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Bacterial laccase of *Anoxybacillus ayderensis* SK3-4 from hot springs showing potential for industrial dye decolorization

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Abstract

Introduction: Laccases are green biocatalysts that possess attractive for the treatment of resistant environmental pollutants and dye effluents.

Purpose: To exploit the laccase of *Anoxybacillus ayderensis* SK3-4 that possesses dye decolorization ability at room and higher temperature, we characterized the enzyme in considerable detail and investigated its ability to decolorize different dyes.

Results: A bacterial laccase gene designed as *LacAn* from *Anoxybacillus ayderensis* SK3-4 of hot springs was cloned and expressed in *Escherichia coli*. LacAn is a monomeric protein with a molecular weight of 29.8 kDa. The optimum pH and temperature for syringaldazine oxidation were 7.0 and 75 °C, respectively. LacAn was stable at pH values ranging from 6.5 to 8.5 above 65 °C. The enzyme activity was significantly enhanced by Cu^{2+} and Mg^{2+} but inhibited by Zn^{2+} and Fe^{2+}. Furthermore, LacAn showed high decolorization capability toward five dyes (direct blue 6, acid black 1, direct green 6, direct black 19, and acid blue 93) in the absence of redox mediators. It also demonstrated a wide temperature range, and it can retain its high decolorization ability even at high temperatures.

Conclusions: These properties including better enzymatic properties and efficiency to decolorize dyes demonstrate that the bacterial laccase LacAn has potentials for further industrial applications.

Keywords: *Anoxybacillus ayderensis* SK3-4, Laccase, Purification, Characterization, Decolorization

Introduction

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are a family of multicopper oxidases widely distributed in the bacteria, fungal, insect, plant kingdoms, and particularly in basidiomycetes polyphenols. They catalyze the one-electron oxidation of a wide range of aromatic compounds including phenols and amines by coupling to the reduction of oxygen to water (Hoegger et al., 2006; Baldrian, 2006). Laccases have been considered as green and environmental-friendly biological catalysts and have been the focus of an increasing contemporary investigation due to their diverse potential biotechnological applications, such as dye decolorization, biopulping, biobleaching, xenobiotic degradation, food processing, biopolymer modification, ethanol production, biosensor development, drug synthesis, and organic synthesis (Telke et al., 2011; Kudanga and Le Rose-Hill, 2014; Mate and Alcalde, 2016).

In the early stage of laccase application, the biological roles of fungal and plant laccases have been thoroughly studied and shown to be related to the degradation and synthesis of lignin (Leonowicz et al., 2001). However, recent studies have discovered that laccases are widespread in bacteria, such as *Bacillus tequilensis* (Sonica et al., 2014), *Caldalkalibacillus thermarum* (Sunil et al., 2018), *Streptomyces griseorubens* (Feng et al., 2015), and *Agaricus bisporus* (Othman et al., 2018), and their application is common in the industrial field. Industrial processes

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usually include harsh conditions, such as high temperature, acidic or alkaline pH, and high salt and detergents; thus, some laccases that are resistant to these harsh conditions are preferable (Hilden et al., 2009; Gunne and Urlacher, 2012). Of the reported bacterial laccases, several possess distinctive properties, including excellent activity and stability under alkaline conditions. For example, the *Tth* laccase from *Thermus thermophilus* exhibits extreme stability against heat with a half-life of more than 14 h at 80 °C (Miyazaki, 2005). The Ssl1 laccase from *Streptomyces sviceus* is highly alkali stable and resistant to detergents and organic solvents (Gunne and Urlacher, 2012). In addition, the SN4 laccase from *Bacillus tequilensis* is thermo-alkali stable and metal tolerant (Sonica et al., 2014). Bioinformatics analysis has demonstrated the high diversity of laccase or laccase-like enzymes in bacteria (Ausec et al., 2011), but bacterial laccase-like enzymes have yet to be exploited as promising laccase resources.

In this study, a putative laccase-like gene (designated *lacAn*) from *Anoxybacillus ayderensis* SK3-4 was cloned and heterologously expressed in *Escherichia coli* BL21 (DE 3). *Anoxybacillus ayderensis* SK3-4 was isolated from the Sungai Klah (SK) and Dusun Tua (DT) hot springs in Malaysia (Kahar et al., 2013). To exploit the laccase of *Anoxybacillus ayderensis* SK3-4 that possesses dye decolorization ability at room and higher temperature, we characterized the enzyme in considerable detail and investigated its ability to decolorize different dyes. LacAn possessed great enzymatic properties in terms of biochemical characteristics. The potential of the enzyme in the decolorization of industrial dyes in the absence of mediators was also evaluated.

