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ORIGINAL ARTICLE

Cloning and characterization of CotA laccase from *Bacillus subtilis* WD23 decoloring dyes

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Abstract CotA protein is a component of the endospore coat of *Bacillus subtilis* and it exhibits the activities of laccase. CotA protein is known as CotA laccase. A CotA laccase gene from *B. subtilis* WD23 was cloned and expressed in *Escherichia coli*. The expressed CotA laccase was observed in an active form. The molecular weight of CotA laccase was estimated to be 67.5 kDa. Optimal laccase activity was detected at pH 7.2 and 45 °C with syringaldazine as substrate. The half-life of the laccase was 1.5 h at 80 °C at the optimum pH. Half of the laccase activity was lost after 8 h at 45 °C and pH 9.0. The CotA laccase exhibited high tolerance to acetone, petroleum ether, ethyl acetate and chloroform, like spore laccase. Purified CotA laccase was activated 157 % by Cu^{2+} and remained stable to Fe^{2+}. The purified CotA laccase could decolorize 87 % of Remazol Brilliant Blue R (RBBR) and 81 % of Congo Red in 6 h in absence of any mediator.

Keywords *Bacillus subtilis* · CotA Laccase · Dye decolorization · Expression · Mediator

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Introduction

Laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) are blue multicopper oxidases that normally contain four copper atoms. These enzymes catalyze the oxidation of a broad range of organic and inorganic substrates, including diphenols, polyphenols, diamines, aromatic amines, and synthetic dyes, coupled with the reduction of molecular oxygen to water (Giardina et al. 2010; Majeau et al. 2010). The broad substrate range makes laccases candidates for many industrial and biotechnological applications (Rodríguez Couto and Toca Herrera 2006), such as bioremediation (Mayer and Staples 2002), decolorization of synthetic dyes (Baldrian 2006), and biosensors (Vianello et al. 2006). Laccases widely distribute among fungi, plants, and bacteria. Only fungal laccases are currently used in industrial processes (Rodríguez Couto and Toca Herrera 2006). However, fungal laccases are usually unstable at high temperatures and alkaline conditions, limiting their practical applications in wastewater treatment (Sharma et al. 2007). Although bacterial laccases are less frequently reported, they usually possess high stability under drastic conditions.

The bacterial laccases have high potential in commercial applications (Dubé et al. 2008). Most bacterial laccases are CotA laccases. CotA protein is an abundant component of the outer coat layer of *Bacillus subtilis*. CotA is a highly thermostable laccase, and its assembly into the coat is required for spore resistance to hydrogen peroxide and UV light (Enguita et al. 2003). The *cotA* gene of *Bacillus* sp. HR03 (Mohammadian et al. 2010), *Bacillus pumilus* W3 (Guan et al. 2014), and *Bacillus licheniformis* DSM 13 (Koschorreck et al. 2008), respectively, was expressed. Heterologously expressed laccases possess thermostability and alkaline activity, and can provide plentiful amounts of water soluble enzyme. The most common host for heterologous

protein expression is *Escherichia coli*, which is characterized for its fast growth rate, easy genetic manipulation, and lack of post-translational modification, such as glycosylation (Lu et al. 2013). In this study, we cloned the CotA laccase gene of *B. subtilis* WD23 to investigate its expression in *E. coli*. The expressed enzyme was purified, characterized and tested for its ability in dye decolorization.

Materials and methods

Strains and vectors

Bacillus subtilis WD23 was maintained on Luria–Bertani (LB) slants at 4 °C and conserved in our laboratory. *E. coli* DH5α competent cells (BioDev, China) were used for subcloning procedures. *E. coli* BL21(DE3) competent cells (Tiangen, China) were used for expression of CotA laccase. The pMD18-T vector (TaKaRa, China) was used for *cotA* gene cloning. The expression vector pET-22b(+) was conserved in our laboratory.

