Solid-state Fermentation on Poplar Sawdust and Corncob Wastes for Lignocellulolytic Enzymes by Different *Pleurotus ostreatus* Strains

Mei-Ling Han,[a,c] Qi An,[a,b,*] Sai-Fei He,[a] Xiao-Lin Zhang,[a] Ming-Hui Zhang,[a] Xin-Hua Gao,[a] Qian Wu,[a] and Lu-Sen Bian [d]

Solid state fermentation with different lignocellulolytic materials as inducers was used for lignocellulolytic enzyme production in this study. *Pleurotus ostreatus* strains were assessed by measuring laccase, CMCase, and xylanase activities. The secretion potential of the lignocellulolytic enzymes by wild and cultivated strains was analyzed for the first time. The wild and cultivated strain showed their unique capacities for secreting lignocellulolytic enzymes on solid-state fermentation with different lignocellulosic materials. The wild *P. ostreatus* strain preferred corncob for the secretion of laccase and xylanase activity, but the cultivated strain preferred poplar sawdust. The wild strain and cultivated strain showed a consistent preference for poplar sawdust for the secretion of CMCase activity. The wild strain was advantageous because it achieved the maximum hydrolytic enzyme activities within a short time period. Poplar sawdust and corncob were conducive to laccase secretion by the wild or cultivated strains and the rapid accumulation of laccase on solid-state fermentation. Additionally, continuous, stable laccase production was an extremely important advantage by solid-state fermentation of poplar sawdust, particularly in the wild strain. These findings are helpful in selecting the appropriate strain that corresponds to suitable lignocellulosic materials. The optimization of integrated industrial lignocellulolytic enzyme production can also be achieved.

Keywords: Pleurotus ostreatus; Corncob; Poplar wood; Wild and cultivated strains; Lignocellulolytic enzymes

Contact information: a: College of Life Science, Langfang Normal University, Langfang 065000, Hebei, China; b: Technical Innovation Center for Utilization of Edible and Medicinal Fungi in Hebei Province, Langfang 065000, Hebei, China; c: Edible and Medicinal Fungi Research and Development Center of Universities/Colleges in Hebei Province, Langfang 065000, Hebei, China; d: Experimental Centre of Forestry in North China, Chinese Academy of Forestry, Beijing 102300, China;
** Corresponding author: fungiqian@yahoo.com*

INTRODUCTION

Lignocellulosic biomass, the most abundant and renewable eco-friendly resource on earth, has attracted attention for producing bioenergy and other value-added industrial products (Pinar *et al*. 2017). Due to the rapid development of agriculture and forestry, huge amounts of biomass waste are produced. Based on dry weight, the annual global primary production of biomass is about 220 billion tons (Chandra *et al*. 2012; Saratale *et al*. 2017). In addition, rational use of biomass is also conducive to solving the problem of environmental pollution because of the prohibited burning of agricultural and forestry waste. The production of corn was about 257 million tons in China, and the planting area of poplar exceeded 7 million ha. Corn and poplar related wastes have very little to no

economic value. They are quite abundant and have become heavily under-valued resources in China. One major product obtained from the bioconversion of lignocellulosic biomass is bioethanol. The process of converting lignocellulosic biomass to bioethanol is facilitated by hydrolytic enzymes. Another major way lignocellulosic biomass is used is to produce edible and medicinal fungi. Edible and medicinal white rot fungi can utilize a variety of lignocellulosic residues by producing several extracellular secreted enzymes. These enzymes grow fruiting bodies for human consumption. Therefore, cultivation of edible fungi on corn and poplar byproducts may be one of the possible solutions for converting these agricultural and forestry wastes into accepted edible food of high and useful market value (Xie *et al.* 2017). Additionally, there is also a process of producing lignocellulolytic enzymes by solid fermentation.

