

POTENTIAL OF *TRAMETES HIRSUTA* MYCELIUM FOR SELENIUM ABSORPTION

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ABSTRACT

Biologically active substances, especially polysaccharides, isolated from fruiting bodies of *Trametes* species have immunomodulating and antitumor activities. Selenium is a trace element which at nutritional levels has antioxidant and numerous anticarcinogenic or preventive effects, while at higher levels it could be toxic. The purpose of this study was to resolve the question of whether various selenium concentrations affect ability of *T. hirsuta* mycelium to absorb and accumulate this trace element. Selenium was used in the form of Na₂SeO₃ and in the following concentrations: 0.3 mg/l, 0.7 mg/l, 1.0 mg/l, and 1.3 mg/l. The absorbed selenium concentration was determined by Atomic Absorption Spectrophotometer. Content of this trace element in the mycelium increased with enlargement of its concentration in the medium. Amounts of absorbed selenium were ranged from 7.54 µg/g (in the enriched medium with 0.3 mg/l) to 55.17 µg/g (in the enriched medium with 1.3 mg/l). Potential of mycelium to absorb this trace element was good. If the selenium concentration in the mycelium is presented as a percentage of its content in the medium it can be seen that absorption level was ranged from 15% to 25%.

Keywords: *Trametes hirsuta*; Mycelium; Selenium absorption

INTRODUCTION

Dynamic industrial and economic development has numerous advantages but also social and environmental consequences. Environment pollution by numerous chemical compounds (metal, metalloids, pesticides, toxic xenobiotics, halogenated and polycyclic aromatic hydrocarbons etc), connecting with higher intensity of UV light and ionizing radiation, climate change, loss of biodiversity, and decrease of natural resources present causal agents of numerous disorders (cancer, heard diseases, hypertension, diabetes, cataract, atherosclerosis, neurodegenerative disorders, etc.), which trigger is oxidative stress [1]. Selenium (Se) is a trace element which participates in biosynthesis of important selenoproteins and selenoenzymes that are parts of the organism defense system [2]. Unfortunately, Se is distributed in different forms and concentrations worldwide. Likewise, Se presence and amount are not good indicators of its bioavailability [3]. This element is easily absorbed in the forms of soluble selenates and selenites and incorporated in the cells in the forms of selenocysteine, selenomethionine, selenoproteins, polysaccharides, and nucleic acids [2]. Numerous studies demonstrated that

mushrooms are good but variable Se sources, depending on the species and substrate [4]. Due to mushroom ability to absorb inorganic Se forms and convert them to bioactive cell compounds they could be used as dietary supplements.

The aim of this study was to research the influence of Se added to the medium on the ability of *Trametes hirsuta* mycelium to absorb and accumulate this trace element.

MATERIALS AND METHODS

Trametes hirsuta BEOFB 30 was collected from *Prunus* sp. in Belgrade. Culture on malt agar medium is maintained in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade (BEOFB). Se was used in the form of sodium selenite (Na_2SeO_3) and in the following concentrations: 0.3 mg/l; 0.7 mg/l; 1.0 mg/l; 1.3 mg/l.

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. 5 ml of the prepared inoculum was used for inoculation of 70 ml modified synthetic medium (with glucose in the amount of 65.0 g/l and peptone as nitrogen source in the concentration of 2.0 g/l) enriched with tested Se concentrations. The medium without Se was used as the control. Submerged cultivation was carried out at room temperature on rotary shaker for 21 days. The obtained biomass was filtered, washed 3 times with 50 ml of dH_2O at magnetic stirrer and temperature of 30°C with the aim of removing the remaining Se on cell wall, and dried at 50°C to constant weight. Three repetitions for each Se concentration were made.

Dried mycelium (0.09 g), as well as Se-enriched media (2 ml) before inoculation and after mushroom cultivation, were digested with 10 ml of 100% HNO_3 and 3 ml of 100% HCl . The obtained samples were diluted with Milli-Q water, to the final volume of 20 ml, and cooled at 4°C. Se concentration was measured by hydride generation Atomic Absorption Spectrophotometer (HG AAS) Model SP190 (Pye Unicam, England). A standard curve was obtained from solutions containing Se in the concentrations of 0, 10, 25 and 50 ppb. The obtained values are presented as $\mu\text{g/g}$ of dried biomass or mg/l of the medium.

RESULTS AND DISCUSSION

Se content in the mycelium increased with enlargement of its concentration in the medium. Amounts of absorbed Se ranged from 7.54 $\mu\text{g/g}$ (in the enriched medium with 0.3 mg Se/l) to 55.17 $\mu\text{g/g}$ (in the enriched medium with 1.3 mg Se/l) (Fig. 1). Potential of mycelium to absorb this trace element was good if it is compared with the Se concentrations in the Se-enriched liquid media after sterilization and before inoculation (Table 1). If the Se concentration in the mycelium is presented as a percentage of its content in the medium it can be seen that absorption level was ranged from 15% to 25%.

