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Growth and Tolerance of *Pleurotus ostreatus* at Different Selenium Forms

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Abstract

Selenium is an important element in physiological and metabolic processes. Due to low Se concentration in most of the soils, strategies as enrichment and biofortification have been used to increase its incorporation in food. The fungus has capacity to absorb, accumulate and transform Se inorganic into organic compounds. However, the concentration and chemical forms of Se used for enrichment can affect the mycelial growth and mushrooms production. Thus, the aim of this study was to analyze the capacity of *Pleurotus ostreatus* in absorb, accumulate and tolerate growing concentrations of different Se chemical forms. In the disc of agar with mycelium was added 20 mL of PDA medium and Se concentration (0-200 mg L^{-1}) in the forms of sodium selenite, sodium selenate or selenomethionine (SeMet). The greatest inhibition of mycelial growth and biomass production were observed in highest Se concentration. Regardless of the Se level, SeMet and sodium selenite were more harmful to the *P. ostreatus* growth than sodium selenate. However, the highest Se accumulation in the mycelium was observed in culture medium with sodium selenite. Thus, Se supplementation in the forms of sodium selenite was more indicated to enrichment of *P. ostreatus* mushrooms than sodium selenate and SeMet.

Keywords: fungal biomass, selenium inorganic, selenium organic, mushrooms, mycelium growth rate

1. Introduction

Selenium (Se) is an essential element to human health. It has antioxidant function and is an immunosystem modulator, and have participation in biosynthesis of ubiquinone, ATP and proteins (Viaro et al., 2001). In the case of chronic diseases such as cancer, cardiovascular, oxidative stress or inflammatory conditions, the Se can act as protective element (Rayman, 2000). However, the Se concentration in food is low (Ferreira et al., 2002). Therefore, enrichment or biofortification have been strategies used to increase the concentration and availability of this element in food (da Silva et al., 2012; Silva et al., 2010; Solovyev et al., 2018).

The Se-enrichment of mushrooms using agriculture residue has been an alternative for accumulation of Se and production of organic Se (da Silva et al., 2012; Falandysz, 2008; Fang et al., 2018; Hu et al., 2018; Nunes et al., 2012; Solovyev al., 2018). Furthermore, mushrooms are much appreciated food, which contain protein, essential aminoacids, fibers, fatty acid and minerals (Manzi et al., 1999). This food is indicated to feeding of people with malnutrition problem (Kane et al., 2017).

The high Se concentration can inhibit the mycelial growth and production of *Pleurotus ostreatus* mushrooms (Da Silva et al., 2012, 2013). Therefore, for Se-enrichment of mushrooms it would be interesting to investigate the effect of different Se chemical forms on growth and morphology of mycelium of *P. ostreatus* and determine the best concentration to be used for enrichment. Furthermore, *P. ostreatus* is one of the most produced and consumed mushrooms in the world (Azevedo et al., 2012; Furlani & Godoy, 2007) that shows its economic and nutritional importance.

Thus, the aim of this study was to analyze the capacity of *Pleurotus ostreatus* in absorb, accumulate and tolerate growing concentrations of different chemical forms of Se.

2. Material and Method

2.1 Microorganisms

The isolate of *P. ostreatus* (PLO 02) of the Department of Microbiology of the Federal University of Viçosa/BIOAGRO, MG, Brazil was used in this study. This isolate was also used in enrichment of mushrooms with selenium, lithium and zinc (da Silva et al., 2012; de Assunção et al., 2012; Vieira et al., 2013).

For inoculum production, this isolate was grown in a Petri dish containing potato dextrose agar (PDA) culture medium, pH 5.8, and incubated at 25±2 ºC for seven days.

2.2 Enrichment of P. ostreatus

Two assays were done. In the first test, *P. ostreatus* was inoculated in PDA media containing 12.5; 25; 50 or 75 mg L^{-1} of Se, as sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4) or selenomethionine (SeMet). These concentrations were obtaining on previous studies (da Silva et al., 2012, 2013; Silva et al., 2010). In the second, the fungus was inoculated in PDA with 25, 50, 100, 150 and 200 mg L^{-1} of Se, as Na_2SeO_4. In both tests, the control was PDA without Se. The cultures were incubated for seven days at 25±2 ºC.

