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Full Length Research Paper

Decolorization of dyes by recombinase CotA from *Escherichia coli* BL21 (DE3) and characterization of the purified enzyme

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Dyes are usually difficult to be decolorized due to their complex chemical structures. In this work, recombinant CotA laccase was purified from *Escherichia coli* to evaluate its application in dye decolorization. Factors influencing laccase expression, such as induction temperature, phosphate buffer (pH), copper concentration and isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration, were investigated. The recombinant laccase was purified to electrophoretic homogeneity, and was estimated to have a molecular mass about 67.5 kDa. The CotA protein was stained red by syringaldazine after Native-PAGE. The purified enzyme showed a similar behaviour to the spores laccase produced by *Bacillus subtilis* WD23. Using syringaldazine as the substrate to determine the CotA laccase activity, the optimum pH and temperature were 7.2 and 45°C, respectively. High laccase activity was maintained in a pH range from 6.0~7.6. The temperature half-life of the CotA laccases was 95 min at 80°C. The pH half-life was 8 h at pH 9.0 he CotA laccase was strongly inhibited by ethylenediaminetetraacetic acid (EDTA) and methyl alcohol. The CotA laccase could efficiently decolorize anthraquinone and azo dyes in 24 h. The decolourization capacity of this recombinant laccase suggested that it could be a useful biocatalyst for the treatment of dye-containing effluents.

Key words: Recombinant CotA laccase, *Escherichia coli*, purification, dye decolorization.

INTRODUCTION

Laccases (EC 1.10.3.2) are polyphenol oxidases that catalyse the oxidation of phenols and aromatic amines, reducing molecular oxygen to water (Baldrian, 2006). Classical laccases are considered to be associated exclusively with plants and fungi (Claus, 2004; Baldrian, 2006). However, laccases are also widespread among bacteria, based on homology searches in protein databases and bacterial genomes (Alexandre and Zhulin, 2000). The first bacterial laccase was reported in 1993 (Givaudan et al., 1993). The *B. subtilis* outer endospore coat protein CotA also shows laccase activity (Hullo et al., 2001). Laccases have biotechnological applications in fields of delignification, plant fiber derivatization, textile dye or stain bleaching, and contaminated water or soil detoxification. Laccases also be implicated in a variety of functions pertaining to detoxification, pigmentation of fruiting bodies, sexual differentiation, lignolysis and so on (Thurston, 1994). Synthetic dyes are extensively used in the textile dyeing, printing, drug and food processing industries (Padamavathy et al., 2003). Unfortunately, 1 to10% of the dyes are lost in use, and thus causes considerable environmental pollution (Forgacs et al., 2004). Synthetic dyes exhibit considerable diversity in structure, and their existence in water may be toxic to human and animals (Robinson et al., 2001). So the colored wastewater must be treated before release into the natural environment. Conventional wastewater treatment is not efficient to remove synthetic dyes from effluents, because

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Abbreviations: EDTA, Ethylenediaminetetraacetic acid; **IPTG,** isopropyl -d-1-thiogalactopyranoside; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis; **VE,** vertical electrophpresis; **pH,** phosphate buffer; **DTT,** dithiothreitol; **RBBR,** remazol brilliant blue R.

most of the conventional methods are low efficiency, high cost and intensive energy requirements (Eichlerová et al., 2005). Currently, the application of laccase for the removal of synthetic dyes from industrial effluents offers many advantages (Wesenberg et al., 2003).

The successful applications of laccases in this field require the production of high amounts of laccases at low cost. However, most classical laccases have an acidic pH optimum, and few have a near neutral pH optimum, for example, Lcc1 of *Coprinus cinereus* (Yaver et al., 1999) and the laccase of *Melanocarpus albomyces* (Kiiskinen et al., 2002). Therefore, bacterial laccases were more suitable to dye decolorization. In previous studies, higher decolorization rates were reported for the laccases from a number of fungi. The process of dye decolorization based on laccase was an efficient method and attracted increasing interest (Couto and Herrera, 2006). However, the potential of bacterial laccases for this purpose has rarely been addressed and no decolorization activity was observed at pH values higher than pH 7 (Kandelbauer et al., 2004). A possible solution to these problems is the production of recombinant bacterial laccase in a heterologous host. In a previous work, we have cloned the *cotA* gene of *B. subtili* WD23. By upstream and downstream primers was designed according to *B. subtilis cotA* gene on GenBank. The *cotA* gene was transformed to *E.coli* BL21 (DE3) competent cell successfully, obtaining recombinant engineering strains. In this paper, we described the heterologous production of *B. subtilis* CotA laccase in *E.coli*, the recombinant CotA laccase was purified and characterized. The CotA laccase, which has a much higher thermostability and pH-stability than fungal laccases, may have advantageous properties compared to classical laccases in industrial applications. De-colourization of several chemically different synthetic dyes was investigated using the purified recombinant CotA enzyme to assess its applicability in the treatment of dye-containing wastewater.