Pleurotus ostreatus (Jacq.) P. Kumm., an edible basidiomycete, is both a white rot fungus and an edible fungus. *P. ostreatus* grows on lignocellulosic biomass and is one of the well-known producers of lignocellulolytic enzymes (Knežević *et al*. 2013; An *et al*. 2016b). All basidiomycetes belonging to the genus *Pleurotus* can secrete extracellular hydrolytic and oxidative enzymes. These enzymes relate to the degradation of lignocellulose with different efficiencies (Reddy *et al.* 2003; Elisashvili *et al.* 2006; Kachlishvili *et al.* 2006). Lignocellulolytic enzymes contain two groups: the ligninolytic enzymes and the cellulolytic enzymes. The ligninolytic enzymes contain peroxidases and oxidases, while the cellulolytic enzymes contain cellulases and hemicellulases (An *et al*. 2016b; Nguyen *et al.* 2018). Lignocellulolytic enzymes are highly specific, safe, and efficient biocatalysts that are exploited in different industrial processes (Asgher *et al.* 2016). They carry importance and have vast applications in many fields, especially in the area of industry and environmental protection. Utilizing low-cost biological residues from agriculture and forestry as substrates for growing microorganisms may constitute an interesting alternative in the enzyme industry (Lamia *et al*. 2017).

Many factors affect the secretion of lignocellulolytic enzyme production by fungi, such as the fermentation method, the type and complexity of carbon and nitrogen sources, ion concentration, temperature, and more (Stajić *et al.* 2006; Sohail *et al.* 2009; Zhang *et al.* 2013; Bentil *et al.* 2018; Ferraz *et al.* 2019; Filipe *et al.* 2019; Akpinar and Urek 2020; Rajavat *et al.* 2020). The types of substrates and the mode of cultivation of fungal species play an essential role in enzyme productivity. These factors also affect the resulting enzyme activity profile (Elisashvili *et al.* 2008a). The influences of co-cultivation (Metreveli *et al.* 2017), nutrient medium (An *et al.* 2016b), and ion type (Saparrat *et al.* 2010) also have been investigated. Compared to submerged fermentation (SmF), solid-state fermentation (SSF) is a procedure in which the substrate barely appears in water (Nguyen *et al.* 2019). The low moisture content in the fermentation of microorganisms is limited primarily to yeasts, fungi, and bacteria (Acharya *et al.* 2010). Fungi are highly adaptable to SSF because the fungal hyphae features are spread across the surface. They easily penetrate inter-particle spaces where their colonization is typically more efficient than other organisms in solid substrates (Sukumaran *et al.* 2005; Nguyen *et al.* 2019). More importantly, using solid state fermentation can help avoid the dilution of enzyme products due to the high content of water in submerged fermentation (Oostra *et al.* 2000).

The production of laccase using an alkaline lignin inducer is different in cultivated and wild strains of *P. ostreatus* (An *et al*. 2018). However, the effects of different lignocellulosic materials on hydrolytic enzyme production in cultivated and wild strains have not been reported in fungi, particularly in white rot fungi. This study aimed to measure the effects of different lignocellulosic materials on lignocellulolytic enzymes production

in *P. ostreatus* and lay a foundation for selecting lignocellulosic materials suitable for different strains producing lignocellulolytic enzymes. This was accomplished using cultivated and wild strains tested in the conventional solid-state fermentation method.

EXPERIMENTAL

Materials

Two white-rot fungal strains, one cultivated strain *P. ostreatus* CCEF99 and one wild strain CY568, were obtained from the Institute of Microbiology, Beijing Forestry University (Beijing, China). All organisms were maintained on a Complete Yeast Medium (CYM) agar medium (glucose 20 g per L, peptone 2 g per L, yeast extract 2 g per L, $MgSO_4 \cdot 7H_2O$ 0.5 g per L, $K_2HPO_4 \cdot 3H_2O$ 1 g per L, KH_2PO_4 0.46 g per L, and agar 20 g per L) at 4 °C in the College of Life Science, Langfang Normal University.

Poplar wood and corncob were collected from Langfang Normal University (Hebei, China) and farmers in Chengde city, Hebei province, China. All biomass was chopped into small pieces. All residues were air-dried and ground. The particles of size 20 to 80-mesh were prepared for subsequent use.

Methods

Organism and inoculum preparation

The microorganism was transferred to new CYM agar medium plates and incubated at 26 °C for 7 days. Inoculants with a diameter of 5 mm were then made on a flat plate covered with mycelium by a hole punch. Under aseptic conditions, 6 inoculants were placed in 250 mL flasks containing 100 mL of the CYM medium (glucose 20 g per L, peptone 2 g per L, yeast extract 2 g per L, $MgSO_4 \cdot 7H_2O$ 0.5 g per L, $K_2HPO_4 \cdot 3H_2O$ 1 g per L, and KH_2PO_4 0.46 g per L). The medium was then cultured on a rotary shaker (ZHICHENG, Shanghai, China) at 26 °C with a speed of 150 rpm. After 7 days, mycelial pellets were harvested and homogenized with a laboratory blender (Tianjin hengao Technology Development Co., Ltd, Tianjin, China) for 2 min at 5000 rpm. The resulting suspension was used as an inoculum.

