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Enhanced hydrolysis of lignocellulosic biomass with doping of a highly thermostable recombinant laccase

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ABSTRACT

A highly thermostable laccase from *Geobacillus* sp. strain WSUCF1 was cloned into *Escherichia coli* (*E. coli*) using pRham N-His SUMO expression system. The thermostable laccase with a molecular weight ~30 kDa had a $t_{1/2}$ (pH 6.0) of 120 h at 50 °C. The homology modelling for laccase structure showed the presence of Cu active centers with His and Cys residues involved in the active site and ligand binding activity of the enzyme, respectively. The K_m, V_{max}, K_{cat} and K_{cat}/K_m values of the purified enzyme with ABTS were found to be 0.146 mM, 1.52 U/mg, 1037 s^{-1} and 7102.7 s^{-1} mM^{-1}, respectively. The doping of recombinant WSUCF1 laccase to commercial enzyme cocktails Accellerase® 1500 and Cellic CTec2 improved the hydrolysis of untreated, alkali and acid treated corn stover by 1.31–2.28 times and bagasse by 1.32–2.02 times. Further, in-house enzyme cocktails with laccase hydrolyzed untreated, alkali and acid treated bagasse and gave 1.44, 1.1, and 0.92 folds higher sugar, respectively, when compared with Accellerase 1500. The results suggested that thermostable laccase can aid in the improved hydrolysis of lignocellulosic biomass.

1. Introduction

The abundantly and economically available lignocellulosic biomass (LCB) is touted as a substitute for the current starch-based substrates for biofuel and bio-based chemical production [1]. However, the benefits of using LCB have not been reaped fully yet, due to the recalcitrance challenges posed by high lignin content, cellulose crystallinity etc., because of which carbohydrates cannot be accessed, hydrolyzed and fermented efficiently without pretreatment [2]. Several pretreatment methods viz. physical (fragmentation, radiation etc.), chemical (acid, alkali, oxidation etc.), biological (bacterial and enzymatic) methods had been employed to use LCB efficiently, among which the biological methods are considered best due to their lower energy requirements, environmental, and work safety benefits [3].

Laccases are multicopper oxidases that can oxidize a broad spectrum of organic compounds viz. phenols, polyphenols, anilines etc. via a single electron transfer mechanism, and had been used to remove lignin-one of the major contributors to LCB recalcitrance [4,5]. Several have asserted positively that the laccases could play an important role in the degradation of lignocellulosic biomass by (1) acting on the lignin directly and (2) removing the inhibitors produced during pretreatment [6]. The reduction of the inhibitor concentration by laccase to effectively utilize rice straw for bioethanol production by Kumar and coworkers [7]; improved the phenolic tolerance of xylanase enzymes during sugarcane bagasse hydrolysis by laccase [8], and 80% delignification and 587 mg/g sugar with laccase mediated hydrolysis of LCB [9], are some of the recent studies done on the laccase application in LCB hydrolysis.

One of the requirements for biological pretreatment methods is ability of the microorganisms and enzymes to perform in extreme conditions viz. pH, temperature, inhibitors, substrates etc. to make the bioprocess effective and economical. Previously, laccases produced by white-rot fungi such as *Phlebia floridensis* [10], *Trametes versicolor* [11], and *Phanerochaete chrysosporium* [12] dominated the literature studies and industrial applications but, the lower thermostability of the fungal laccases makes them unfavorable for high temperature industrial processes [13]. High temperature offers several advantages such as improved hydrolysis performance due to long half-lives at high temperatures, higher mass transfer rates, low viscosity that may lead to increased solubility of reactants and products; lower risk of contamination from mesophilic microbes, and structural and functional stability at higher temperatures [14]. Hence, bioprospecting had been done for thermostable laccases that can withstand higher temperatures for extended periods of time. Most of the thermostable laccases studied had been of bacterial origin as they offer better thermostability at higher

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temperatures than their fungal counterparts. Laccases from *Bacillus* sp. strain PC-3 had a half-life ($t_{1/2}$) of 3.75 h at 60 °C [15]; *Geobacillus* sp. strain JS12 — $t_{1/2}$ > 120 min at 65 °C [16]; and *Geobacillus thermoglucosidasius* — $t_{1/2}$ 3 h (approximately) at 80 °C [17], include some of studied thermostable laccases of bacterial origin.

