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Selenium Induces Manganese-dependent Peroxidase Production by the White-Rot Fungus *Bjerkandera adusta* (Willdenow) P. Karsten

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Abstract In this study, selenium (Se) induction of the ligninolytic enzyme manganese-dependent peroxidase (MnP) production, and the effects on the oxidative state in the white-rot fungus *Bjerkandera adusta* (Willdenow) P. Karsten were demonstrated. Low concentration of Se (0.5 mM) caused up to a twofold increase in MnP production (0.81±0.05 U/ml) when compared to control (0.39±0.07 U/ml), whereas higher concentrations of Se (200 mM) inhibited (0.03±0.01 U/ml) MnP production. Addition of high concentration of Se also caused up to a twofold increase in lipid peroxidation levels. These results demonstrate for the first time that Se may induce or reduce MnP production and lipid peroxidation levels which play a significant role in lignin degradation by white-rot fungi.

Keywords Lipid peroxidation · Manganese peroxidase · Selenium · White-rot fungi

Abbreviations

Ag	Silver
Cd	Cadmium
Cu	Copper
Hg	Mercury
Lac	laccase
LiP	lignin peroxidase
MDA	Malondialdehyde
Mn	Manganese
MnP	Manganese-dependent peroxidase
Se	Selenium
Zn	Zinc

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Introduction

White-rot fungi play a unique role in degradation of aromatic, phenolic, and nonphenolic substrates as well as lignin, which is one of the main components of wood and the most abundant aromatic polymer in nature [1]. Various aspects of the biotechnological uses of these fungi have been studied regarding the nonspecific ligninolytic system of white-rot fungi such as the degradation of industrial textile dye effluents and various xenobiotics [2–4]. Most white-rot fungi, such as *Phanerochaete chrysosporium*, *Trametes hirsuta* [5], *Bjerkandera adusta* [6], *Physisporinus rivulosus* [7], and *Trametes versicolor* [2], secrete several lignin-degrading enzymes including lignin peroxidase (LiP), laccase (Lac), and manganese-dependent peroxidase (MnP). *B. adusta* (Willdenow) P. Karsten is a white-rot basidiomycete whose ligninolytic system consists mainly of MnP [8]. This enzyme catalyzes the oxidation of Mn (II) to Mn (III), which in turn can oxidize phenolic substrates [3]. The ability of the fungus to grow in soil and its excellent performance in MnP production makes this fungus an interesting subject for biotechnological studies. However, up to now, there is only scattered information about the physiological effects of heavy metals on white-rot fungi. Toxicity of heavy metals to microorganisms has drawn considerable attention in recent years, especially as a result of their increasing dispersal into the environment [9]. Although some heavy metals are essential for the fungal metabolism, others have no known biological role.

Essential or nonessential heavy metals can be toxic for white-rot fungi when present in excess and affect their growth and extracellular enzyme systems. The fungi should be able to sequester essential trace metal ions from various sources, where the metals can be present in concentrations ranging from trace to toxic levels. Low concentrations of essential heavy metals are necessary for the development of ligninolytic enzyme system [10]. For example, Cu and Mn directly participate in the process of lignin degradation involving in the catalytic cycle of MnP and the cofactor of laccase, respectively. Other heavy metals such as Cd, Hg, and Pb are important enzyme-activity modulators, and their inductor effects on ligninolytic enzymes were previously addressed [11]. The positive effects of some metals such as Cu and Zn on the production of LiP, MnP, and laccase were reported in *P. chrysosporium*, *T. versicolor*, and *T. hirsuta*. These enzymes are strongly regulated on the transcription level [12]. Although the inducer effects of some metals on ligninolytic enzyme production were published, the effects of metals on oxidative stress situation in white-rot fungi have not been thoroughly researched. Selenium accumulation in the white rot fungus *Pleurotus ostreatus* has been reported [13], but there has been no information about the physiological effects of Se on the white-rot fungal enzymatic system.