Culture conditions

The solid-state fermentation (SSF) of the poplar sawdust and corncob was performed individually at 26 °C in 250 mL flasks. The flasks contained 3 g of the substrate and were also moistened with 12 mL of basal medium (glucose 15 g per L, yeast extract 6 g per L). All flasks were autoclaved at 121 °C for 30 min. After being cooled, 3 mL of the homogenized mycelium was used to inoculate every flask containing the substrate that was moistened with the basal medium. All flasks were incubated at 26 °C.

To determine the enzymes activities under different substrate and strain conditions, the laccase activities were tested on which fungi grew from day 1 to day 10, day 15, day 20, day 25, day 30, and day 35. CMCase and xylanase activities were tested on day 5, day 10, day 15, day 20, day 25, day 30, and day 35 of the fungi growth. The extracellular enzymes were extracted with 50 mM sodium acetate buffer (pH 5.5). Briefly, 100 mL of the sodium acetate buffer mentioned above was added into SSF flasks for the extraction process. The extractions were performed on a rotary shaker at 10 °C with a speed of 150 rpm for 5 h (An *et al*. 2016b). The extracted liquid was filtered by Whatman No. 1 filter paper, then centrifuged at 4 °C with a speed of 12,000 rpm for 20 min. The supernatant

was the crude enzyme liquid, which was used for measuring the enzyme activity.

Enzyme activities assays

The laccase (EC 1.10.3.2) activity was determined by the changes in the absorbance at 415 nm, related to the rate of oxidation of 1 mM 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) in a 50 mM sodium acetate buffer (pH 4.2). The assay mixture was measured using an iMark^{TM} Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute ($\varepsilon_{415} = 3.16 \times 10^4$ M^{-1} cm^{-1}).

The endoglucanase (CMCase) activity was determined in accordance with previously described methods (Ghose 1987). Briefly, 0.5 mL of the appropriately diluted enzyme was extracted with 1.5 mL of 1% w per v carboxymethyl cellulose, which dissolved in a sodium citrate buffer (50 mM, pH 5.0) at 40 °C for 30 min. The reaction was terminated by adding 3 mL of 3,5-dinitrosalicylic acid reagent and boiled for 10 min. The xylanase activity was determined in accordance with previously described methods (Bailey *et al*. 1992). The process was like the CMCase method. The absorption was 540 nm. One unit of endoglucanase or xylanase activity was defined as the amount of enzyme releasing 1 μmol of reducing sugar per minute under the assay conditions.

All measurements were derived from three independent experiments and the mean values were taken. The standard deviations for the experiments were less than ± 10%.

Statistical analysis

To examine the effects of substrates and strains on laccase, CMCase, and xylanase activities, a two-way analysis of variance followed by the Tukey post hoc test was applied to these variables. The substrates and strains were the fixed factors (PROC GLM, IBM SPSS software version 22.0; Armonk, NY, USA). All statistical figures were generated using the Origin Pro 8.0 program (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

The Effects of Strains and Substrates on the Production of Laccase Enzymes

Many studies indicate that the particle size, concentration, type, and nature of substrates used as inducer show a notable effect on laccase production (Han *et al.* 2017, 2018; Leite *et al.* 2019). Similarly, laccase production is also significantly affected by different species or different strains that belong to the same species (p-value < 0.001) (Janusz *et al.* 2015; An *et al.* 2016a, 2018; Huang *et al.* 2019). As shown in Table 1, the effect that the substrates have on the laccase activities of *P. ostreatus* was significant most of the time (p-value < 0.05), except on day 5. Meanwhile, the effect of the strains on the laccase activities of *P. ostreatus* was also significant most of the time (p-value < 0.05), except on day 3, day 8, and day 25.

Obviously, the CY568 wild strain and the CCEF99 cultivated strain showed their unique capacities for secreting laccases on solid-state fermentation with different lignocellulosic material (Fig. 1). The laccase production trend for the CY568 wild strain was different under the substrates of sawdust and corncob. The laccase activity for the CY568 wild strain under sawdust fermentation and corncob fermentation conditions ranged from 36.77 ± 2.17 U/L to 353.83 ± 11.94 U/L and 9.64 ± 0.52 U/L to 440.73 ± 8.36

U/L, respectively (Fig. 1). The maximum laccase activity for the CY568 wild strain obtained from the corncob substrate was higher than that obtained from the sawdust substrate (approximately 1.25-fold as seen in Table 2).