The laccases reported though are thermostable but, their thermostability is of short duration. High temperature bioprocesses require use of enzymes that can retain their thermostability for longer duration. Wild-type organisms usually have higher thermostability, but due to lower yield of the enzymes in the natural hosts they are expressed in a heterologous host. During heterologous expression the thermostability of the enzymes reduce, unless additional efforts are made to improve the thermostability in the heterologous host. Bhalla and coworkers observed that overexpression of xylanase gene in *E. coli* reduced the thermostability of the recombinant xylanase by 65% compared to the wild type [18]. Hence, to maintain the thermostability or keep the losses at minimum, cloning an enzyme that can maintain thermostability for longer duration is advantageous.

Hence, laccase from *Geobacillus* sp. strain WSUCF1, a thermophile obtained from the compost pile in Washington State University, Pullman, Washington, USA (46.7319° N, 117.1542° W) and known to produce enzymes with very high thermostability [18,19], was overexpressed and characterized. The efficacy of laccase towards lignocellulosic biomass was determined with doping it in the commercial hydrolytic enzymes (Accellarase 1500 and Cellic CTec2). Further, comparison was done for lignocellulosic biomass hydrolysis by secretome from a lab strain-*Penicillium* sp. RMT9 and commercial counterparts for the hydrolysis of untreated and pretreated bagasse.

2. Materials and methods

2.1. Enzyme production and activity assay

For laccase production *Geobacillus* sp. strain WSUCF1 was grown in 50 mL minimal media developed previously in our laboratory [20]. 250 mL Erlenmeyer flasks with 50 mL minimal media were autoclaved, and untreated (UT), alkali (AL) and acid treated (AC) rice straw, wheat straw, bagasse, corn stover and prairie cord grass prepared (1% w/v) as carbon source were added to the autoclaved media using the method described previously [20,21]. The flasks were inoculated with 10% (v/v) of freshly grown WSUCF1 culture and incubated at 60 °C for 120 h under shaking conditions (150 rpm). After incubation, the supernatant obtained after centrifugation at 8800 rpm for 20 min of the culture extracts was used for determination of the laccase activity. The reaction mixture (1 mL) contained equal volumes of enzyme extract and 1 mM ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) (Sigma-Aldrich, St. Louis, MO, U.S.A.) prepared in 0.1 M sodium phosphate buffer (pH 7). The reaction mixture was incubated at 60 °C for 10 min, and the increase in absorbance was determined at 420 nm. One IU (International Unit) of laccase activity represents the amount of enzyme required to oxidize 1 μmol of ABTS per minute under given assay conditions. The total protein content in the enzyme extracts was determined following Folin-Ciocalteu reagent method [21]. Proper substrate and cell-free controls were prepared, and all the experiments were performed in triplicates.

2.2. Cloning and expression of Geobacillus sp. WSUCF1 laccase

The genomic DNA was extracted from *Geobacillus* sp. strain WSUCF1 cells using the DNA isolation kit as per the manufacturer's instructions (Qiagen, Germantown, MD, U.S.A). PCR primers (F 5′-CGCGAACAGATTGGAGGTGAGGGTTGACGTGAATGCCG-3′ and R 5′-GTGGCGGCCGCTCTATTAGTCATGCCGTCTCCTCCCTT-3′) containing sequences homologous to the ends of pRham N-His SUMO Kan Vector (Lucigen, Middleton, WI, U.S.A.) were designed for the amplification of the laccase gene (825 bp) of *Geobacillus* sp. strain WSUCF1. The conditions used for PCR amplification were as follows: initial denaturation at 94 °C for 2 min followed by 30 cycles of 94 °C for 45 s, 63 °C for 40 s and 72 °C for 45 s in 50 μL reaction with final extension of 2 min at 72 °C. The amplified gene was ligated to the vector and transformed into chemically competent *E.* cloni 10G cells as per the manufacturer's instruction manual. The resultant transformants were plated on agar plates containing terrific broth (TB), rhamnose (0.2% w/v) and kanamycin (30 μg/mL), and incubated at 37 °C for 36 h. The positive clones were screened through colony PCR as per the manufacturer's instructions and confirmed by assaying laccase activity as described in the previous section.