2.3 Mycelial Growth Velocity and Biomass

The mycelial growth was verified by taken two measurements, perpendicular to each other, at seventh days. The growth rate was calculated by the ratio between colony diameter and time of incubation. This diameter was measured by taken two measurements, perpendicular to each other at seven days.

To evaluate the mycelium dry biomass, all the content of Petri dish was transferred to a flask with 200 mL of distilled water and boiled, in microwave oven, to liquefy agar (Da Silva et al., 2012), followed by filtration and rinsing in distilled water. The retained mycelium was dried at 60 ºC, until constant weight.

2.4 Hyphae Diameter and Distance Between Septa

Theses parameters were measured after stained with calcofluor and observed under epifluorescence microscopy (Olympus BX 50). The images were capture by digital camera FUJIX HC-300Z and processed with the software Image Pro Plus.

2.5 Selenium Determination in Mycelium

All solutions were prepared from analytical reagent grade chemicals using high-purity deionized water obtained from a Milli-Q water purification system (Millipore, Belford, USA). Selenium analytical solution (Na_2SeO_3) of 1000 mg L^{-1} was used as standard and its determination by Graphite furnace atomic absorption spectrometry (GF AAS). In the last case, a volume of 10 μL of chemical modifier solution of 5 mg of palladium and 3 mg of magnesium was co-injected with 10 μL of samples or analytical solutions into the graphite furnace.

The acid digestion of mycelium was done using 65% (v/v) nitric acid (HNO_3) and 30% (v/v) hydrogen peroxide (H_2O_2) from Merck (Darmstadt, Germany). A buffer solution of 0.2 mol L^{-1} Tris/HCl was prepared by dissolving Tris(hydroxymethyl)aminomethane (USB Corporation) in deionized water and adjusting the pH to 7.5 with chloridric acid (Merck).

The mycelium of *P. ostreatus* were subjected to acid digestion in a microwave oven, using diluted oxidant mixture (2.0 mL HNO_3 + 1.0 mL H_2O_2 + 3.0 mL H_2O). The microwave heating program presented four steps (Temperature/1C; ramp/min; hold/min): 1 (140; 5; 1), 2 (180; 4; 5), 3 (200; 4; 10), 4 (0; 0; 20). The final volume was 15 mL.

Selenium concentration in the digested samples was determined (GF AAS) according to Silva et al. (2010).

2.6 Statistics

The experiment was a completely randomized design, with three replicates. The assay was for twice. The data were subjected to analysis of variance and mean values were compared by Tukey's test ($p < 0.05$).

3. Results and Discussion

Selenium affect the color of colony and the mycelial growth rate (Figures 1 and 2). The change of color from white to orange (Figure 1), mainly in culture medium with sodium selenite, may be due to the precipitation of selenium elemental. In anaerobic conditions, it occurs the reduction of selenite in selenium (Sylvia et al., 1999). Therefore, during *P. ostreatus* growth in the culture medium may have occurred the anaerobiosis microsites that favor the selenite reduction. Furthermore, the changing in the color of colony is due to metabolites production in

culture medium with selenium (Da Silva et al., 2013). Thus, studies for identification of theses metabolites are important for understand the transformation of selenium inorganic to organic forms by the fungus.

