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Multiple Applications of Enzymes Induced by Algal Biomasses from a New *Bacillus* Isolate to Saccharify Algae and Degrade Chemical Dyes

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Abstract

To find a multifunctional lignocellulolytic enzyme-producing strain, ten bacterial isolates from paper mill wastewater were tested for their carboxymethyl cellulose (CMC) hydrolytic ability. *Bacillus* sp. TPF-1, which exhibits the highest hydrolytic ability, was selected to produce lignocellulolytic enzymes using various biomass types as carbon sources. The highest CMCase (9.12 U/mL) and xylanase (102.55 U/mL) activities were obtained by green algae, and the maximum laccase activity (7037.28 U/L) was induced by *Sargassum fusiforme*. CMCase and xylanase showed the highest activities at 55 and 50 °C, respectively, with the same optimum pH of 5.4. The laccase exhibited optimum temperature of 40 °C and retained 60% more activity at 80 °C in extreme acid conditions (pH 2.2). To explore the multiple applications of these enzymes, crude enzymes induced by green algae were used to saccharify untreated algae. The reducing sugar produced by crude enzymes and commercial cellulase was 23 and 14% higher than that of the control, respectively, and it was 48% higher using crude enzymes with commercial cellulase (72 h). Additionally, the laccase induced by *S. fusiforme* was tested to decolorize two chemical dyes under an acidic condition (pH 2.2). The highest decolorization rates were 56.13 and 62.14% for Coomassie brilliant blue R-250 and Congo Red, respectively, in the presence of hydroxybenzotriazole monohydrate.

Keywords *Bacillus* · CMCase · Algae · Laccase · Xylanase

Introduction

The increase in fossil fuel consumption causes the urgent need to find alternative sources of traditional energy. Lignocellulosic biomass, which is broadly distributed in nature, has been proposed as one of the best alternative energy sources [1]. As an ecofriendly method, enzymatic saccharification is widely used in the conversion of lignocellulosic biomass [2]. As is well known, 5- and 6-carbon sugars lock up approximately 70% of plant biomass [3]. These sugars are present in the lignocellulosic biomass, which are comprised mainly of cellulose and hemicelluloses as well as lignin to a lesser extent [2]. Therefore, the complexes of cellulase (EC 3.2.1.4; 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase), xylanase (EC 3.2.1.8; 4-β-D-xylan xylanohydrolase), and laccase (EC 1.10.3.2; benzenediol: oxygen oxidoreductase) are desirable for efficient degradation of lignocellulosic biomass [3]. However, the high cost of commercial enzymes severely restricts their application in bioenergy production. To solve this problem, fungi and yeasts are being currently used to produce lignocellulolytic

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enzymes on an industrial scale [4]. Whereas, bacteria should be considered more appropriate for the industrial production of lignocellulolytic enzymes due to their shorter generation time [2], simpler applicable purification technology [3], easy of artificial cultivation [5], and excellent tolerance to environmental stresses [6].

According to previous findings, bacterial cellulases are mainly produced by *Bacillus, Paenibacillus, Lysinibacillus*, and *Psychrobacter* sp. [7, 8]. Xylanase activities have been reported in several bacterial species of *Bacillus, Pseudomonas*, and *Thermoanaerobacterium* sp. [9–11]. Moreover, laccase activities were found in *Escherichia coli, Azospirillum, Bacillus*, and *Serratia* sp. etc. [12–14]. Laccases are blue multicopper oxidases, which have the capacity to catalyze the oxidation of a variety of aromatic substrates [15]. It has been proven that the enzymatic systems involving the enzymes of lignin degradation are able to transform and remove the industrial dyes released into the environment [16]. The laccases from many fungal strains have been induced, characterized, and purified for the application of dye decolorization [17]. Nevertheless, fungal laccases are easy to lose their activities in extreme pH conditions ($pH < 2.5$ or $pH > 8.5$) [18, 19]. Bacterial laccases are more pH stable than fungal laccases, and this stability is advantageous for the biodegradation of industrial dyes [20]. Therefore, laccases from bacterial strains not only have the capacity to destroy the lignin structure but also show a significant positive effect on the biodegradation of chemical dyes [21]. Thus far, most of the studies on the lignocellulolytic enzyme-producing bacterial strains have focused on only one aspect, either the decomposition of lignocellulosic biomass [1] or the decolorization of chemical dyes [12]. Conversely, the studies on efficient bacteria that secrete multiple enzyme complexes to both saccharify lignocellulosic biomasses and degrade chemical dyes are limited.

In this study, we focused on the isolation and characterization of highly efficient lignocellulose-decomposing bacterial strains from paper mill wastewater in the hopes of finding the strain with excellent lignocellulosic enzyme production, a strong tolerance to severe environments, and a greater potential for multiple applications in industrial manufacturing. An efficient *Bacillus* strain TPF-1 exhibiting a significant ability to hydrolyze carboxymethyl cellulose (CMC) was selected. Low-cost natural biomasses were used as the sole carbon sources to economically produce cellulases, xylanases, and laccases from strain TPF-1. By conducting saccharification assays and measuring the reducing sugars released from untreated green algae, the hydrolytic efficiency of crude enzymes induced by green algae from strain TPF-1 was evaluated. In order to develop more application value of strain TPF-1, the laccase induced by *Sargassum fusiforme* from this strain was tested for its ability to decolorize synthetic dyes.