2.3. Production and purification of the WSUCF1 recombinant laccase

A positive clone 'RAILC13' was selected and grown in 50 mL TB medium prepared as explained above. The flasks were incubated at 37 °C under shaking conditions (250 rpm) for 36 h. 25 mL of the supernatant obtained after centrifugation was loaded onto a Ni-NTA agarose resin column (1.5 × 5 cm, Thermo Scientific) pre-equilibrated with equilibration buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0). The column was washed with 5 column volumes of washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0) to remove any unbound proteins. The protein of interest was eluted using elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0) and 2 mL of the eluted fractions were collected at a flow rate of 0.5 mL/min. The fractions with laccase activity were treated with SUMO specific protease to remove the His Tag as per the manufacturer's instruction. The laccase was separated from His Tag and SUMO protease by repeating the metal affinity chromatography (Ni-NTA agarose resin column). The fractions with laccase activity were pooled and concentrated five folds using Amicon Ultra-15-Millipore (10 kDa cut off) for further physico-chemical characterization.

2.4. Laccase characterization

To determine the molecular weight the protein sample (purified laccase) was resolved on SDS-PAGE (10% resolving gel and 4% stacking gel) [22]. 10 μL of the concentrated protein was mixed with 10 μL of sample denaturing buffer (2×) and boiled for 5 min. The SDS-PAGE gel was run in the Mini-Protean II system (Bio-Rad, Hercules, CA) first at 90 V and 120 V respectively, for stacking and resolving gel regions. The SDS-PAGE gel was stained using silver staining solution. The conserved domain analysis was done using NCBI's conserved domain database [20].

The optimal pH of laccase was determined by incubating the reaction mixture (1 mL) containing purified enzyme (100 μL) and 1 mM ABTS (900 μL) prepared in different buffers (0.1 M): sodium acetate (pH 3.0–5.0), sodium citrate (pH 6.0), sodium phosphate (pH 7.0–8.0), and glycine NaOH (pH 9.0), at 60 °C for 30 min. The optimum pH was used to determine optimal temperature by incubating the reaction mixture at different temperatures (30–90 °C). To determine thermal stability, 2 mL of the purified laccase was incubated at 50, 60, 70, 80 and 90 °C and the samples were withdrawn at 0, 3, 6, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h for enzyme assay. To determine the effect of additives enzyme aliquots were pre-incubated with 2 mM $CuSO_4$, $FeSO_4$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, $CaCl_2$, NaCl, KCl, DTT, EDTA, and SDS for 30 min at room temperature, and the residual activities were determined using 1 mM ABTS at 50 °C and pH 6.0. Further, different concentrations of $CuSO_4$ (0.1–1, 2, 3, 4 and 5 mM) were evaluated to determine optimal concentration for laccase activity.

The Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) of the purified laccase were determined by assaying enzyme activity between 0 and 1 mM concentrations of ABTS in sodium citrate buffer (0.1 M, pH 6.0) at 50 °C for 30 min. The apparent values of K_m and V_{max} were used to determine turn over number (K_{cat}) and catalytic efficiency (K_{cat}/K_m) of the purified enzyme.

2.5. Enzymatic hydrolysis

The potential of recombinant laccase to enhance hydrolytic efficiency of commercial enzyme preparations Accellerase 1500 (DuPont) and Cellic CTec2 (Novozymes) was evaluated using untreated, alkali and acid treated corn stover (CS) and bagasse (BG) (at 7% w/v loading rate). The laccase was added to the commercial enzyme preparations in the ratio 1:9. The acid treated, alkali treated, and untreated substrates, respectively, were prepared as described by Rai et al. [21] and Bibra et al. [20]. *Penicillium* sp. strain RMT9 (an in-house strain) was grown on cellulose, wheat bran and rice straw (CWR) and secretome was used to obtain in-house enzymes [21]. The hydrolytic potential of these in-house enzyme cocktails was compared with the potential of commercial enzyme preparations (Accellerase 1500 and Cellic CTec2) to hydrolyze untreated, alkali and acid treated bagasse (7% w/v). The hydrolysis was carried in 5 mL glass vials with screw caps using an enzyme loading of 5 mg protein/g dry substrate (gds). The total volume of the reaction was adjusted to 1 mL with sodium acetate buffer (50 mM, pH 5.0). The sugars in the hydrolysates were analyzed using the DNS method and HPLC (Shimadzu Scientifics, Columbia, U.S.A.) equipped with Aminex column HPX-87P (Bio Rad, Hercules, CA, U.S.A.) and RI detector (50 °C) and 0.5 mM H_2SO as mobile phase.

