congo red	45.18
Acid red 27	45.11
Methyl orange	36.53
Acid blue 25	15.51
Disperse blue 60	16.45
Reactive brilliant blue KN-R610 nm	19.21

purified *LfuLac* protein kept more than 70% of its activity at pH 7–10, which was not prominent compared with other reported alkali-stable laccases. However, *LfuLac* retained 47% of its activity at pH 11, suggesting its strong alkali resistance. Most of the known laccases are unstable at concentrations above 10% of organic solvents [34, 35]. However, *LfuLac* was stable and retained more than 80% of its activity in the presence of 10% ethanol, acetone, methanol, and chloroform. *LfuLac* kept about 60% activity in 20% organic solvents, indicating that it was an organic solvent-stable enzyme. Moreover, *LfuLac* decolorized triarylmethane, azo, and anthraquinone dyes. Therefore, *LfuLac* might have potential to industrial applications, such as pulp biobleaching and textile dye decolorization, due to its remarkable tolerance to alkali-organic solvents and decolorizing ability.

In conclusion, this study reported the laccase *LfuLac* obtained from *L. fusiformis* W11. Alkali tolerance, stability in organic solvents, and decolorizing ability were the important features of *LfuLac*. Overall, *LfuLac* could be a promising enzyme for biotechnological applications.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42770-023-01091-2>.

Funding This study was funded by the Key Scientific Projects for colleges of Henan Province (Grant number 21A180004), the Henan Provincial Science and Technology Research Project (Grant number 222102320250), the Doctoral Scientific Research Start-up Foundation from Henan University of Technology (Grant number 2018BS080), and Natural Science Foundation of Henan Province (Grant number 232300420206).

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval This study does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication The participant has consented to the submission of the case report to the journal.

Competing interests The authors declare no competing interests.

References

- Khatami SH, Vakili O, Movahedpour A, Ghesmati Z, Ghasemi H, Taheri-Anganeh M (2022) Laccase: various types and applications. *Biotechnol Appl Biochem* 69(6):2658–2672. <https://doi.org/10.1002/bab.2313>
- Arregui L, Ayala M, Gomez-Gil X, Gutierrez-Soto G, Hernandez-Luna CE, Herrera de Los Santos M, Levin L, Rojo-Dominguez A, Romero-Martinez D, MCN S, Trujillo-Roldan MA, Valdez-Cruz NA (2019) Laccases: structure, function, and potential application in water bioremediation. *Microb Cell Fact* 18(1):200. <https://doi.org/10.1186/s12934-019-1248-0>
- Schmitz E, Leontakianakou S, Norlander S, Nordberg Karlsson E, Adlercreutz P (2022) Lignocellulose degradation for the bioeconomy: the potential of enzyme synergies between xylanases, ferulic acid esterase and laccase for the production of arabinoxylo-oligosaccharides. *Bioresour Technol* 343:126114. <https://doi.org/10.1016/j.biortech.2021.126114>