Cloning of the *cotA* gene

Genomic DNA from *B. subtilis* WD23 was isolated by Tris-phenol (pH 8.0)/chloroform/isoamyl alcohol (25:24:1, by vol.) extraction and subsequent absolute alcohol precipitation. Amplification of the *cotA* gene was performed by polymerase chain reaction (PCR) with the primers 5′-CGG*GGATCC*GACACTTGAAAAAT-3′ (forward) and 5′-GCG*AAGCTT*TTATTTATGGGGATC AGT-3′ (reverse). Recognition sites for *Bam*HI and *Hind*III endonucleases are indicated in italics. The pair of primers was designed by Primer Premier 5.0 software according to the sequences of *B. subtilis cotA* genes on GenBank.

PCR was carried out in a 20-μl reaction mixture containing 1 μl of genomic DNA as the template, 10 pmol each of primer, 4 nmol of each of the dNTPs, as well as 1 U of Taq polymerase and 2 μl 10×PCR buffer (15 mM $MgCl_2$ plus) (BioDev, China). The PCR was run in a Gene Amp PCR system 9700 (Applied Biosystems, Singapore). After the initial denaturation at 93 °C for 5 min, 30 cycles consisting of denaturation at 94 °C for 18 s, annealing at 45.7 °C for 1 min, and extension at 72 °C for 78 s and then a final extension at 72 °C for 7 min were carried out to amplify the *cotA* gene.

Amplified products purified using the DNA Gel Extraction Kit (TaKaRa) were cloned using a commercially available pMD18-T vector cloning kit and transformed into *E. coli* DH5α competent cells. The positive transformants were screened on 5-bromo-4-chloro-3-indoly-β-D-galactopyranoside (X-Gal)-isopropyl-β-D-thiogalactopyranoside (IPTG)-ampicillin-indicator plates by color-based selection. Positive colonies were identified by colony-PCR technique. The DNA extracts were sequenced by Shanghai Sangon Biotechnology Company.

Expression vector construction

The expression vector pET-22b(+) carrying the strong bacteriophage T7 promoter and terminator and the recombinant pMD18-T vector containing the *cotA* gene were digested with *Bam*H I and *Hind* III, and the resulting products were purified as described above. The purified *cotA* gene was ligated with the digested pET-22b(+) fragment using T4 DNA ligase overnight at 16 °C. The ligation mixture was transformed into *E. coli* DH5α competent cells. The positive transformants were screened and identified as described above. The recombinant pET-22b/*cotA* vector was confirmed by restriction enzyme digestion, agarose gel electrophoresis and sequence analysis. Recombinant pET-22b/*cotA* vector was transformed into *E. coli* BL21(DE3) competent cells.

Optimization of CotA synthesis in *E. coli* BL21(DE3)

Escherichia coli BL21(DE3) cells carrying pET-22b/*cotA* vector were grown in 5 ml LB medium supplemented with 50 μg ml^{-1} ampicillin at 37 °C in a shaking incubator overnight. A 250-ml shake flask containing 50 ml LB medium with ampicillin was inoculated with 0.5 ml of the prepared culture and incubated at 37 °C in a shaking incubator. Single factor experiments were performed with other conditions invariant. At a culture broth OD_{600} of 0.6, 0.8, 1.0, 1.2, or 1.4, CotA protein expression was induced by supplemented with isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.5 mM, 1.0 mM, 1.5 mM, 2.0 mM, or 2.5 mM. The cells were incubated at different temperatures of 20 °C, 25 °C, 30 °C, 35 °C, or 40 °C in a shaking incubator (200 rpm) for 3 h, 6 h, 9 h, 12 h, or 15 h. The cells were harvested by centrifugation at 3600× *g* for 20 min and resuspended in 20 mM buffer, pH 7.5, containing 200 g l^{-1} sucrose. After an ice-bath for 30 min, the cells were collected by centrifugation at 12,000× *g* for 10 min and resuspended in 20 mM Tris–HCl buffer, pH 7.5. After an ice-bath for 60 min, the cell debris was removed by centrifugation at 12,000× *g* for 20 min and the supernatant containing the expressed CotA protein was collected. The uninduced strain was the control. All experiments were performed in triplicate.