3. Results and discussion

3.1. Laccase production

The laccase production started after 12 h with corn stover as the substrate (1% w/v). The production increased gradually onwards, with maximum activity observed at 96 h (Fig. 1a). When various lignocellulosic substrates (1% w/v) subjected to different treatment (acid, alkali or untreated) were used for laccase production untreated corn stover gave highest laccase production (37 U/L) followed by acid treated corn stover (32.4 U/L) and alkali treated wheat straw (25.8 U/L) (Fig. 1b). Higher laccase production was observed with untreated substrates compared to the acid treated and alkali treated substrates due to higher amount of lignin present in the untreated substrates. Acid and alkali treatment also remove lignin in the lignocellulosic biomass to a certain extent thus reducing the amount of lignin available compared to the untreated substrates [23]. Higher enzyme activity was observed with alkali treated wheat straw as post treatment wheat straw had the highest lignin and hemicellulose content. Lignin is coupled with hemicellulose in native form and more hemicellulose presence in alkali pretreated wheat straw could have resulted in higher laccase activity in wheat straw [3].

3.2. Expression, purification and characterization

Laccase gene (WP_011230630.1) from *Geobacillus* sp. strain WSUCF1 was amplified, cloned and expressed into chemically competent *E. coli* cells using the pRham N-His SUMO Kan vector (Fig. 2a). The recombinant laccase was purified with the metal affinity chromatography (Ni-NTA agarose residue) and ran on SDS-PAGE (10%) gel where a single prominent band of ~30 kDa was observed (Fig. 2b). A laccase with molecular weight of ~30 kDa was also reported from *Geobacillus thermoglucosidasius* [17]. Another laccase reported from *Geobacillus thermocatenulatus* showed two bands of 42.5 and 65 kDa [24], whereas one reported from *Geobacillus thermopakistaniensis* had a molecular weight of 60 kDa [25]. Laccases with different molecular weights had also been reported viz. 56 kDa from *Geobacter metalloreducens* [26], 65 kDa from *Bacillus pumillus* [27], and *Bacillus* sp. HR03 [28].

The multiple sequence alignment of the laccase from *Geobacillus* sp. WSUCF1 showed >95.0% sequence similarity to laccase from *Geobacillus* sp. strain C56-T3, *Geobacillus thermolevorans*, but exhibited only 61.0% sequence similarity to laccases from *Geobacillus thermoglucosidasius*. The alignment of amino acids also showed that the His^{155} was conserved