Table 1. Effects of Strains, Substrates, and Strains × Substrates Interactions on the Laccase Activities of *Pleurotus ostreatus* (Two-Way ANOVA)

Incubation Period (d)	Strain	Substrate	Strain × substrate
1	276.898***	872.222***	60.469***
2	346.975***	108.649***	18.787**
3	4.140	13.766**	11.169*
4	7.261*	27.830***	56.657***
5	68.426***	0.894	10.005*
6	62.684***	6.239*	42.173***
7	577.230***	343.197***	162.031***
8	0.344	1172.586***	220.569***
9	98.218***	752.755***	956.274***
10	86.547***	528.421***	0
15	203.222***	2207.150***	686.116***
20	9.824*	486.024***	47.810***
25	2.068	550.832***	42.880***
30	354.384***	2234.061***	85.313***
35	10.715*	575.145***	91.759***
*Note: df = 1, 1, 1; *P < 0.05, **P < 0.01, ***P < 0.001			

The maximum laccase activity for the CY568 strain from sawdust was on day 5, and that of the corncob was on day 7. According to Fig. 1, the laccase activity of the CY568 strain was high and stable from the beginning to day 35 in the presence of sawdust. However, in corncob the laccase activity of the CY568 strain was higher in the early stage and decreased in the later stage. At 35 days, it was only 17.08 ± 1.06 U/L from the corncob fermentation, which was about 1/7 of the laccase activity from the sawdust fermentation. The laccase production trend for the CCEF99 cultivated strain was similar under the sawdust and corncob substrates. The laccase activity for the CCEF99 cultivated strain under sawdust fermentation and corncob fermentation conditions ranged from 67.21 ± 3.67 U/L to 548.72 ± 19.59 U/L and 16.88 ± 1.51 U/L to 286.12 ± 25.80 U/L, respectively (Fig. 1). The maximum laccase activity for the CCEF99 cultivated strain obtained from the sawdust substrate was higher than that obtained from the corncob substrate (approximately 1.92-fold as can be seen in Table 2). This was distinguished from the CY568 wild strain. The maximum laccase activity for the CCEF99 strain from the sawdust was on day 9, and that of the corncob was on day 5 (Table 2). The laccase activity of the CCEF99 strain was high and relatively stable from the beginning to day 35 when sawdust was used as the inducer (Fig. 1). The result was similar to the laccase activity from the CY568 strain under the sawdust fermentation. On the other hand, the laccase activity from the CCEF99 strain under fermentation with corncob was relatively low and unstable. Overall, the laccase production of the CCEF99 cultivated strain on poplar sawdust was more efficient than that of the CY568 wild strain. In other words, the CCEF99 cultivated strain showed higher laccase production than the CY568 wild strain when poplar sawdust moistened with a basal

medium was used as the inducer on the solid-state fermentation. However, the wild *P. ostreatus* strain showed higher laccase activity than the cultivated *P. ostreatus* strain when only alkaline lignin or poplar sawdust was used as the inducer in submerged fermentation. The advantage is relatively obvious (An *et al.* 2018). It seemed the results were opposite, while this phenomenon was understandable. A possible reason was that the cultivated strains were all domesticated. The domesticated edible fungi grew better on the related cultivated substrates, such as sawdust and cottonseed hull. On the corncob substrate, the laccase production of the CY568 wild strain was higher than that of the CCEF99 cultivated strain. The dominant strains on the two substrates were just the opposite. To some extent, it also reflected the selective specificity of the strain to the substrate.