Materials and Methods

Isolation, Screening, and Identification of Cellulase-Producing Strain TPF-1

Cellulase-producing bacteria were isolated from paper mill wastewater in Thunder Bay, ON, Canada. The isolation of bacteria was conducted with the standard serial dilution plate method using the Luria–Bertani (LB) medium [22]. The cellulase-producing strains were visualized by using the information on halos in the CMC-containing agar by staining the plates with Gram's iodine solution [23]. The hydrolytic ability was tested and calculated by the square of the halo diameter to colony diameter ratio according to the description of Guo et al. [23]. The strain TPF-1, which showed the greatest hydrolytic ability, was selected for further study.

To identify strain TPF-1, its genomic DNA was extracted following the protocol of the Geneaid DNA Extraction Kit (Toronto, Canada). The 16S rRNA gene primers, HAD-1 (5′-GACTCCTACGGGAGGCAG CAGT-3′) primers, and E1115R (5′-AGGGTTGCGCTC GTTGCGGG-3′) primers were used in the polymerase chain reaction (PCR) to amplify the 16S rRNA gene fragments. The PCR products were verified by using 1% agarose gel, which was purified with a PCR purification kit (FroggaBio, Toronto, Canada), and then sequenced. The resultant sequencing was imported into the NCBI (http://blast.ncbi.nlm.nih.gov/) database to identify the possible genus using the basic local alignment search tool (BLAST). The phylogenetic relationship was analyzed, and a phylogenetic tree was constructed using MEGA 7.0 software.

Lignocellulolytic Enzyme Productions with Various Biomasses by Strain TPF-1

To evaluate the production ability of lignocellulolytic enzymes by strain TPF-1 using various biomasses as a carbon source, the strain was first cultured in an LB liquid medium overnight at 37 °C. The bacterial suspension was then inoculated (2%, v/v) in 50 mL of minimal medium (1.0 g/L $NaNO_3$, 1.0 g/L K_2HPO_4, 1.0 g/L KCl, 0.5 g/L $MgSO_4$, 0.5 g/L yeast extract, and 3 g/L peptone) containing 0.5% (w/v) different low-cost natural biomasses, which included barley straw, wheat bran, *Miscanthus*, agave, green algae (*Chlorella zofingiensis*), and *S. fusiforme*. The biomasses were dried at 50 °C until constant weight, cut into 2–5 cm (except green algae), and then ground through a 50 mesh sieve for further study [1]. The flasks were incubated at 37 °C with agitation at 200 rpm,

and then the culture broth was collected each day and centrifuged at 12,000×*g* for 3 min. The supernatants were taken as crude enzymes for CMCase, xylanase, and laccase activity analysis.

Enzyme Activity Assay

The CMCase and xylanase activities were measured using carboxymethyl cellulose and xylan as substrate, respectively, according to the description of Guo et al. [23]. The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) reagent, as described by Miller [24]. The laccase activity was determined according to the description of Guo et al. [23]. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of substrate per minute.

SDS-PAGE and Zymogram Analysis

To determine their molecular weights, CMCase, xylanase, and laccase enzymes from strain TPF-1 were run along with a protein marker using 12% (w/v) polyacrylamide gel and 5% stacking gel according to the method of Laemmli [25]. Gels containing 0.25% (w/v) corresponding substrates were used to confirm the activity of CMCase and xylanase. After electrophoresis, the gel was divided into two parts. The part of the gel containing the samples and molecular markers was stained with Coomassie brilliant blue R-250 (CBBR-250) to visualize the protein. The remainder of the gel was washed with 1% Triton X-100 for 30 min and then incubated in 0.05 M CH_3COOH–CH_3COONa buffer (pH 5.4) to detect the cellulase (at 55 °C for 30 min) and xylanase (at 50 °C for 30 min). Thereafter, the gel was flooded with 0.1% Congo Red (CR) solution for 30 min and de-stained with 1 M NaCl solution for a zymogram analysis. The gel was dipped in a 1% (v/v) acetic acid solution to stop the reaction. To detect laccase activity, after elution with the Triton X-100, the gel was immersed in a 0.05 M glycine–HCl buffer (pH 2.2) containing 2.0 mM ABTS. A protein band with laccase activity was then visualized.

Effect of Temperature and pH on Crude Enzyme Activity

The effect of temperature, which ranged from 30 to 80 °C at 5 °C intervals, was observed. The influence of pH was measured in the range of 2.2–9.0 at intervals of 0.4. The buffer solutions used were as follows: pH 2.2–3.0 in a 0.05 M glycine–HCl buffer, pH 3.4–7.4 in a 0.05 M CH_3COOH–CH_3COONa buffer, and pH 7.8–9.0 in a 0.05 M glycine–NaOH buffer. To confirm the effect of pH on laccase activity, ABTS, 2, 6-dimethoxyphenol (2, 6-DMP), and guaiacol were used as substrates.