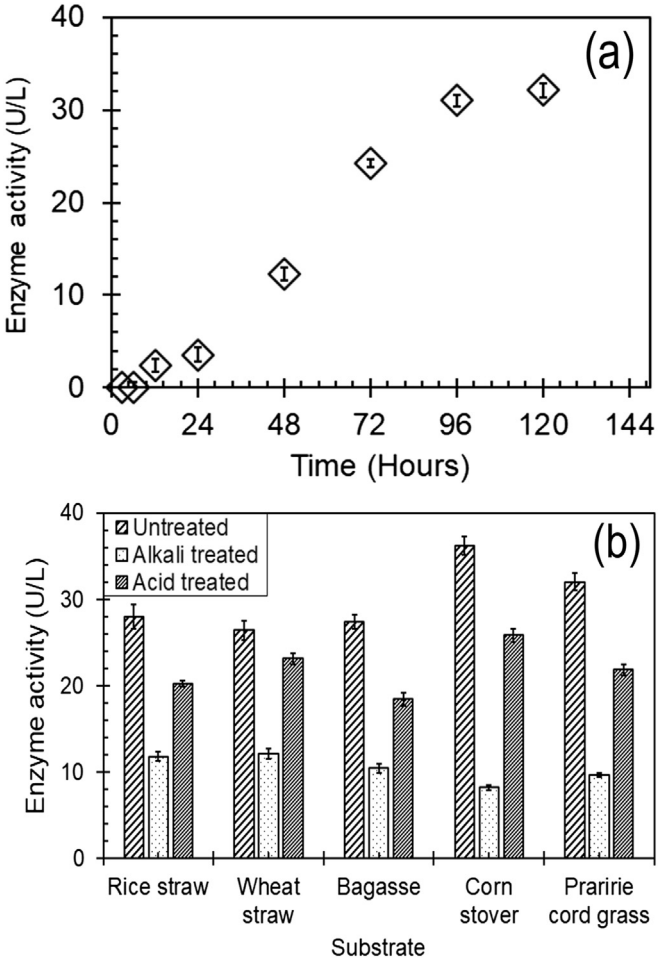

Fig. 1. (a) Production profile of laccase by *Geobacillus* sp. strain WSUCF1 in untreated corn stover (1% w/v) supplemented minimal medium, and (b) effect of different complex carbon sources on the production of laccase by *Geobacillus* sp. strain WSUCF1.

residue among all the *Geobacillus* spp. but was substituted by glutamate in *Geobacillus thermoglucosidasius* at position 155. Instead of His^{155}, Asn^{152} was the active site residue in *Geobacillus thermoglucosidasius*.

The purified laccase (specific activity 1.73 U/mg) showed optimal activity at pH 6.0 and temperature 50 °C (100%) (Fig. 3a and b). Thermostable laccases obtained from different bacterial sources showed optimal activity at variable temperatures and pHs, respectively, such as: *Geobacter metallireducens* — 75 °C and 6.0 [26]; *Geobacillus thermopakistaniensis* — 60 °C and pH 7.0–7.5 [25]; *Bacillus amyloliquefacians* strain — LC02 60 °C and 3.8 [29]; and *Geobacillus thermoglucosidasius* — 50 °C and 3.0 [17]. The recombinant laccase had a half-life of 108, 96 and 60 h at 50, 60 and 70 °C respectively at the optimal pH (Fig. 3c). Several thermostable laccases had also been reported previously viz. *Geobacter metalloreducens* — $t_{1/2}$ 70 min (approximately) (60 °C) [26]; *Bacillus amyloliquefacians* strain LC02 — $t_{1/2}$ 7 h (60 °C) [29], *Geobacillus* sp. strain JS12 — $t_{1/2}$ > 120 min (min) at 65 °C [16]; and *Geobacillus thermoglucosidasius* — $t_{1/2}$ 3 h (approximately) at 80 °C [17]. However, none of the reported laccase is as thermostable as reported in the current work. Hence, to the best of our knowledge, this is the most thermostable laccase reported so far.

The effect of different metal ions and reducing agents (1 mM) on laccase activity is shown in Table 1. $CuSO_4$ (122%) and $FeSO_4$ (109%) increased the laccase activity compared to control, whereas DTT (dithiothreitol) and SDS, respectively, resulted in 72 and 79% loss of laccase activity. A similar effect of $CuSO_4$ and $FeSO_4$ supplementation on the laccase activity was reported by Muthukumarasamy [30] where both the metal ions significantly enhanced laccase activity. Basheer and