Here, Se was evaluated as a model agent because of its unique role as a trace element having both anti-oxidative and toxic characteristics in biological systems [14–16]. In the present study, the effects of Se on MnP production were examined in the white-rot fungus. Furthermore, extracellular lipid peroxidation and biomass levels were examined in cultures of the fungus to understand the link between oxidative stress and MnP production, in addition to demonstrating Se-induced MnP production and lipid peroxidation in the white-rot fungi for the first time.

Materials and Methods

Culture Conditions

The wood-rotting fungus was kindly provided by H. Jung from Sunchon National University, Korea. The organism was identified and characterized via the ITS region sequence determination as *B. adusta* (Willdenow) P. Karsten. The fungus was grown on potato dextrose agar plates for 1 week at 28°C. Agar cubes cut from the growing edge of the mycelia were homogenized to produce an inoculum and added into 500 ml Erlenmeyer flasks containing 200 ml of culture medium. One liter of the culture medium consisted of mycological peptone (17 g), glucose (50 g), KH_2PO_4 (2.5 g), $MnSO_4$ (50 mg), $MgSO_4$ (0.5 g), $CuSO_4$ (20 mg), and thiamine-HCl (10 mg). The fungal cultures were grown at 28°C in a rotary shaker (150 rpm, Φ 50 mm) for 13 days. On the fourth day of cultivation, various concentrations of sodium selenate (control group, 0 μM; experimental groups, 0.5, 1, 10, 100, and 200 μM) were added into the cultures. Samples were taken daily for MnP production analysis. Samples collected on the 13th day were also used for the determination of extracellular lipid peroxidation, total protein, and total biomass. Samples were centrifuged at 14,000 rpm for 7 min, and supernatants were used for the assays. All the chemicals used were reagent grade and obtained from commercial sources.

Enzyme Assay and Lipid Peroxidation Measurements

Manganese-dependent peroxidase (EC 1.11.1.13, MnP) activity was determined by measuring the increase of Mn^{3+}-malonate formation at 270 nm ($\varepsilon_{max}=11{,}590\ M^{-1}\ cm^{-1}$) at 25°C. Assay mixtures (1 ml) contained sodium malonate buffer (70 mM, pH 4.5), $MnSO_4$ (2 mM), H_2O_2 (6 mM), and 25 μl of the samples to be assayed. One unit of MnP was defined as 1 μmol of product formed per minute under the assay conditions. Extracellular total protein concentrations were determined according to the Bradford method using bovine serum albumin as the standard. To assess the amount of lipid peroxidation, products of lipid peroxidation were measured as extracellular thiobarbituric acid reactive substances produced in thiobarbituric acid reaction, as previously described [15]. 1,1,3,3-Tetraetoxypropan was used as the standard, and all lipid peroxidation product, malondialdehyde (MDA), was calculated with respect to total protein concentrations. The fungal biomass in each flask was collected at the end of the experiment and dried at 80°C overnight and kept in a desiccator for 30 min.

Statistical Analysis

All experiments were done in triplicate. Data were expressed as mean±SE in all graphs. All the result groups were evaluated statistically via one-way analysis of variance followed by the Kruskal–Wallis and via Scheffe for multiple comparisons of the controls against the experimental groups using Statistical Package for the Social Sciences (SPSS) for Windows (ver 10.0). The results were considered significant at the level of $p<0.05$.

Results

Manganese-Dependent Peroxidase Levels

The MnP activity reached maximum on day 13 in both control and experimental groups treated with Se (Fig. 1). The most prominent difference between the MnP levels in control (0.39±0.07 U/ml) and experimental groups occurred in the 8-day old cultures that received 0.5 or 1.0 μM Se (0.81±0.05 and 0.67±0.00 U/ml, respectively; $p<0.0001$). In 100 and 200 μM Se-treated cultures, MnP production was inhibited significantly in comparison to the controls (0.84±0.05 U/ml; $p<0.05$). Manganese-dependent peroxidase induction occurred only when the Se concentration was lower than 10 mM. To understand whether