Enzymatic Saccharification

A commercial cellulase (Celluclast 1.5 L, Novozymes, Franklinton, NC, USA) was used for comparison with the enzymes secreted from strain TPF-1. Green algae were used as the sole carbon source to cultivate strain TPF-1 under submerged cultivation. The supernatants were sterile filtered with a 0.22 μm Millipore filter and used as crude enzymes enriched with CMCase and xylanase (CECX) after 24 h of cultivation. Enzymatic saccharification was conducted in the 0.05 M CH_3COOH–CH_3COONa buffer (pH 5.4) containing 1% (w/v) green algae incubated at 50 °C with an agitation of 200 rpm. Three treatments, including 2.0 mL of CECX, 8.5 U of commercial cellulase, and 1.0 mL of CECX supplemented to 4.25 U of commercial cellulose, were set. The CH_3COOH–CH_3COONa buffer (0.05 M, pH 5.4) without enzymes was set as the control. The supernatants (25 μL) were collected at intervals of 12 h to analyze the amount of reducing sugars using the DNS method described in "Enzyme Activity Assay" section.

Decolorization of Dyes

To obtain the maximum amount of laccase for dye decolorization, the strain was cultivated using 0.5% (w/v) *S. fusiforme* as the sole carbon source (Fig. S1), and the crude enzymes enriched with laccase (CEL) were obtained after 72 h of cultivation. The decolorization of 0.05% (w/v) chemical dyes was conducted at 45 °C with an agitation of 200 rpm, and 2.0 mL of CEL was added to 5.0 mL 0.05 M glycine–HCl buffer (pH 2.2). To evaluate the effect of 1-hydroxybenzotriazole monohydrate (HOBt; Bio Basic Canada Inc., Ontario, Canada) on the dye decolorization, HOBt was added to a final concentration of 1.0% (w/v) to initiate the enzymatic reaction. Decolorization abilities were determined every 6 h by using the xMark Microplate Spectrophotometer (Epoch, BioTek Instruments, Inc., USA) to record the decrease in absorbance of CBBR-250 at 563 nm and CR at 643 nm [26].

The decolorization ratio was calculated as follows:

$$\text{Decolorization ratio} = \frac{\text{Initial absorbance} - \text{Absorbance after reaction}}{\text{Initial absorbance}} \times 100\%$$

The same volume of glycine–HCl buffer (pH 2.2) without CEL was set as the control.

Statistical Analysis

To quantify the effect of different carbon sources, a one-way ANOVA was conducted in each experimental treatment through Fisher's least significant difference test ($P < 0.05$).

Results

Isolation, Screening, and Identification of Strain TPF-1

A total of ten strains showed the hydrolytic ability of CMC in the preliminary screening of cellulolytic bacteria (Fig. 1a). The hydrolytic ability values of these ten strains ranged from 2.25 to 21.53 (Table S1). The bacterial strain TPF-1 selected for further experimentation exhibited the greatest hydrolytic ability value (21.53), which was 2.3-fold higher than that of the positive control strain *C. xylanilytica* (9.42).

To identify the strain, the 16S rRNA gene sequence of TPF-1 (accession number: KY906990) was amplified and sequenced. According to the BLAST results showing100% similarity with the known *Bacillus* strain (accession number: FN666885) and to the phylogenetic tree (Fig. 1b), this strain belonged to *Bacillus*. Thus, TPF-1 was identified as *Bacillus* sp. according to the morphological and phylogenetic characteristics of this strain and to the comparative analysis of the 16S rRNA gene sequence.

Effects of Different Biomasses on the Production of Lignocellulolytic Enzymes

The lignocellulolytic enzymes produced by TPF-1 using six different biomasses as the carbon source were significantly different (Fig. 2). The highest CMCase and xylanase activities were detected in the medium containing green algae. The peak activities of two enzymes at 9.12 and 102.55 U/mL for CMCase and xylanase, respectively, were observed at 24 h. Compared with other biomasses, green algae were