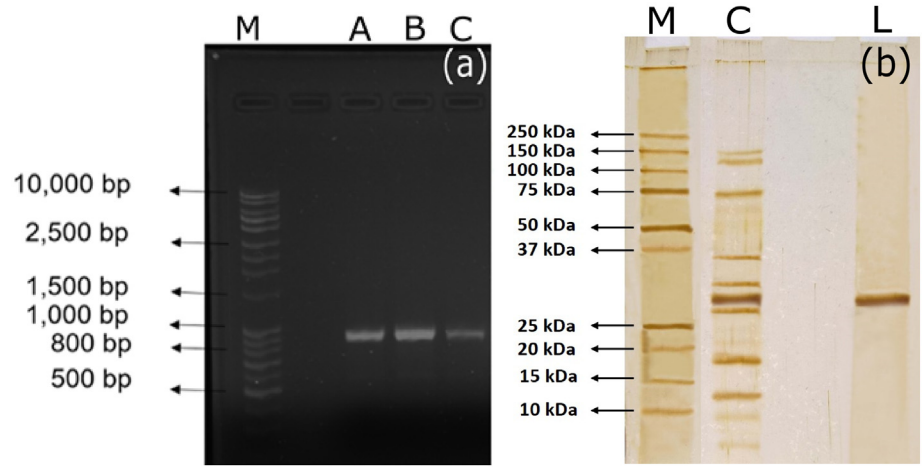

Fig. 2. (a) Agarose gel (1%) showing amplified products of ~824 bp (predicted length of laccase gene) through colony PCR of laccase positive clones; Lane M: 1 kb plus DNA ladder, Lanes A, B and C: laccase positive clones; (b) SDS-PAGE (10%) showing molecular weight of the purified laccase (Lane L), Lane M: marker.

coworkers also showed that $CuSO_4$ addition (100 μM) increased the activity of laccase produced by *Geobacillus thermopakistaniensis* five times [25]. However, the results for positive modulation of laccase activity in the presence of Fe^{2+} are in contradiction to the report by Wang and coworkers where Fe^{+2} addition inhibited laccase (Novozymes) activity [31]. Further investigation of $CuSO_4$ concentrations showed that a concentration of 0.1–1 mM positively modulated the enzyme with maximal activity (134%) observed at 0.5 mM concentration. However, the activity was inhibited at concentrations higher than 2 mM (Fig. 3d). The determination of kinetic parameters showed that enzyme followed classic Michaelis-Menten kinetics. The apparent values of K_m, V_{max} and K_{cat} were determined to be 0.146 mM, 1.52 U/mg and 1037 s^{-1}, respectively.

Laccase from *Geobacillus* sp. strain WSUCF1 had comparatively higher affinity for ABTS than *Bacillus pumilus* (0.252 mM) [27], *Mycena pupureofusca* (0.3 mM) [32] and *Trametes versicolor* (0.43 mM) [33] but, lower than *Geobacillus thermopakistaniensis* (0.0068 mM) [25]. Further, the enzyme showed quite high catalytic efficiency with K_{cat}/K_m value of 7102.7 mM^{-1} s^{-1} that was higher than the laccase reported from *Trametes polyzona* (3960 mM^{-1} s^{-1}) [34], *Bacillus pumilus* (152 mM^{-1} s^{-1}) [27], and *Geobacillus thermopakistaniensis* (1179 mM^{-1} s^{-1}) [25]. Hence, with high catalytic efficiency and thermostability the laccase from *Geobacillus* sp. strain WSUCF1 can be a success for longer duration high temperature industrial bioprocesses.

3.3. Hydrolysis

During hydrolytic studies it was found that the doping of recombinant laccase to commercial enzyme preparations (Accellarase 1500 and Cellic CTec2) in a ratio of 9:1 improved the hydrolysis of both

