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SHORT COMMUNICATION

Selenium speciation and biological characteristics of selenium-rich Bailing mushroom, *Pleurotus tuoliensis*

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ABSTRACT

Nowadays the study of selenium-rich mushrooms is very popular. In the present study, selenium speciation in fruiting body of *Pleurotus tuoliensis* was investigated in cultivation substrates with different concentrations of sodium selenite, as well as mycelia growth and mushroom development. The results showed that the *P. tuoliensis* mycelia appeared good tolerance to selenium at all test concentrations. A selenium concentration of 10 mg/kg promoted fruiting of *P. tuoliensis*; the fruiting bodies were of good quality and had a low malformation rate. HPLC–ICP-MS determined that organic seleniums enriched in stipes and caps existed mainly in the form of selenoCystine and selenoMethionine at selenium concentrations of 10-100 mg/kg. These findings suggest that *P. tuoliensis* could be developed as a selenium-rich mushroom product for use as a novel dietary source of bioavailable supplemental selenium.

Keywords: Mycelia growth rate; *Pleurotus tuoliensis*; Selenium speciation; Selenium-rich product

INTRODUCTION

Selenium is an important trace element in the environment and a necessary component of selenocysteine and selenium enzymes, such as peroxidase, which have important biological functions, including cancer prevention (Pd 2004), antioxidation (Whanger 2002), immune stimulation (Silva et al., 2010), and HIV inhibition (Yu et al., 2007). Insufficient or excessive intake of selenium can lead to many diseases. Selenium poisoning causes symptoms such as nausea, upper abdominal discomfort, constipation and pain in the shoulder and foot (Hamilton 2004). However, an insufficient supply of selenium may cause growth retardation and dysfunctional bone metabolism, which can lead to abnormal thyroid function (Köhrle et al. 1992). Many diseases in humans and animals are closely associated with selenium deficiency, such as Kashin-Beck disease (Jie et al., 2017), atherosclerotic heart disease (Alehagen and Aaseth 2014), or diarrheal disease (Amare et al., 2011). Although selenium may be acquired from foods such as eggs, onions, malts, meat, mushrooms and nuts, its abundance in most natural and processed foods is low. It has been reported that certain endemic diseases such as Keshan disease and Kashin-Beck disease are prevalent in northeastern China and southeastern Siberia due to the insufficient amount of selenium in locally produced food. Therefore, it is necessary to add selenium to the diet in such regions to meet the requirements of the human body.

Mushrooms are excellent accumulators of minerals from the environments in which they grow. Mushrooms can accumulate selenium in their fruiting bodies when cultivation substrates are supplemented with selenium in the form of organic salt or inorganic salt. Cultivation of *Agaricus bisporus* on substrates supplemented with 0.6 mmol L^{-1} Se resulted in a 2.5-fold increase in the selenium content of the fruiting bodies (Rzymski et al., 2016). Moreover, selenium enrichment by *Cordyceps militaris* (Dong et al., 2013), *Pleurotus ostreatus* (Yan and Chang 2012) and *Lentinula edodes* (Ogra et al., 2004) was enhanced remarkably when they were grown on a selenium-enriched substrates. However, the ability of different species of mushrooms to accumulate selenium varies widely. For example, Jerzy Falandysz reported that the total selenium content in *Hericium erinaceus* and *Ganoderma lucidum* fruiting bodies grown on substrates with 0.1 mM inorganic selenium was 17.1 mg/kg DW and 28.3 mg/kg DW, respectively, while the control mushrooms had total selenium content of 14.1 mg/kg DW and 8.5 mg/kg DW, respectively (Niedzielski et al., 2014).

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Pleurotus tuoliensis, commercially known as Bai Ling Gu, is a precious edible fungus with extremely high nutrient and medicinal value (Zhao et al., 2016). Initially, *P. tuoliensis* restrictly located in the northwest part of China, i.e., Xinjiang Autonomous Region (Mou 1987), with independent intellectual property rights in China. In recent years, the factory cultivation of *P. tuoliensis* has been achieved. Based on the selenium-enriched study in other kinds of mushroom, selenium enrichment cultivation should also increase the nutrient value of *P. tuoliensis* fruit body and make it as a new selenium supplementation food. However, to our knowledge, there is no report on selenium-enriched *P. tuoliensis*. Therefore, we were to evaluate enrichment ability of *P. tuoliensis* inoculated in cultivation substrates with different concentrations of selenium. Furthermore, Selenium speciation and biological characteristics in selenium-rich Bailing mushroom were also explored to provide a scientific basis for developing selenium-enriched *P. tuoliensis* products.