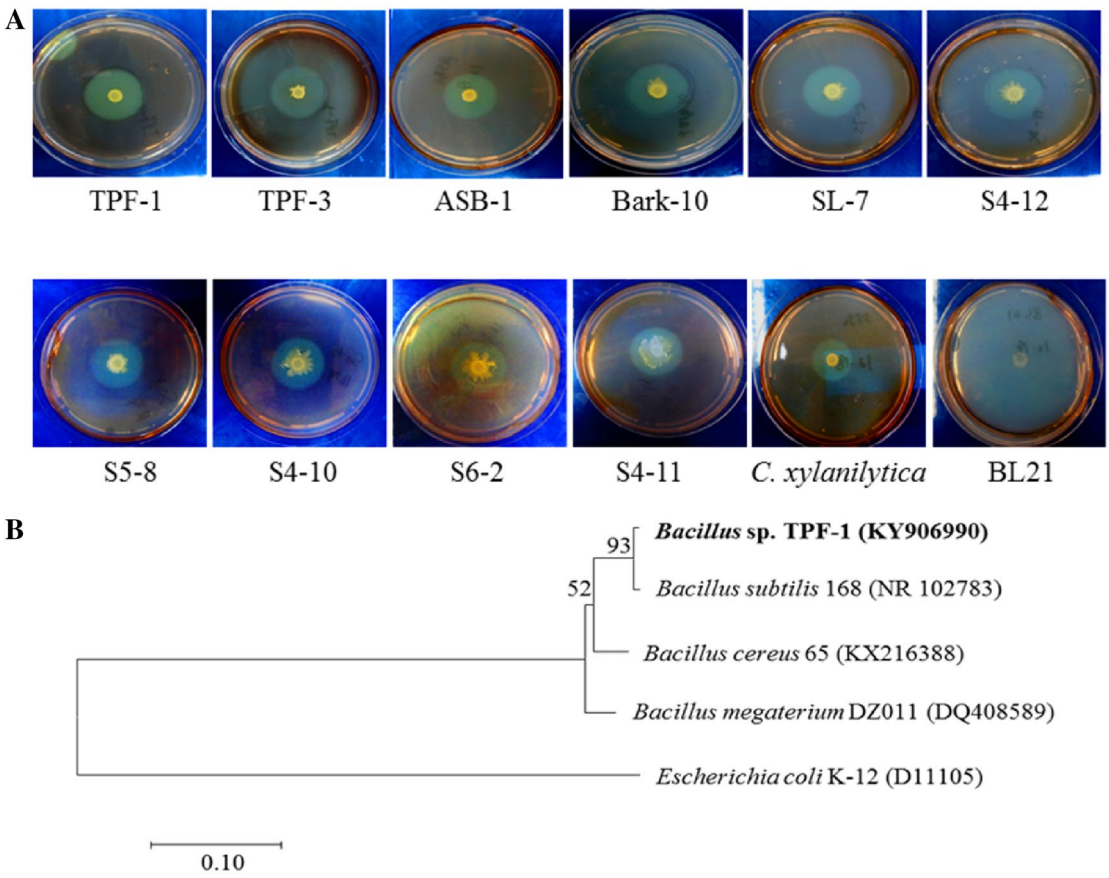

Fig. 1 Hydrolysis halos of 10 CMC-degrading isolates compared with the positive (*Cellulomonas xylanilytica*) and negative (*Escherichia coli* BL 21) controls. **a** Neighbor-joining phylogenetic tree reconstructed on the basis of 16S rDNA sequences (**b**) showing the phylogenetic relationships between strain TPF-1 and related type bacteria. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. The scale bars represent 0.10 substitutions per nucleotide position

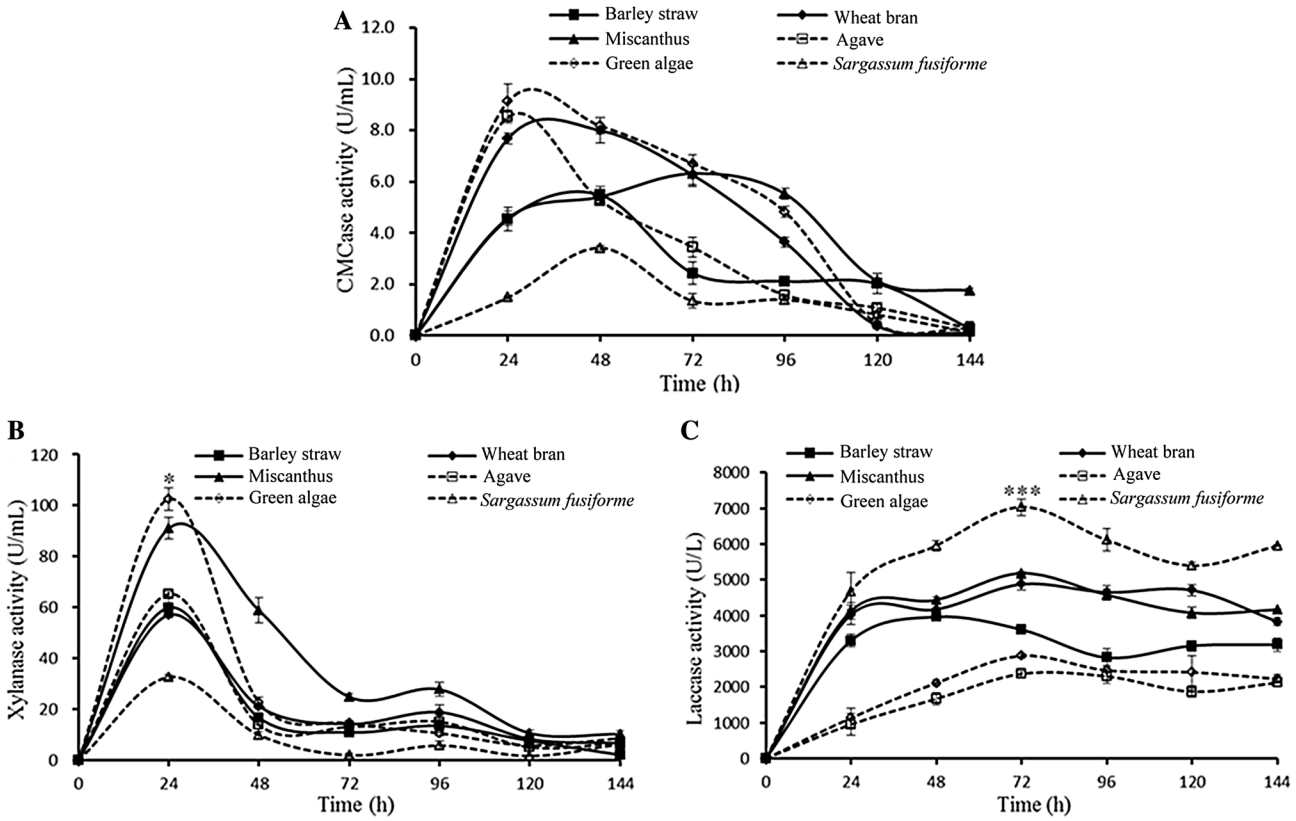

Fig. 2 CMCase (**a**), xylanase (**b**), and laccase (**c**) activities of *Bacillus* sp. TPF-1 using different biomasses as substrate in submerged cultivation with shaking for 6 days at 37 °C. Bars indicate the standard deviation (n = 3). The symbol * indicates significant difference at $P < 0.05$, and *** indicates significant difference at $P < 0.001$

