Efficient Lignin Degradation of Corn Stalk by *Trametes* with High Laccase Activity and Enzymatic Stability in Salt and Ionic Liquid

Shi-Jia Dong,[a,1] Bi-Xian Zhang,[b,1] Fu-Ling Wang,[a] Liu Xin,[a] Yun-Fei Gao,[b] Wei Ding,[a,*] Xin-Miao He,[b,c] Di Liu,[b,c,*] and Xiao-Mei Hu [a,*]

The screening of new potential microbes for the selective degradation of lignin makes agricultural straws available to achieve the complete conversion to biofuel. Because of the capability of laccase to oxidize phenolic compounds and to reduce the molecular oxygen of water, laccase has attracted much interest in recent years for its industrial applications. In this study, a fungal strain with a relatively high laccase activity was isolated from corn farm residue and identified as *Trametes* KS-2. The maximum laccase activity was 631 U/L for *Trametes* KS-2 when glucose/corn stalk (1/1, w/w) and peptone were used as the carbon source and nitrogen source, with 0.09 mmol of Cu^{2+} at pH 5.5 and 28 °C for 10 days. Laccase activity of *Trametes* KS-2 was relatively stable in the presence of salt and an ionic liquid. Scanning electron microscopy analysis indicated the morphological alteration of lignocelluloses *via Trametes* KS-2 treatment. Remarkable degradation of lignin in corn stalk was achieved with *Trametes* KS-2. After 15 days, the lignin was noticeably reduced to 76 mg, and the degradation rate was increased to 65.4%. *Trametes* KS-2 could be potentially utilized in the microbial degradation of lignin for lignocellulosic biomass and the industrial production of laccase.

Keywords: Lignin degradation; Trametes; Laccase; Corn stalk

*Contact information: a: Northeast Agricultural University, Harbin, 150030, China; b: Key Laboratory of Combining Farming and Animal Husbandry, Ministry of Agricultural and Rural Affairs, 150086, P.R. China; c: Heilongjiang Academy of Agricultural Sciences, Harbin, 150086,China; [1]These authors contributed equally to this work; *Corresponding author: huxiaomei1982@163.com;*

INTRODUCTION

Lignocellulosic biomass is a potentially valuable resource for biofuels and bioproducts. Agricultural straws are the most available lignocellulosic biomass and non-food resource. An annual total of 700 million tons of agricultural straws are produced in China, accounting for 20% to 30% of the world's production.

Lignocellulosic biomass consists mainly of three polymeric components, which are lignin, hemicelluloses, and cellulose. Lignin is a three-dimensional aromatic polymer in the form of the phenylpropanoids p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). Lignin is considered to be a physical barrier to the biological and chemical hydrolysis of cellulose, making lignocellulose inherently recalcitrant (Himmel *et al.* 2007). Lignin can be depolymerized by thermochemical methods such as pyrolysis, hydrogenolysis, gasification, hydrolysis, and chemical oxidation under supercritical conditions (Pandey and Kim 2011). However, these pretreatment technologies require significant energy inputs, environmentally harmful processes, or produce hazardous waste. Biodegradation serves as an attractive energy-saving and environmentally friendly option. The use of microbial

degradation of lignin makes the lignocellulose available to achieve the complete conversion to biofuel.

Microbial degradation of lignin has been primarily studied with the use of white-rot fungi, such as *Phanerochaete chrysosporium*, *Ganoderma lucidum*, *Ceriporiopsis subvermispora*, *Lentinus edodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus* (Potumarthi *et al.* 2013; Cianchetta *et al.* 2014; Ma and Ruan 2015), which are capable of producing several extracellular ligninolytic enzymes including laccase, manganese peroxidase (MnPs), lignin peroxidase (LiPs), and versatile peroxidase (Xu *et al.* 2017). Moreover, laccases have attracted much interest in recent years because of their low substrate specificity and good oxidative abilities for their industrial applications, such as dye decolorization (Abadulla *et al.* 2000), pulp biobleaching in the paper industry (Arias *et al.* 2003), the production of valuable compounds from lignin, soil bioremediation, biodegradation of environmental phenolic pollutants, and removal of endocrine disruptors (Fukuda *et al.* 2001; Kidwai *et al.* 2012; Sole *et al.* 2012; Divya *et al.* 2013a).

Biological decomposition is a more acceptable, feasible, and economical process in the production of biofuels. However, white rot fungi that are suitable for biodelignification of wood may not be suitable for biodelignification of agricultural straws. More efforts on selecting the most effective strain for different lignocelluloses are necessary. In addition, the selective degradation of lignin should be improved due to the ability of some white rot fungi to degrade the lignin simultaneously with cellulose. Furthermore, most of the corn straw has not been well utilized in China, and straw burning is still used, which causes environment pollution and haze weather. In this study, a strain with selective degradation of lignin in corn stalk was isolated and identified. The culture conditions for the enzyme production of the strain including carbon and nitrogen sources, incubation time and temperature, Cu^{2+} concentration, and initial pH were optimized. The enzyme activity in the presence of salt and ionic liquids was determined. The degradation of lignin from corn stalk by this strain was also investigated.

