

EFFECT OF SELECTED NITROGEN SOURCES ON Mn- OXIDIZING PEROXIDASES ACTIVITY IN *TRAMETES* *GIBBOSA*

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ABSTRACT

Trametes gibbosa is an efficient lignin-degrading species due to its ability to produce laccase and Mn-dependent peroxidase that enable degradation of lignin and of a wide range of structurally similar compounds. Wheat straw is a very common worldwide agricultural residue that contains a certain amount of soluble carbohydrates and inducers of lignocellulolytic enzyme synthesis. Whether nitrogen source and concentration could affect activity of Mn-dependent peroxidase and versatile peroxidase in *T. gibbosa*, thereby also affecting wheat straw fermentation, was the question that provided the goal for the present study. *T. gibbosa*, originated from *Fagus moesiaca*, was used for the study. Analyzed inorganic nitrogen sources were NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ in nitrogen concentrations ranged between 10 and 40 mM, and the organic source was peptone in concentrations from 0.25 to 4.0%. The enzyme activity was determined spectrophotometrically. $(\text{NH}_4)_2\text{SO}_4$ was the most appropriate nitrogen source for the Mn-dependent peroxidase activity. The maximal activity level was noted at a nitrogen concentration of 15 mM (4479.5 U/l). Slightly lower activity was noted in 1% peptone-enriched medium (4035.5 U/l), and higher peptone amounts caused rapid activity decrease and production was not noted at a concentration of 3%. In the case of NH_4NO_3 , Mn-dependent peroxidase activity was increased with enlargement of nitrogen concentration reaching peak at concentration of 40 mM (3669.5 U/l). Production of versatile peroxidase was significantly lower in all studied nitrogen sources and concentrations.

Keywords: *Trametes gibbosa*; Wheat straw; Laccase; Mn-oxidizing peroxidases

INTRODUCTION

Trametes gibbosa degrades lignin and a broad range of structurally similar aromatic pollutants [1-3] due to its ability to produce laccase and Mn-dependent peroxidase [4]. Therefore this species could take participation in various processes, such as biopulping, biobleaching, textile dye discolouration, treatment of agricultural residues, of industrial wastewater, etc. and contribute to the pollution problem solving [5, 6]. Agricultural residues represent prospective substrates for the bioconversion into fungal biomass and production of lignocellulolytic enzymes, but also they could be potential environmental pollutants [7]. Wheat straw is an abundant residue in numerous countries worldwide and a prospective substrate for the bioconversion into fungal biomass and lignocellulolytic enzymes, due to their appropriate chemical composition [8].

The aim of this study was concerned with different production of Mn-oxidizing peroxidases by *T. gibbosa* depending on nitrogen sources and concentrations.

MATERIALS AND METHODS

Trametes gibbosa BEOFB 310 was collected from *Fagus moesiaca* on Suva Mountain (Serbia). The culture on malt agar medium is maintained in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOFB).

The inoculum preparation was contained from several steps: (i) inoculation of 100 ml of synthetic medium (glucose, 10.0 g/l; NH_4NO_3 , 2.0 g/l; K_2HPO_4 , 1.0 g/l; $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$, 0.4 g/l; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0.5 g/l; yeast extract, 2.0 g/l, pH 6.5) with 25 mycelial discs of 7-day-old culture; (ii) incubation at room temperature on a rotary shaker during 7 days; (iii) washing of obtained biomass 3 times by sterile distilled water (dH_2O); and (iv) homogenization of the biomass with 100 ml of sterile dH_2O in a laboratory blender. The ligninolytic enzyme activities were studied after solid-state fermentation of wheat straw. Cultivation was performed at 25°C in 100 ml flasks containing 2g of wheat straw soaked with 10 ml of the modified synthetic medium (without glucose, with one of inorganic nitrogen sources, NH_4NO_3 or $(\text{NH}_4)_2\text{SO}_4$, in nitrogen concentrations of 0, 10, 15, 20, 25, 30, and 40 mM, or peptone as an organic source in the concentrations of 0, 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0%). In this way prepared flasks were inoculated with 3 ml of homogenized inoculum.

Samples were harvested after 7 days of cultivation and ligninolytic enzymes were extracted with 50 ml of dH_2O . The obtained extracts were separated by centrifugation and the supernatants were used for determination of the activity of Mn-oxidizing peroxidases [Mn-dependent peroxidase (MnP) and versatile peroxidase (VP)]. Five replicates for each nitrogen source and concentration were prepared to decrease statistical error.

Activities of Mn-oxidizing peroxidases were determined spectrophotometrically (CECIL CE2501 Spectrophotometer) with phenol red ($\epsilon_{610} = 22000 \text{ M}^{-1} \text{ cm}^{-1}$) as a substrate with or without MnSO_4 (for MnP and VP, respectively). Enzymatic activity of 1 U is defined as the amount of enzyme that transforms 1 μmol of substrate/min.

RESULTS AND DISCUSSION

Activities of Mn-oxidizing peroxidases were detected after 7 days of solid-state fermentation of wheat straw by *T. gibbosa* BEOBF 310 in the presence of all tested nitrogen sources and concentrations (Fig. 1A-C). The nitrogen sources and concentrations showed significantly different potentials for stimulation of peroxidase production ($P < 0.01$).