Assay of CotA laccase activity

Laccase activity was determined at 40 °C using syringaldazine (dissolved in absolute alcohol, Oxiod, England) as the substrate. The oxidation of syringaldazine was detected by measuring the absorbance increase at 525 nm (ε_{525}= 65000 M^{-1} cm^{-1}) after 3 min using a spectrophotometer (U-2800, Hitachi, Japan). The reaction mixture (3 ml)

contained 100 μl of enzyme sample, 2.4 ml of citrate–phosphate buffer (0.1 M, pH 5.0), and 0.5 ml of 0.5 mM syringaldazine. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of substrate per minute. All assays were carried out in triplicate.

Purification of expressed CotA protein

Crude extract of liquid cultures was precipitated by the salting out method of 80 % saturated $(NH_4)_2SO_4$. The precipitate was dissolved by 20 mM Tris–HCl (pH 6.8) and then dialysed to the same buffer for 24 h. The sample was concentrated with polyethylene glycol 6000 (PEG 6000) and was applied to a DEAE-Sepharose Fast Flow column equilibrated with 20 mM Tris–HCl (pH 6.8). The column was washed with the same buffer, and absorbed proteins were eluted by a linear concentration gradient of NaCl (0–0.6 M) at a flow rate of 1 ml min^{-1}. The fractions containing laccase activity were pooled and concentrated. The concentrated enzyme was applied to a Sephadex G-75 column equilibrated with 20 mM Tris–HCl (pH6.8). Proteins were eluted with the same buffer at a flow rate of 1 ml min^{-1}. The fractions possessing laccase activity were pooled, concentrated by PEG 6000 as described above, and stored at −20 °C for further use.

SDS-PAGE was carried out with 5 % (w/v) stacking gel and 15 % (w/v) resolving gel using a mini vertical electrophoresis system (Liuyi Instrument Factory, Beijing, China). The molecular mass of the expressed CotA laccase was determined by calculating the relative mobility of standard protein markers (TaKaRa, Dalian, China). All experiments were performed in triplicate.

Characterization of CotA laccase

The effect of pH on the activity of CotA laccase towards syringaldazine was determined within a pH range of 5.6–8.0 in 0.1 M citrate-phosphate buffer. Optimum temperature of the purified laccase was examined over the temperature range of 0–100 °C, with syringaldazine as the substrate at its optimal pH value. Enzyme thermostability was measured at 60 °C and 80 °C by incubating the enzyme solution in 0.1 M citrate-phosphate buffer, pH 7.2. At appropriate time, samples were withdrawn, and residual activity was determined using syringaldazine as the substrate. 10 ml of petroleum ether, xylene, chloroform, acetone, ethyl acetate, formaldehyde and methanol were added to 10 ml of spore suspension in Erlenmeyer flasks, respectively, and mixed for 30 min. The laccase activities were determined after water bath at 50 ° C for 10 min. The effects of potential inhibitors on the laccase activities were also determined as previously described (Wang et al. 2011). All experiments were performed in triplicate.

Dye decolorization

The purified laccase was estimated for its ability to decolorize four synthetic dyes, Remazol Brilliant Blue R (RBBR) (λmax=590 nm), Congo Red (λmax=561 nm), Indigo Carmine (λmax=610 nm) and Crystal Violet (λmax=582 nm). Stock solutions of the dyes were prepared to the required concentration. Dye decolorization was determined by the decrease in absorbance under the maximum wavelength of each dye and expressed in terms of percentage. All measurements were taken in triplicate.