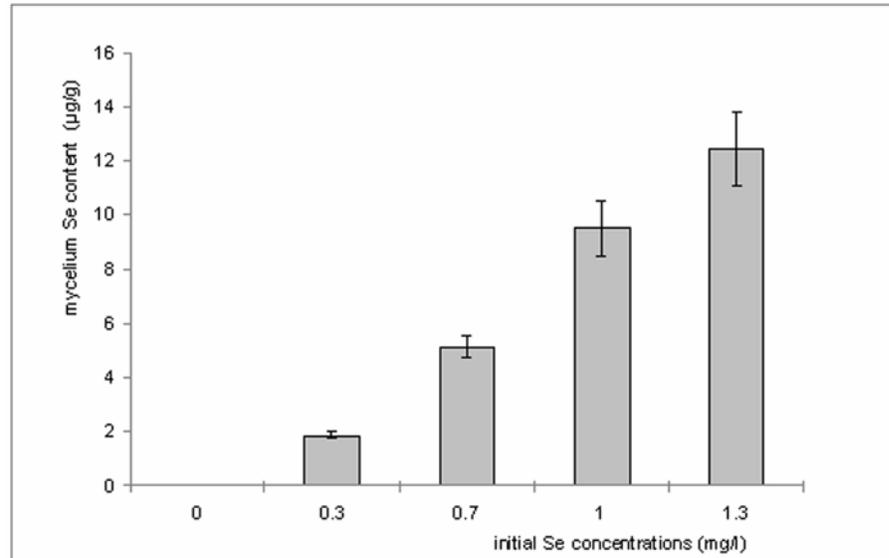


Figure 1. Concentration of absorbed Se in the mycelium of *Trametes hirsute*

The amount of remaining Se in the medium after 21 days of the cultivation was ranged between 6.23 ppb (in the enriched medium with 0.3 mg Se/l) and 23.13 ppb (in the enriched medium with 1.3 mg Se/l).

Table 1: Se concentration in liquid medium

Before sterilization (mg/l)	After sterilization (ppb)	After cultivation period (ppb)
0	0	0
0.3	096.3	6.23
0.7	210.0	12.4
1.0	296.3	17.67
1.3	373.0	23.13

Numerous studies have shown that concentration of the absorbed and accumulated Se depends on its form and amount in the medium, as well as mushroom species. Thus, selenized yeast was Se form with more bioavailability than Na₂SeO₃ for absorption and accumulation by *Agaricus bisporus* [5]. Values of incorporated Se in fruiting bodies were 160 µg/g dw, after cultivation in 10 µg selenized yeast-enriched compost, and 110 µg/g dw, in 10 µg Na₂SeO₃-enriched compost. Significant capacity for Se absorption was also noted in *Ganoderma lucidum* (up to 72 µg/g dw) as well as in species of the genus *Boletus* (absorbed Se amount was up to 40 µg/g dw) [2, 6-8]. However, the aim of only few studies was assay of mycelium capacity for Se accumulation [9, 10]. These authors reported that mycelium of *Pleurotus ostreatus* HAI 387 absorbed the highest Se amount after 28 days of submerged cultivation in 1.3 mg/l Na₂SeO₃-enriched synthetic medium. Compared to this species, *T. hirsute* is a poor Se absorber. However, regarding to the fact that it contains up to 22.6% of the dietary reference intake of Se for health adults, man and woman (55 µg/day) recommended by the European Scientific Committee on Food [7, 8], it could be concluded that it should be used as a food supplement.

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REFERENCES

- [1] Limón-Pacheco J. and Gonsebatt M. E. (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutat. Res.* 674:137-147.
- [2] Falandysz J. (2008). Selenium in edible mushrooms. *J. Environ. Sci. Heal.C*, 26, 256–299.
- [3] Barceloux D. G. (1999). Selenium. *Clin. Toxicol.* 37 (2): 145-172.
- [4] Kalač P. (2010). Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000-2009. *Food Chem.* 122: 2-15.
- [5] Dernovics, M., Stefanka, Z., Fodor, P. (2002). Improving selenium extraction by sequentive enzymatic process for Se-speciation of selenium-enriched *Agaricus bisporus*. *Analyt. Bioanalyt. Chem.* 372: 473-480.
- [6] Zhao, L., Zhao, G., Zhao, Z., Chen, P., Tong, J., & Hu, X. (2004). Selenium distribution in a Se-enriched mushroom species of the genus *Ganoderma*. *J. Agr. Food Chem.* 52: 3954–3959.
- [7] Cocchi, L., Vescovi, L., Pertini, L.E., Pertini, O. (2006). Heavy metals in edible mushrooms in Italy. *Food Chem.* 98: 277-284.
- [8] Costa-Silva, F., Marques, G., Matos, C.C., Barros, A.I.R.N.A., Nunes, F.M. (2011). Selenium contents of Portuguese commercial and wild edible mushrooms. *Food Chem.* 126: 91-96.
- [9] Stajić M., Milenković I., Brčeski I., Vukojević J., Duletić-Laušević S. (2002). Mycelial growth of edible and medicinal oyster mushroom [*Pleurotus ostreatus* (Jacq.: Fr.) Kumm.] on selenium-enriched media. *Int. J. Med. Mushr.* 4: 241-244.
- [10] Stajić M., Brčeski I., Wasser S. P., Nevo E. (2006). Screening of selenium absorption ability of mycelia of selected *Pleurotus* species. *Agro Food Ind. Hi. Tec.* 17: 33-35.