proved to be the best carbon source for *Bacillus* sp. TPF-1-producing CMCase and xylanase (Fig. 2a, b). When *S. fusiforme* was used as the carbon source, no significant increase occurred in the CMCase and xylanase activities until the end of this experiment. However, the maximum value of laccase activity reaching 7037.28 U/L was detected after culturing strain TPF-1 for 72 h. The least laccase activity of 2371.45 U/L (72 h) was observed when agave was used as the carbon source. Compared with that of the other two enzymes described above, the laccase activity remained stable after reaching the highest activity in each medium (Fig. 2c).

SDS-PAGE and Zymogram Analysis

SDS-PAGE and zymogram analysis of CECX showed two protein bands with CMCase activity. The molecular weights of these two bands were approximately 15 and 10 kDa, respectively (Fig. 3a). The xylanase activity of CECX was confirmed through SDS-PAGE and zymogram analysis, in which a single protein band appeared (Fig. 3b). The molecular weight of this band was approximately 10 kDa. The CEL was tested by electrophoretic analysis for laccase activity and exhibited one protein band with a molecular weight of 55 kDa (Fig. 3c).

Optimum Temperature and pH for Enzymatic Activity

The optimum temperatures for CMCase, xylanase, and laccase activities were 55, 50, and 45 °C, respectively (Fig. 4a). The activities of both CMCase and xylanase rapidly decreased with increasing temperature and were almost denatured at 80 °C while losing 96.63 and 89.55% of activity, respectively. Laccase showed a broad temperature activity profile at a temperature range of 30–80 °C, with a slight decline after reaching the highest value. The optimum pH for both CMCase and xylanase was 5.4 (Fig. 4b, c). The laccase activity of CEL exhibited the highest activity under extremely acidic conditions, and the enzymatic activity significantly decreased beyond pH 3.0 (Fig. 4d). When ABTS was used as the substrate, the optimum pH of laccase activity was below 2.2, and the maximum value of laccase activity was detected at pH 3.0 using 2, 6-DMP and guaiacol as substrates.

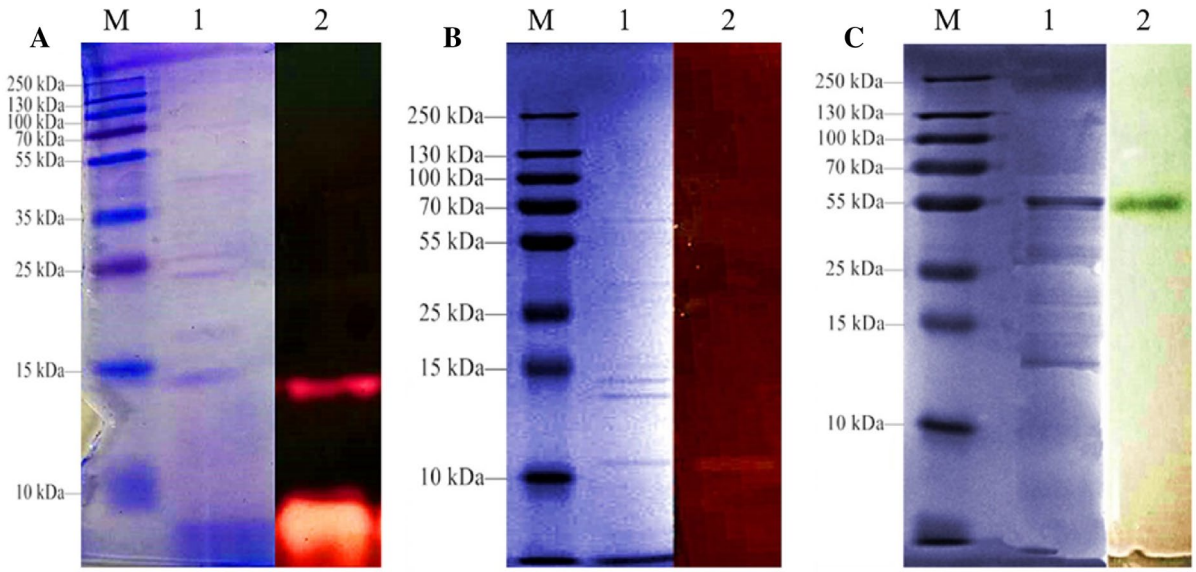

Fig. 3 SDS-PAGE and zymogram of the supernatant expression of *Bacillus* sp. TPF-1. CMCase zymogram. Standard protein molecular weight markers (M), Coomassie blue staining of the SDS-PAGE gel (lane 1), and CR staining of the SDS-PAGE gel containing 0.25% (w/v) CMC (lane 2) (**a**). Xylanase zymogram. Standard protein molecular weight markers (M), Coomassie blue staining of the SDS-PAGE gel (lane 1), and CR staining of the SDS-PAGE gel containing 0.25% (w/v) beechwood xylan (lane 2) (**b**). Laccase zymogram. Standard protein molecular weight markers (M), Coomassie blue staining of the SDS-PAGE gel (lane 1), and ABTS staining of the SDS-PAGE gel (lane 2) (**c**)