EXPERIMENTAL

Materials

Screening procedure

The surface soil (0 to 5 cm) was collected from a corn farm of Keshan County in the Heilongjiang province in China. A total of 10 g of the soil sample was added into 90 mL of enrichment medium (sucrose 30 g, $CuSO_4$ 0.5 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.5 g, $NaNO_3$ 2 g, KCl 0.5 g, $FeSO_4$ 0.01 g, and water 1000 mL). The medium was incubated at 150 rpm and 28 °C for 3 days. Following that, 1 mL of the suspension was mixed with 9 mL of deionized water and the suspension was diluted into 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} (mL/mL). Then, 200 μL of dilution was applied on the potato dextrose agar (PDA) medium (potato 200 g, glucose 20 g, agar 15 g, and water 1000 mL) and incubated at 28 °C for 7 days. The single colony of fungi was isolated on the PDA medium.

Next, the single colony was incubated on the PDA medium at 28 °C for 7 days. Then, 1 cm of agar piece was removed and transferred into 30 mL of PDA medium with the addition of guaiacol (0.1%, w/w) or aniline blue (0.1%, w/w). The solutions were cultured at 28 °C for 7 days with shaking at 150 rpm. Each sample was cultured three times. The strains with reddish brown zones were selected as potential microbes for producing

laccase. The strains with color fading zones were selected as potential microbes for producing manganese peroxidase or lignin peroxidase.

Enzyme assay

A total of 1 cm of agar piece was added into 100 mL of fermentation medium (glucose 10 g, ammonium tartrate 0.2 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, $CaCl_2$ 0.1 g, vitamin B_1 100 mL [0.1 g/L], $CuSO_4$ 0.007 g, $MnSO_4$ 0.035 g, $ZnSO_4$ 0.006 g, $FeSO_4$ 0.005 g, $CoCl_2$ 0.001 g, Tween 80 (Aladdin, Shanghai, China) 1 mL, acetic acid/sodium acetate buffer 100 mL [0.1 mol/L], water 1000 mL) at 28 °C with an inoculation size of 3% at 150 rpm (Shaker: ZQLY-108S; Shanghai Zhichu Instrument Co., Shanghai, China) for an appropriate time. The resulting solution was then centrifuged at 10,000 rpm (Allegra TMX-22R; Beckman Coulter Inc., Brea, CA, USA) for 5 min at 4 °C to give the crude enzyme solution. Enzyme assay was performed in 4 mL of the solution containing 0.5 mL ABTS (2,2′-azino-bis[3-ethylbenzthiazoline-6-sulfonate], 0.5 mmom/L) (Wang *et al*. 2013), the crude enzyme (200 μL), and acetic acid/sodium acetate buffer (3.3 mL, 0.1 mol/L) at 30 °C for 3 min. The absorbance was measured at 420 nm on a UV-mini-1240 spectrometer (Shimadzu, Kyoto, Japan). Three replicates were performed. One unit (U) of enzyme activity was defined as the amount of the enzyme that oxidized 1 μmol of ABTS/min under the conditions indicated.

Molecular identification

A DNA kit (Omega, Georgia, USA) was used to extract the DNA of the fungi. Fungal analysis was performed with the universal primers ITS1 (5'-TCCGTAGGT-GAACCT GCGG-3') and ITS4 (5'-TCCTCGCCTTATTGATATGC-3'). The sequence was amplified *via* polymerase chain reaction (PCR) with 50 μL of the mixture containing template DNA (2 μL), forward primer (2 μL), reverse primer (2 μL), ddH_2O (19 μL), and 2× Taq PCR MasterMix (25 μL, Tiangen, Beijing, China). The PCR products were sequenced by the Huada Company (Beijing, China). The bioinformatics tools were available online *via* BLAST and the MEGA program (Molecular Evolutionary Genetics Analysis Company, version 5.0, USA) with the neighbor-joining (NJ) algorithm, were employed to build phylogenetic trees (Felsenstein 1985). The ITS sequence data for the fungal samples were deposited into the National Center for Biotechnology Information (NCBI) GenBank database.

Methods

Optimization of the conditions for laccase production

Individual carbon sources, including starch, glucose, sucrose, lactose, xylose, corn stalk powder, poplar powder, and bamboo powder, at a concentration of 1% (w/w) were measured with peptone as the nitrogen source with inoculation size of 3% (w/w) after incubating at 150 rpm and 28 °C for 7 days on the basis of fermentation medium. Nitrogen sources, including peptone, ammonium sulfate, ammonium chloride, urea, and ammonium nitrate, at a concentration of 0.2% (w/w) were measured with glucose/corn stalk (1/1, w/w) as the carbon source with inoculation size of 3% (w/w) after incubating at 150 rpm and 28 °C for 7 days. In addition, pH (pH 3.0, pH 4.0, pH 4.5, pH 5.0, pH 5.5, pH 6.0, and pH 6.5), Cu^{2+} concentration (0 mmol/L, 0.03 mmol/L, 0.06 mmol/L, 0.09 mmol/L, 0.12 mmol/L, and 0.20 mmol/L), and incubation temerature (18 °C to 38 °C) with 5 intervals were investigated with glucose/corn stalk (1/1, w/w) as the carbon source and peptone as the nitrogen source for 7 days at 28 °C.

