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The effect of trace elements on wheat straw degradation by *Trametes gibbosa*

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ABSTRACT

The aim of the study was comparative analysis of ligninolytic enzyme productions of *Trametes gibbosa* and its ability to degrade wheat straw, as well as effect of trace elements on those processes. Mn-oxidizing peroxidases were the main enzymes and the tested trace elements affected their activities mainly inhibitory. Stimulatory effect on Mn-independent peroxidase had Mn^{2+} in all concentrations, while Zn^{2+} concentrations of 5.0 mM and 10.0 mM led to the maximum Mn-dependent peroxidase activity. The trace elements affected laccase activity differently. Zn^{2+} and Fe^{2+} (0.5 mM and 10.0 mM, respectively) had the greatest effect on the activity, while Mn^{2+} and Cu^{2+} at the amount of 10.0 mM completely stopped the enzyme production. The tested enzymes acted simultaneously on wheat straw degradation. The presence of 1.0 mM of Mn^{2+} caused the highest level of dry matter loss (43.1%), which was the consequence of hemicellulose, cellulose and lignin degradation in the rate of 62.6%, 35.6% and 61.2%, respectively. Selective lignin degradation was noted especially in the presence of Fe^{2+} at the concentration of 0.5 mM.

Introduction

Biodegradation resistance of wood and other lignified materials is directly related to the presence of lignin, one of the most recalcitrant natural compounds (Peláez et al., 1995; Martínez et al., 2005). Lignin mineralization in nature is process catalyzed by complex ligninolytic enzyme system composed of extracellular oxidoreductases, such as laccase and peroxidases (Glenn et al., 1983; Tien and Kirk, 1983, 1984; Saparrat et al., 2008). Wood-rotting fungi are the only eukaryotic organisms producing ligninolytic enzymes and take crucial part in wood degradation (Tomšovský and Homolka, 2003; Himmel et al., 2007). White-rot fungi are currently being used not only for the lignin degradation but also in bioremediation of other phenolic compounds, such as industrial dyes, aromatic pollutants etc. (Bumpus et al., 1985; Gadd, 2001; Tamagawa et al., 2005; Baldrian and Snajder, 2006; Kokol et al., 2007; Vargas-García et al., 2007). However, the presence of trace elements in toxic concentrations represents a serious problem because of influences on fungal efficiency (Jellison et al., 1997). The microelements could be found in both water effluents and contaminated soil and could reduce the mycelial colonization, decrease microbial respiration, affect the degradation reactions, modulate enzyme activity, delay enzymatic reactions, etc. (Baldrian, 2003).

Species of the genus *Trametes* posses a potent ligninolytic enzyme system and represent a promising lignin degraders (Knežević et al., 2013a). Although the effect of trace elements on ligninolytic enzyme activity has received much attention (Baldrian and Gabriel, 2002; Stajić et al., 2013), there is less number of studies attempting to explain a relationship between fungal exposure to trace elements and their degradation ability (Baldrian, 2003). Furthermore, there are only a few papers available in literature where these enzymes have been studied simultaneously during the degradation of natural lignocellulosic residues (Valmaseda et al., 1991; Vares et al., 1995; Arora et al., 2002; Knežević et al., 2013b).

This research was particularly focused on comparative analysis of ligninolytic enzyme activities of *Trametes gibbosa* (Pers.) Fr. and its ability to degrade wheat straw, with the aim to obtain clear insight into the rate of lignin degradation in the presence of even toxic concentrations of trace elements.

Materials and methods

Organism

The basidiocarps were collected on Suva Mt., Serbia, and identified as *Trametes gibbosa* (Pers.) Fr. according to the macroscopic features and the micromorphology of the reproductive structures using

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identification keys (Phillips, 1981; Courtecuisse, 1999). The culture of *T. gibbosa* BEOFB 310 isolated from the fruiting body is maintained on malt agar medium, at 4 °C, in the Culture Collection of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOFB).

Growth conditions

The inoculum was prepared by inoculation of 100 mL of synthetic medium (glucose, 10.0 g L^{-1}; NH_4NO_3, 2.0 g L^{-1}; K_2HPO_4, 1.0 g L^{-1}; $NaH_2PO_4 \times H_2O$, 0.4 g L^{-1}; $MgSO_4 \times 7H_2O$, 0.5 g L^{-1}; yeast extract, 2.0 g L^{-1}; pH 6.5) in 250-mL Erlenmayer flasks with 25 mycelial agar discs (Ø 0.5 cm) of 7-day-old *T. gibbosa* culture on Malt agar and incubation at room temperature (22 ± 2 °C) on a rotary shaker (100 rpm) for 7 days. The obtained biomass was washed 3 times with sterile distilled water (dH_2O) and homogenized with 100 mL of dH_2O in laboratory blender.

Ligninolytic enzyme production was studied after solid-state fermentation of wheat straw, which was washed, dried at 50 °C and chopped up to 0.5–1.0 cm in a laboratory blender.

Solid-state cultivation was carried out at 25 °C in 100-mL flasks containing 2.0 g of wheat straw as the carbon source and 10 mL of the modified synthetic medium (without glucose, with $(NH_4)_2SO_4$ in a previously determined optimal nitrogen concentration of 10 mM and with tested trace elements). The trace elements were Mn^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+} in the forms of $MnSO_4 \times H_2O$, $CuSO_4 \times 5H_2O$, $ZnSO_4 \times 7H_2O$ and $FeSO_4 \times 7H_2O$, respectively, and in the concentrations of 0.5, 1.0, 5.0 and 10.0 mM. Flasks were then inoculated with 3.0 mL of homogenized inoculum. The medium without trace elements was used as the control.