- Mayolo-Deloisa K, Gonzalez-Gonzalez M, Rito-Palomares M (2020) Laccases in food industry: bioprocessing, potential industrial and biotechnological applications. *Front Bioeng Biotechnol* 8:222. <https://doi.org/10.3389/fbioe.2020.00222>
- Feng H, Zhang D, Sun Y, Zhi Y, Mao L, Luo Y, Xu L, Wang L, Zhou P (2015) Expression and characterization of a recombinant laccase with alkalistable and thermostable properties from *Streptomyces griseorubens* JSD-1. *Appl Biochem Biotechnol* 176(2):547–562. <https://doi.org/10.1007/s12010-015-1594-2>
- Neifar M, Chouchane H, Mahjoubi M, Jaouani A, Cherif A (2016) *Pseudomonas extremorientalis* BU118: a new salt-tolerant laccase-secreting bacterium with biotechnological potential in textile azo dye decolorization. *3 Biotech* 6(1):107. <https://doi.org/10.1007/s13205-016-0425-7>
- Chandra R, Chowdhary P (2015) Properties of bacterial laccases and their application in bioremediation of industrial wastes. *Environ Sci Process Impacts* 17(2):326–342. <https://doi.org/10.1039/c4em00627e>
- Chaoua S, Chaouche NK, Songulashvili G, Gares M, Hiligsmann S, Flahaut S (2023) Yellow laccase produced by *Trametes versicolor* K1 on tomato waste: a comparative study with the blue one produced on semi-synthetic medium. *J Biotechnol* 361:99–109. <https://doi.org/10.1016/j.jbiotec.2022.12.001>
- Nandal P, Ravella SR, Kuhad RC (2013) Laccase production by *Corioliopsis caperata* RCK2011: optimization under solid state fermentation by Taguchi DOE methodology. *Sci Rep* 3:1386. <https://doi.org/10.1038/srep01386>
- Yan J, Chen Y, Niu J, Chen D, Chagan I (2015) Laccase produced by a thermotolerant strain of *Trametes trogii* LK13. *Braz J Microbiol* 46(1):59–65. <https://doi.org/10.1590/S1517-838246120130895>
- Margot J, Bennati-Granier C, Maillard J, Blázquez P, Barry DA, Holliger C (2013) Bacterial versus fungal laccase: potential for micropollutant degradation. *AMB Express* 3(1):63. <https://doi.org/10.1186/2191-0855-3-63>
- Siroosi M, Amoozegar MA, Khajeh K (2016) Purification and characterization of an alkaline chloride-tolerant laccase from a halotolerant bacterium *Bacillus* sp. strain WT. *J Mol Catal B: Enzym* 134:89–97. <https://doi.org/10.1016/j.molcatb.2016.10.001>
- Dasgupta R, Gupta K, Nami F, de Groot HJM, Canters GW, Groenen EJJ, Ubbink M (2020) Chemical exchange at the trinuclear copper center of small laccase from *Streptomyces coelicolor*. *Biophys J* 119(1):9–14. <https://doi.org/10.1016/j.bpj.2020.05.022>
- Unuofin JO (2020) Sustainability potentials of novel laccase tinctures from *Stenotrophomonas maltophilia* BIJ16 and *Bordetella bronchiseptica* HSO16: from dye decolorization to denim bioscouring. *Biotechnol Rep (Amst)* 25:e00409. <https://doi.org/10.1016/j.btre.2019.e00409>
- Morioka H, Oka K, Yamada Y, Nakane Y, Komiya H, Murase C, Iguchi M, Yagi T (2022) *Lysinibacillus fusiformis* bacteremia: case report and literature review. *J Infect Chemother* 28(2):315–318. <https://doi.org/10.1016/j.jiac.2021.10.030>
- Zhu L, Guo J, Sun Y, Wang S, Zhou C (2021) Acetic acid-producing endophyte *Lysinibacillus fusiformis* orchestrates jasmonic acid signaling and contributes to repression of cadmium uptake in