Effect of CECX on the Enzymatic Saccharification of Green Algae

To assess the hydrolytic efficiency of CMCase and xylanase secreted from TPF-1, saccharification experiments were conducted by incubating CECX and commercial cellulase with green algae. The production of reducing sugars in different treatments increased gradually along with incubation time (Fig. 5). After 72 h of incubation, the highest yield of reducing sugars was obtained when both CECX and commercial cellulase were present, the value of which was 20.3 and 29.8% higher than using CECX and commercial cellulase, respectively (Fig. 5). These results are a synergistic effect of CECX and commercial cellulase on the yield of reducing sugars.

Effect of CEL on the Dye Decolorization of *S. fusiforme*

The laccase from strain TPF-1 efficiently decolorized CBBR-250 and CR, with both final decolorization ratios being higher than 50% in the presence of a redox mediator (HOBt). When only CEL was used, the decolorization ratios were 29.22 and 32.56% for CBBR-250 and CR, respectively, after 24 h of incubation (Fig. 6). Apparently, the addition of HOBt significantly increased the decolorization efficiency of both dyes, and the final decolorization ratios of CBBR-250 and CR were 56.13 and 62.14% in the presence of CEL and HOBt together, respectively (Fig. 6).

Discussion

In the present study, a novel *Bacillus* sp., TPF-1, was isolated from the wastewater of a paper mill and was proved to be an effective lignocellulolytic enzyme-producing bacterial strain. The lignocellulolytic enzymes from strain TPF-1 were characterized through incubation under different temperatures and various pH levels. The conditions of 55 °C with pH 5.4 and 50 °C with pH 5.4 gave rise to the greatest CMCase and xylanase activities, respectively. These conditions are similar to those of most reported enzymes, such as the cellulases and xylanases in soil [27], white-rot fungus [28], and *B. subtilis* CK-2 [29]. For laccase, the maximum activity was obtained at 45 °C with pH 2.2 when ABTS was used and at 45 °C with pH 3.0 when 2, 6-DMP and guaiacol were used. The difference in pH optima of these three substances is typical for laccases and is mainly due to the different oxidation mechanisms that depend on the substrate [30]. The molecular weights of CMCase, xylanase, and laccase determined by SDS-PAGE and zymogram analysis were 15, 10, and 55 kDa, respectively. Two bands of cellulase were shown, and the appearance of smaller species (< 10 kDa) could represent a partially unfolded form of the enzyme caused by incomplete denaturation with the SDS treatment