Materials and methods

Chemicals

2,6-Dimethoxyphenol and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS), syringaldazine (SGZ), and 4-fluoro-2-methylphenol were from Sigma. Restriction enzymes and DNA ligase were from New England Biolabs (MA, USA). Agarose gel DNA extraction kit was from Qiagen, Hilden, Germany. Taq DNA polymerase and isopropyl-β-D-thiogalactoside (IPTG) were purchased from TaKaRa (Dalian, China). Direct blue 6 (DB6), acid black 1 (AB1), direct green 6 (DG6), direct black 19 (DB19), and acid blue 93 (AB93) were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Microorganisms and maintenance

E. coli BL21(DE3) was purchased from TransGen (Beijing, China) and was used as the host strain for plasmid propagation and protein expression. The *E. coli* strain was routinely grown in Luria-Bertani medium.

Cloning, expression, purification, and verification of identity

A series of potential laccase sequences were obtained on NCBI through bioinformatics technology (the access no. EPZ38526.1 from GeneBank). The target laccase-like gene (designated *lacAn*) was selected from *Anoxybacillus* sp. and optimized according to *E. coli* preference. Then the sequence was sent to General Biosystems (Anhui, China) for synthesis (Fig. S1). Finally, the synthesized sequence is inserted into the vector pUC18.

The open reading frame (a 804-bp fragment) of LacAn was amplified using the primer pair of GATA TA*CATATG*AACGATATATTTCGCCAAG (*Nde* I site italicized) and GTGGTG*CTCGAG*CCTCCAGC CAATAAGCGC (*Xho* I site italicized) based on the optimized gene sequence (pUC18-*LacAn*). Construction of plasmid pET22b-LacAn was performed according to the methods of Fang et al. (Fang et al., 2011). *E. coli* BL21(DE3) cells carrying pET22b-LacAn were grown at 37 °C in 200 mL of LB medium containing 100 μg/mL ampicillin. The cultivation temperature was reduced to 16 °C, and IPTG at a final concentration of 0.2 mM was added into the culture to induce enzyme expression when the OD_{600} of culture medium reached 0.6. After an additional incubation for 16 h, the cells were collected by centrifugation. The pellets were resuspended in cold 20 mM Tris-HCl buffer (pH 7.9) containing 500 mM NaCl and 5 mM imidazole, disrupted by sonication, and then centrifuged at 30,000×*g* for 30 min. The supernatant was applied to Ni-NTA (Novagen, Darmstadt, Germany) affinity chromatography to purify the recombinant LacAn. The purified protein was stored at 4 °C.

The molecular mass of the denatured protein was estimated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Proteins were stained with Coomassie brilliant blue R-250. Activity staining of LacAn was performed in 50 mM Na_2HPO_4-KH_2PO_4 buffer (pH 7.0) containing different substrates and 10 μM $CuSO_4$. Protein concentration was determined using the BAC method (Bio-Rad, USA).

Sequence analysis of *lacAn*

The ORF of *lacAn* was determined using the ORF Finder provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Similar sequence searching was performed using BlastP at NCBI. The module structure of the enzyme was analyzed with simple modular architecture research tool SMART (http://smart.embl-heidelberg.de/). Multiple sequence alignment of *lacAn* with other related laccase sequences was performed using Clustal X 2.0.

Enzyme assay

The assay mixture consisted of 10 μL of appropriately diluted LacAn stock and 990 μL of 50 mM Na_2HPO_4-

KH_2PO_4 buffer (pH 7.0) containing 100 μM SGZ (65, 000 M^{-1} cm^{-1}) and 10 μM $CuSO_4$. The reaction was initiated by adding SGZ and enzyme into the solution. After incubation at 45 °C for 5 min, the mixture was transferred into ice-water bath for 30 s to stop the reaction and the absorbance was measured at 525 nm using a UV-visible spectrophotometer (Shimadzu uv-vis 1700, Japan). Alternative substrates for the measurement of laccase activity were 2 mM 2,6-DMP. Reactions with heat-treated LacAn were used as control. One activity unit (U) was defined as the amount of LacAn for oxidizing 1 μmol of substrate per minute. Protein concentration of LacAn was determined at 595 nm using the Modified Bradford Protein Assay Kit (Sangon, China) as bovine serum albumin as the standard.