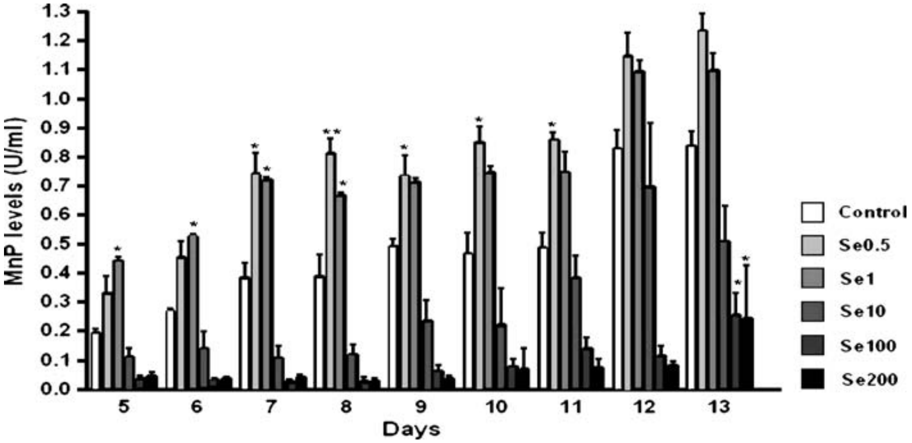


Fig. 1 MnP activities during the 13-day incubation for control and Se. *Single asterisk* $p<0.05$ compared to control group, *double asterisk* $p<0.0001$ compared to control group. $p \leq 0.05$ was considered significant

or not MnP was inhibited directly by sodium selenate, the enzyme assays were conducted in the absence and presence of various concentrations of this compound in the assay mixture. However, in separate experiments, no enzyme inhibition by Se was observed, demonstrating that MnP production, but not the enzyme itself was inhibited (data not shown).

Extracellular Assays and Lipid Peroxidation Levels

Extracellular lipid peroxidation levels exhibited interesting results. Although no significant difference was found in lipid peroxidation levels between the flasks containing Se (0.5 μM) or not, a further increase of Se concentration from 1.0 to 100 mM resulted in a gradual increase in lipid peroxidation. However, 200 mM Se increased lipid-peroxidation levels dramatically (Fig. 2).

Biomass Levels

Biomass production was determined at the end of the cultivation (Fig. 3). Compared to the control group (0.78 g), the biomass concentration was slightly higher (0.84 g) in cultures

Fig. 2 Extracellular lipid peroxidation levels (nmol MDA/mg protein) at the 13th day of cultivation for Se treatment. Control; *Se0.5* 0.5 μM Se treatment; *Se1* 1 μM Se treatment; *Se2.5* 2.5 μM Se treatment; *Se10* 10 μM Se treatment; *Se100* 100 μM Se treatment; *Se200* 200 μM Se treatment. *Asterisk* $p<0.05$ compared to control group and Se0.5 group. $p \leq 0.05$ was considered significant)

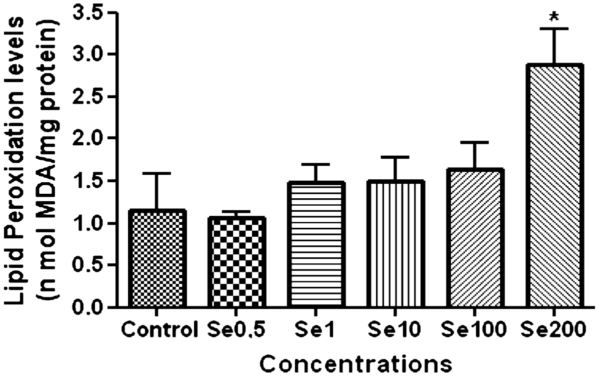
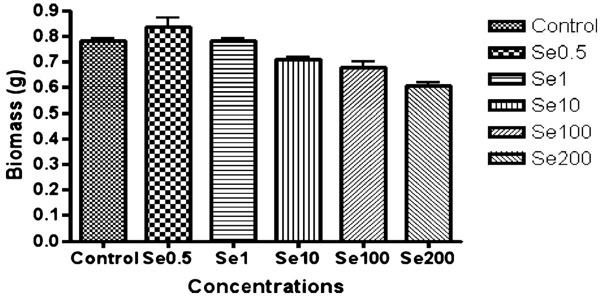


Fig. 3 Biomass production on the 13th day of cultivation by control and Se treatment



that received 0.5 µM Se. Further increase of the Se concentration (to 200 µM) resulted in a slight decrease of biomass levels, to 0.61 g. These results indicated that Se administration in these concentrations had an insignificant effect in fungal growth; therefore, changes in MnP and lipid-peroxidation levels was not attributed to biomass production.