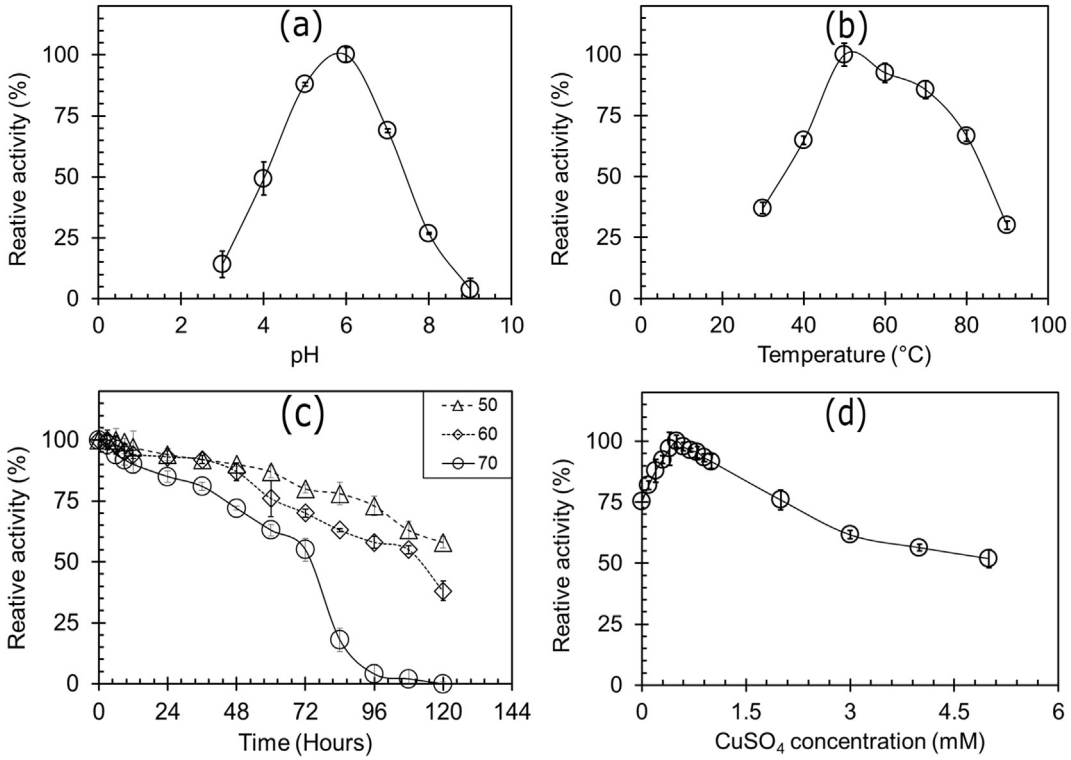

Fig. 3. Characterization of purified laccase (a) pH optimum, (b) temperature optimum, (c) thermostability, and (d) effect of different Cu^{+2} concentrations on laccase activity.

Table 1
The effect of metal ions and other additives on the activity of purified laccase.

Metal ions	Relative activity (%)
Control	100 ± 1.63
$CuSO_4$	122 ± 1.91
$ZnSO_4$	66 ± 2.01
$MnSO_4$	53 ± 1.15
$FeSO_4$	109 ± 0.96
$MgSO_4$	43 ± 1.75
$CaCl_2$	79 ± 0.86
KCl	90 ± 2.19
NaCl	85 ± 1.83
DTT	28 ± 2.06
SDS	21 ± 1.99
EDTA	72 ± 0.85

±SE @ 5% level.

untreated and pretreated lignocellulosic biomass. The hydrolysis of untreated, alkali and acid treated corn stover was increased by 1.31–2.28 folds and bagasse by 1.32–2.02 folds, compared to hydrolysis by commercial enzymes alone (Fig. 4a). The supplementation of laccase to Cellic CTec2 increased the sugar released (mg/g of used biomass) by 1.19, 1.06 and 1.1 folds, respectively, with untreated, acid-treated and alkali-treated corn stover. Similarly, the doping of laccase to Accellerase 1500 increased the sugar content by 1.12 times with acid treated bagasse as the substrate. The lignocellulosic biomass in untreated form has cellulose and hemicellulose surrounded by lignin. The acid pretreatment is less effective in removing lignin, but removes hemicellulose