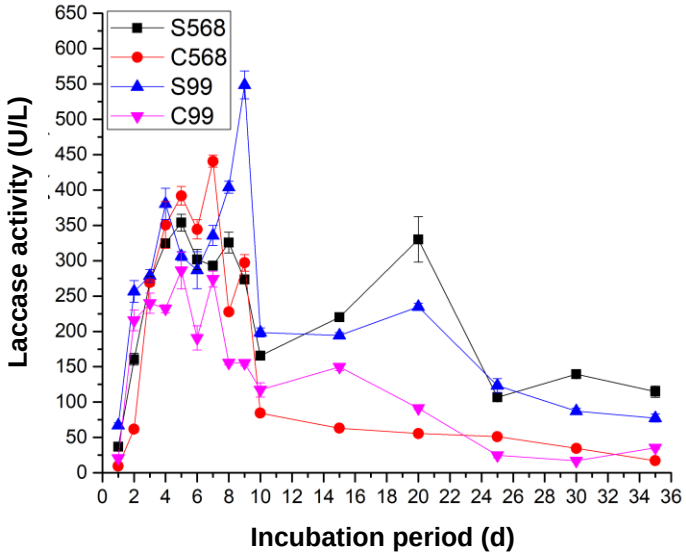

Fig. 1. The effects of different strains and substrates on the laccase production by *Pleurotus ostreatus*. The average values were calculated from individual measurements for each of the three parallel cultures of the two strains. S568 indicates poplar sawdust with a wild *P. ostreatus* strain CY568; C568 indicates corncob with a wild *P. ostreatus* strain CY568; S99 indicates poplar sawdust with a cultivated *P. ostreatus* strain CCEF99; and C99 indicates corncob with a cultivated *P. ostreatus* strain CCEF99.

Table 2. Maximum Laccase Activities, Lignocellulosic Material, Strains, and the Time of *Pleurotus ostreatus* Strains

Maximum Laccase Activity (U/L)	Lignocellulosic Material	Strain	Time (day)
353.83 ± 11.94	Poplar sawdust	CY568	5
548.72 ± 19.60	Poplar sawdust	CCEF99	9
440.73 ± 8.36	Corncob	CY568	7
286.12 ± 25.80	Corncob	CCEF99	5
*Note: data are presented as the mean ± standard deviation for triplicates and are expressed as U per L.			

The presence of poplar wood is conducive to secreting laccase by *P. ostreatus* strains in submerged fermentation, but this is a slow process for accumulating laccase (An *et al.* 2018). Similar to a previous study, poplar sawdust could promote the laccase production of wild or cultivated strain on solid state fermentation, as found in this study.

Additionally, continuous and stable laccase production was found to be an extremely important advantage by solid-state fermentation of poplar sawdust. This was previously a nonexistent phenomenon of wild or cultivated *P. ostreatus* strains in liquid fermentation. The maximum laccase activity with a lignin-rich walnut shell as the inducer by *Funalia trogii* is higher than with corncob as the inducer on solid-state fermentation (Birhanli and Yeşilada 2013). The maximum laccase activity with a powdered walnut shell as the inducer by *F. trogii* or *Trametes versicolor* is higher than with powdered wheat straw as the inducer in submerged fermentation (Birhanli and Yeşilada 2013). The maximum laccase activities secreted by *P. ostreatus* strains used corncob and complete yeast media as the inducer are higher than using sawdust and complete yeast media as the inducer in submerged fermentation (Han *et al.* 2017). The laccase activity in tree leaves solid-state fermentation by *P. ostreatus* 2175, *P. ostreatus* IBB8, *P. ostreatus* IBB108, and *P. ostreatus* 2191 is 15 U per flask, 7 U per flask, 14 U per flask, and 14 U per flask, respectively (Elisashvili *et al.* 2008b). The laccase activity in wheat straw solid-state fermentation by *P. ostreatus* 2175, *P. ostreatus* IBB8, *P. ostreatus* IBB108, and *P. ostreatus* 2191 is 12 U per flask, 7 U per flask, 10 U per flask, and 17 U per flask, respectively (Elisashvili *et al.* 2008b). Additionally, the lignin content of leaves and wheat straw varied greatly from 0% and 16 to 21%, respectively (Sánchez 2009). The laccase activity in tree leaves solid-state fermentation or in wheat straw solid-state fermentation by *P. ostreatus* 2175, *P. ostreatus* IBB8, *P. ostreatus* IBB108, and *P. ostreatus* 2191 has almost no difference (Elisashvili *et al.* 2008b). All the above studies indicate that the lignin content is not directly proportional to the laccase production secreted of fungi induced by these lignocellulosic materials. A roughly similar phenomenon was also found in this study. The lignin content of corncobs and hardwood stems was 15% and 18 to 25% (Sánchez 2009). The laccase activity on the corncob substrate was sometimes higher than on the sawdust substrate (Fig. 1).