Determination of laccase activity in the presence of salt and ionic liquid

1-Ethyl-3-methylimidazolium acetate ([EMIM]CH_3COOH) was prepared using a published method in the literature (Wasserscheid and Welton 2003). The laccase activity of the crude enzyme was determined under different concentrations of NaCl and ionic liquid in the presence of 0%, 2.5%, 5.0%, and 7.5% (w/v) of NaCl and 0%, 2.5%, 5.0%, and 7.5% (w/v) of ionic liquid after 1 h.

SEM analysis

Scanning electron microscopy (SEM) analysis was conducted on a Hitachi S-3400N microscope (Hitachi, Tokyo, Japan). Prior to acquiring images, the samples were mounted with double sided carbon tape on corn stalk sample stubs and sputter coated (Hitachi, Tokyo, Japan) with approximately 30 angstroms of Au/Pd with a 5 kV accelerating voltage.

Lignin degradation

The corn stalk powder was incubated with *Trametes* KS-2 at 28 °C and pH 5.5 with glucose/corn stalk (1/1, w/w) and peptone for 5 days, 10 days, and 15 days. The remaining solid was filtered and dried under vacuum at 60 °C for 24 h. The components of corn stalk was determined according to National Renewable Energy Laboratory standard procedure (NREL) (Sluiter *et al.* 2011; Zhang *et al.* 2015). Lignin degradation was determined as the ratio of the division of the lignin (mg) before and after *Trametes* KS-2 treatment to the lignin (mg) in corn stalk according to Eq. 1:

$$\text{Lignin degradation (\%)} = \frac{\text{Original Lignin (mg)} - \text{Lignin after } \textit{Trametes} \text{ KS-2 Treatment (mg)}}{\text{Original Lignin (mg)}} \quad (1)$$

RESULTS AND DISCUSSION

Isolation and Identification of the Microbes

If microbes had the ability to degrade lignin, a reddish brown zone could be produced in the medium containing guaiacol due to the oxidation of guaiacol by this strain (Wang 2013). If the diameter of the zone was larger and the red color was deeper, the capability of the strain to produce laccase was stronger. The strains that produced a reddish brown zone were isolated as the positive microbes, indicating their ability to secrete laccase. The enzyme activities of these microbes were then determined.

The supernatant of the microbes became visibly green following exposure to ABTS (2,2′-azino-bis[3-ethylbenzthiazoline-6-sulfonate]) and a linear increase in the absorbance at 420 nm was observed. Laccase activity was measured based on the ABTS oxidation at 420 nm. As shown in Fig. 1, the laccase activity noticeably increased as time increased from 4 days to 10 days. A high laccase activity (107 U/L) was observed with glucose and peptone at 28 °C after 10 days. After that, the enzyme activity was reduced. Initially, nutrients were efficiently utilized by microbes, which resulted in a high yield of laccase, and then the laccase production was decreased due to the depletion of nutrients in the fermentation medium.

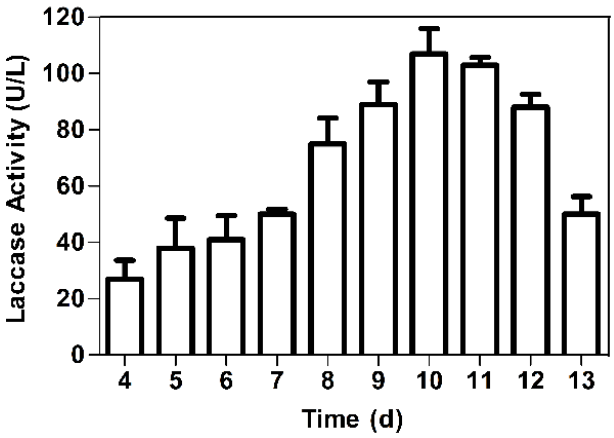

Fig. 1. Effect of fermentation time on the laccase production

Molecular Identification of Microbes

The strain was determined based on its ITS sequence of DNA. A phylogenetic tree is shown in Fig. 2. The ITS sequence of KS-2 showed 99% sequence similarity with *Trametes hirsuta* SYBC-L8(HQ891292), *Trametes hirsuta* 5154(EF546240), *Trametes hirsuta* A19(KC414249), *Trametes hirsuta* NBRC 6477(AB733168), *Trametes hirsuta* SICAU SDT36(KJ028001), and *Trametes hirsuta* XSD-65(EU326211) (Fig. 2). Therefore, the strain was identified as *Trametes* KS-2.The obtained nucleotide sequence has been submitted to the NCBI GenBank under the accession number MK182788. Laccase production occurs in various fungi over a wide range, for example, white rot fungi such as *Lentinus tigrinus* (Ferraroni *et al*. 2007), *Pleurotus ostreatus* D1 (Pozdniakova *et al.* 2006), *Trametes sp*. Strain AH28-2 (Xiao *et al.* 2003), *Trametes pubescens* (Shleev *et al.* 2007), and *Trametes versicolor* (Arora and Gill 2000). *Trametes* KS-2 might be a new effective strain for laccase production.