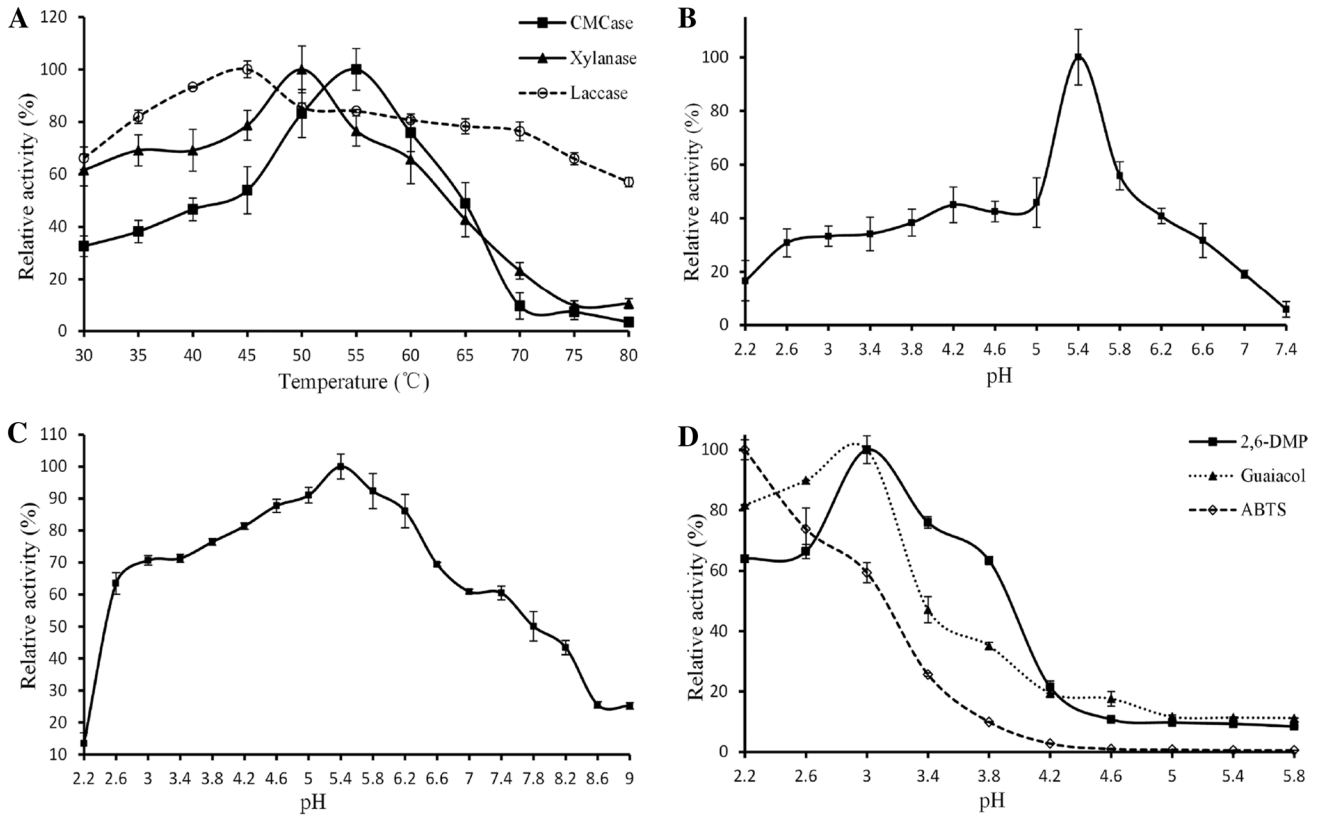

Fig. 4 Effects of different temperatures and pH on enzymatic activity. The optimum temperature for enzymatic activity was determined under temperature conditions of 30–80 °C. **a** The optimum pH for CMCase activity was determined under pH conditions of 2.2–7.4. **b** The optimum pH for xylanase activity was determined under pH conditions of 2.2–9.0. **c** The optimum pH for laccase activity was determined under pH conditions of 2.2–5.8. **d** Bars indicate the standard deviation (n = 3)

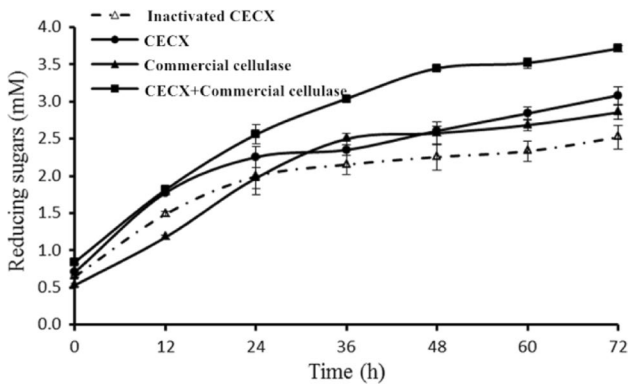

Fig. 5 Enzymatic saccharification of green algae. Bars indicate the standard deviation (n = 3)

[31]. The molecular weight of xylanase in this study is consistent with that in previous research identifying microbial xylanases as generally single subunit proteins with molecular weights in the range of 8–145 kDa [32]. Additionally, the SDS-PAGE and zymogram analysis results of laccase are the same as those in Chaurasia et al. [33].

Different biomasses were used as the sole carbon source for the enzyme production experiments. Carbon sources in bacterial cultivation can provide effective components to induce the expression of enzymes [34]. The maximum activities of CMCase and xylanase were detected when green algae were used. As green algae contain rich polysaccharides, *Bacillus* sp. TPF-1 utilized polysaccharides directly for its growth in the first 24 h. After the polysaccharides were depleted, CMCase and xylanase were secreted from *Bacillus* sp. TPF-1 to degrade the celluloses and hemicelluloses in the green algae, and then oligosaccharides (main products) were released for the strain's growth. These results indicate that green algae can be applied as a low-cost carbon source in the CMCase and xylanase production of *Bacillus* sp. TPF-1. Although some previous studies on *Bacillus* sp. strains proved that these strains have the ability to produce cellulases or xylanases [35–39], few studies have focused on the production of multiple enzymes by bacterial strains. The CMCase activity measured in these experiments was higher than that of other *Bacillus* sp. strains, such as *Bacillus pumilus* EB3 (0.77 U/mL) and *Bacillus* sp. 313SI (4.15 U/mL using pretreated substrate under an optimized condition) [38,