Characterization of LacAn

The effect of pH on laccase activity was examined in the pH range of 4.5–9.0. Sodium citrate buffer (pH 4.5–5.5), Na_2HPO_4-KH_2PO_4 buffer (pH 5.5 to 8.0), and Tris-HCl buffer (pH 8.0–9.0) were used at a final concentration of 50 mM. The enzyme stability against pH was determined by measuring the residual activities of LacAn after incubation at 4 °C for 1 h in the aforementioned buffers. The effect of temperature on the activity was measured by incubating LacAn at pH 7.5 and a temperature range of 40–80 °C. Thermostability was determined by incubating LacAn at various temperatures (15–55 °C) at pH 7.5 for 15 min. The values and standard deviations are calculated based on three independent experiments.

The effects of 0.1 mM Mg^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Fe^{2+}, EDTA 5% dimethyl sulfoxide (DMSO), 0.5 mM SDS, and 5% ethanol on LacAn activity were investigated by incubating LacAn with each effector for 15 min at 4 °C prior to substrate SGZ addition. The laccase assays were carried out under the aforementioned conditions. Control was carried out under the conditions with Na_2SO_4, KCl, or without NaCl in the normal manner.

Decolorization of textile azo dyes

The LacAn was used to oxidize 5 synthetic dyes of different classes which were shown in Table 1. The chemical structures of these dyes were shown in an additional figure (Fig. S2). Initial solutions (10 mM) of these dyes were prepared in DMSO and diluted to the required concentration and then used for the reaction. Initially, the reaction was mixture 1 mL contained 50 mM citrate-phosphate buffer (pH 7.5), crude enzyme (200 U/L), and dye solution (100 μM).

To evaluate the effect of the conditions on dye decolorization, the pH (5.5–8.5), temperature (55–80 °C), enzyme amount (50–250 U/L), and time (20–140 min) were optimized in turn. The reactions were incubated for 2 h, and the boiled LacAn as the control. The decolorization of these dyes by LacAn was monitored by the decrease in absorbance at the wavelength of each dye. The decolorization ratio was calculated according to the following equation:

Decolorization ratio (%) = $(A - A0)/A0 \times 100\%$

A0 and A represented the initial and final absorbance of the dye, respectively.

Results and discussion

Analysis of the LacAn sequence

The putative ORF of *lacAn* consists of 804 nucleotides that encode a protein containing 267 amino acid residues. Putative conserved domains were detected according to the Pfam database (Pfam PF02578), demonstrating that LacAn belongs to the Cu-oxidase_4 superfamily (Beloqui et al., 2006). The relationship between *lacAn* and multiple selected laccase genes is shown in Fig. 1. Among the 12 identified copper sites in RL5, all sites were conserved in *lacAn*, which proved that LacAn is a bacterial laccase (Beloqui et al., 2006).

Purification of LacAn

After preliminary optimization of expression and purification, a protein with laccase activity and molecular mass of about 29.8 kDa (Fig. 2), named LacAn, was obtained. Under the corresponding optimum condition, the specificity of LacAn was 3.2 U mg^{-1} with substrate SGZ. At present, the molecular mass of most fungi and bacterial laccases range from 50 to 100 kDa (Brijwani et al., 2010; Sharma et al., 2007). However, the molecular mass of LacAn is only 29.8 kDa. Similar conditions were also found in the laccase protein SN4LAC of *B. tequilensis* (Sonica et al., 2014). Sondhi et al. suggested that this difference in molecular mass makes SN4LAC an interesting protein for studying the structure-function relationship of laccases (Sonica et al., 2014).

