International Biodeterioration & Biodegradation 110 (2016) 108–112

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Changes in lignocellulolytic enzyme activity during the degradation of *Picea jezoensis* wood by the white-rot fungus *Porodaedalea pini*

Sunardi [a, b, c], Jun Tanabe [a, b], Futoshi Ishiguri [a], Jyunichi Ohshima [a], Kazuya Iizuka [a], Shinso Yokota [a, *]

[a] Faculty of Agriculture, Utsunomiya University, Utsunomiya, Tochigi, 321-8505, Japan
[b] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 183-8509, Japan
[c] Faculty of Mathematic and Natural Sciences, Lambung Mangkurat University, South Kalimantan, 70714, Indonesia

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form
29 January 2016
Accepted 29 February 2016
Available online 22 March 2016

Keywords:
Porodaedalea pini
Picea jezoensis
Lignocellulolytic enzyme
Wood degradation

ABSTRACT

The extracellular lignocellulolytic enzymes secreted by *Porodaedalea pini* were investigated for their ability to degrade the wood of *Picea jezoensis* over various time periods. In addition, changes in wood chemical component contents were also investigated. Enzyme production and wood degradation by *P. pini* significantly increased starting at 60 days of incubation. The total lignin, holocellulose, and α-cellulose contents, as well as pH, decreased during the degradation process, while hot water, 1% NaOH, and ethanol-toluene extract contents significantly increased. These results indicate that *P. pini* simultaneously degraded the lignin and polysaccharides of *P. jezoensis* wood. Additionally, *P. pini* continuously produced xylanase, β-glucosidase, and endoglucanase with higher activities than those of exoglucanase and cellobiose dehydrogenase. Manganese(II)-dependent peroxidase showed the highest ligninolytic activity, followed by lignin peroxidase and laccase. These results indicate that *P. pini* produces a variety of lignocellulolytic enzymes, and that the produced enzymes contribute to the degradation of *P. jezoensis* wood components.

1. Introduction

The great capability of white-rot fungi to degrade wood components is mainly results from the activites of different kinds of extracellular enzymes (Baldrian, 2003; Levin et al., 2007; Dashtban et al., 2009; Irbe et al., 2014). Some white-rot fungi removed lignin, cellulose, and hemicelluloses simultaneously, while others selectively degraded lignin and hemicelluloses first, followed by cellulose (Eriksson et al., 1990; Yoshizawa et al., 1992; Machuca and Ferraz, 2001; Tanaka et al., 2009). These fungi secrete oxidative enzymes, such as lignin peroxidase, manganese(II)-dependent peroxidase, and laccase, to degrade lignin, as well as hydrolytic enzymes, such as cellulase, hemicellulases, and pectinase (Machuca and Ferraz, 2001; Ferraz et al., 2003; Levin et al., 2007).

Much research is needed to fully understand the degradation of wood by white-rot fungi, especially of fungal enzyme production. Such information could help to elucidate the biochemical mechanisms of wood degradation by fungi and, consequently, to facilitate fungal strain selection for biopulping and other industrial biotechnological applications (Machuca and Ferraz, 2001).

Porodaedalea is a genus of white-rot fungi that belongs to the *Hymenochaetaceae* family, and it is widely distributed in North America, East Asia, and Europe (Tomšovský et al., 2010). The fungus is a conifer parasite (Szewczyk et al., 2014) and an economically damaging pathogen of *Pinus* and *Picea* trees (Tomšovský et al., 2010). *Porodaedalea pini* is distributed in East Asia on indigenus *Picea jezoensis* (Siebold et Zucc.) Carrière (Dai, 1999), which is an important plantation tree species in Hokkaido, Japan (Narukawa et al., 2003).

Studies of wood degradation by *P. pini* and its enzyme production have been conducted by several researchers (Yamashita et al., 1978; Blanchette, 1980; Yoshizawa et al., 1992). Yoshizawa et al. (1992) examined the degradation of *Chamaecyparis obtusa* Endl sapwood by *P. pini*. They found that this fungus produces a cellulase whose activity is greater than that of ligninolytic enzymes, whereas it degrades lignin to a greater extent than carbohydrates (Yoshizawa et al., 1992). However, detailed relationships between the amounts of remaining wood chemical components and

* Corresponding author.
E-mail address: yokotas@cc.utsunomiya-u.ac.jp (S. Yokota).

http://dx.doi.org/10.1016/j.ibiod.2016.02.022
0964-8305/

enzymatic activities have not been established, especially for cellulose, hemicellulose, and ligninolytic enzymes during wood degradation by this fungus.

In the present study, *P. jezoensis* wood was degraded by *P. pini* under laboratory conditions. During the degradation, the types and amounts of enzymes produced were assayed. In addition, the relationships between enzymes and mass loss, wood chemical components, and pH change are discussed.

2. Materials and methods

2.1. Microorganism and culture conditions

A white-rot fungus, *P. pini* strain WD1174, which was originally provided from the Forestry and Forest Products Research Institue, Tsukuba, Japan, was used in this study. The fungus was pre-cultured on potato-dextrose-agar (PDA; Difco, Becton, Dickinson and Company, USA) medium in 9-cm diameter Petri dishes at 26 °C for 27 days. To study enzyme production and wood degradation, polypropylene bottles (volume, 850 ml; mouth diameter, 58 mm) containing 100 ml of medium (4% glucose, 0.3% peptone, 1.5% malt extract, and 2% agar) were sterilized by autoclaving (HV-110, Hirayama, Japan) at 121 °C and 1.2 atm for 20 min. Seven-millimeter-diameter mycelial disks were punched from pre-cultured PDA plates using a cork borer, and inoculated into the medium in bottles. Then, the fungus was incubated at 26 ± 2 °C and 70% relative humidity.