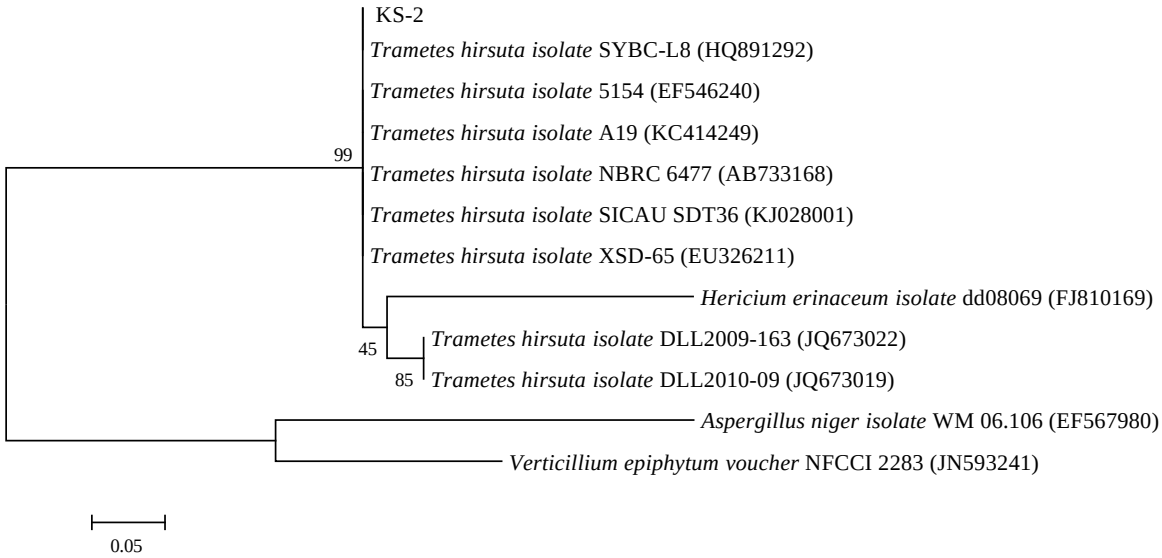

Fig. 2. Phylogenetic relationship of KS-2 strain

Optimization of the Conditions for Laccase Production

Effects of carbon sources

Generally, an appropriate carbon source is important for the successful production of laccase. In this work, the favorable carbon sources, including starch, glucose, sucrose, lactose, and xylose, and the natural lignocellulosic materials, including corn stalk powder, poplar powder, and bamboo powder, were investigated at 30 °C and pH 7.2 with peptone at 150 rpm for 10 days. The results are shown in Fig. 3.

Both corn stalk and glucose were the most effective carbon sources, while starch slightly reduced laccase production. Glucose is a widely used carbon source for most microbes, and a good enzyme yield was obtained at 107 U/L with glucose in this study. In addition, lignin was required by *Trametes* KS-2; thus, higher laccase activity was observed at 118 U/L in the presence of corn stalk as the sole carbon source. The chemical composition, the chemical or physical associations, and the accessibility of lignin and other valuable components in corn stalk increased the enzyme hydrolysis by *Trametes* KS-2. However, a low activity was observed when poplar and bamboo powder were used, as their chemical constitutions and fiber characteristics are different from agricultural straws (Scurlock *et al.* 2000). A selective degradation of corn stalk was found for *Trametes* KS-2.

Furthermore, the laccase activity was increased in the glucose-containing medium as compared to those produced in the glucose-free medium. The maximum laccase activity (321 U/L) was achieved when glucose and corn stalk were added at the ratio of 1/1 (w/w). This result was in agreement with earlier reports that the presence of glucose as a readily metabolizable substrate could enhance laccase activity (Couto and Herrera 2006; Mechichi *et al.* 2006). Laccase production was dependent on the nature of the carbon source used in the culture medium. Both corn stalk and glucose provided the appropriate nutrients for the fermentation process of *Trametes* KS-2.