MATERIALS AND METHODS

Microbial strains and chemicals

E. coli BL21 (DE3) containing expression vector pET22b-*cotA* was maintained on LB agar slants at 4℃. The GenBank accession number for pET22b-*cotA* was GQ184294. Syringaldazine and IPTG were purchased from Sigma (St. Louis, MO, USA). Sephadex G-75 column (1.6×50 cm) was purchased from Amersham Phamarcia (Uppsala, Sweden). DEAE-Sepharose Fast Flow column (1.6×50 cm) was purchased from Dingguo (Beijing, China). The standard protein marker was purchased from TaKaRa (Dalian, China). All other chemicals were from Guangfu (Tianjin, China) and were analytical grade.

Optimization of recombinant laccase production in *E. coli*

The control and engineering strains were grown in a 250 ml Erlenmeyer flask containing 120 ml LB medium supplemented with 0.2 mmol/L $CuSO_4$ (220 rev/min). At an OD_{600} of 0.8, 1 mmol/L IPTG was added for induction at 30℃ (200 rev/min). Incubation was continued for further 24 h. After breaking the cell wall, the extraction of the fermentation broth was assayed for CotA laccase activity. The ability of engineering strains producing the recombinant laccase was selected for investigating the effects of induction temperature, pH, copper concentration and IPTG concentration. All experiments were performed in triplicate.

The crude extract of CotA laccase

The fermentation broth was harvested by centrifugation at 4000 g for 10 min under sterile conditions at 4℃. The cell sediment was washed and suspended in Tris-HCl (20 mmol/L, pH 7.5) containing 20% sucrose (*w/v*), and were then in ice bath for 30 min, centrifuged at 13000 g for 20 min. The cell pellets were washed and resuspended in osmotic shock buffer 2 and centrifuged at 13000 g for 20 min, obtaining supernatant. So the supernatant was the crude extract of CotA laccase.

Laccase assay

Assay of the CotA laccase activity was determined at 40℃ using 4-hydroxy-3,5- dimethoxybenzaldehyde azine (syringaldazine) as the substrate. The oxidation of syringaldazine was detected by measuring the change of OD_{525} after 3 min using spectrophotometer (U-2800, Hitachi, Japan). The reaction mixture (3 ml) contained 100 µl of CotA laccase, 2.4 ml of citrate-phosphate buffer (0.1 mol/L, pH 5.0), and 0.5 ml of 0.5 mmol/L syringaldazine. One unit of enzyme activity was defined as the value of OD_{525} required to change 0.001 per minute. All assays were carried out in triplicate for each sample. The standard deviation did not exceed 5% of the average values.

Purification of CotA laccasse

The extraction of the fermentation broth (200 ml) was centrifuged at 3000 g for 5 min at 4℃. The purification of CotA laccase was carried out using ultrafiltration. The supernatant was precipitated by ammonium sulfate (80% saturation) for 12 h, and the precipitate was redissolved using Tris-HCl (pH 6.8) buffer. The homogenate was dialyzed against distilled water for 24 h with periodic transfer into distilled water. The dialysis bag with the solution was embedded polyethylene glycol 20,000 for concentration until remaining 10 ml liquid. This liquid was defined as crude laccases. The sample was applied to a DEAE-Sepharose Fast Flow column that was equilibrated with 20 mmol/L (pH 6.8). Absorbed proteins with the same buffer washed were eluted by a linear gradient of NaCl (0~0.6 M, 150 ml) at a flow rate of 1 ml/min. The CotA laccase activity fractions were collected together and concentrated by polyethylene glycol 20,000 with dialysis bag. The concentrated enzyme solution was used for a Sephadex G-75 column equilibrated with 20 mmol/L (pH 6.8). The bound proteins were eluted with the same buffer at a flow rate of 0.8 min/ml. The CotA laccase positive fractions were pooled and stored at -20℃ for further research.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was based on the protocol of (Laemmli, 1970) with 5% stacking gel and 15% resolving gel using a Hoefer mini VE vertical electrophpresis system (Amersham Biosciences, San Francisco, CA, USA). Protein bands were stained with Coomassie brilliant blue R-250 (Fluka, Buchs and Switerland). The molecular weight of the purified enzyme was estimated by the standard protein marker. To prove the enzyme was completely purified and can catalyze the