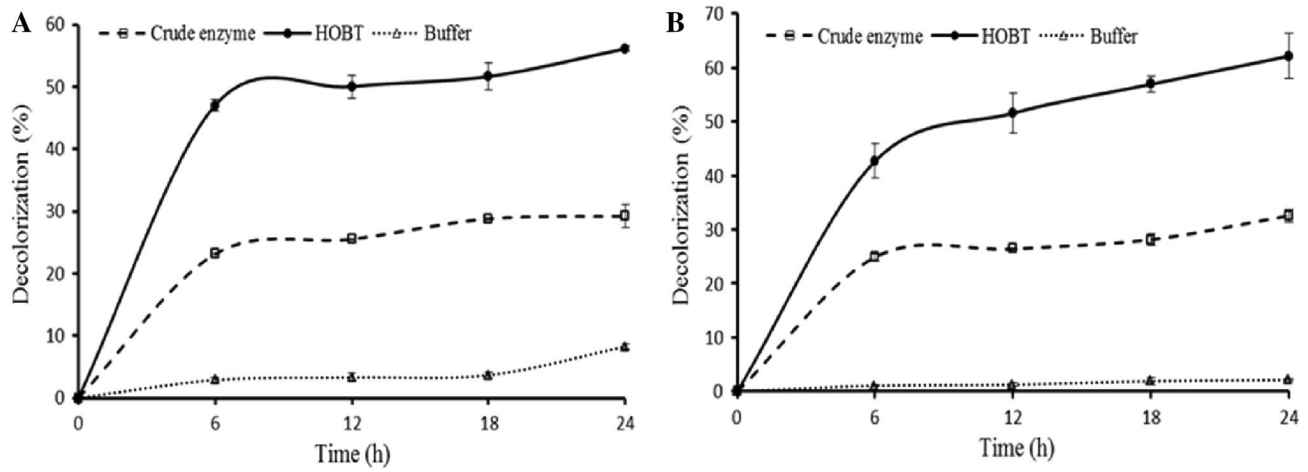

Fig. 6 Dye decolorization by crude laccase and decolorization catalyzed by HOBt. **a** The decolorization of CBBR-250, and **b** The decolorization of CR. Bars indicate the standard deviation (n = 3)

40]. The maximum value of xylanase activity of *Bacillus* sp. TPF-1 was 3.5-fold higher than that of *Bacillus amyloliquefaciens* MIR 32 [41] and was even higher than that of the multiple enzyme-producing fungus *Aspergillus fumigatus* N2 (91.90 U/mL) [42].

The improvement in the saccharification rate of celluloses and hemicelluloses in biomass plays a key role in industrial bioconversion [43]. In this study, the reducing sugars released from green algae by different enzymatic complexes were compared. The greatest yield of reducing sugars was detected in the enzyme cocktail containing crude enzymes and commercial cellulase, and the additive effect was observed in the saccharification of green algae with the enzyme cocktail. Inefficient saccharification is mainly caused by limited substrate accessibility [44], and xylanase can facilitate cellulose saccharification by breaking down hemicellulose and providing cellulose accessibility [1]. Therefore, synergistic action could be found when the xylanase released from *Bacillus* sp. TPF-1 was combined with commercial cellulases during the saccharification of green algae. In the process of industrial production of biofuels, saccharification of the biomass without pretreatment can save time and reduce economic costs [4].

The greatest enzymatic activity of laccase was obtained by cultivating with *S. fusiforme*. As a type of polyphenol oxidase, laccase is of particular interest in the textile, paper, and pulp industries' biodegradation and biotransformation of pollutants because of its ability to utilize a broad range of substrates [13, 45]. A previous study found that the extracts of *S. fusiforme* have remarkable antimicrobial and antioxidant effects because of the existence of rich polyphenols [46]. The enzymatic activities of polyphenol oxidases have been reported to increase significantly when polyphenols are supplemented as substrates [47, 48]. This may be the reason why *S. fusiforme* obviously improved the laccase production of *Bacillus* sp. TPF-1. *S. fusiforme* is a type of marine algae classified as brown algae, and it has been studied as an efficient ingredient in Chinese herbal medicine [49]. Most studies on this alga were focused on the multiple applications of its polysaccharide extracts in nutriology and pharmacology [50–52]. However, to the best of our knowledge, investigations on enzyme induction in *S. fusiforme* are still limited.

The laccase produced by *Bacillus* sp. TPF-1 showed a remarkably low pH tolerance for the oxidation of ABTS (below 2.2). Although most classical laccases have an acidic pH optimum [14], only some fungal laccases have shown good ability in a very acidic range (pH < 3.0) [53], and reports about *Bacillus* sp. strains are few. As the laccase activity in this study could be increased up to 7037.28 U/L under pH 2.2, this pH value was used for dye decolorization. Laccases have the ability to degrade various recalcitrant compounds [54–56]. In this study, the laccase from *Bacillus* sp. TPF-1 had a continuous and stable decolorization effect on CBBR-250 and CR. Additionally, the effect was significantly enhanced by using HOBt as a mediator, which has been proved to have a promoting function in dye decolorization [57]. The high efficiency of dye decolorization under extremely acidic conditions indicated a promising application of the laccase–mediator system in extremely acidic dye effluents.

Conclusion

In this study, *Bacillus* sp. TPF-1 was isolated and selected as a high lignocellulolytic enzyme-producing bacterial strain. Green algae were found to be a well-suited carbon source for the high production of CMCase and xylanase from *Bacillus*

sp. TPF-1. Therefore, unpretreated green algae were efficiently saccharified by using CECX, and the supplementation of commercial enzyme showed a synergistic effect in the process of saccharification. Moreover, *S. fusiforme* was the optimum substrate to improve the production of laccases. The laccase from *Bacillus* sp. TPF-1 could efficiently decolorize different synthetic dyes (CBBR-250 and CR) in combination with a mediator (HOBt) under acidic conditions. Considering all of the above, *Bacillus* sp. TPF-1 is a multifunctional bacterium having the potential to utilize algal biomasses to produce lignocellulolytic enzymes for the bioconversion of algal biomass and the biodegradation of chemical dyes in strongly acidic industrial wastewater.

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Compliance with Ethical Standards

Conflict of interest No conflict of interest exits in the submission of this manuscript. We declare that we do not have any commercial or associative interest that represents a conflict of interest in the work submitted.

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