Table 1 Characteristics of dyes used in this study

Dye	Abbreviation	Classification	Wave length (nm)	Molecular weight
Direct green 6	DG6	Azo	514	812.70
Direct black 19	DB19	Azo	514	839.77
Direct blue 6	DB6	Azo	520	932.76
Acid black 1	AB1	Azo	520	616.49
Acid blue 93	AB93	Anthraquinone	600	799.79

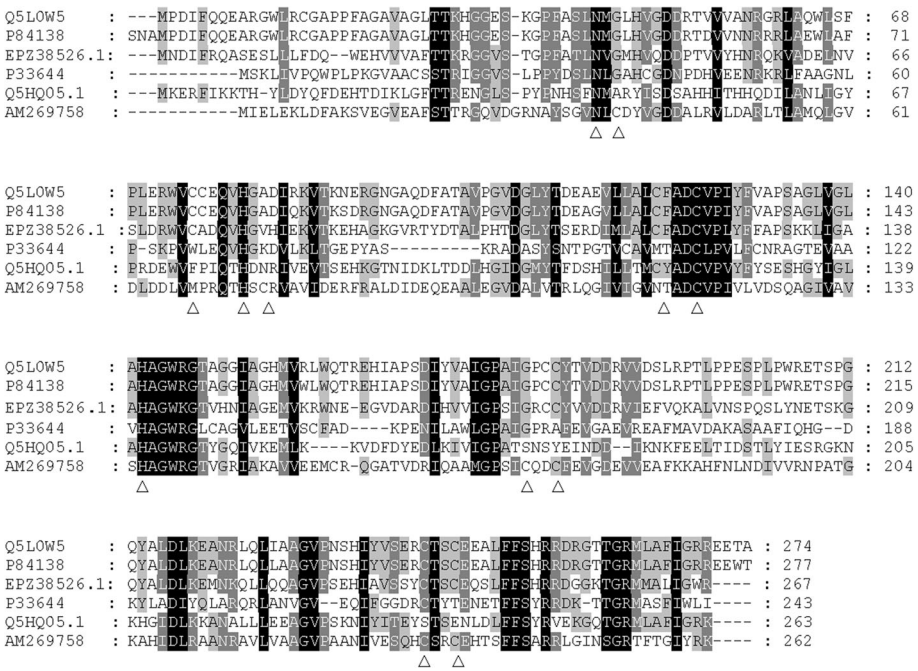

Fig. 1 Multiple sequence alignment of LacAn with selected LacAn-related proteins. Amino acid sequences were retrieved from NCBI or the UniProt database. LacAn, a copper oxidase in this study (GeneBank accession no. EPZ38526.1); yfiH, a laccase from *E. coli* (UniProt accession no. P33644); 1t8h (UniProt accession no. P84138), Q5HQ05.1, Q5L0W5 were uncharacterized proteins from *Bacillus stearothermophilus*, *Staphylococcus epidermidis RP62A*, and *Geobacillus kaustophilus*; RL5, a laccase from the bovine rumen metagenome (NCBI accession no. AM269758). The amino acid residues binding to the three copper sites in RL5 are indicated with **Δ**

Characterization of LacAn

The optimum temperature of the purified LacAn was 75 °C (Fig. 3a). LacAn displayed more than 60% of the maximal activity with 180 min from 25 to 45 °C. In addition, the enzyme was stable at relatively high temperatures, with a half-life of 155 min at 65 °C when syringaldazine was used as a substrate (Fig. 3b). Obviously, LacAn, similar to many discovered bacterial laccases, showed great thermostability even at temperatures above 60 °C (Table S1). In addition, environmental factors also affected its thermostability. *Anoxybacillus ayderensis* SK3-4 originated from hot springs, which have a high temperature environment, explaining the stable thermostability of LacAn.

Conversely, LacAn displayed the maximum activity at pH 7.0 (Fig. 3c). It was stable at pH values ranging from 6.5 to 8.5 at 75 °C and was the most stable at pH 7.5, retaining more than 80% of the original activity after incubation at 75 °C for 180 min while catalyzing syringaldazine (Fig. 3d). In general, bacterial laccases are functional in an alkaline environment and are active at pH 7.0–8.5, while fungal laccases are partially active in an acidic environment (Claus, 2003; Brander et al., 2014). LacAn is an extracellular thermo-alkali-stable laccase. The alkaline activity of LacAn is similar to those of other bacterial laccases in previous studies (Table S1). Thus, it may be further applied in industrial and biotechnological field. Additional, K_m and V_{max} of LacAn for the substrate SGZ were 14.2 μM and 10.6 μmol min^{-1} mg^{-1}, respectively.