Results

Cloning of the *cotA* gene and heterologous expression

A product of about 1.5 kb was amplified from *B. subtilis* WD23 genomic DNA with the primers of *cotA* (Fig. 1). The sequenced *cotA* gene was 1542 bp in length and was submitted to NCBI databases under the accession number GQ184294. There was 99.9 % sequence similarity of *cotA* between *B. subtilis* WD23 and *B. subtilis* (AB007638) according to the GenBank database. The *cotA* gene was subcloned into the pET22b(+) expression vector by T4 DNA ligase, and the pET22b/*cotA* expression vector was transformed to competent *E. coli* BL21(DE3) cells. The expression of CotA laccase was induced with IPTG at 30 °C. Laccase activity of CotA protein was found in the supernatant of the cell lysis solution, not in the supernatant of the culture broth, indicating that CotA laccase was intracellularly expressed in *E. coli* BL21(DE3) cells in an active form. Laccase activity wasn`t detected in the supernatant of th cell lysis solution of the control culture.

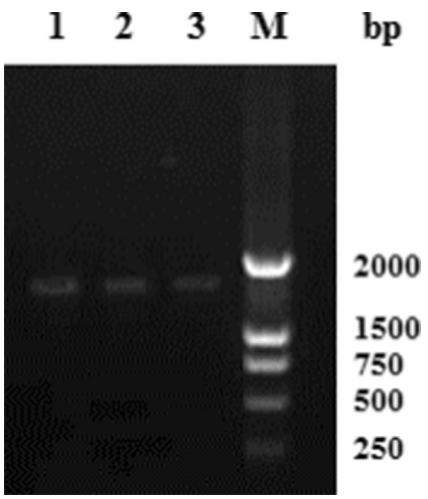

Fig. 1 PCR result of the *cotA* gene from *E. coli* WD23. M: Molecular Marker, Lane 1–3: PCR result of *cotA* gene

Expression optimization of CotA protein in *E. coli* BL21(DE3)

The transformant strain *E. coli* BL21(DE3)/pET22b/cotA was cultured in LB medium to optimize the expression of CotA laccase under various cultivation conditions. The effect of inducing temperature on laccase expression was analyzed between 20 and 40 °C for 12 h, adding 1.0 mM IPTG when the OD_{600} of the culture broth was 1.2. Maximum activity was observed at 25 °C. For temperatures higher than 25 °C, the synthesis speed of CotA protein may have been too fast and the CotA protein couldn't be folded to the right conformation due to limited molecular chaperones in the host cells. Therefore, most protein lost natural activity and aggregated as inclusion bodies (Fig. 2a). IPTG concentrations varying between 0.5 and 2.5 mM were added to induce the expression of CotA protein at 30 °C for 12 h when OD_{600} of the culture broth was 1.2. The highest level of laccase activity was observed in the presence of 1.0 mM IPTG. Laccase activity then decreased with increasing of IPTG concentration. Although IPTG could induce CotA protein expression, IPTG possessed toxicity to the bacterial cells. Too much IPTG might inhibit cellular growth or damage host cells, so CotA protein expression was less when the concentration of IPTG was more than 1.5 mM (Fig. 2b).

The influence of induction time on CotA protein expression was determined from 3 to 15 h at 30 °C, supplemented with 1.0 mM IPTG when OD_{600} of the culture broth was 1.2. The laccase activity was enhanced by prolonging induction time. The highest activity was found when the cells were induced by IPTG for 15 h (Fig. 2c). The effect of OD_{600} of the culture broth on CotA protein expression when IPTG was supplemented was detected during induction by 1.0 mM IPTG at 30 °C for 12 h. The laccase activity was the highest when OD_{600} was 1.0 and the activity decreased with the increase of OD_{600} (Fig. 2d).

Purification of recombinant CotA protein

We purified 9.64 mg of CotA laccase from supernatant after removing cell debris; the final purification fold was 5.26 and the CotA laccase activity was 14,322.54 U mg^{-1} after DEAE-Sepharose Fast Flow and Sephadex G-75. The purified CotA protein was checked on SDS-PAGE, and the enzyme showed a single band of approximately of 67.5 kDa (Fig. 3). The purified CotA protein was incubated for 5 min at 100 °C and then centrifuged before electrophoresis. This method may be attributed to the single band of CotA protein being heat tolerant. The other thermolabile protein was denatured at 100 °C and precipitated by centrifugation.