The Effects of Strains and Substrates on the Production of CMCase Enzymes

Cellulase is a generic name for the group of enzymes that catalyze the hydrolysis in cellulose. It is a class of an inducible enzymes produced by organisms, especially fungi. These enzymes can either be free or grouped into a multienzyme complex. The cellulase enzyme system mainly involves three components: endo-β-1, 4-glucanase (EC 3.2.1.4), exo-β-1, 4-glucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Zhang *et al.* 2006). Fewer articles have considered the effects of wild strains and cultivated strains on cellulose production. Thus, in this research a comparative study of endoglucanase production by wild and cultivated *P. ostreatus* strain on different lignocellulose materials was conducted.

The results demonstrated that the CMCase activities of the two *P. ostreatus* strains were significantly different ($P < 0.001$), except on day 15 (Table 3). Various lignocellulose substrates remarkably affected the CMCase activities of different *P. ostreatus* strains ($P < 0.001$), except on day 5, 10, and 25 (Table 3). The extracellular CMCase activity varied from 19.16 ± 0.32 U/L to 35.52 ± 2.79 U/L and 13.03 ± 0.32 U/L to 24.93 ± 0.56 U/L by *P. ostreatus* CY568 on poplar sawdust and corncob, respectively (Fig. 2). The corresponding maximum CMCase activity appeared on days 5 and 10 (Table 4). However, the CCEF89 strain was different from the CY568 strain. The difference was mainly reflected in the maximum enzyme activity and occurrence time. The extracellular CMCase activity varied from 7.27 ± 0 U/L to 45.16 ± 4.65 U/L and 5.98 ± 0.32 U/L to 32.76 ± 0.49 U/L by *P. ostreatus* CCEF99 on poplar sawdust and corncob, respectively (Fig. 2). The corresponding maximum CMCase activity appeared on days 30 and 35 (Table 4). In terms of the maximum CMCase activity, the CCEF99 cultivated strain showed superiority to the

CY568 wild strain. In terms of the time of maximum CMCase activity, the CY568 wild strain performed higher than the CCEF99 cultivated strain (Table 4). On the other hand, the maximum CMCase activity and occurrence time of the two strains were higher on the poplar sawdust solid-state fermentation than on the corncob. Fungi cultivation in identical culture conditions reveal wide differences among both species and strains of the same species (Elisashvili *et al.* 2008b). In this respect, our results agreed with data of other authors reporting on the quantitative variations of the CMCase activity during solid state cultivation of *P. ostreatus* strains (Reddy *et al.* 2003; Elisashvili *et al.* 2008b; Membrillo *et al.* 2008; An *et al.* 2016b).

Table 3. Effects of Strains, Substrates, and Strains × Substrates Interactions on the CMCase Activities of *Pleurotus ostreatus* (Two-Way ANOVA)

Incubation Period (d)	Strain	Substrate	Strain × substrate
5	52.296***	0.024	574.903***
10	152.893***	4.794	102.037***
15	1.757	711.345***	0.224
20	64.424***	300.017***	1.729
25	27.453***	0.788	13.712**
30	61.316***	43.648***	24.212**
35	205.714***	173.145***	47.424***
*Note: df = 1, 1, 1; *P < 0.05, **P < 0.01, ***P < 0.001			