Metal ions bind to laccases and alter their stability. Cu^{2+} was important for LacAn activity because no activity was detected for the purified as isolated protein (Fig. 4a). The stimulation of laccase activity by Cu^{2+} observed in the study occurred probably due to the filling of type I or II copper binding sites with copper ions, highlighting the importance of Cu^{2+} ion in laccase function (Nagai et al., 2002; Kaushik and Thakur, 2013; Sonica et al., 2014). Zn^{2+} and Fe^{2+} decreased LacAn activity to 90% compared with that without metal ions. Inhibition of LacAn in the presence of Zn^{2+} was in accordance with the results from previously characterized fungal laccases (Murugesan et al., 2006; Sunil et al., 2018). The inhibition effect of Fe^{2+} may be due to its interaction with the electron transport system of laccase. The blockage of the access of the substrate or the transfer of electron at the T1 site results in inhibition in laccase activity (Murugesan et al., 2009). LacAn was almost stable in the presence of other metal ions. For example, Mg^{2+} increased the activity of LacAn to 133%, and Co^{2+} and Ca^{2+} increased the enzyme activity to 122% and 106%,

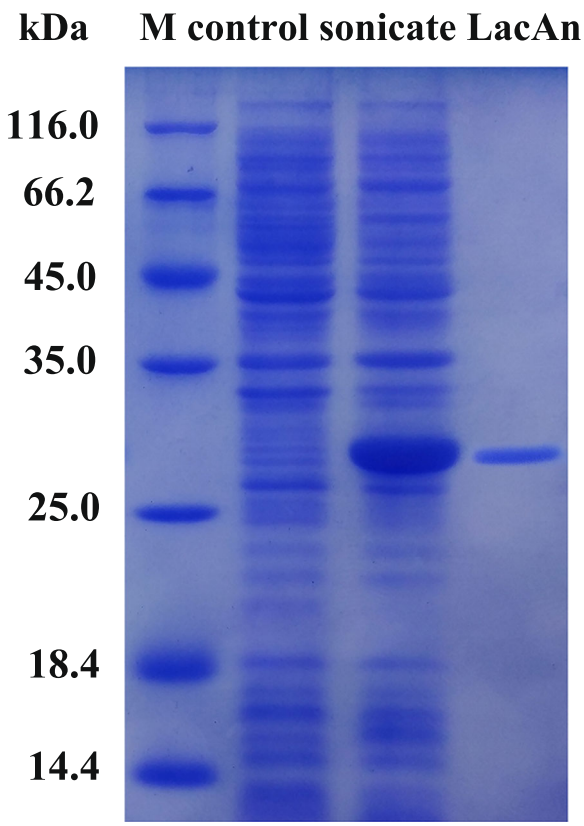

Fig. 2 SDS-PAGE of overexpressed and Ni^{2+}-NTA purified LacAn. Lane M, standard molecular weight marker; lane control, BL21(DE3) without pET22b-lacAn; lane sonicate, sonication product precipitate of BL21(DE3)-pET22b-lacAn; lane LacAn, purified LacAn

respectively, similar to the results reported for *B. tequilensis*, may be due to their interaction with electron transport system of laccase (Sonica et al., 2014, Murugesan et al., 2006). The stability of LacAn in the presence of some metal ions makes LacAn suitable for applications where metal ions are present in high concentrations, such as in the pulp and paper industry and in wastewater containing heavy metals (Shraddha et al., 2011).

In addition, potential inhibitors exerted various effects on LacAn activity. Ionic surfactants reportedly inhibit laccase activity (Robles et al., 2002; Zhang et al., 2013). Under the above reaction conditions, 0.5 mM SDS significantly inhibited the enzyme activity, and only 13% of the enzyme activity was retained. This result may be due to binding of the ionic surfactant [below Critical Micelle Concentration (CMC)] to the enzyme which may cause the alterations in its enzymatic and physical characteristics (Robles et al., 2002; Sonica et al., 2014; Zhang et al., 2013). The effect of 10 mM EDTA was slightly weaker than SDS with 22% of the activity was retained, due to the deprivation of the Cu^{2+} ions present at type 1 copper center and inhibit the enzyme activity (Kaushik and Thakur, 2013). When DMSO and ethanol organic solvents were added, about 50% of the enzyme activity was retained (Fig. 5). However, as a comparison, ionic surfactants reportedly stimulate the activity of laccase from *Azospirillum lipoferum* and *B. tequilensis* (Diamantidis et al., 2000; Sonica et al., 2014).