Discussion

The effect of certain heavy metals on the activity of ligninolytic enzymes has recently been described in different white-rot fungi [10]. One of the ligninolytic enzymes, laccase, was induced by several metals. For example, Mn and Cu enhance Lac formation, whereas Ag, Cd, Hg, and Zn are not effective in enhancing Lac formation in *Trametes pubescens* [16]. Copper (0.5–5 mM) and Cd (1–5 mM) caused an important increase in Lac activities in liquid cultures of *P. ostreatus* [11]. A significant increase in the production of MnP in liquid cultures of litter-decomposing fungi was reported in the presence of Mn [17]. Addition of Cu increases the activities of MnP and glyoxal oxidase in *Trametes trogii* [18]. Previously, it was reported that Se could bind to polysaccharides and specifically to chitin in the cell walls of *P. ostreatus*, and a low concentration of Se (2.5 mg/l) stimulates the growth of fungi, whereas the mycelial growth is inhibited by high concentration (5 mg/l) [13]. However, our biomass results show that Se does not significantly affect the growth of the fungi. A low concentration of Se strongly induces MnP production, whereas its high concentration increases lipid peroxidation in the fungus. Similarly, it was reported that MnP production and lignin degradation by white-rot fungi are related to the extracellular lipid peroxidation [19].

The important role of lipid peroxidation in toxicity of heavy metal ions to microorganisms and white-rot fungi was investigated in recent studies [9, 11]. The accumulation of lipid peroxidation products, such as lipid hydroperoxides, within the hydrophobic core of plasma membranes might result in disturbances in the arrangement of phospholipid moieties and impairment of membrane function [20]. The susceptibility of microorganisms is dependent on the plasma membrane permeability and toxicity on plasma membrane fatty-acid composition [9], causing oxidative stress by reactive oxygen species which are agents in lignin degradation and wood decay [21]. Induction of lipid peroxidation by Cu and Cd in yeast was previously reported [9]. It was proposed that lipid peroxidation is a potent oxidative process strong enough to decompose nonphenolic lignin structures in the presence of Cu in the cultures of white-rot fungus *Cerioporiopsis subvermispora* [19]. Lipid peroxidation depends on the presence of MnP and is involved in the nonphenolic lignin-degradation processes [19]. It was also stated that lipid peroxidation-promoting activity of the fungal cultures depended on the presence of MnP [22]. In the current work, it can also be seen that Se controls both MnP activity and lipid peroxidation in *B. adusta* (Willdenow) P. Karsten.

As a result, the MnP increase due to Se treatment appears to be highly advantageous, as MnP is considered as a potential enzyme of biotechnological implications. The results shown above might be valuable in terms of bioremediation applications such as the conversion of Se into a less toxic form. It can be concluded that Se induces MnP activity increasing in the cultures. The addition of trace amounts of Se created a twofold increase in MnP activity, whereas higher concentrations (200 μM) of Se decreased the MnP production while increasing lipid peroxidation levels. Our results exhibited for the first time the Se-induced lipid peroxidation and MnP induction in white-rot fungi. Here, we could demonstrate that higher concentrations of Se caused oxidative stress by inducing lipid peroxidation, whereas MnP production was induced only when low concentrations were applied. Yet, it is interesting that no direct relationship was observed between the MnP produced and lipid peroxidation levels, as opposed to what was earlier reported with other fungi [19].