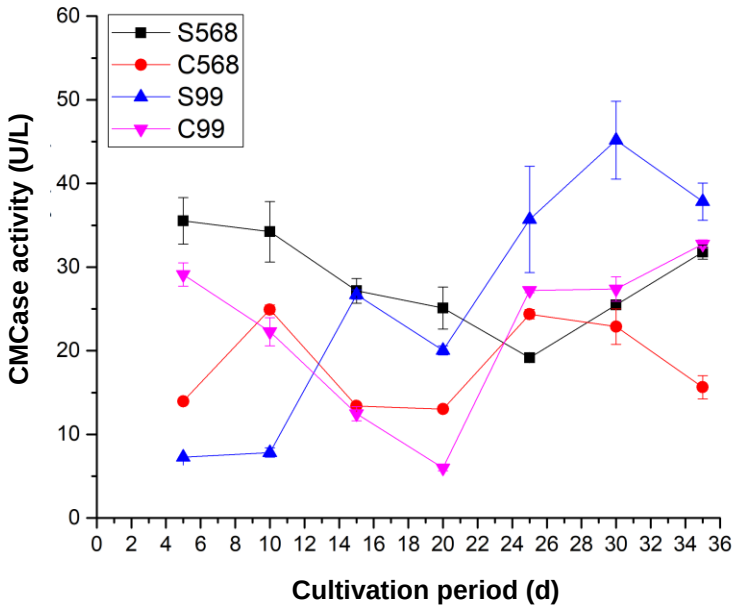

Fig. 2. The effects of different strains and substrates on CMCase production by *Pleurotus ostreatus*. The average values were calculated from individual measurements for each of the three parallel cultures of the two strains. S568 indicates poplar sawdust with a wild *P. ostreatus* strain CY568, C568 indicates corncob with a wild *P. ostreatus* strain CY568, S99 indicates poplar sawdust with a cultivated *P. ostreatus* strain CCEF99, and C99 indicates corncob with a cultivated *P. ostreatus* strain CCEF99.

Table 4. Maximum CMCase Activities, Lignocellulosic Material, Strains, and the Time of *Pleurotus ostreatus* Strains

Maximum CMCase Activity (U/L)	Lignocellulosic Material	Strain	Time (day)
35.52 ± 2.79	Poplar sawdust	CY568	5 th
45.16 ± 4.65	Poplar sawdust	CCEF99	30 th
24.93 ± 0.56	Corncob	CY568	10 th
32.76 ± 0.49	Corncob	CCEF99	35 th
*Note: data are presented as the mean ± standard deviation for triplicates and are expressed as U per L.			

The Effects of Strains and Substrates on the Production of Xylanase Enzymes

Although similar enzymes are involved for cellulose and hemicellulose biodegradation, more enzymes are required for the complete degradation of hemicellulose because of its greater heterogeneity (Malherbe and Cloete 2002). Hemicelluloses are biodegraded to monomeric sugars and acetic acid. Xylan is the main carbohydrate found in hemicellulose (Sánchez 2009). Complete degradation of xylan requires the cooperative action of a variety of hydrolytic enzymes. Hemicellulases are frequently classified according to their action on distinct substrates. Endo-1,4-β-xylanase (EC 3.2.1.8) generates oligosaccharides from the cleavage of xylan and xylan-1,4-β-xylosidase (EC 3.2.1.37) produces xylose from the oligosaccharides (Sánchez 2009). In this research, xylanase activities produced by the wild and cultivated *P. ostreatus* strains were used to compare the performance of different lignocellulose materials.

The effect of strain on xylanase activities of *P. ostreatus* was significant in the early days of cultivation before day 25 (p-value < 0.01). Meanwhile, the effect of the lignocellulose material on xylanase activities of *P. ostreatus* was less significant most of the time (p-value > 0.05) (Table 5). Two *P. ostreatus* strains maintained a higher xylanase activity than the CMCase activity in the detection stage (Fig. 3). The xylanase activity of the CY568 strain on day 5 in the poplar sawdust and corncob substrates were at approximately equivalent levels of 1279.59 ± 15.19 U/L and 1263.17 ± 27.76 U/L, respectively. Similarly, the xylanase activity of the CCEF99 strain was also approximately equivalent in both the poplar sawdust and corncob substrates on day 5 at 1110.22 ± 40.25 U/L and 1113.22 ± 110.8 U/L, respectively.

Previous research showed that the xylanase activity of *P. ostreatus* IBB 108 from tree leaves and wheat straw solid-state fermentation was approximately equivalent, about 220 ± 19 U/flask and 260 ± 20 U/flask (Elisashvili *et al.* 2008b). That was similar to our results. Interestingly, two strains showed distinct trends in their xylanase activities. However, for one strain the trend of xylanase activity was similar on different lignocellulose materials. The maximum xylanase activity of the CY568 strain was 1324.74 ± 46.31 U/L and 1339.11 ± 18.73 U/L on the poplar sawdust and corncob substrates on day 10 (Table 6). After the maximum xylanase activity, the trend of xylanase activity decreased and then increased (Fig. 3). The maximum xylanase activity of the CCEF99 strain on the poplar sawdust and corncob substrates was 1362.53 ± 66.52 U/L and 1305.68 ± 30.70 U/L on day 35 and 25, respectively (Table 6).