Decolorization of azo dyes by LacAn

The ability of LacAn to oxidize dyes was tested by single-factor optimization. When the five dyes were oxidized, the decolorization rates remained high when the pH was at 8.5 (Fig. 5a). The highest decolorization rates were achieved at pH 7.5, which was then chosen as the optimum pH and suitable for industrial applications. The temperatures were set at the range of 55–80 °C. Results showed that LacAn had good catalytic properties at high temperature and remained active within a wide temperature range. A high decolorization rate was achieved even at 80 °C (Fig. 5b). In addition, all five dyes were efficiently decolorized and the highest decolorization rates were observed even when 200 U/L LacAn was added (Fig. 5c). The decolorization rates of the dyes increased when the reaction time was extended. Finally, the highest decolorization rates of the five dyes showed that direct blue 6 and acid black 1 reached 33.15% ± 2.69% and 31.08% ± 1.31%, respectively, at 100 min of reaction. At 60 min of reaction, direct green 6 and direct black 19 reached 99.64% ± 1.13% and 51.34% ± 1.66%, respectively. Acid blue 93 reached 34.45% ± 1.52% at 80 min (Fig. 5d, Table 2).

Laccase can oxidize a variety of substrates for industrial applications, especially for the oxidation of dyes in industrial wastewater treatment, which has important environmental significance (Verma and Madamwar, 2003; Rai et al., 2005). LacAn is a bacterial laccase with high catalytic activity in alkaline pH. In this study, LacAn was able to oxidize five dyes under neutral to alkaline conditions and still had higher decolorization rates even at pH 8.5 (Fig. 5a). This result indicates that the bacterial laccase has better decolorization characteristics than fungal laccases under neutral alkaline conditions.

Most of the reported decolorization reaction temperatures of bacterial laccase are between 30 and 45 °C. With the increase in temperature, the activity of laccase decreased and the decolorization ability decreased, which limited the application of laccase decolorization (Kalmea et al., 2009; Molina-Guijarro et al., 2009; Pereira et al., 2009; Liu et al., 2011; Lu et al., 2012). LacAn can still decolorize dyes effectively at 80 °C, especially direct green 6 with a high decolorization rate of 99% (Fig. 5b). It enables LacAn to oxidize dyes at low and high temperatures and also shortens the reaction time at high temperatures, revealing its advantage of high temperature decolorization. LacAn has the