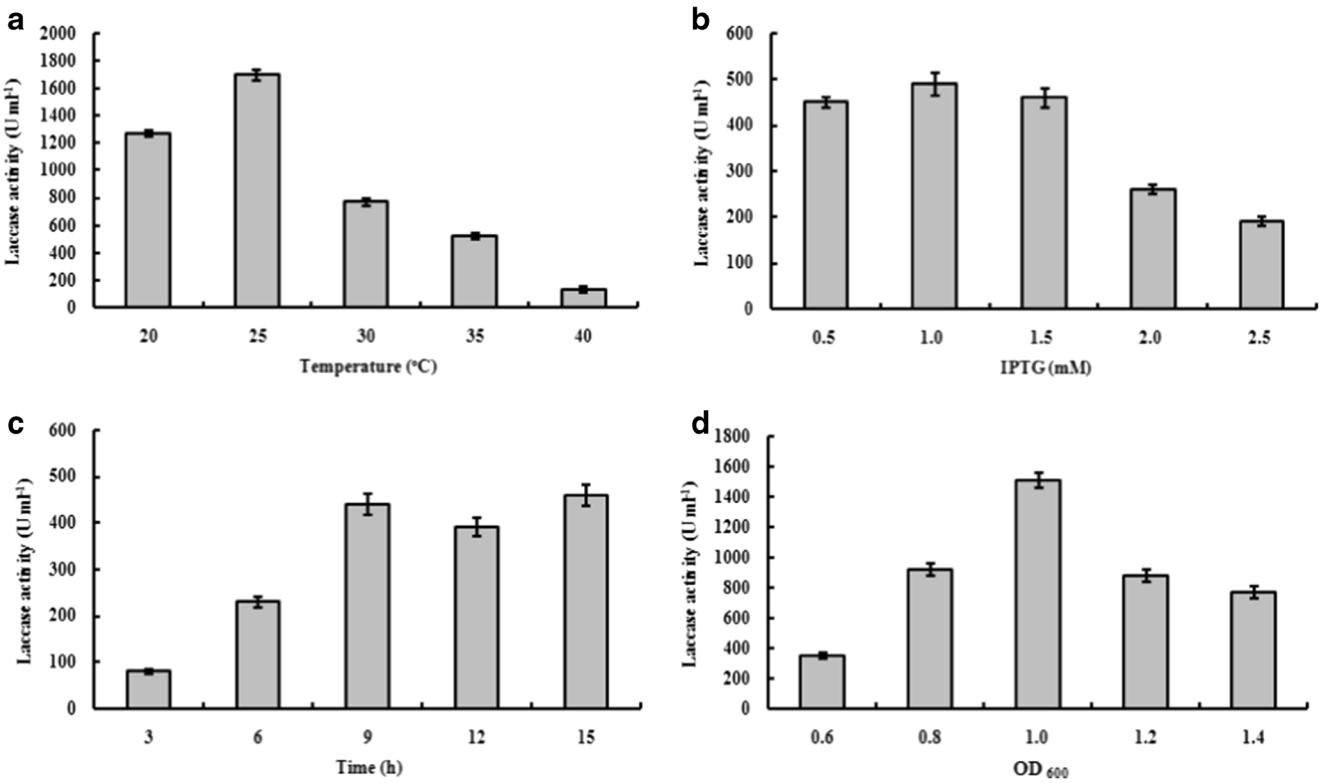

Fig. 2 Effects of temperature (**a**), IPTG concentration (**b**), time (**c**), and OD_{600} (**d**) on the production of laccase in LB medium supplemented with 50 μg ml^{-1} ampicillin. Cultivation conditions: **a** 1.0 mM IPTG, 12 h, 1.2 of OD_{600}; **b** 30 °C, 12 h, 1.2 of OD_{600}; **c** 30 °C, 1.0 mM IPTG, 1.2 of OD_{600}; **d** 1.0 mM IPTG, 30 °C, 12 h