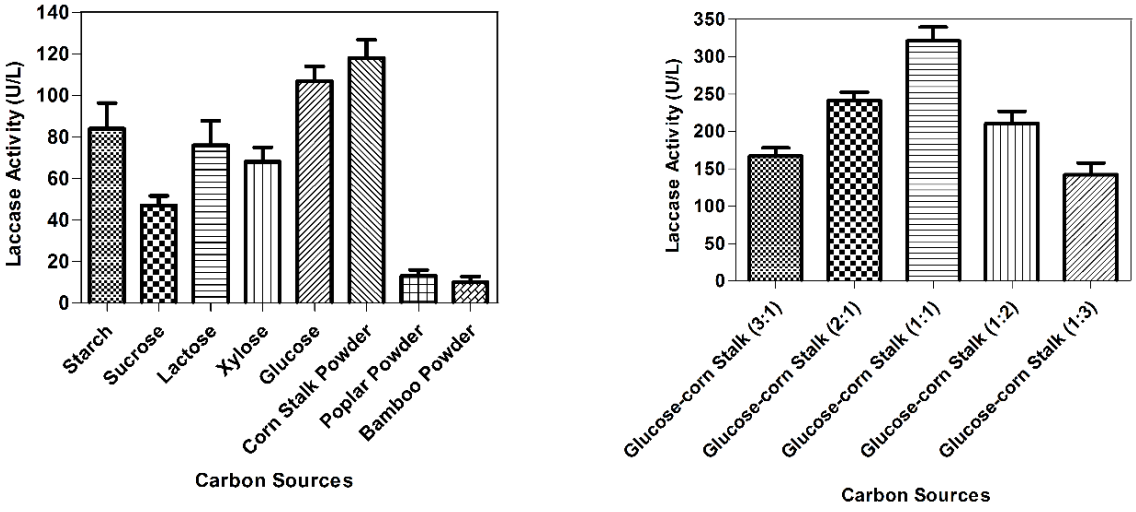

Fig. 3. Effects of carbon sources on the laccase production

Effects of nitrogen sources

The investigation of nitrogen sources was performed at 30 °C and pH 7.2 with corn stalk/glucose (1/1, w/w) at 150 rpm for 5 days. As shown in Fig. 4, peptone was the suitable organic nitrogen source for *Trametes* KS-2, which supported the high level of laccase

activity up to 574 U/L. Good enzyme yield was obtained when ammonium sulfate was used as the inorganic nitrogen at 485 U/L.

Laccase production occurs during the secondary metabolic phase of the fungi and is often triggered by nitrogen depletion. Thus, variation in the nitrogen source has a considerable effect on the metabolic processes of *Trametes* KS-2. Peptone as the effective nitrogen source supported high enzyme yield for *Trametes* KS-2.

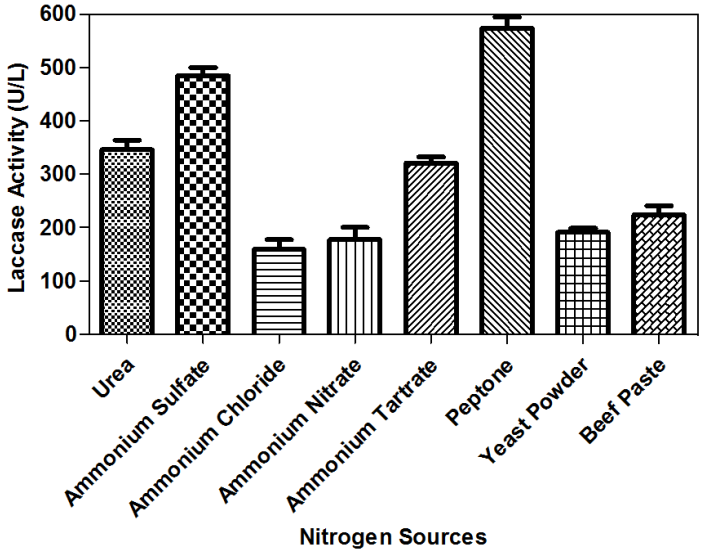

Fig. 4. Effects of nitrogen sources on the laccase production

Effect of Cu^{2+} concentration

Laccases are oxidoreductases that contain copper ions at the catalytic center and play a crucial role in lignin degradation (Kiiskinen *et al.* 2002). The investigation of Cu^{2+} concentration was performed at 30 °C and pH 4.0 for 10 days. As shown in Fig. 5, a high laccase activity of *Trametes* KS-2 of 598 U/L was obtained with Cu^{2+} concentration at 0.09 mmol/L.

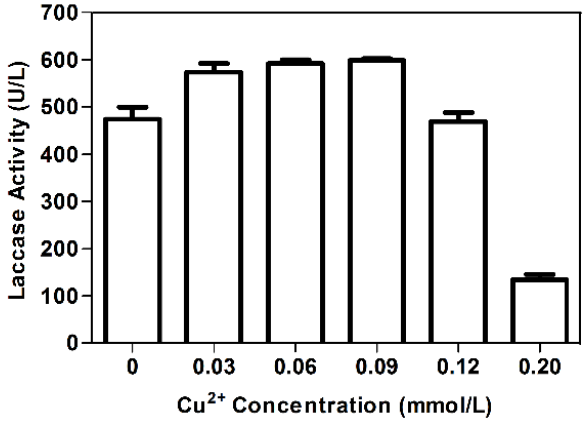

Fig. 5. Effect of Cu^{2+} concentration on laccase production

The investigation of Cu^{2+} concentration was performed at 30 °C and pH 4.0 for 10 days. As shown in Fig. 5, a high laccase activity of *Trametes* KS-2 of 598 U/L was obtained with Cu^{2+} concentration at 0.09 mmol/L. Laccase activity was noticeably inhibited at concentrations above 0.2 mmol/L. There is no significant difference in laccase activity under the Cu^{2+} concentration of 0.03-0.09 mmol/L.

Effect of initial pH

Analysis of pH was completed at 30 °C for 5 days. As shown in Fig. 6, the laccase activity exhibited good stability in the range of pH 4.5 to 5.5. The optimal pH of *Trametes* KS-2 appeared at pH 5.5 and the laccase activity was 626 U/L.

The initial pH of the medium is one of the important factors that affects microbe growth, enzyme production, and transport of various components across the cell membrane. A pH value that is lower or higher than the optimum affects the metabolic activities of the organism. It also influences the stability of the enzyme and may lead to protein denaturation (Kalra and Sandhu 1986).