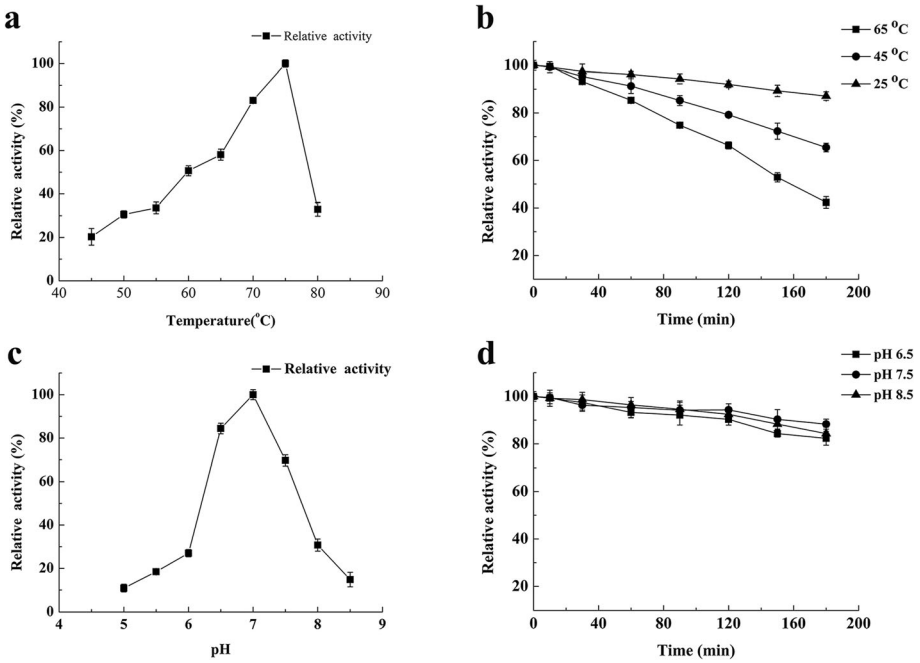

Fig. 3 Effects of pH and temperature on the activity and stability of LacAn. **a** Optimum temperature of LacAn for enzyme activity was measured in 50 mM Na_2HPO_4-KH_2PO_4 at pH 7.0 with syringaldazine as a substrate. **b** Thermostability was investigated at 25 °C (triangle), 45 °C (circle), and 65 °C (square) by determining the enzyme activity under the same conditions as above. **c** Optimum pH of LacAn for enzyme activity was measured in 50 mM Na_2HPO_4-KH_2PO_4 at 75 °C with syringaldazine as a substrate. **d** pH stability was investigated at pH 8.5 (triangle), pH 7.5 (circle), and pH 6.5 (square) by determining the enzyme activity under the same conditions as above. Error bars represent standard deviations

advantage of high temperature application even in laccase derived from thermophilic bacteria. Compared with a laccase named TthLAC from *Thermus thermophilus*, when the azo dyes were oxidized by the TthLAC, the efficient decolorization was at temperature of 60–65 °C. It made the LacAn more widely used (Kumari et al., 2018).

The spatial structure of the dye macromolecule hinders its binding with the active center of the enzyme. Most laccases have low redox potential. Mediators such as ABTS and syringaldehyde must be added to oxidize dye substrates to complete the catalytic reaction and increase the decolorization efficiency (Guan et al., 2012; Yang et al., 2018). For example, the laccase LaclK from *Kurthia huakuii* was used to oxidize azo dyes (Guo et al., 2016). Only when ABTS was added as a mediator, LaclK showed obvious decolorization ability (Guo et al., 2016). A laccase derived from *Bacillus subtilis* cjp3 was used for dye oxidative decolorization, and results showed

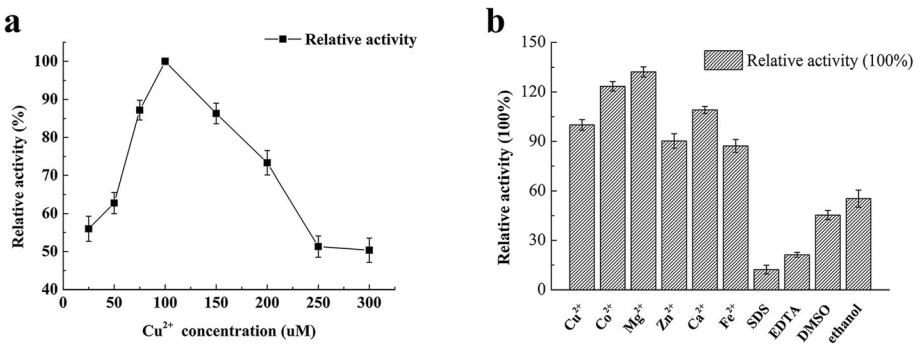

Fig. 4 Effects of Cu^{2+}, metal ions, inhibitors, and organic solvent on the enzyme activity of LacAn. **a** Optimum Cu^{2+} concentration of LacAn for enzyme activity was measured in 50 mM Na_2HPO_4-KH_2PO_4 at 75 °C and pH 7.0 with syringaldazine as a substrate. **b** Effects of metal ions, inhibitors, and organic solvent on the enzyme activity of LacAn. The enzyme activity was measured in 50 mM Na_2HPO_4-KH_2PO_4 at 75 °C and pH 7.0 with syringaldazine as a substrate, supplemented with 0.1 mM $CuSO_4$. Error bars represent standard deviations

