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Lignocellulose degradation patterns, structural changes, and enzyme secretion by *Inonotus obliquus* on straw biomass under submerged fermentation

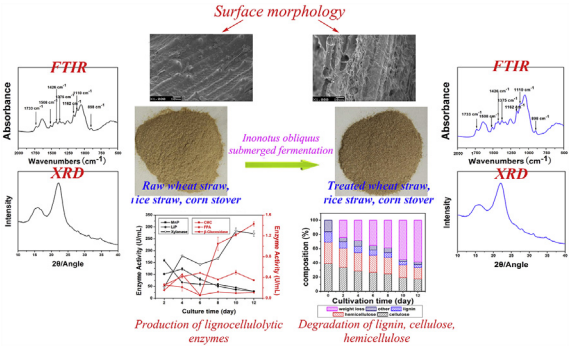
Xiangqun Xu *, Zhiqi Xu, Song Shi, Mengmeng Lin

College of Life Sciences, Zhejiang Sci-Tech University, China

HIGHLIGHTS

- Degradation patterns and structures of three straw biomass were compared.
- Lignin in wheat straw was selectively and effectively degraded.
- Crystalline cellulose in rice straw was significantly decreased.
- Degradation was in line with the production of ligninolytic and hydrolytic enzymes.

GRAPHICAL ABSTRACT



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ABSTRACT

This study examined the white rot fungus *I. obliquus* on the degradation of three types of straw biomass and the production of extracellular lignocellulolytic enzymes under submerged fermentation. The fungus process resulted in a highest lignin loss of 72%, 39%, and 47% in wheat straw, rice straw, and corn stover within 12 days, respectively. In merely two days, the fungus selectively degraded wheat straw lignin by 37%, with only limited cellulose degradation (13%). Fourier transform infrared spectroscopy revealed that the fungus most effectively degraded the wheat straw lignin and rice straw crystalline cellulose. Scanning electronic microscopy showed the most pronounced structural changes in wheat straw. High activities of manganese peroxidase (159.0 U/mL) and lignin peroxidase (123.4 U/mL) were observed in wheat straw culture on Day 2 and 4, respectively. Rice straw was the best substrate to induce the production of cellulase and xylanase.

1. Introduction

Agricultural residues are the most available sources of lignocellulosic biomass in the world and are therefore the materials of highest interest for the production of bio-fuels, chemicals, and polymeric materials. In China, an annual total of 700 million tons of agricultural residuals are produced originating from, for example, rice, wheat, corn, and bean, accounting for 20%-30% of the world total production.

Generally, the conversion of lignocellulose to glucose and further production of ethanol has to undergo three main steps: delignification, depolymerization and enzymatic hydrolysis (or fermentation). Two major facts hinder the lignocellulose degradation and conversion: (1) the highly protective lignin surrounding it that acts as a physical barrier against enzymatic attack, and (2) the

* Corresponding author.
E-mail address: xuxiangqun@zstu.edu.cn (X. Xu).

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recalcitrance of crystalline cellulose itself. Pretreatment is therefore required to alter the biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be more efficient with greater yields. A number of analyses have been developed to investigate the pretreatment effects on the lignocelluloses. These analyses indicate alterations in composition (e.g., by Fourier transform infrared spectroscopy, FTIR), crystallinity (e.g., by X-ray diffraction, XRD), pore size (e.g., by Simon staining), surface structure (e.g., by Scanning electron microscopy, SEM), enzymes adsorption/desorption, and degree of polymerization (Castoldi et al., 2014; Karimi and Taherzadeh, 2016).

Biological pretreatments have the advantage of being less expensive and environmentally friendly compared with mechanical, chemical, thermal, and thermo-chemical treatment (Sindhu et al., 2016). White rot fungi that can degrade lignin seem to be the most promising microorganisms for biological pretreatment (Rouches et al., 2016). Many studies reported the ability to ferment different crop residues of white rot fungi such as *Phanerochaete chrysosporium, Lentinus edodes, Coriolus versicolor, Ceriporiopsis subvermispora, Ganoderma lucidum, Pleurotus eryngii*, and *Pleurotus ostreatus* etc (Cianchetta et al., 2014; Ma and Ruan, 2015; Potumarthi et al., 2013). White rot fungi mainly degrade polysaccharides by hydrolytic enzymes like cellulases and xylanase, and lignin by oxidative ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) (Rouches et al., 2016).

Nonetheless, for an efficient pretreatment, the use of white rot fungi still has its limitations, such as the few lignin-degrading microbes, low activity of decomposing enzymes, difficult colonization of the substrate by the inoculum, and long cycle in solid state fermentation (weeks to months) (Rouches et al., 2016). Additional studies are also required to improve the selective degradation of lignin using white rot fungi due to the ability of some white rot fungi to degrade lignin selectively but some to do simultaneously with cellulose. In other words, the selective lignin degrading white rot fungi hold immense importance in enhancing the utilization of agro-residuals. The examples are *Lentinus edodes*, *Pycnoporus cinnabarinus*, *Crinipellis* sp. RCK-1, *Ceriporiopsis subvermispora*, *Phlebia brevispora, Pleurotus ostreatus* (Kuijk et al., 2015). However, more efforts on selecting the most effective strain for different lignocelluloses are needed to make the process more efficient by reducing the treatment time and carbohydrate loss (Kuijk et al., 2015).