The optimum pH for the microbes with high laccase production was reported to be between pH 4.5 and 6.0 (D'Souza *et al.* 2006; Sadhasivam *et al.* 2008). The optimum pH for *Trametes* KS-2 was obtained in the range of pH 4.5 to 5.5.

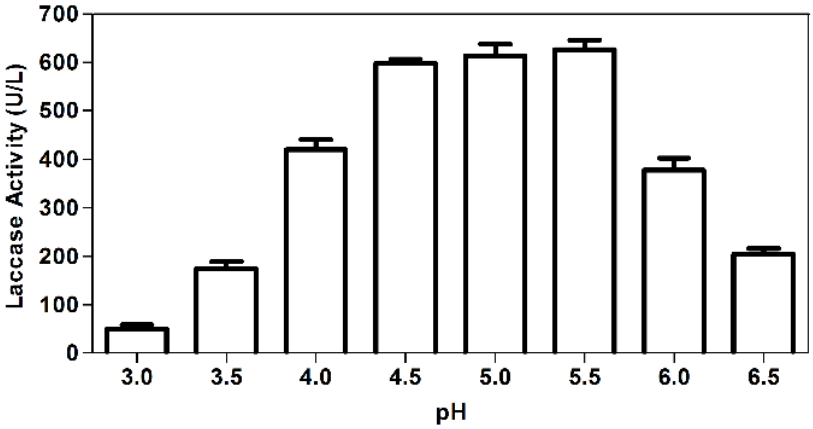

Fig. 6. Effect of pH on laccase production

Effect of incubation temperature

As presented in Fig. 7, the laccase activity was increased from 18 °C to 28 °C and decreased from 28 °C to 38 °C. High enzyme production was found at 28 °C for 10 days at pH 5.5 and the resulting laccase activity was 631 U/L.

Incubation temperature is a major process parameter of a fermentation system due to alterations in microbial protein structure and properties with temperature variations. At lower or higher temperatures, metabolic activities are reduced with consequent inhibition in growth and enzyme synthesis, resulting in the reduction of enzyme production.

Therefore, the maximum laccase activity was 631 U/L for *Trametes* KS-2 in the optimum conditions with glucose/corn stalk (1/1, w/w) and peptone for 10 days at 28 °C at pH 5.5. The culture conditions of a particular strain were well optimized for the maximum production of laccase and subsequent industrial implementation.

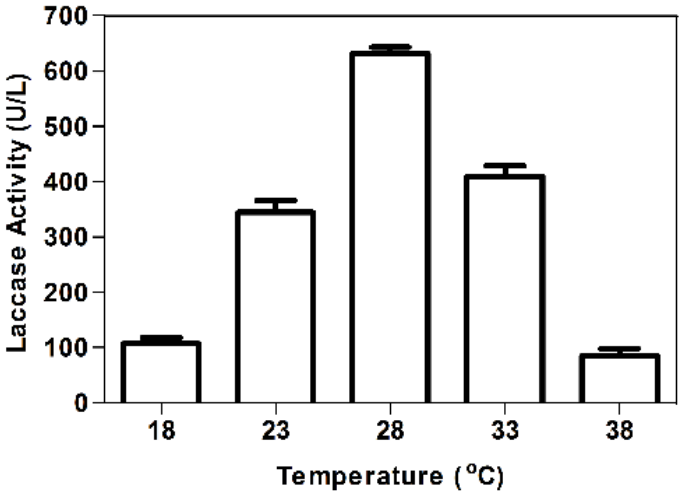

Fig. 7. Effect of temperature on the laccase production

Laccase Activity of *Trametes* KS-2 in the Presence of Salt

Laccase activity of *Trametes* KS-2 was evaluated under different NaCl concentrations of 0% to 7.5% (w/v). Maximum activity was achieved at 2.5% (w/v) NaCl concentration (Fig. 8); 687 U/L of original laccase activity was observed. The enzyme was found to be stable at NaCl concentrations up to 5% (w/v), and 96% of its original activity was retained. A slight reduction in laccase stability was observed at NaCl concentrations up to 7.5% (w/v).

In high salt concentrations, proteins contain an excessive number of negatively charged acidic amino acids on their surface, possibly to form a hydrated ion network or to prevent the protein aggregation through electrostatic repulsive charges at the protein surface to keep the protein soluble (Zhang *et al*. 2011).

Salt stability is one of the preferred characteristics for enzymes. Laccase from *Trametes* KS-2 has better prospects for application purposes.

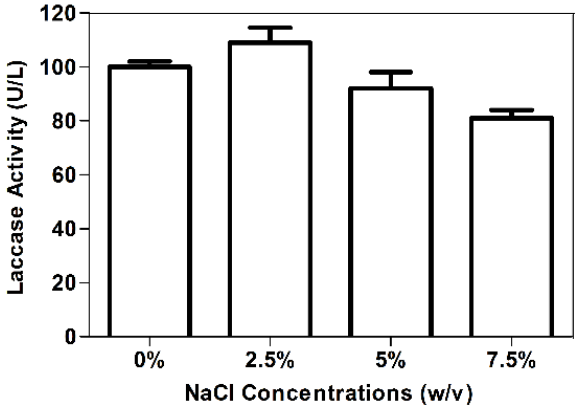

Fig. 8. Laccase activity of *Trametes* KS-2 in the presence of salt

Laccase Activity of *Trametes* KS-2 in the Presence of ILs

Ionic liquid (IL)-assisted pretreatment of lignocellulosic biomass has been extensively studied and [EMIM]CH_3COOH was widely reported as an effective pretreated solvent. However, because the fungal enzymes were inhibited by ILs (Turner *et al.* 2003; Kamiya *et al.* 2008), extensive water washing was required to remove any residue from ILs, which increased the difficulty of IL recovery and its ability to be reused. It is important to identify the relatively stable enzymes in the presence of ILs to decrease the amount of washing and subsequently reduce the extra cost.