Samples were harvested after 19 days of cultivation. Extraction of synthetized ligninolytic enzymes were performed by sample stirring with 50 mL of dH_2O on magnetic stirrer at 4 °C for 10 min. The obtained extracts were separated by centrifugation (3000 rpm, 10 min, 4 °C) and the supernatants were used for measurement of laccase (EC 1.10.3.2) and Mn-oxidizing peroxidases (EC 1.11.1.13) activities, as well as total protein content. Five replicates for each trace element and concentration were analyzed.

Enzyme activity assays

Activity of the ligninolytic enzymes was determined spectrophotometrically.

Laccase activity was estimated by monitoring the A_{436} change related to the rate of oxidation of 50 mM 2,2'-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS) (ε_{436} = 29,300 M^{-1} cm^{-1}) in 0.1 M phosphate buffer (pH 6.0) at 35 °C. The reaction mixture (V_{tot} = 1.0 mL) contained buffer, ABTS, and sample (Stajić et al., 2010).

Activities of Mn-oxidizing peroxidases [Mn-dependent peroxidase (MnP) and Mn-independent peroxidase (MnIP)] were determined with 3.0 mM phenol red (ε_{610} = 22,000 M^{-1} cm^{-1}) as a substrate, in a buffer with the following content: succinic acid disodium salt, albumin from bovine serum, and DL-lactic acid sodium salt (pH 4.5). The reaction mixture (V_{tot} = 1.0 mL) contained: buffer, sample, 2.0 mM H_2O_2, and phenol red, with or without 2.0 mM $MnSO_4$ (for MnP and MnIP, respectively). The reaction was stopped by adding 2.0 M NaOH (Stajić et al., 2010).

Enzymatic activity of 1 U is defined as the amount of enzyme that transforms 1.0 μmol of substrate per min. The presented values correspond to mean value ± S.E of five replicates.

Determination of wheat straw fibres

Content of hemicelluloses, cellulose, and lignin in wheat straw was determined by quantitative methods. The effect of tested trace elements on level of fiber degradation was analyzed only in the media with their optimum concentrations for enzyme activity.

Hemicellulose content determination

Determination of hemicellulose content was carried out using Van Soest fibre analysis (Goering and Van Soest, 1970; Van Soest et al., 1991). The air dried and ground sample (1.0 g) was placed in a crucible containing 100 mL of a neutral detergent solution (NDS), 0.5 g of Na_2SO_3 and some drops of n-octanol, heated to boiling and refluxed for an hour. NDS as a water solution of $Na_2B_4O_7 \times 10H_2O$, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), 2-ethoxyethanol and Na_2HPO_4 (pH between 6.9 and 7.1) was used to dissolve pectins, proteins, sugars and lipids. The residues were filtered, washed three times with hot water and twice with cold acetone, dried at 105 °C for 8 h, cooled in a desiccator and weighed as neutral detergent fibre (NDF). After that, acid detergent solution [ADS, 20 g of cetyltrimethylammonium bromide (CTAB, technical grade) in 1 L of 0.5 M H_2SO_4] was added to the samples at room temperature, the mixtures were heated to boiling, than temperature was reduced to avoid foaming and refluxion lasted an hour. The next steps were: sample filtration in a tared 50-mL coarse porosity Gooch crucible; washing thoroughly with hot water (90–100 °C) and then with acetone; drying overnight at 105 °C and weighing. Acid detergent fibre (ADF) is determined gravimetrically as the residue remaining after extraction. Hemicellulose content is than expressed as NDF −ADF.

Cellulose and lignin content determination

ADF was used for determination of cellulose and lignin content. Lignin content was defined using the Klason or 72% H_2SO_4 method (Kirk and Obst, 1988). 1.0 mL of 72% H_2SO_4 was added for each 100 mg of sample. The mixture was incubated at 30 ± 0.5 °C in a water bath for an hour and stirred frequently. After that, the solution was diluted using 28 mL of water per each 1.0 mL of acid. Secondary hydrolysis was performed in an autoclave at 120 °C for an hour. The hot solution was filtered through a tared Gooch alundum and the Klason lignin residues were washed with hot water. The alundums containing the sample were dried to constant weight at 105 °C and lignin content (LC) in samples is expressed as a percentage of the original sample ± S.E. of three replicates. Cellulose content is expressed as ADF − LC.

Statistical analysis

The results were expressed as the mean ± standard error of data obtained from the replicates. One-way analysis of variance (ANOVA) followed by LSD post-hoc determinations were used to test the significance of differences among trace elements and their concentrations, using STATISTICA software, version 5.0 (StatSoft, Inc). *P*-values less than 0.01 were considered statistically significant.

Results

Effect of trace elements on laccase activity

Laccase activity was measured in water extracts of 19-day-old culture of *T. gibbosa* BEOFB 310, which was grown in the wheat straw medium, as well as in that enriched with tested trace elements in selected concentrations (Fig. 1A). The presence of selected trace elements affected laccase activity differently compared to the control, from significant increase to even complete absence of the activity depending on concentration. Mn^{2+}, Zn^{2+} and Fe^{2+} caused increase of the activity in the range between 50% and 61% in the concentrations of 1.0 mM, 0.5 mM and 10.0 mM, respectively.