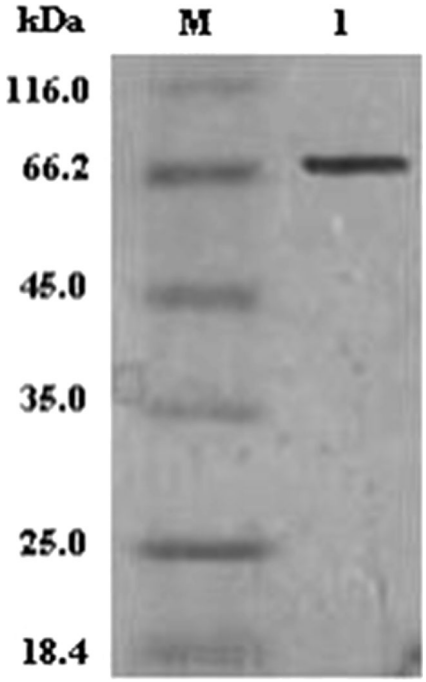

Fig. 3 The SDS-PAGE result of the purified CotA laccase from recombinant *E. coli* BL21(DE3). The purified CotA laccase (Lane 1) was incubated for 5 min at 100 °C and then centrifuged before electrophoresis

Characterization of CotA laccase

The pH profile for laccase activity of purified CotA laccase against syringaldazine showed a peak of maximum activity at pH 7.2. The optimal temperature for the purified CotA laccase was determined to be 45 °C. The half-life of the laccase was 1.5 h at 80 °C at the optimum pH.Half of the activity was lost during preincubation of the enzyme for 8 h at the optimum temperature at pH 9.0. The CotA laccase showed high thermal and alkali stability. The optimal temperature of the purified CotA laccase was 15 °C lower than that of the spore laccase from *B. subtilis* WD23. The temperature and pH stability of the expressed laccase was lower than that of spore laccase. Other spore coat components might protect the spore laccase from harm by heat.

The activities of the purified CotA laccase and spore laccase from *B. subtilis* WD23 were strongly inhibited by 1 mM EDTA and dithiothreitol (DTT) (Table 1). The metal ion chelator EDTA could combine Cu^{2+}, while copper ion was necessary for laccase activity. The CotA laccase exhibited high tolerance to acetone, petroleum ether, ethyl acetate and chloroform, like the spore laccase. However, methanol and xylene strongly inhibited the activity of purified CotA laccase. Methanol and formaldehyde strongly inhibited the activity of spore laccase (Table 2). The effects of several metal ions on laccase activity were tested with syringaldazine as the substrate (Table 3). The activity of purified CotA laccase was strongly activated by Cu^{2+}, Ca^{2+} , was moderately activated by Mn^{2+} and Mg^{2+}, and was strongly inhibited by Zn^{2+}.

Table 1 Effects of inhibitors on the activity of CotA laccase and spore laccase

Inhibitor	Concentration (mM)	Residual activity (%)	
		CotA laccase	Spore laccase[a]
None	-	100	100
Dithiothreitol	0.1	90.32±2.30	94.84±3.52
	1	16.41±0.23	0
NaN3	0.1	96.77±3.27	77.52±1.23
	1	60±1.63	23.57±0.83
L-Cysteine	0.1	87.1±3.85	60.39±2.45
	1	52.5±1.42	22.62±0.41
EDTA	0.1	80.65±1.56	34.72±1.03
	1	3.75±0.14	2.72±0.07

[a] From Wang et al. (2011)

Dye decolorization

The purified CotA laccase was able to efficiently decolorize different synthetic dyes at pH 5.0. All the tested dyes could be decolorized by the expressed CotA laccase without the addition of any mediators. As shown in Fig. 4, about 80 % of RBBR and Congo Red were decolorized after 1 h, then the decolorization percentages were improved to 87 % and 81 % after 6 h of treatment. Thirty-six percent of Crystal Violet was decolorized after 1 h and the decolorization percentage was improved to 57 % at 6 h. In contrast, the indigo dye Indigo Carmine was more resistant to decolorization by the expressed CotA laccase. Less than 50 % decolorization for Indigo Carmine was observed after 6 h.