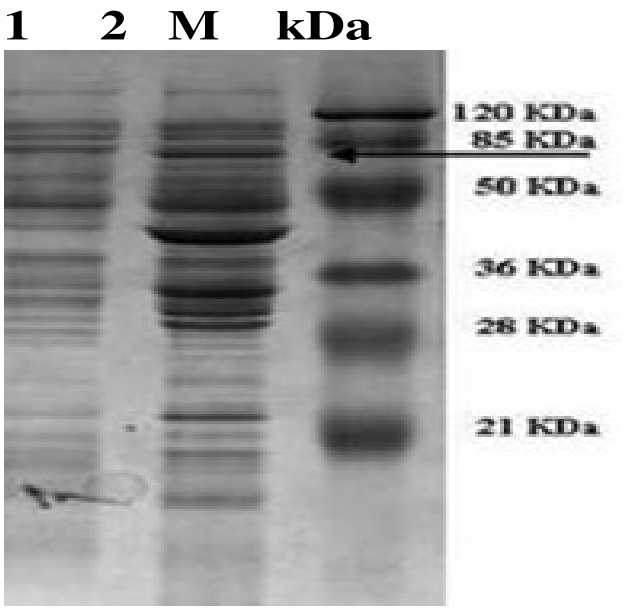

Figure 1. Expression of the recombinant CotA in *E. coli.* M, molecular weight markers Lane 1, supernatant of a crude extract of an uninduced *E. coli* BL21(DE3) culture; Lane 2, supernatant of a crude extract of an IPTG-induced *E. coli* BL21(DE3) culture. The band indicated by the arrow is the CotA protein.

oxidation of syringaldazine. The gel was stained with citrate- (pH 6.8) and 0.5 mmol/L syringaldazine after native PAGE.

Optimum pH and temperature of purified recombinant CotA enzyme

Determination of the optimum pH was conducted in 0.1 mol/L citrate- of pH 5.6~8.0. The optimum temperatures of the CotA laccase was determined over the range of 0~100°C with syringaldazine as the substrate at their optimum pH values.

Thermal and pH stability of purified recombinant CotA enzyme

Thermal stability of the CotA laccase was determined by preincubation 0.1 mol/L citrate- optimum pH of The CotA protein at 45 and 80°C and measured the remaining activity. The pH stability was examined similarly at 45°C in buffers ranging from pH 5.0 to 9.0.

Kinetic property

Substrate specificity of the purified enzyme was measured using syringaldazine. The rate of substrate oxidation was calculated by measuring the absorbance change at each wavelengths and optimum pH. The Michaelis-Menten constant was determined from double-reciprocal plots of the initial oxidase rates and concentrations of substrates (Lineweaver and Burk, 1934).

Effects of organic solvents and inhibitors on purified recombinant CotA enzyme

The effects of organic solvents and potential inhibitors on the laccase were investigated with 0.5 mmol/L syringaldazine as the substrate in 0.1 mol/L citrate–phosphate buffer at optimum pH. Ten milliliters of petroleum ether, xylene, aether, chloroform, acetone, ethyl acetate, formaldehyde and methanol were added to 10 ml CotA laccase in Erlenmeyer flasks, respectively, and mixed for 30 min. The laccase activities were determined after removing the organic solvents by incubation in a hot water bath at 50°C. The effects of L-cysteine, sodium azide, dithiothreitol (DTT), and EDTA on laccase activity were determined after 3 min of incubation of the enzyme with the various inhibitors at 25°C. All experiments were carried out in triplicate.

Determination of dye decolorization efficiency of CotA laccase

The general dyes, Remazol Brilliant Blue R (RBBR), Alizarin Red, Congo red, Isatin and crystal violet were prepared individually with the concentration of 25 mg/L. The prepared dye solution was supplemented with CotA laccase (325 U/ml) and incubated at 40°C under mild shaking conditions for 1 d. Dye samples without laccases were as the control. Control samples were done in parallel without CotA laccase under identical conditions. The absorption spectrum of each dye between 200 and 800 nm was measured with a U-2800 spectrophotometer (Hitachi, Japan). Dye decolorization was assessed by the decrease in absorbance under the maximum wavelength of the dye. All measurements were done in triplicate. The decolorization of RBBR was determined by CotA laccase. The effects of temperature, pH, dye concentration, and enzyme activity on the decolorization of RBBR were examined, respectively. The 4 ml reaction system contained 0.1 mol/L Tris-HCl buffer (pH 3.5-9.5), indigo carmine (final concentration 50 to 200 mg/L), and enzyme solution (final enzyme activity 108 to 325 U/ml). The reaction temperature was 30 to 50°C. Control samples were prepared in parallel with inactivated enzyme under the same conditions, and all cultivations maintained in triplicate.

RESULTS

Expression of the recombinant CotA in *E. coli*

Expression of *cotA* in *E. coli* BL21 (DE3) could be driven upon IPTG induction of the strong T7lac promoter. SDS-PAGE analysis of supernatant of crude extracts from *E.coli* BL21 (DE3) revealed that the addition of IPTG to the culture resulted in the accumulation of an extra band at 67.5 kDa (Figure 1, lane 2). The 67.5-kDa band was absent from extracts of uninduced engineering strains. So adding IPTG can make engineering strains expressed.