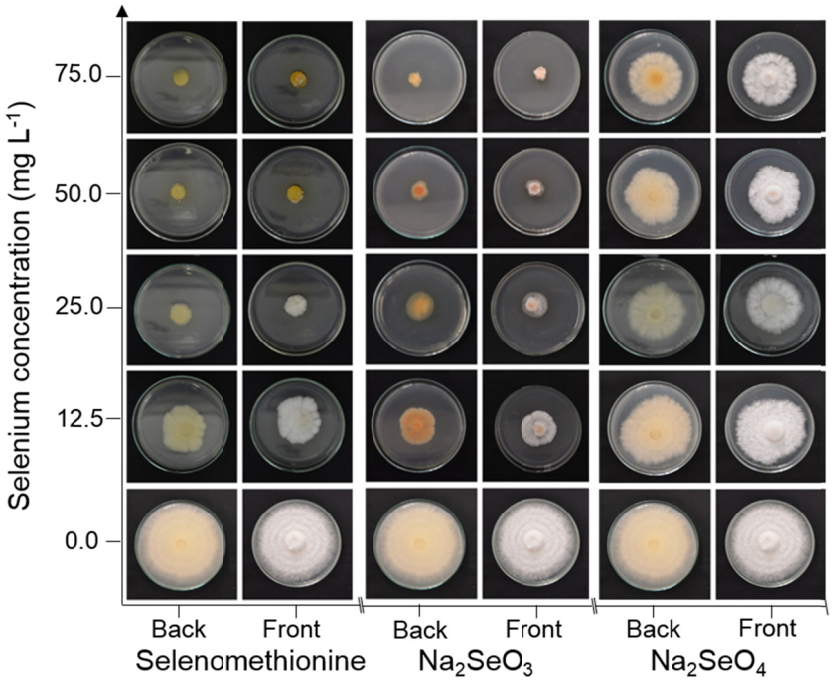

Figure 1. Micelyal growth of *Pleurotus ostreatus* (Plo 02) in PDA medium with selenomethione (SeMet), sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) in differ selenium concentration (0, 12.5, 25, 50 and 75 mg L^{-1})

The inhibition in mycelium growth rate may be due to the selenium oxidation (Figures 1 and 2). Selenomethionine and selenite were more toxic for *P. ostreatus* than selenate (Figure 2). These different effects on the mycelial growth and biomass may be due to the metabolic mechanisms of Se forms (Sors et al., 2005).

We also observed strong odor in culture medium with selenite during fungus growth that may be due to the production of dimethyl selenide [$(CH_3)_2Se$] (Sylvia et al., 2004; Amouroux et al., 2000). According to Zhang and Chasteen (1994), the methylation of selenium inorganic by microorganism produce volatile organic compounds that are less toxic than inorganic forms. Thus, the [$(CH_3)_2Se$] production by fungus can be a detoxification mechanism (Milovanovic et al., 2014; Zhang & Chasteen, 1994). Therefore, more studies have be done to evaluate this mechanism.

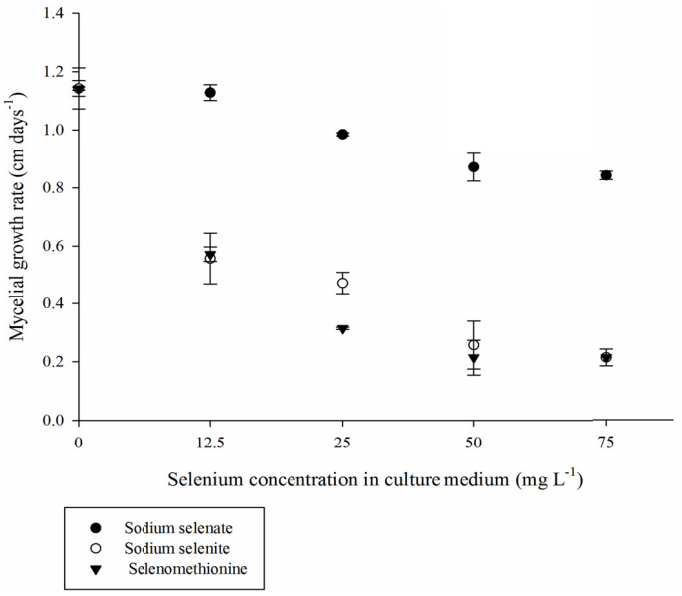

Figure 2. Mycelial growth rate of *Pleurotus ostreatus* (Plo 02) in PDA medium with selenomethione (SeMet), sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) in differ selenium concentration (0, 12.5, 25, 50 and 75 mg L^{-1})

Similar to mycelial growth rate, the biomass decreased in function of increasing of Se concentration and oxidation state (Figure 3). The biomass with selenate was not affected until 25 mg L^{-1} ($p < 0.05$), while to others chemical forms the biomass was affected by all tested selenium concentrations (Figure 3). Da Silva et al. (2013) observed that the increase in Se concentration in form of sodium selenite decreased form 2.5 at 8 folds in fungal biomass.