2.2. Wood samples

Small wood blocks (approximately 2.0 cm × 2.0 cm × 1.0 cm in radial, tangential and longitudinal directions, respectively) without any defects, such as resin, gum, or knots, and no visible evidence of decay, were prepared from wood samples collected from the heartwood of a single *P. jezoensis* tree. Before degradation, the specimens were dried at 60 °C for 48 h and then weighed to determine their initial dry mass. The specimens were sterilized with propylene oxide gas for 2 days in a desiccator. After sterilization, the moisture content of the blocks was adjusted to 50–70% by dipping them into sterilized distilled water. The blocks were put onto the medium in the bottles (three blocks per bottle) and then incubated at 26 ± 2 °C in the dark.

After certain period of incubation (30, 60, 90, or 120 days), the mycelium covering the wood blocks was carefully removed. The blocks were air-dried at room temperature for 24 h and then dried at 60 ± 2 °C for 48 h. The percentage of mass loss was calculated by dividing the mass of the wood block after degradation by its initial mass.

2.3. Analysis of wood chemical components

The amounts of wood chemical components were determined before and after degradation. The wood samples were ground with a rotary speed mill (P-14; Fritsch, Germany) and then sieved through 42–82 mesh. The amounts of hot water extracts, 1% NaOH extracts, ethanol-toluene extracts, Klason lignin, acid soluble lignin, holocellulose, and α-, β-, and γ-cellulose were determined by standard methods (Dence, 1992; The Japan Wood Research Society, 2000; Carrier et al., 2011). The ash content was also determined by heating the wood meal in a muffle furnace (FO 100; Yamato, Japan) at 600 °C for 2 h. After cooling in a desiccator, it was heated again at 600 °C for 1 h, followed by cooling and weighing the residue. All chemical quantifications were performed in triplicate.

2.4. pH measurement

The pH was measured according to the method described by Koenigs (1974). Air-dried wood meal (0.5 g) was placed in a test tube (1.6-cm diameter and 10-cm length) containing 3.5 ml of distilled water. The tubes with wood meal were heated in boiling water for 20 min and then cooled to room temperature. The pH was determined with a pH meter (pH meter M-12, Horiba, Japan). The measurements were performed in triplicate.

2.5. Enzyme extraction and enzymatic activity assay

Enzymes were extracted according to the method described by Elissetche et al. (2007). For the enzyme extraction, the wood blocks were collected from the bottles after incubation (30, 60, 90, or 120 days). The collected blocks were cut into small chips. The chips were transferred to a 300-ml Erlenmeyer flask, and then the enzymes were extracted with 80 ml (4 ml per g of wood chips) of 50 mM sodium acetate buffer (pH 5.0) supplemented with 0.01% Tween-80. The extraction was conducted using a rotary shaker (NR-150, TAITEC Corp., Japan) at 120 rpm for 24 h at 20 ± 2 °C. The crude extract was obtained by filtration through a glass filter (1G3) and subsequently through a 0.45-μm membrane filter (Advantec, Japan).

Endoglucanase, exoglucanase, and xylanase were assayed using carboxymethylcellulose, Avicel, and xylan as substrates, respectively (Wood and Bhat, 1988; Bailey et al., 1992). The reducing sugars were determined by dinitrosalicylic acid (DNS) method (Miller, 1959). β-Glucosidase activity was determined by measuring the *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (Wood and Bhat, 1988). For oxidizing enzymes, cytochrome C, vanillylidene acetone, veratryl alcohol, and syringaldazine were used to assay cellobiose dehydrogenase (Samejima and Eriksson, 1992), manganese(II)-dependent peroxidase (Paszczyñski et al., 1988), lignin peroxidase (Tien and Kirk, 1988), and laccase activities (Bollag and Leonowicz, 1984; Cho et al., 2008), respectively. All enzymes were assayed photometrically, in triplicate, using a spectrophotometer (V-650, Jasco, Japan), and the enzymatic activities were expressed as nkat/mg of enzyme.

3. Results

3.1. Wood degradation

A thin mycelial mat was first observed on the wood on the 15th day of incubation. Wood blocks were completely covered with a white mycelial mat after incubation for 60 days. After 60 days of incubation, the mycelial mat on the wood block surface began to turn reddish-brown, which was similar to the color of the mycelia grown in the culture medium.

Table 1 shows the percentage of mass loss of *P. jezoensis* wood blocks after degradation by *P. pini*. Significant mass loss of the wood was first observed at 60 days of incubation, and it increased with increasing incubation time. Finally, the mass loss was 21.2 ± 3.0% after incubation for 120 days.

Table 1 also shows the changes in the amounts of wood chemical components of *P. jezoensis* after degradation by *P. pini*. No significant differences were found in the amounts of wood chemical components between days 0 and 30 of incubation. The amounts of Klason lignin, total lignin, holocellulose, and α-cellulose significantly decreased with increasing degradation time. The increasing trends at the later stages of degradation were similar to those of acid soluble lignin and β-cellulose. However, the amount of γ-cellulose did not significantly change.

Table 2 shows the changes in the amounts of the extracts, as