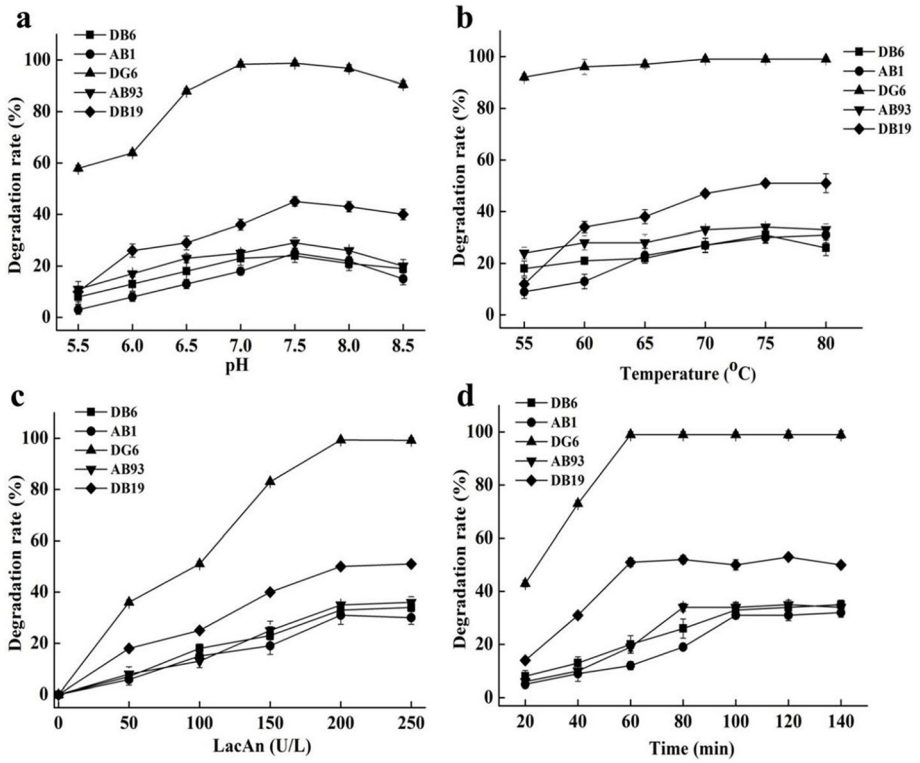

Fig. 5 Effect of pH (**a**), temperature (**b**), concentration of LacAn (**c**), and time (**d**) on the decolorization rate of LacAn oxidation dyes. The values and standard deviations were calculated based on three independent experiments

that reactive blue 19, reactive black 5, and indigo carmine can be effectively decolored by purified laccase with ABTS as a mediator. More than 86% of the dyes tested were removed at 4 h (Qiao et al., 2017). For the above laccases, without a mediator, there was no decolorization ability. But LacAn had an effective decolorization ability when the mediator was absent. Although the mediator could improve the decolorization efficiency, it would cause more pollution and increase the cost of decolorization. Due to the lack of mediators in decolorization, LacAn will have more important application value.

The LacAn used in this study still had a good decolorization effect on the selected dyes within a short reaction time even without any mediators (Fig. 5, Table 2).

Table 2 The conditions of dyes oxidized with LacAn

Dyes	pH	Temperature, °C	Laccase, U/L	Time, min	Decolorization rate, %
DG6	7.5	70	200	60	99.64 ± 1.13
DB19	7.5	70	200	60	51.34 ± 1.66
DB6	7.5	70	200	100	33.15 ± 2.69
AB1	7.5	70	200	100	31.08 ± 1.31
AB93	7.5	70	200	80	34.45 ± 1.52

This result may be due to the better catalytic activity and stability of LacAn. Thus, LacAn has a wide application prospect in industrial wastewater treatment.

Conclusion

A bacterial laccase (LacAn) from *Anoxybacillus ayderensis* SK3-4 was cloned and successfully expressed in *E. coli*. The purified enzyme is smaller than other known laccases. In terms of biochemical characteristics, the optimum pH and temperature for SGZ oxidation were 7.0 and 75 °C, respectively. LacAn was stable at pH values ranging from 6.5 to 8.5 above 65 °C. Moreover, LacAn showed desirable stability at high temperatures and alkaline pH, and dye decolorization ability. These properties render LacAn a prospect for further industrial applications.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13213-020-01593-6.

Additional file 1. Figure S1. DNA sequence of LacAn after codon optimizaion

Additional file 2. Figure S2. The chemical structures of the 5 synthetic dyes

Additional file 3. Table S1. Characteristics of LacAn compared with reported prokaryotic laccases

Research involving human participants and/or animals
N/A

Informed consent
N/A

Authors' contributions
The author(s) read and approved the final manuscript.

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Competing interests
The authors declare that they have no conflict of interest.

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