The medicinal mushroom *Inonotus obliquus* (*I. obliquus*) is a white rot fungus that belongs to the family Hymenochaetaceae of Basidiomycetes. Found in Europe, Asia, and North America, it is well known as one of the most popular medicinal species due to its various pharmacological activities (Balandaykin and Zmitrovich, 2015). The mushroom produces a diverse range of bioactive metabolites including triterpenoids, polysaccharides, and polyphenols. However, in nature, *I. obliquus* is restricted to very cold habitats (45–50° N altitude) and grows very slowly on the trunks of Betula trees. Due to host specificity, rarity in nature and slow growth, the sclerotia of *I. obliquus* are not a reliable source for industrial production of these bioactive metabolites, when submerged culture of *I. obliquus* is a promising alternative for efficiently producing bioactive polysaccharides and polyphenols. Furthermore, a strategy has been developed: the lignocellulose bioconversion of wheat straw, rice straw, corn stover, sugarcane bagasse, and peanut shell to these compounds using *I. obliquus* under submerged fermentation. *I. obliquus* was capable of degrading these lignocellulosic materials (Chen et al., 2011; Xiang et al., 2012; Xu and Zhu, 2011; Zhu and Xu, 2013; Xu et al., 2014). However, the lignocellulosic degradation patterns and enzyme secretion by *I. obliquus* under submerged fermentation are unknown.

This present study, for the first time, attempted to answer the questions of (1) whether *I. obliquus* is able to produce lignocellulolytic enzymes induced by straw biomass under submerged fermentation; (2) whether it is a selective lignin degrading white rot fungus for the biodegradation of straw biomass. The objective was to discover *I. obliquus* as a new lignocellulolytic white rot fungus and to understand the efficacy for the pretreatment of straw biomass. The efficiency of the fungal process was studied in detail in order to clarify if the fungal performance presents any dependence with the substrate origin (wheat straw, rice straw, and corn stover). Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used to detect the main structural transformations.

2. Methods

2.1. Submerged fermentation of wheat straw, rice straw, and corn stover

2.1.1. Substrates

Rice straw, wheat straw, and corn stover were obtained from local farms of Jiangxi (rice straw) and Shandong provinces, China. The washed and dried substrate was ground and passed through 60-mesh sieve and trapped in a 100-mesh screen. The particles with sizes between 60 and 100 mesh were collected for submerged fermentation.

2.1.2. Inoculum preparation and fermentation

I. obliquus (CBS314.39) was maintained on malt extract agar slants containing (g/L): malt extract 30, peptone 3, and agar 15 at pH 5.6 ± 0.2. The fungus was cultivated at 25 °C for about 2 weeks, then stored at 4 °C, and subcultured every 3 months. One square centimeter of malt extract agar with mycelia was chipped off and transferred into a 250-mL Erlenmeyer flask with 100-mL medium (g/L): glucose 20, peptone 3, yeast extract 2, KH_2PO_4 1, $MgSO_4$ 1.5, and $CaCl_2$ 0.1 and cultured for 4–5 days in a rotary shaker (150 rpm) at 28 °C.

The harvested seed culture in the inoculum concentration of 8% was added into a 250-mL Erlenmeyer flask that contained 100-mL medium and incubated and at 28 °C in a rotary shaker at 140 rpm. The medium contained (g/L): ground wheat straw, or rice straw, or corn stover 30; corn starch 35, peptone 3, KH_2PO_4 1, $ZnSO_4{\cdot}2H_2O$ 0.01, K_2HPO_4 0.4, $FeSO_4{\cdot}7H_2O$ 0.05, $MgSO_4{\cdot}7H_2O$ 0.5, $CuSO_4{\cdot}5H_2O$ 0.02, $CoCl_2$ 0.01, and $MnSO_4{\cdot}H_2O$ 0.08, pH = 6.0 (Chen et al., 2011).

2.1.3. Sample preparation and determination of mycelial biomass

The fermentation broth was filtered from the residue at each time interval. The mycelia were separated from solid substrates and dried at 45 °C for 48 h for biomass dry weight (DW) determination. Although the methods such as ergosterol or 18 s rDNA quantification are recommended for fungal quantification in solid state fermentation in which fungal mycelia cannot easily be separated from solid substrates (van Kuijk et al., 2015), the biomass under submerged fermentation was determined by dry weight with simple steps in this study. The samples were thoroughly washed for 4–5 times through a 60-mesh sieve to make sure that the mycelia were free from solid substrates. The mycelium colonization much larger than 60-mesh aperture and substrates smaller than the aperture were collected for biomass determination, SEM images, and polymer loss analysis, respectively. It was noticed that a very small amount of wheat straw residue integrated with the mycelia on Day 2 even though after the separating process, so the determination on biomass and polymer loss of the wheat