Laccase activity of *Trametes* KS-2 was investigated under different concentrations of 1-ethyl-3-methylimidazolium acetate ([EMIM]CH_3COOH) in the range of 0% to 7.5% (w/v). As shown in Fig. 9, laccase activity was 580U/L in the medium containing 2.5% (w/v) of [EMIM]CH_3COOH. As the concentration of [EMIM]CH_3COOH was increased to 5% (w/v), 377 U/L of laccase activity was retained.

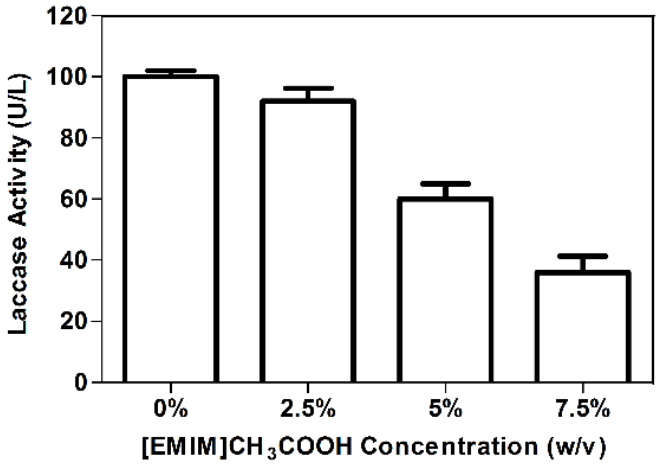

Fig. 9. Laccase activity of *Trametes* KS-2 in the presence of ILs

SEM Analysis

The morphological modification of the corn stalk that was treated by *Trametes* KS-2 for an appropriate time was analyzed. The original corn stalk showed the compact ordered and intact structures (Fig. 10). A relatively smooth surface was observed. After the treatment with *Trametes* KS-2, it became cracked and porous. Some holes appeared in the corn stalk. The morphological alteration indicated the disruption of linkages in corn stalk and the remarkable reduction of lignin content after fungal treatment.

Lignin Degradation of Corn Stalk

The results of lignin degradation of corn stalk are shown in Table 1. Corn stalk consist 42.8% cellulose, 18.7% hemicellulose, 20.8% lignin and 0.7% ash. Initially, lignin was measured as 100% (208 mg) in the original corn stalk according to a National Renewable Energy Lab (NREL) method (2011). After 5 days, the lignin content was reduced to 136 mg and lignin degradation was 40.1%. As the fermentation time was increased, the lignin degradation was increased. After 10 days, the lignin was decreased to 97 mg and the degradation was 53.3%.

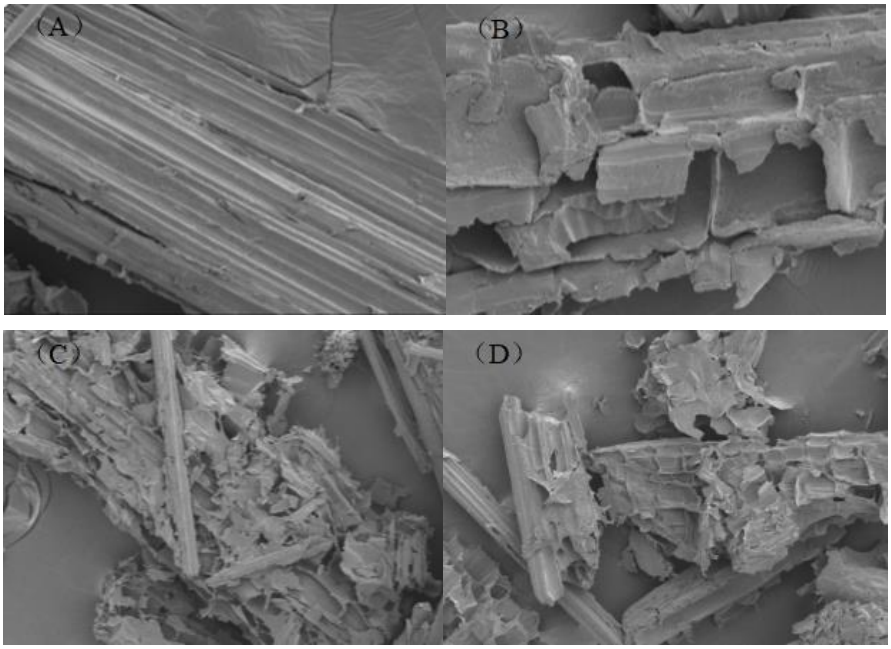

Fig. 10. SEM analysis: (A) original corn stalk (100 μm), (B) after 5 days (100 μm), (C) after 10 days (500 μm), and (D) after 15 days (500 μm)

After 15 days, the lignin was reduced to 76 mg and the degradation was increased to 65.4%.