Optimization of the recombinant laccase production

The ability of engineering strains producing the recombinant laccase was selected to optimize the heterologous expression of *cotA* gene under various cultivation conditions. The culture medium (LB) was for investigating the effects of temperature, pH, copper concentration and IPTG concentration. All experiments were performed in triplicate. LB medium (pH 7.0) containing 0.2 mmol/L $CuSO_4$ were set up with initial pH values from 6.0 to 10.0 to study the effect of pH on laccase production. A higher laccase activity was observed in cultures with initial pH

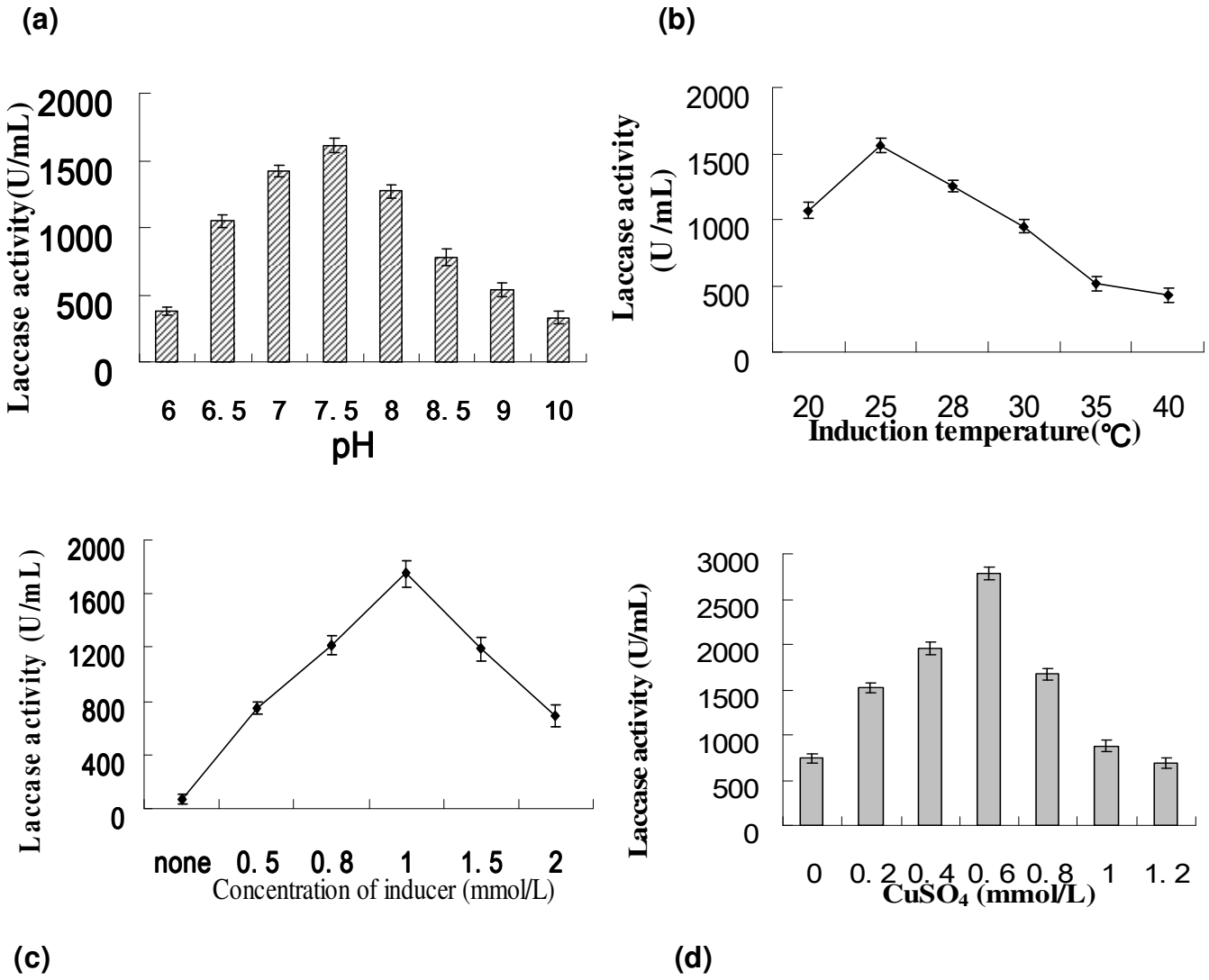

Figure 2. Effects of pH; **a** Induce temperature; **b** IPTG concentration; **c** and copper concentration; **d** on the production of laccase in LB medium supplemented with 0.2 mmol/L $CuSO_4$. Cultivation conditions; **a** 0.2 mmol/L $CuSO_4$, 1 mmol/L IPTG, 30°C; **b** 0.2 mmol/L $CuSO_4$, pH 7, 30°C; **c** 0.2 mmol/L $CuSO_4$, 1 mmol/L IPTG, 30°C; **d** 1 mmol/L IPTG, pH 7, 30°C.