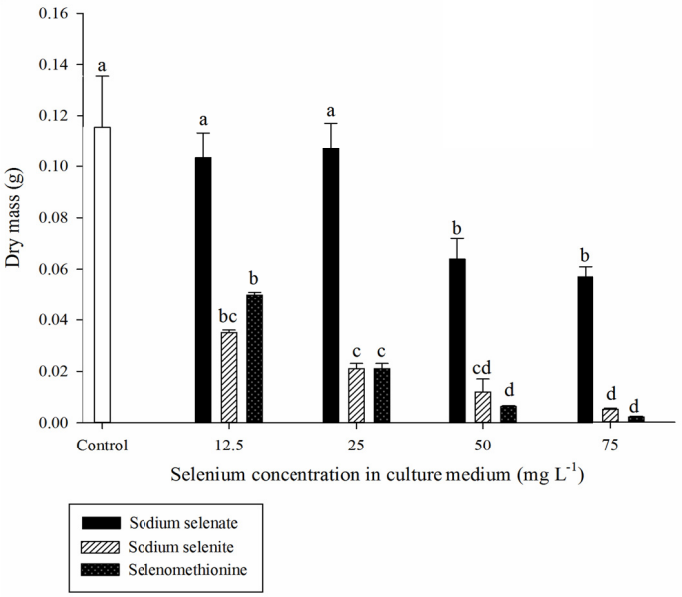

Figure 3. Dry mass (Biomass) of *Pleurotus ostreatus* (Plo 02) in PDA medium with selenomethione (SeMet), sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) in differ selenium concentration (0, 12.5, 25, 50 and 75 mg L^{-1}). Means followed by different letters differ at Tukey's test ($p < 0.05$)

This is the first study using selenomethionine in mycelial growth of *P. ostreatus*. The aim of the addition of this amino acid was to analyze the ability of this fungus to incorporate it directly into selenoprotein. The production of selenium organic by microorganisms have been shown (Da Silva et al., 2012; de Assunção et al., 2014; Fang et al., 2018; Hu et al., 2018; Milovanovic et al., 2014). Furthermore, the selenium inorganic was added to proteins by replacing sulfur (Rayman, 2008).

To quantify selenium was used the mycelium produced in culture medium with selenite and selenate in 12 and 25 mg L^{-1} of Se due to highest mycelial growth rate and biomass production (Figures 2-4). The trace concentration of selenium in the control can be derived from the reagents, PDA or water (Figure 4).

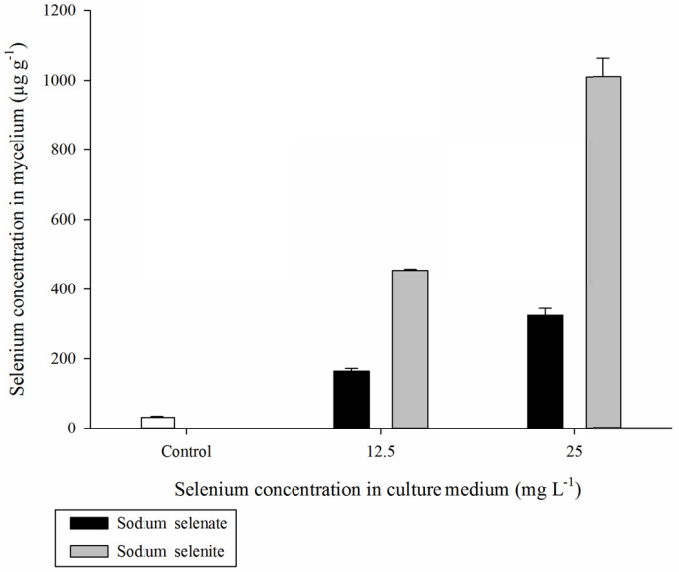

Figure 4. Selenium concentration in *Pleurotus ostreatus* (Plo 02) mycelium that was growth in PDA medium with sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) in differ selenium concentration (0, 12.5 and 25 mg L^{-1})

The accumulation of selenium in the mycelium was higher in *P. ostreatus* grown with selenite than selenate (Figure 4), because the incorporation of selenium in protein is faster as selenite than selenate (Rayman 2008). Therefore, for production of selenoprotein it is important to choose the fungal species/isolate, the chemical form, and the maximum concentration of selenium, which allows its growth, as well as the absorption, accumulation and biotransformation. The capacity of *P. ostreatus* and *Lentinula edodes* in absorb, accumulate and incorporate Se inorganic in mushrooms have been shown when these fungi were grown in agroindustrial residues containing different sodium selenite levels (Da Silva et al., 2012; Hu et al., 2018; Nunes et al., 2012; Silva et al., 2010; Solovyev et al., 2018).