Table 5. Effects of Strains, Substrates, and Strains × Substrates Interactions on the Xylanase Activities of *Pleurotus ostreatus* (Two-Way ANOVA)

Incubation Period (d)	Strain	Substrate	Strain × substrate
5	20.529**	0.036	0.076
10	101.428***	0.186	0.107
15	71.176***	7.330*	0.021
20	37.799***	11.822**	0.370
25	0.380	3.450	3.450
30	1.073	0.366	1.378
35	0.612	14.579**	14.579**
*Note: df = 1, 1, 1; *P < 0.05, **P < 0.01, ***P < 0.001			

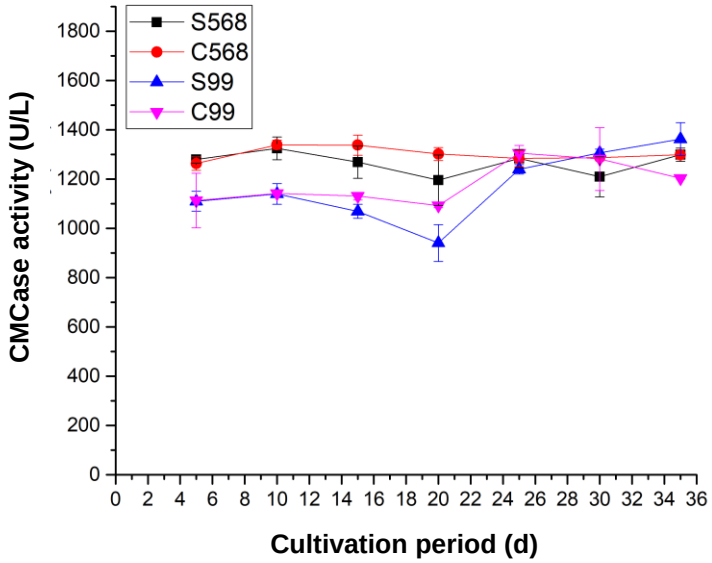

Fig. 3. The effects of different strains and substrates on the xylanase production by *Pleurotus ostreatus*. The average values were calculated from individual measurements for each of the three parallel cultures of the two strains. S568 indicates poplar sawdust with a wild *P. ostreatus* strain CY568, C568 indicates corncob with a wild *P. ostreatus* strain CY568, S99 indicates poplar sawdust with a cultivated *P. ostreatus* strain CCEF99, and C99 indicates corncob with a cultivated *P. ostreatus* strain CCEF99.

Table 6. Maximum Xylanase Activities, Lignocellulosic Material, Strains, and the Time of *Pleurotus ostreatus* Strains

Maximum Xylanase Activity (U/L)	Lignocellulosic material	Strain	Time (day)
1324.74 ± 46.31	Poplar sawdust	CY568	10
1362.53 ± 66.52	Poplar sawdust	CCEF99	35
1339.11 ± 18.73	Corncob	CY568	10
1305.68 ± 30.70	Corncob	CCEF99	25
*Note: data are presented as mean ± standard deviation for triplicates and are expressed as U/L.			

CONCLUSIONS

1. This study indicated that the biosynthetic potential of basidiomycetes could be highly dependent on the type of fungal and type of lignocellulosic substrate. The preference of wild strain and cultivated strain to lignocellulosic biomass was different.
2. In terms of the laccase and xylanase activity of *P. ostreatus*, the wild strain preferred corncob and the cultivated strain preferred poplar sawdust. The wild strain and cultivated strain showed a consistent preference to poplar sawdust for the secretion of CMCase activity.
3. The wild *P. ostreatus* strain showed exceptional potential for hydrolytic enzyme synthesis through solid-state fermentation with poplar sawdust or corncob as the inducer. The main advantage was that the wild strain could reach the maximum hydrolytic enzyme activities in both substrates within a short period of time.
4. The presence of poplar sawdust and corncob was conducive to secreting laccase by the wild or cultivated *P. ostreatus* strain on solid-state fermentation. Furthermore, it was a fast process for accumulating laccase. Additionally, the continuous and stable laccase production was an extremely important advantage by solid-state fermentation of poplar sawdust. This phenomenon was evident in the case of the wild strain.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (31700024), Science Technology Research and Guidance Project of Colleges and Universities in Hebei Province (Z2019001), and the Top-notch Youth Project of Colleges and Universities in Hebei Province (BJ2019007).

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Article submitted: March 3, 2020; Peer review completed: May 3, 2020; Revised version received: May 6, 2020; Accepted: May 7, 2020; Published: May 13, 2020.
DOI: 10.15376/biores.15.3.4982-4995