7.5 demonstrated (Figure 2a). The effect of induction temperature on laccase expression was analysed at 20, 25, 28 30, 35 and 40°C, using LB medium (pH 7.0) containing 0.2 mmol/L $CuSO_4$. Increasing the induction temperature did not led to an enhancement of laccase activity beyond 25°C (Figure 2b). The activity in the culture kept at 25°C was 5.5 times higher than that kept at 40°C. Variation of IPTG concentration between 0 and 2.0 mmol/L was used to induce the expression of laccase in LB (pH 7.0) supplemented with 0.2 mmol/L $CuSO_4$. The laccase activity decreased with the increasing of IPTG concentration (Figure 2c). The laccase activity was none in the absence of IPTG. IPTG concentration of 1.0 mmol/L produced the highest level of laccase activity. Under the optimal conditions, laccase activity reached the highest.

The induction of laccase activity in the recombinant *E. coli* cultures was enhanced using different amounts of copper in the LB medium (pH 7.0). The laccase activity was relatively low in the absence of copper. The highest laccase activity was obtained in the presence of 0.6 mmol/L $CuSO_4$ (Figure 2d).

Purification CotA

As described in Table 1, 9.64 mg of CotA laccase was purified from 200 ml culture supernatant, final purification fold was 5.26 and recovery of total enzyme activity was 27.83%. The purified enzyme was a protein band corresponding to a molecular mass of 67.5 kDa on SDS-PAGE gel (Figure 3a). The CotA protein was stained red by syringaldazine after Native-PAGE (Figure 3b).

Table 1. Purification of CotA laccase from *E. coil.*

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	9.64	26,250	2,723.30	1.00	100
80%$(NH_4)_2SO_4$ precipitation	4.76	18,003	3,782.14	1.39	68.58
DEAE-Sepharose Fast Flow	1.04	11,044	10,619.23	3.90	42.07
Sephadex G-75	0.51	7,304	14,322.54.	5.26	27.83

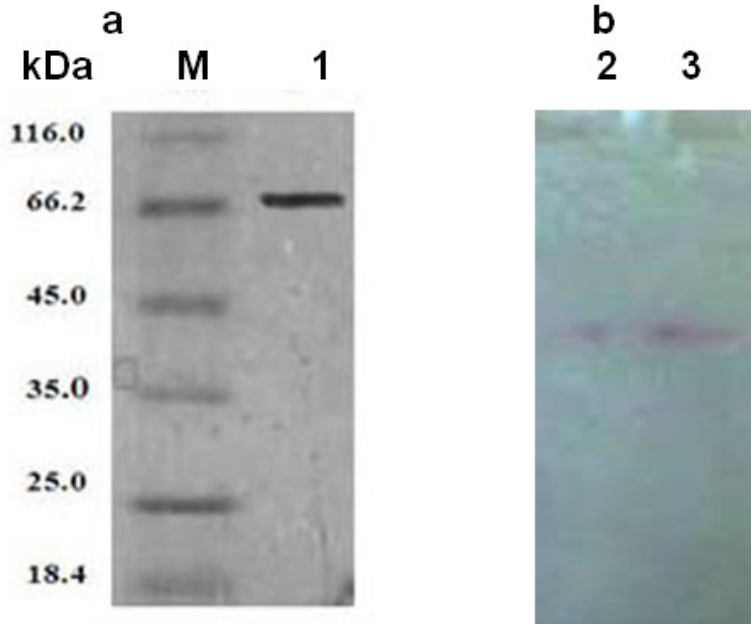

Figure 3. The SDS-PAGE result of the purified CotA protein: SDS-polyacrylamide gel analysis of CotA purified from recombinant *E. coli* BL21 (DE3). Samples of purified CotA were incubated for 5 min at 100°C (lane 1) before electrophoresis. The Native-PAGE result of the purified CotA protein was stained with syringaldazine (lane 2 and 3).

Effect of pH and temperature on purified recombinant CotA enzyme activity and stability

The CotA laccase exhibited high thermal and pH-stability. The temperature half-life of the laccase was 95 min at 80°C. In addition, the laccase has a high stability at the optimum temperature ($t_{1/2}$=76 h at 45°C). The laccase activity showed higher stability over a broad pH range. Within a pH range from 5.0 to 7.0, the half-life was more than 23 h at 45°C. In contrast to fungal laccases, CotA laccase activity showed a high stability at alkaline pH values ($t_{1/2}$=8 h at pH 9.0).

Kinetic properties

The recombinant laccase exhibited *K*m and Vmax values of 1.10 mmol/L and 0.26 mmol/min, respectively (Figure 4), for syringaldazine at pH 7.2 and 45°C.

Effects of organic solvents and inhibitors on purified recombinant CotA enzyme activity

The effects of several organic solvents and putative inhibitors on laccase activity were tested with syringaldazine as the substrate Table 2. Methanol and formaldehyde strongly inhibited the purified CotA laccase activity, and the activity also was inhibited by 0.1 mmol/L EDTA, as well as by 1 mmol/L L-cysteine, sodium azide, and DTT. 0.1 mmol/L DTT, NaN_3 and other organic solvents only showed a slight inhibitory effect.