- tomato plants. *Front Plant Sci* 12:670216. <https://doi.org/10.3389/fpls.2021.670216>
17. Reyes-Cervantes A, Robles-Morales DL, Tellez-Jurado A, Huerta-Ochoa S, Jimenez-Gonzalez A, Medina-Moreno SA (2021) Evaluation in the performance of the biodegradation of herbicide diuron to high concentrations by *Lysinibacillus fusiformis* acclimatized by sequential batch culture. *J Environ Manag* 291:112688. <https://doi.org/10.1016/j.jenvman.2021.112688>
 18. Ouyang B, Xu W, Zhang W, Guang C, Mu W (2022) Efficient removal of sulfonamides and tetracyclines residues by the laccase-mediator system employing a novel laccase from *Lysinibacillus fusiformis*. *J Environ Chem Eng* 10(6). <https://doi.org/10.1016/j.jece.2022.108809>
 19. Karittapattawan P, Benchawattananon R (2021) Evaluation of laccase production by monokaryotic strains of edible mushrooms. *Pak J Biol Sci* 24(4):454–460. <https://doi.org/10.3923/pjbs.2021.454.460>
 20. Qu JH, Ma WW, Li HF, Wang XF, Lu BB, Luo Y (2019) *Altererythrobacter amylolyticus* sp. nov., isolated from lake sediment. *Int J Syst Evol Microbiol* 69(4):1231–1236. <https://doi.org/10.1099/ijsem.0.003299>
 21. Liao H, Li Y, Zhang M, Lin X, Lai Q, Tian Y (2017) *Altererythrobacter mangrovi* sp. nov., isolated from mangrove sediment. *Int J Syst Evol Microbiol* 67(11):4851–4856. <https://doi.org/10.1099/ijsem.0.002393>
 22. Sun Y, Liu ZL, Hu BY, Chen QJ, Yang AZ, Wang QY, Li XF, Zhang JY, Zhang GQ, Zhao YC (2021) Purification and characterization of a thermo- and pH-stable laccase from the litter-decomposing fungus *Gymnopus luxurians* and laccase mediator systems for dye decolorization. *Front Microbiol* 12:672620. <https://doi.org/10.3389/fmicb.2021.672620>
 23. Jiang YP, Cai JL, Pei JJ, Li Q, Zhao LG (2021) Cloning, overexpression, and characterization of a thermostable, organic solvent-tolerant laccase from *Bacillus pumilus* ARA and its application to dye decolorization. *ACS Omega* 6(14):9741–9749. <https://doi.org/10.1021/acsomega.1c00370>
 24. Si J, Ma H, Cao Y, Cui B, Dai Y (2021) Introducing a thermo-alkali-stable, metallic ion-tolerant laccase purified from white rot fungus *Trametes hirsuta*. *Front Microbiol* 12:670163. <https://doi.org/10.3389/fmicb.2021.670163>
 25. Sharma N, Leung IKH (2021) Characterization and optimisation of a novel laccase from *Sulfitobacter indolifex* for the decolourisation of organic dyes. *Int J Biol Macromol* 190:574–584
 26. Park JH, Jeon SJ (2021) Production and characterization of crude laccase from *Irpex* sp. JS7 that decolorizes synthetic and natural melanin. *Folia Microbiol (Praha)* 66(6):1039–1046. <https://doi.org/10.1007/s12223-021-00904-x>
 27. Passera A, Rossato M, Oliver JS, Battelli G, Shahzad GI, Cosentino E, Sage JM, Toffolatti SL, Lopatriello G, Davis JR, Kaiser MD, Delledonne M, Casati P (2021) Characterization of *Lysinibacillus fusiformis* strain S4C11: in vitro, in planta, and in silico analyses reveal a plant-beneficial microbe. *Microbiol Res* 244:126665. <https://doi.org/10.1016/j.micres.2020.126665>
 28. Khadka S, Adhikari S, Thapa A, Panday R, Adhikari M, Sapkota S, Regmi RS, Adhikari NP, Proshad R, Koirala N (2020) Screening and optimization of newly isolated thermotolerant *Lysinibacillus fusiformis* strain SK for protease and antifungal activity. *Curr Microbiol* 77(8):1558–1568. <https://doi.org/10.1007/s00284-020-01976-7>
 29. Valle JS, Vandenberghe LP, Oliveira AC, Tavares MF, Linde GA, Colauto NB, Socol CR (2015) Effect of different compounds on the induction of laccase production by *Agaricus blazei*. *Genet Mol Res* 14(4):15882–15891. <https://doi.org/10.4238/2015.December.1.40>
 30. Guetsky R, Kobiler I, Wang X, Perlman N, Gollop N, Avila-Quezada G, Hadar I, Prusky D (2005) metabolism of the flavonoid epicatechin by laccase of *Colletotrichum gloeosporioides* and its effect on pathogenicity on avocado fruits. *Phytopathology* 95(11):1341–1348. <https://doi.org/10.1094/PHYTO-95-1341>
 31. Jeon SJ, Lim SJ (2017) Purification and characterization of the laccase involved in dye decolorization by the white-rot fungus *Marasmius scorodonius*. *J Microbiol Biotechnol* 27:1120–1127. <https://doi.org/10.4014/jmb.1701.01004>
 32. Vantamuri AB, Kaliwal BB (2016) Purification and characterization of laccase from *Marasmius* species BBKAV79 and effective decolorization of selected textile dyes. *3 Biotech* 6:189. <https://doi.org/10.1007/s13205-016-0504-9>
 33. Sondhi S, Sharma P, Saini S, Puri N, Gupta N (2014) Purification and characterization of an extracellular, thermo-alkali-stable, metal tolerant laccase from *Bacillus tequilensis* SN4. *PLoS One* 9(5):e96951. <https://doi.org/10.1371/journal.pone.0096951>
 34. Liu X, Deng W, Yang Y (2021) Characterization of a novel laccase LAC-Yang1 from white-rot fungus *Pleurotus ostreatus* strain Yang1 with a strong ability to degrade and detoxify chlorophenols. *Molecules* 26(2). <https://doi.org/10.3390/molecules26020473>
 35. Motamedi E, Kavousi K, Sadeghian Motahar SF, Reza Ghaffari M, Sheykh Abdollahzadeh Mamaghani A, Hosseini Salekdeh G, Ariaeenejad S (2021) Efficient removal of various textile dyes from wastewater by novel thermo-halotolerant laccase. *Bioresour Technol* 337:125468. <https://doi.org/10.1016/j.biortech.2021.125468>

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

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.