$(\text{NH}_4)_2\text{SO}_4$ was the most appropriate nitrogen source for the MnP activity. The maximal activity level was noted at a nitrogen concentration of 15 mM (4479.5 U/l). Further increases of nitrogen concentration up to 40 mM caused a gradual decrease of activity, while concentration of 40 mM showed stimulation effect to the enzyme production (Fig. 1B). Slightly lower activity was noted in 1% peptone-enriched medium (4035.5 U/l), and higher peptone amounts caused rapid activity decrease and production was not noted at a concentration of 3% (Fig. 1C). In the case of NH_4NO_3 , MnP activity was increased with enlargement of nitrogen concentration reaching peak at concentration of 40 mM (3669.5 U/l) (Fig. 1A). Production of VP was significantly lower in all studied nitrogen sources and concentrations (Fig. 1A-C). The maximum

activity level was noted in 10 mM (NH₄)₂SO₄- and 1.0% peptone-enriched medium (1555 U/l and 1506.5 U/l, respectively).

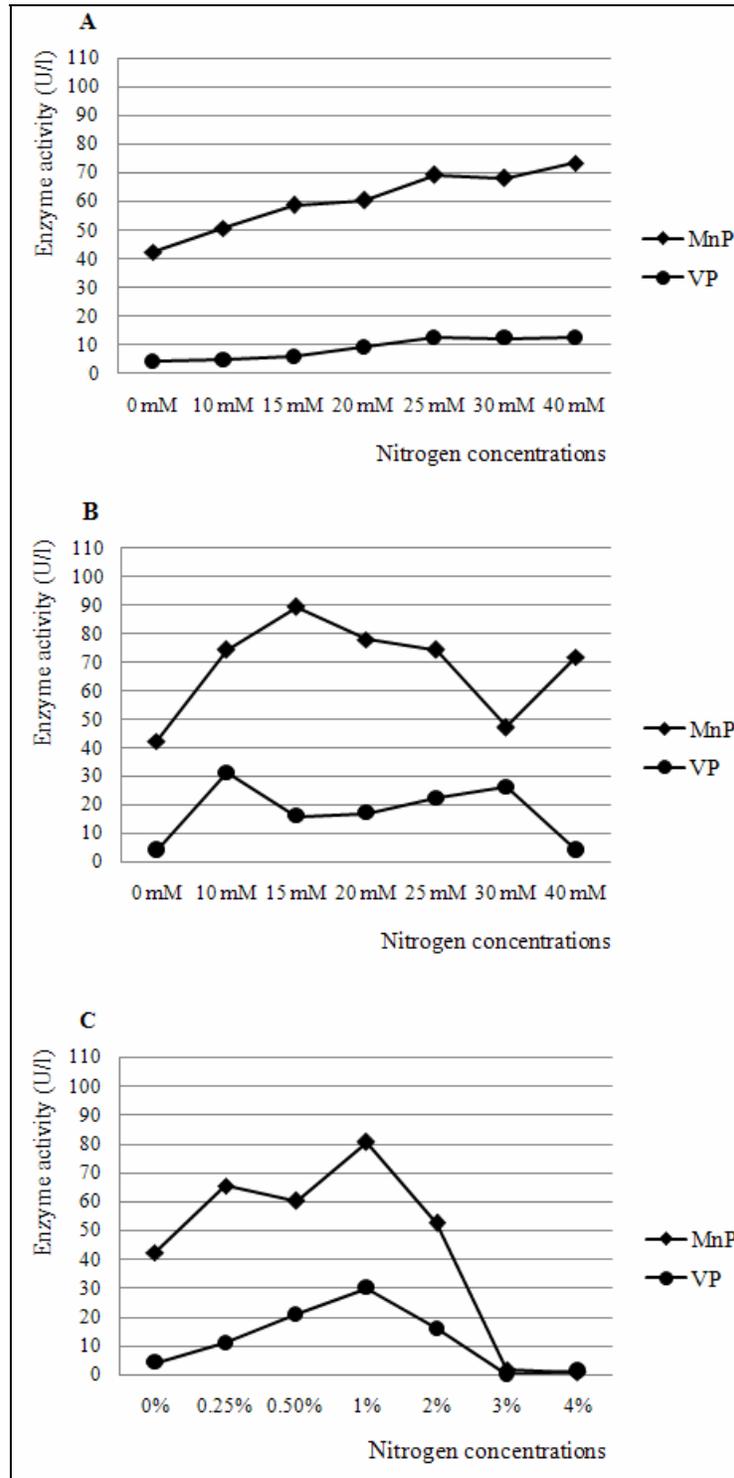


Figure 1: Activity of Mn-oxidizing peroxidases in *Trametes gibbosa* BEOFB 310 depending on nitrogen sources and concentrations: A. NH₄NO₃; B. (NH₄)₂SO₄; C. peptone

The obtained results confirmed previous data that enzyme system of *Trametes* species is effective in the process of wheat straw fermentation [9, 10]. According these authors, NH_4NO_3 was an appropriate nitrogen source for the MnP activity. However, regarding to the optimum nitrogen source, *T. gibbosa* showed different responses compared to the other mushroom species. Thus, $(\text{NH}_4)_2\text{SO}_4$ was an unfavorable nitrogen source and NH_4NO_3 was the appropriate one for *Ganoderma lucidum* [11]. These authors noted a MnP activity twice higher in the NH_4NO_3 -enriched medium than in $(\text{NH}_4)_2\text{SO}_4$ -enriched medium. In the case of the observed VP activity level during wheat straw degradation, *T. gibbosa* was a significant producer compared to numerous other white rot species, for example *G. lucidum* [11]. However, peroxidase activity may be enhanced because of higher biomass production and fact that $(\text{NH}_4)_2\text{SO}_4$ is a better nitrogen source than NH_4NO_3 , since ammonium-sulphate can be rapidly used for biomass production *via* amino acids synthesis. Nevertheless, biomass is a parameter that should be monitored in further studies.

According to the obtained results, it could be concluded that *T. gibbosa* could be used in processes of producing feeds and numerous basic commodities for different industrial purposes, as well as in bioremediation processes.

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