Dye decolorization experiments

Anthraquinone-based dyes are difficult to decolorization due to their complicated aromatic ring structures (Fu and Viraraghavan, 2001). In the present study, anthraquinone and azo dyes were used for dye decolorization. In order to

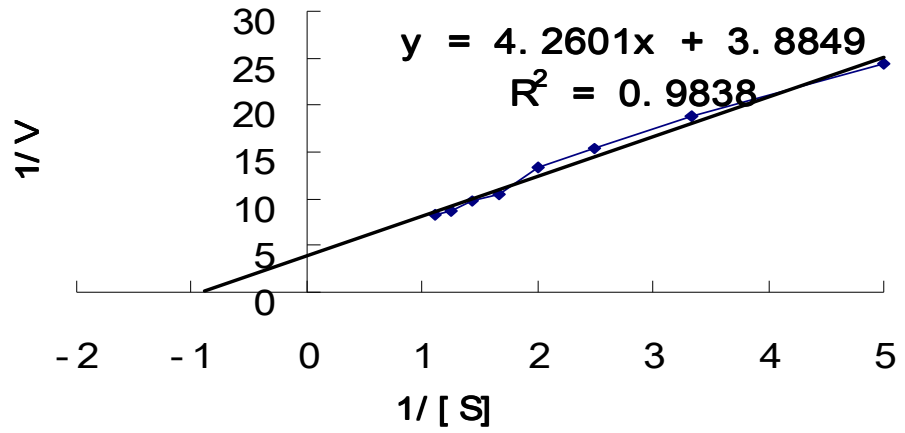

Figure 4. Kinetic constant.

Table 2. Effects of organic solvents and inhibitors on laccase activity.

Organic solvent	Relative activity (%)	Chemical	Conc. (mmol/L)	Residual activity (%)
None	100	None	-	100
Petroleum Ether	86.82	Dithiothreitol	0.1	90.32
Xylene	91.47		1	16.41
Aether	95.25	NaN_3	0.1	96.77
Acetone	86.36		1	30.00
Chloroform	70.45	L-Cysteine	0.1	87.10
Ethyl Acetate	81.82		1	22.62
Formaldehyde	27.25	EDTA	0.1	40.64
Methanol	9.09		1	0.75

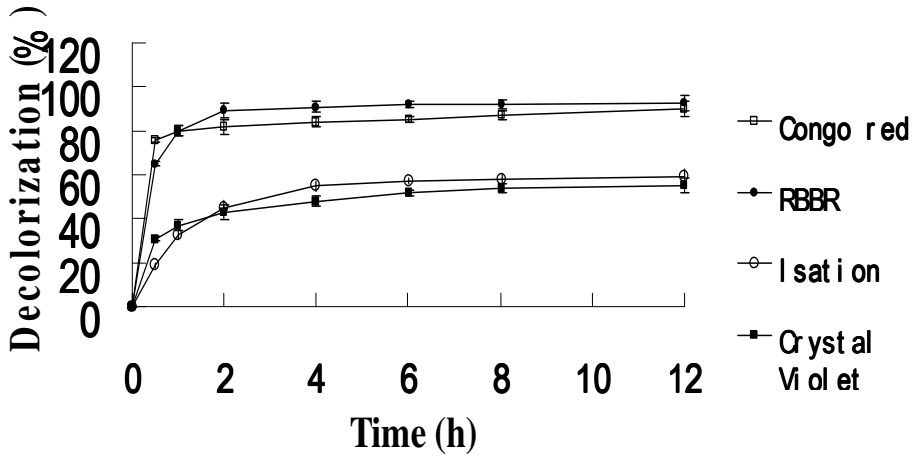

Figure 5. Decolorization of dyes with purified CotA laccase from *E. coli*.

prove the potential application of CotA laccase to the treatment of wastewater containing dyestuff, the CotA laccase was used for the decolorization of RBBR, Alizarin Red, Congo Red, Methyl Orange and Methyl Violet. The decolorization rates were 89% in the treatments of RBBR and Alizarin Red in 8 h, while the final percentage of degradation of other dyes was more than 50% (Figure 5). These results indicated that the CotA laccase could decolorize the most dyes efficiently without additional redox mediators and that the CotA laccase had the potential to be industrial enzyme. Decolorization of RBBR was recorded under 590 nm. The enzyme could efficiently