P. ostreatus had small reduction in mycelial growth rate (Figure 2) and in biomass (Figure 3) in culture medium with at 75 mg L^{-1} of Se in form of selenate. This shows the higher potential of this fungus in grow in substrate containing high selenium concentration in form of selenate than in form of sodium selenite. Therefore, it was analyzed the effect of selenium concentration of 25 at 200 mg L^{-1} in the selenate forms on hyphae diameter and septa distance (Figures 5 and 6). These results are important for clarify the mechanism of tolerance of *P. ostreatus* at high selenium concentration.

The addition of selenate changed only septa distance and it was not observed changes significant entre hyphae diameter (Figures 5 and 6). Da Silva et al. (2013) also observed the decreased septa distance in the *P. ostreatus* and *Pleurotus eryngii in* culture medium containing sodium selenite.

The decrease of septa distance not was proportional the Se concentration (Figure 5) that show the tolerance of *P. ostreatus* at the high Se concentration. Furthermore, none changes in hyphae distance was observed in the fungal growth in PDA containing at 200 mg L^{-1}. These results are important to choose of fungal isolates with high capacity of absorb, accumulate and transform selenium inorganic into organic.

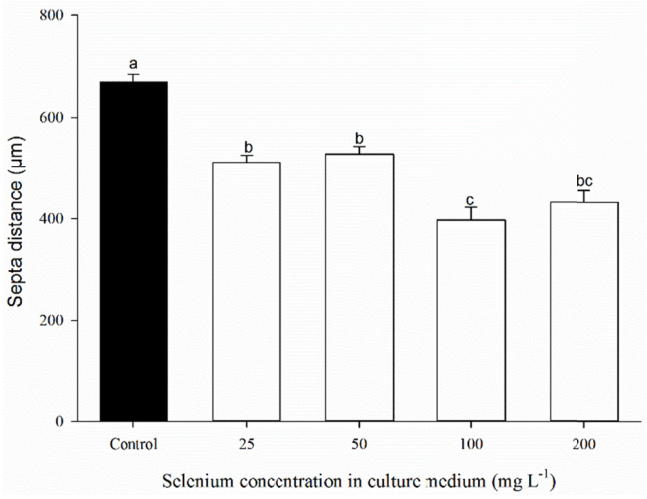

Figure 5. Septa distance of *Pleurotus ostreatus* cultivated in culture medium containing 0.0 (control), 25, 50, 100 and 200 mg L^{-1} of Se in the forms of sodium selenate (Na_2SeO_4).
Means followed by different letters differ at Tukey's test ($p < 0.05$)

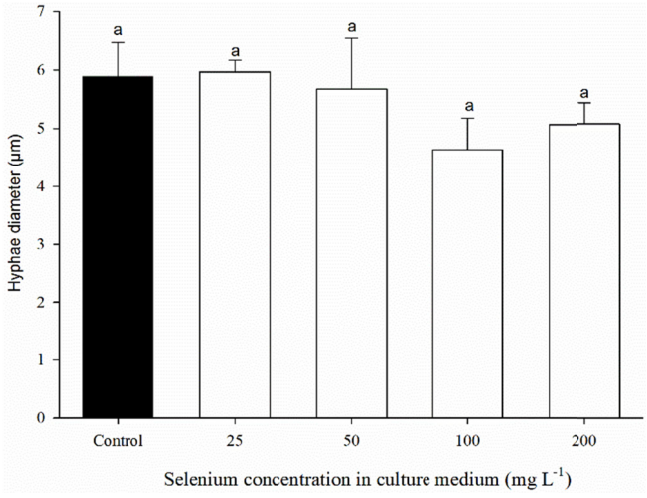

Figure 6. Hyphae diameter of *Pleurotus ostreatus* cultivated in culture medium containing 0.0 (control), 25, 50, 100 and 200 mg L^{-1} of Se in the forms of sodium selenate (Na_2SeO_4).
Means followed by different letters differ at Tukey's test ($p < 0.05$)

4. Conclusions

The sodium selenite is more indicated to enrichment of *P. ostreatus* mushrooms than other selenium forms analyzed, due to highest Se accumulation in the mycelium. The changes of colony in color and morphology can be a fungal mechanism to tolerate high Se concentration.

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