It was reported that 28.3% lignin degradation was achieved with *Phanerochaete chrysosporium*, and 34.7% was obtained with *Fusarium moniliforme* after 10 days fermentation with rice straw as the carbon substrate. Cellulase activities were also observed for *Phanerochaete chrysosporium* (Chang *et al*. 2012). *Myrothecium verrucaria* was reported as an effective fungus for selectively removing lignin and led to lignin degradation that reached 45.50 ± 2.12% in birch sawdust (Wang *et al.* 2017). In this study, high lignin degradation (65.4%) was observed for *Trametes* KS-2 with high laccase production. The cellulase activities were further studied. No filter paper cellulase (FPase), endoglucanase (CMCase), or β-glucosidase was obtained.

Table 1. Lignin Degradation of Corn Stalk

Time (d)	5	10	15
Lignin After KS-2 Treatment (mg)	136	97	76
Lignin Degradation (%)	40.1	53.3	65.4

Discussion

Lignin, the most recalcitrant component of lignocellulosic material, acts as a barrier and prevents access of any lignocellulolytic enzymes to the interior lignocellulosic structure. Microbes are increasingly reported to be responsible for the efficient lignin degradation in lignocelluloses, primarily by producing lignin peroxidase (Lip) and manganese peroxidas (MnP). *Phanerochaete chrysosporium* ME-446 was described to be able to effectively produce Lip and MnP in the medium containing wheat straw (Kapich *et al.* 2004). *Fusarium moniliforme* was found capable of highly selective lignin degradation in rice straw by producing Lip and MnP (Chang *et al*. 2012). *Fusarium concolor* selectively

delignified wheat straw based on the production of Lip, MnP, and laccase (Li *et al.* 2008). In this study, laccase was obtained from *Trametes* KS-2, while neither lignin peroxidase nor manganese peroxidase activities were detected, indicating that the degradation of lignin was specifically dependent on laccase activity. It was reported that the maximum laccase activity for *Pleurotus ostreatus* strains was up to 168.8 U/L (An *et al.* 2018). Laccase activity was obtained at a high production (631 U/L) for *Trametes* KS-2.

In addition, selective degradation on lignin was preferred for the production of biofuels because cellulose and hemicelluloses could be further utilized by cellulase. However, a reduction of hemicellulose and cellulose was observed for some microbes with high laccase activity. Ma *et al.* (2015) described a type of bacteria named *P. ananatis* Sd-1 with 2.59 U/mL of Lip activity and 0.61 U/mL of laccase activity. Lignin, cellulose, and hemicelluloses of rice straw were reduced 35.6%, 75.2%, and 78.8%, respectively, after 6 days by *P. ananatis* Sd-1 (Ma 2016). The ideal pretreatment for enzymatic saccharification should minimize the content of lignin and maximize the retention of cellulose (Ding *et al.* 2012). In this study, high lignin degradation (65.4%) was observed for *Trametes* KS-2. No cellulase activity was detected and no noticeable reduction of holocellulose was observed. A selective degradation of lignin in corn stalk was favorable for *Trametes* KS-2.

Furthermore, salt stability is one of the preferred properties for enzymes for industrial application purposes. Good laccase activity of *Trametes* KS-2 was observed at 2.5% to 5% (w/v) NaCl concentration. A relatively stable capability in the presence of 2.5% of ionic liquid was also found, which would decrease the amount of washing, reduce extra costs, and improve recovery and reuse of ILs.

CONCLUSIONS

1. In this study, a fungal strain was newly isolated and identified as *Trametes* KS-2. Based on the optimal conditions, the maximum laccase activity was 631 U/L when glucose/corn stalk (1/1, w/w) and peptone were used at pH 5.5 and 28 °C for 10 days.
2. Laccase activity of *Trametes* KS-2 was relatively stable in the presence of NaCl (2.5% to 5%). A slight reduction of activity was observed in the presence of an ionic liquid.
3. The SEM analysis indicated the morphological alteration of lignocellulose. Noticeable degradation of lignin in corn stalk was achieved by *Trametes* KS-2. After 15 days, the lignin was reduced to 76 mg and the degradation increased to 65.4%.
4. *Trametes* KS-2 could be attractive for potential biotechnological applications in selectively removing lignin from corn stalks for biofuels. The resulting high laccase production could be utilized in dye decolorization, paper pulp bleaching, or phenolic chemical degradation.

ACKNOWLEDGMENTS

This work was supported financially by the General Financial Grant from the 54th China Postdoctoral Science Foundation (2013M540308), the Heilongjiang Postdoctoral Foundation (LBH-Z12233), and the Harbin Science and Technology Innovative Talents Project (RC2012QN002143).

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Article submitted: March 1, 2019; Peer review completed: May 4, 2019; Revised version received: May 8, 2019; Accepted: May 9, 2019; Published: May 21, 2019.

DOI: 10.15376/biores.14.3.5339-5354