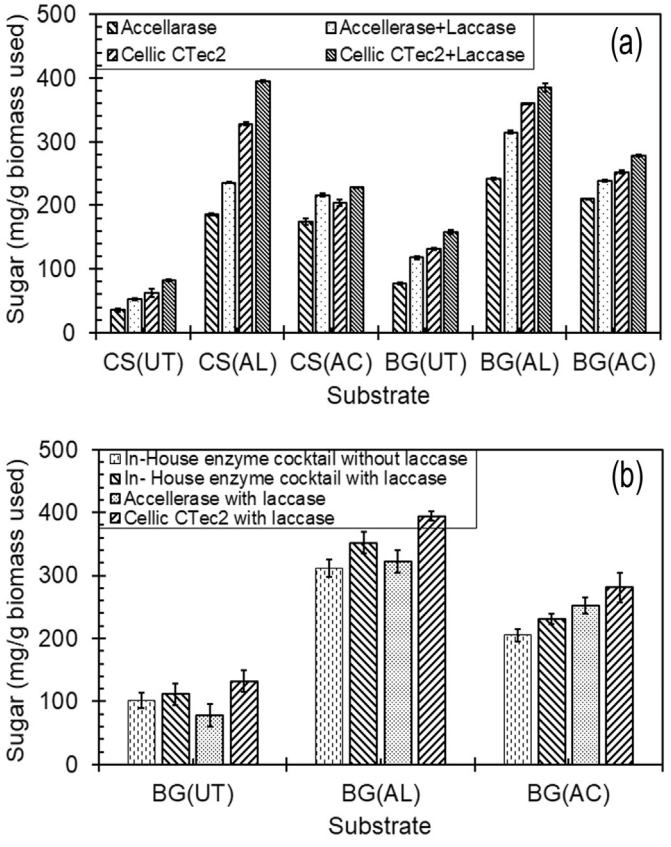

Fig. 4. Hydrolysis (96 h) of untreated (UT), alkali (AL) and acid (AC) treated corn stover (CS) and bagasse (BG) by Accellerase 1500 and Cellic CTec2, with and without supplementation of recombinant WSUCF1 laccase (9:1 v/v), and (b) hydrolysis (96 h) of untreated (UT), alkali (AL) and acid (AC) treated bagasse (at 7% substrate loading rate) by in-house enzyme cocktail, laccase doped in-house enzyme cocktail, laccase doped Accellerase 1500 and laccase doped Cellic CTec2 (5 mg protein/g substrate).

resulting in lower availability of pretreated substrate for hydrolysis. On the other hand, alkaline pretreatment removes lignin and modifies hemicellulose increasing the availability of substrate for hydrolysis compared to untreated and acid pretreated substrate. Hence, the lowest sugar yield was obtained with untreated substrate and highest yield was obtained with alkali treated substrate. Laccases improve hydrolysis by degrading lignin by acting upon phenolic and non-phenolic groups and removing obtained during pretreatment resulting in increased accessibility of cellulolytic/hemicellulolytic glycosyl hydrolases to cellulose/hemicellulose fractions in the biomass and thus increasing the amount of sugar obtained [6,8]. Hence, the addition of laccase improved the sugar yield with untreated, acid treated, and alkali treated substrates. Such improvement in the sugar concentration can be helpful in the production of biobased products using lignocellulosic biomass.

Further, the potential of recombinant laccase for lignocellulosic biomass hydrolysis was also determined with an in-house enzyme cocktail and the results were compared to the commercial counterparts i.e. Cellic CTec2 and Accellerase 1500 (5 mg protein/g substrate). The results showed that laccase doping improved the performance of an in-house enzyme cocktail and was comparable to the commercial counterparts. The amount of reducing sugars released by the laccase doped in-house enzyme cocktail was enhanced by 1.11 folds with different types of substrates (Fig. 4b). When compared to the commercial counterpart, laccase doped in-house enzyme cocktail performed better than laccase doped Accellerase 1500 and gave 1.44 (112 mg/g biomass used), 1.1 (352 mg/g biomass used) and 0.92 folds (231 mg/g biomass used) higher sugar content with untreated, alkali and acid treated bagasse, respectively. However, laccase doped Cellic CTec2 performed better than the laccase doped in-house enzymes. The sugars released for the by laccase doped in-house enzyme cocktails was 78.3, 73.65 and 76.6%, respectively, after hydrolysis of untreated, alkali and acid treated bagasse when compared to laccase doped Cellic CTec2. Cellic CTec2 and Accellerase 1500 have different catalytic behavior in terms of cellulase and xylanases. Higher sugar yield with laccase supplementation to Cellic CTec2 as compared to Accellerase 1500 can be attributed to the higher xylanase activity of the former (38.53 U/mg protein) as compared to the later (6.25 U/mg) [35]. Also, the xylanases are known to mediate cleavage of lignin carbohydrate bonds which results in the removal of solubilized lignin [36]. Hence, the greater synergism between xylanase and laccase in case of Cellic Ctec 2 might have resulted in higher sugar yield compared to Accellerase 1500.

4. Conclusion

In this study, a highly thermostable laccase from *Geobacillus* sp. strain WSUCF1 was cloned and expressed in *E. coli*. The recombinant laccase was characterized and tested for its potential for hydrolysis of lignocellulosic biomass. Owing to its small size (30 kDa), low K_m (0.146 mM) and high thermostability ($t_{1/2}$ of 72 h at 70 °C), the recombinant WSUCF1 laccase could be utilized for various industrial and biotechnological applications that require delignification.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.06.221.

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