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References

1. Brigham JS, Adney WS, Himmel ME (1996) Hemicelluloses: diversity and applications. In: Wyman CE (ed) Handbook on bioethanol: production and utilization. Taylor and Francis, Washington, DC, USA, pp 119–142
2. Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotech Adv 22:161–187
3. Novotny C, Svobodova K, Erbanova P, Cajthaml T, Kasinath A, Lang E, Šašek V (2004) Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol Biochem 36:1545–1551
4. Fenice M, Giovannozzi SG, Federici F, D'Annibale A (2003) Submerged and solid-state production of laccase and Mn-peroxidase by *Panus tigrinus* on olive mill wastewater-based media. J Biotechnol 100:77–85
5. Dominguez A, Rivela I, Couto SR, Sanromán MA (2001) Design of a new rotating drum bioreactor for ligninolytic enzyme production by *Phanerochaete chrysosporium* grown on an inert support. Process Biochem 37:549–554
6. Nakamura Y, Godliving-Sungusia M, Sawada T, Kuwahara M (1999) Lignin-degrading enzyme production by *Bjerkandera adusta* immobilized on polyurethane foam. J Biosci Bioeng 88:41–47
7. Hatakka A (1994) Lignin modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol Rev 13:125–135
8. Bermek H, Gülseren İ, Li K, Jung H, Tamerler C (2004) The effect of fungal morphology on ligninolytic enzyme production by a recently isolated wood-degrading fungus *Trichophyton rubrum* LSK-27. World J Mic Biotech 20:345–349
9. Howlett NG, Avery SV (1997) Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of plasma membrane fatty acid unsaturation. Appl Environ Microbiol 63:2971–2976
10. Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzyme Microb Technol 32:78–91
11. Baldrian P, Gabriel J (2002) Copper and cadmium increase laccase activity in *Pleurotus ostreatus*. FEMS Microbiol Lett 206:69–74
12. Collins PJ, Dobson ADW (1997) Regulation of laccase gene transcription in *Trametes versicolor*. Appl Environ Microbiol 63:3444–3450
13. Munoz AHS, Kubachka K, Wrobel K, Corona JFG, Yathavakilla SKV, Caruso JA, Wrobel K (2006) Se-enriched mycelia of *Pleurotus ostreatus*: distribution os selenium in cell walls and cell membranes/cytosol. J Agric Food Chem 54:3440–3444

14. Tapiero H, Townsend DM, Tew KD (2003) The antioxidant role of selenium and seleno-compounds. Biomed Pharmacother 57:134–144
15. Ledwozyw A, Michalak J, Stepien A, Kadziolka A (1986) The relationship between plasma trygliceride, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clinica Chimica Acta 155:275–284
16. Galhaup C, Haltrich D (2001) Enhanced formation of laccase activity by the white-rot fungus *Trametes pubescens* in the presence of copper. Appl Microbiol Biotechnol 56:225–232
17. Baldrian P, Valaskova V, Merhautova V, Gabriel J (2005) Degradation of lignocellulose by *Pleurotus ostreatus* in the presence of copper, manganese, lead and zinc. Res Microbiol 156:670–676
18. Levin L, Forchiassin F, Papinutti L (2002) Effect of copper on the ligninolytic activity of *Trametes trogii*. Int Biodeterior Biodegrad 49:60
19. Enoki M, Watanabe T, Nakagame S, Koller K, Messner K, Honda Y, Kuwahara M (1999) Extracellular lipid peroxidation of selective white-rot fungus, *Ceriporiopsis subvermispora*. FEMS Microbiol Lett 180:205–211
20. Ginkel VG, Sevian A (1994) Lipid peroxidation-induced membrane structural alterations. Methods Enzymol 233:273–288
21. Hammel KE, Kapich AN, Jensen JKA, Ryan ZC (2002) Reactive oxygen species as agents of wood decay by fungi. Enz Microbiol Tech 30:445–453
22. Kapich AN, Prior BA, Lundell T, Hatakka A (2005) A rapid method to quantify pro-oxidant activity in cultures of wood-decaying white-rot fungi. J Microbiol Methods 61:261–271