Discussion

The optimal pH (7.2) of the purified CotA laccase was 0.4 more than that of the spore laccase (Wang et al. 2011). This

Table 2 Effects of organic solvents on the activity of CotA laccase and spore laccase

Organic solvent (50 %, v/v)	Residual activity (%)	
	CotA laccase	Spore laccase[a]
None	100	100
Acetone	86.36±2.24	84.93±2.31
Petroleum ether	81.82±3.52	96.55±4.20
Ethyl acetate	81.82±3.12	81.98±1.27
Chloroform	70.45±1.25	83.05±2.34
Formaldehyde	56.82±1.29	15.83±0.18
Xylene	14.47±0.16	94.28±2.44
Methanol	9.09±0.21	2.53±0.08

[a] from Wang et al. (2011)

Table 3 Effects of metal ions on the activity of CotA laccase and spore laccase

Metal ion (5 mM)	Residual activity (%)	
	CotA laccase	Spore laccase[a]
None	100	100
Cu^{2+}	157.5±2.35	150.3±3.24
Ca^{2+}	132.5±3.14	107.5±3.87
Mn^{2+}	117.5±2.56	113.2±2.59
Mg^{2+}	107.5±1.69	167.4±4.29
Fe^{2+}	97.5±1.38	160.9±3.56
Na^{+}	97.5±2.36	98.5±2.79
K^{+}	92.5±1.02	97.8±2.56
Zn^{2+}	32±0.85	13.6±0.16

[a] from Wang et al. (2011)

shows that heterologously expressed laccase was more suitable to play a role in alkaline condition. However, the activities of these two forms of laccase from *B. subtilis* WD23 were higher than that of fungal laccase in the pH range of 6.0–9.0. The optimum pH range of many fungal laccases was 2.0–5.0, such as *Hericium coralloides* (pH 2.2) (Zou et al. 2012), *Clitocybe maxima* (pH 3.0) (Zhang et al. 2010), *Fomes fomentarius* (pH 4.0) (Neifar et al. 2010) and *Paraconiothyrium variabile* (pH 4.8) (Forootanfar et al. 2011). The optimal temperature for the activity of purified laccase was at 45 °C, which was 15 °C less than that of the spore laccase (Wang et al. 2011). This indicates that the heterologously expressed laccase had less thermostability for losing the protection of spore protein. However, some fungal laccases had an optimal temperature of 55 °C, such as

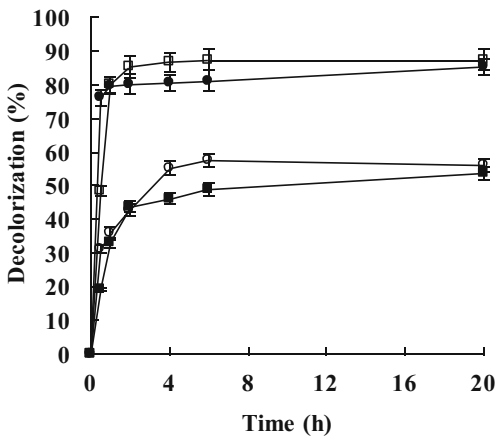

Fig. 4 Decolorization of synthetic dyes by the purified CotA laccase. (■) Indigo Carmine; (□) RBBR; (●) Congo Red; (○) Crystal Violet. The reaction mixture contained 0.1 M citrate–phosphate buffer (pH 5.0), an appropriate amount of dye (final concentration 25 mg l^{-1}), and enzyme solution (final concentration 325 U ml^{-1}). The reactions were initiated with enzyme and incubated at 40 °C under mild shaking conditions. Control tests were done in parallel under identical conditions without enzyme. Dye decolorization was determined by decrease in absorbance at the maximum wavelength of each dye, and was expressed in terms of percentage

Panus tigrinus CBS 577.79 (Quaratino et al. 2007), which was 10 °C higher than that of the purified laccase.