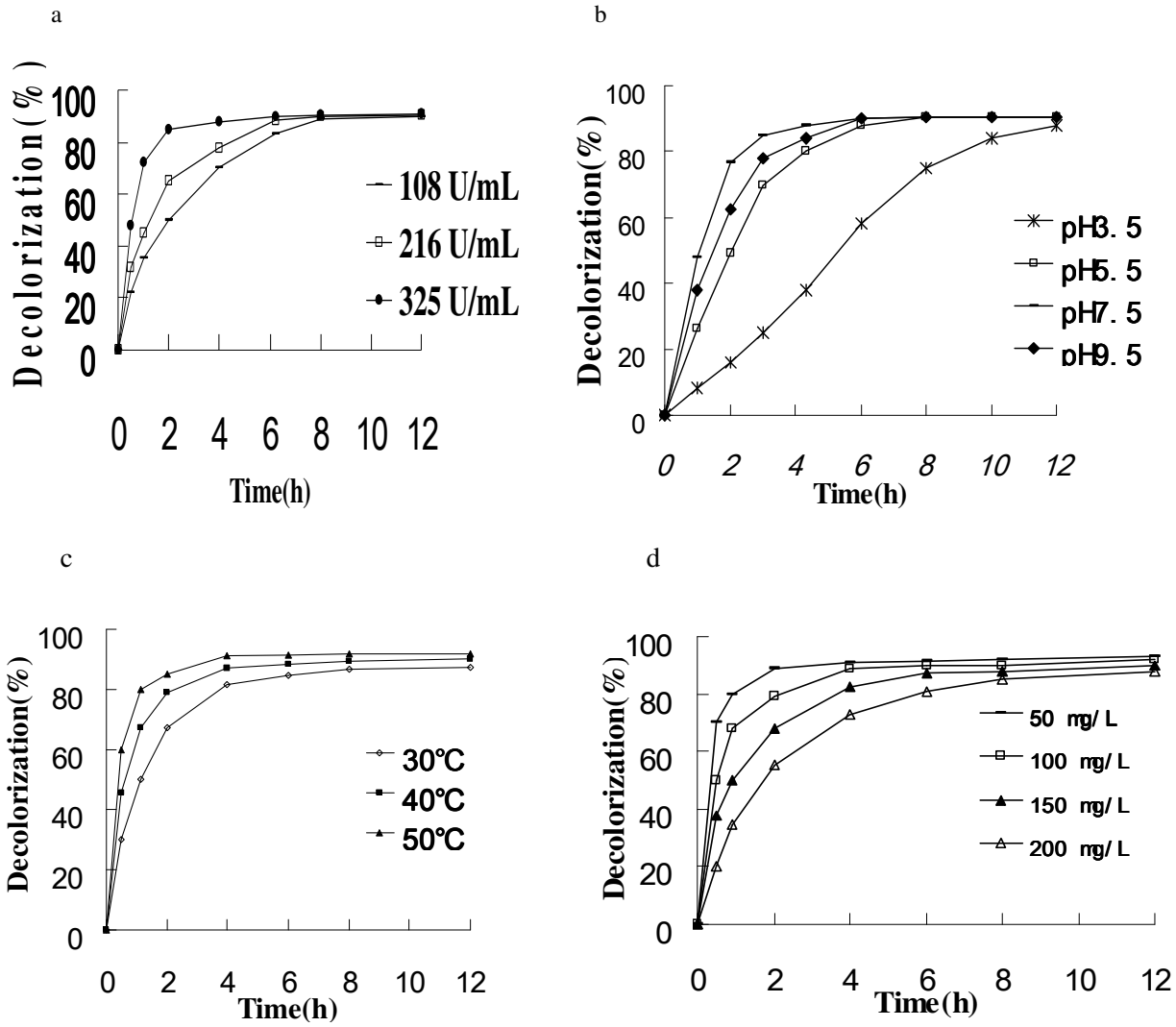

Figure 6. Effects of CotA activity; **a**, pH; **b**, temperature; **c** and dye concentration; **d** on decolorization of RBBR by purified CotA laccase; **a** RBBR (50 mg/L), CotA (108 to 325 U/ml), pH 7.5, 40°C; **b** RBBR (50 mg/L), CotA (325 U/ml), pH (3.5 to 9.5), 40°C; **c** RBBR (50 mg/L), CotA (325 U/ml), pH 7.5 , 30 to 60°C. **d** RBBR (50 to 200 mg/L), CotA (325 U/ml), pH 7.5, 40°C.

decolorize RBBR under different conditions. The decolorization rate increased with the rise of enzyme activity, all the enzyme activity (108 to 325 U/ml) could achieve 91.5% decolorization within 8 h, the reactions were carried out at 40°C with 0.1 mmol/L Tris-HCl buffer (pH 7.5) (Figure 6a). The CotA laccase activity used in the following RBBR decolorization was determined as 325 U/ml. The effects of pH on dye decolorization were shown in Figure 6b, the result revealed the enzyme was capable of decolorizing RBBR over a pH range of 3.5 to 9.5, decolorization rate exceeded 90% after incubation at 40°C for 12 h. At pH 7.5 decolorization process was quicker, while pH 3.5 decolorization process was slightly slow. The effect of temperature on dye decolorization was not quite obvious at pH 7.5 (Figure 6c), 90% decolorization rate was obtained at 50°C within 2 h. Dye decolorization process became slow with the increase of dye concentration (Figure 6d).

DISCUSSION

Compared with fungi laccase, bacterial laccase grew fast, having no glycosylation and higher thermal stability at alkaline conditions (Verma and Madamwar, 2003; Jimenez-Juarez et al., 2005). So bacterial laccase is of important significance in industrial applications such as environmental protection, food industry, paper bio-bleaching, and so on (Mayera and Staples, 2002). For many continuous process applications, it is necessary that the catalysts such as laccases are kept active in the whole process. These characteristics may be of significant

importance for biotechnological applications. The *E. coil* expression system has been used to produce a wide variety of heterologous proteins .The recombinant proteins can be expressed in a high level under the control of promoter, one of the strongest and tightly regulated promoters which can be induced by IPTG. SDS-PAGE displayed that the purified CotA laccase contained a protein band (67.5 kDa). The molecular weight of the purified CotA laccase was consistent with the molecular weight of CotA on spore coat extracts from *B. subtilis* WD23, and similar to other CotA laccase. These authors stated the protein molecular weight was 65 kDa (Donovan et al.,1987; Driks, 2004; Martins et al., 2002).

The molecular mass was deduced from the *cotA* gene sequence as well as the size of the protein previously identified as CotA on spore coat extracts. When *cotA* was expressed in *E.coil*, recombinant CotA laccase appeared a band in SDS-PAGE (Figure 3a). The CotA protein was stained red by syringaldazine after Native-PAGE (Figure 3b). The result suggests that prokaryotic expression of host *Escherichia coli* could express the CotA protein of *B. subtilis* WD23. We found that CotA the purified recombinant CotA enzyme was properly expressed and folded in *E. coli* BL21 (DE3), and the biochemical purified recombinant CotA enzyme (temperature, pH, dye decolorization, metal ions and inhibitors) were similar for the CotA protein of *B. subtilis* WD23. In contrast to fungal laccases, the CotA laccase exhibited a higher thermal stability and pH-stabilitiy. The temperature half-life was 95 min at 80°C.The value was as high as that of other CotA laccases reported at the same temperature and optimum pH values (Held et al., 2005). The pH half-life time was more than 76 h at pH 7.2. Within a pH range from 5.0 to 7.0, the half-life of the CotA laccase was more than 23 h, while the half-life of a laccase from *Trametes hirsute* was 13 h (Abadulla et al., 2000). The CotA laccase activity showed a stability at alkaline pH values ($t_{1/2}$=8h at pH 9.0).

The observation of half-life time indicated that the CotA laccase has a high potential in industrial processes where high temperatures and pH values are common, such as dye decolorization, detoxification and transformation of phenolic and other compounds. The metal ion chelator EDTA was an efficient inhibitor of the CotA laccase because EDTA could deprive copper from the CotA laccase which was copper-dependent (Hullo et al., 2001). Similarly, sodium azide, L-cysteine, and DTT demonstrated strong inhibition towards the laccase activity and those laccases from *Lentinus edodes* (Nagai et al., 2002) and *Daedalea quercina* (Baldrian, 2004). However, the CotA laccase could coexist with many organic solvents. Four synthetic dyes that could be classified as azo, anthraquinone, triphenylmethane and indigo dyes have various structure and extremely stable under exposure to light and washing. These dyes were decolourized by CotA laccase. RBBR is an industrially important anthraquinone dye. Only a few purified laccases could decolorize it without the participation of mediators (Soares et al., 2001). Since RBBR is frequently used in textile industry, its presence in the textile effluents may enhance the range of dyes that could be decolorized by laccase. Thus, the utilization of laccase in the treatment of textile effluents may offer great interest, due to its high activity towards RBBR.

The enzyme exhibited extremely high efficiency in the decolourization of the anthraquinone dye RBBR, which was similar to the spore laccase from *B. subtilis* WD23 (Wang et al., 2010). Azo dyes are the largest chemical class of dyes frequently used in industries (Chang et al., 2010). They are mainly decolourized by bacterial azo reductases to colourless aromatic amines, which may betoxic, mutagenic and carcinogenic to human and animals (Chen, 2006). The oxidation mechanism of laccase may avoid the formation of hazardous aromatic amines during decolourization of azo dyes (Chivukula and Renganathan, 1995). However, many azo dyes are not the substrates of accases and their decolourization depends on the presence of some small redox mediators (Wong and Yu, 1999; Camarero et al., 2005). Indigo carmine was the most recalcitrant to decolourization, indicating it was not the suitable substrate for the CotA laccase. In the presence of mediators, the decolourization of indigo carmine by laccase might be greatly enhanced (Dubé et al., 2008). In the present study, the purified recombinant laccase could also efficiently decolourize most of the azo dye congo red in the absence of any mediators. The decolorization results demonstrate that CotA laccase has potential application in the treatment of wastewater from textile industries.

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