The activities of the purified CotA laccase and spore laccase were strongly inhibited by 1 mM EDTA. Complete inactivation was observed for laccase from *Thermus thermophilus* (Miyazaki 2005) and *Aspergillus ochraceus* (Telke et al. 2010) in the presence of 1.0 mM EDTA. The purified laccase of *P. variabile* was strongly inhibited by 10 mM of EDTA (Forootanfar et al. 2011). Most fungal laccases appeared to be inhibited by this metal chelator (Baldrian 2006). However, 25 mM EDTA showed only a mild inhibitory effect on the laccase from *Pycnoporus sanguineus* (Lu et al. 2007). The inhibitory effect of dithiothreitol on CotA laccase and spore laccase was similar to the laccase from *P. sanguineus* (Lu et al. 2009), *B. licheniformis* LS04 (Lu et al. 2013) and *P. variabile* (Forootanfar et al. 2011). The cell-wall–associated spore laccase showed more resistance to xylene than the soluble CotA laccase, which might be due to the interaction of laccase with other proteins in the cell wall.

Copper ions were necessary for the correct folding and assembly of laccase at post-transcriptional modification (Hoshida et al. 2005) and served as cofactors in the catalytic core of laccase (Majeau et al. 2010). Laccase activity depended on copper, which was supported in bacterial and fungal laccases (Lu et al. 2009, 2013), and laccase from *F. fomentarius* was activated by Cu^{2+} (Neifar et al. 2010). Special attention should be paid to copper and iron ion concentrations, as they are common in wastewater and may interfere with laccase activity if they exceed a certain level (Baldrian 2006). The spore laccase activity from *B. subtilis* WD23 was strongly activated by Fe^{2+} and Cu^{2+} (Wang et al. 2011). Fe^{2+} increased the laccase activity from *Streptomyces psammoticus* (Niladevi et al. 2008). The purified CotA laccase was activated 157 % by Cu^{2+} and remained stable to Fe^{2+}. However, the laccases from *P. variabile* (Forootanfar et al. 2011) and *Pleurotus ostreatus* (Pozdnyakova et al. 2004) were inhibited by Fe^{2+}. The purified CotA laccase was strongly inhibited by Zn^{2+}. The laccases from *Lentinula edodes* (Nagai et al. 2002) and *P. ostreatus* (Pozdnyakova et al. 2004) appeared to be inhibited by this metal ion.

The CotA laccase exhibited extremely high efficiency in the decolorization of the anthraquinone dye RBBR, which was similar to the recombinant laccase from *P. sanguineus* (Lu et al. 2009). Anthraquinone dye was the laccase substrate, which was similar to previous reports for *Polyporus* sp. S133 (Hadibarata et al. 2012a), *Armillaria* sp. F022 (Hadibarata et al. 2012b), *Trametes* sp. SQ01 (Yang et al. 2009) and *F. fomentarius* (Neifar et al. 2010). Many azo dyes were not substrates of laccases and their decolorization depended on some small redox mediators (Camarero et al. 2005). In this study, the expressed CotA laccase could efficiently decolorize most of Congo Red without any mediators. These results suggested a potential application of this expressed CotA laccase in decolorizing industrial synthetic dyes.

Conclusions

The CotA laccase gene from *B. subtilis* WD23 was cloned and expressed in *E. coli*. The expressed laccase was in an active form. The purified CotA laccase possessed high stability towards alkaline pH, high temperature and some organic solvents. High efficiency in dye decolorization by the laccase was obtained in the absence of any mediator.

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Conflict of interest We declare that we have no conflicts of interest.

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