MATERIALS AND METHODS

Cultivation of selenium-rich *Pleurotus tuoliensis*

P. tuoliensis 50869 strain was provided by the Agricultural Culture Collection of China. The cultivation substrates was prepared by 80% cotton seed hull, 18% wheat bran, 1% gypsum and 1% lime. Sodium selenium in cultivation substrates was 10, 20, 40, 60, 100, 150 or 200 mg/kg, respectively. Bottles were filled with the prepared substrates (average of 350 g/bottle with moisture content of 66%), after which the bottles were sterilized at a temperature of 121 °C for 2 h. When the temperature was below 30°C, the sterilized substrates was inoculated with pure culture of *P. tuoliensis*. The inoculated bottles were randomly arranged in incubator with the temperature of 25°C and kept under dark conditions to encourage mycelia growth. After the substrates was fully covered with mycelia, the bottles were remained under dark condition at 25°C for 30 d, called "later maturing stage", after which these bottles were transferred to mushroom house with the light intensity of 800 Lx and relative air humidity at 80%-90%, then subjected to temperature stimulation with 14°C during the daytime and 4°C at night until primordium formation and bud appearance. Then each bottle was left one strong bud and the temperature was maintained at 14°C. Air humidity was increased to 90%-95% and ventilation was enhanced. When caps absolutely unfolded, the fruiting bodies were harvested. During cultivation process, the time from inoculation to bud appearance, the time from bud formation to harvest, fruiting body traits and yield were recorded.

At the same time of cultivation, the cultivation substrates was also added to large test tubes to allow measurement of the growth rate of mycelia.

Mushroom sample collection

All fruiting bodies were collected, weighed, dried in drying oven at 60°C for 24 h, and weighed again for dry weight analysis. Dry stipes and caps were collected respectively, and then separately ground by grinder. The powered samples were carried out in the extraction procedure.

Extraction procedure

The extraction was performed as follows: 0.2 g of stipe and cap sample (homogenized by rubbing and sieving through a 0.02 mm sieve) was accurately weighed into 50-mL centrifuge tubes, respectively. Protease K (4 mg) and pure water (20 mL) were added to centrifuge tubes and the mixture was placed in waterbath oscillator at 37 °C for 4 h, centrifuged at 3000 r/min, filtered with a 0.45-μL filter membrane, and diluted by ten folds for HPLC- ICP-MS analysis. Blank and control samples were prepared at the same manner.

Analysis of selenium speciation by HPLC-ICP-MS

Selenium speciation in stipe and cap were detected by high performance liquid chromatography hyphenated to inductively coupled plasma-mass spectrometry (HPLC–ICP-MS) according to the method described by (Zhu et al., 2017). A Dionex UltiMate 3000 (Dionex, Germering, German) coupled with an X2Series ICP-MSX Series 2 (Thermo Fisher Scientific, German) was used for analysis of selenium speciation. A C18 column (Hypersil ODS2, 250×4.6 mm, 5 μm) was used for the separation of selenium compound. HPLC separation was carried out at 25 °C, and the mobile phase was 50:50 methanol-buffer solution (0.1% mercaptoethanol, 50 mmol L^{-1} NH_4Ac, adjusted to pH 5 with 0.1 mol L^{-1} HAc) with a flow rate of 1.0 mL min^{-1} (isocratic elution). The injection volume was 20 μL. The ICP-MS conditions were seen in (Zhu et al., 2017).

Selenate radical standard solution (GBW10033), selenite radical standard solution (GBW10032), slenomethionine (GBW10034) and selenocystine (GBW10087) (National Institute of Metrology) are purchased from Shanghai yuanye biotechnology Co. Ltd, Shanghai, China. High purity deionized water obtained from Milli-Q element system was used throughout this study.

Data analysis

The experimental data were analyzed using Microsoft Excel and SPSS 17.0. Differences among the means of groups were assessed using Duncan's multiple range tests at 95% confidence level($p<0.05$). Statistical analyses were conducted using SPSS 19.0 (IBM Inc., Armonk, NY, USA).

RESULTS AND DISCUSSION

Effect of selenium on mycelia growth

Cultivation of edible fungi on substrates supplemented with inorganic selenium is a promising method for developing a selenium-rich product, but a high selenium concentration in the substrates can adversely affect mycelia growth and inhibit fruiting body formation (Silva et al., 2012). Indeed, Silva et al. (Silva et al., 2013) reported that a selenium concentration greater than 25.4 mg/L in PDA culture medium drastically decreased the growth rate of *Pleurotus ostreatus* mycelia. In our study, a large test tube filled with cultivation substrates was used to evaluate the growth rate of mycelia during the process of cultivation. Table 1 showed the growth rate of four stages for mycelia, wherein the mycelia growth rates at early stage, and middle and early stage were faster than that at middle and late stage, and late stage at selenium concentrations of 0-200 mg/kg. This was because of good permeability of substrates near bottleneck. The average mycelia growth rate reached 3.23 mm/d at a selenium concentration of 40 mg/kg, which was faster than that of control mycelia (2.66 mm/d). Even when selenium concentration was 200 mg/kg, the mycelia could reach average rate of 2.52 mm/d, equal to the growth rate of control mycelia. This indicated that *P. tuoliensis* mycelia had high selenium tolerance. In addition, mycelia intensity under all selenium irrigation concentrations appeared the same as that of control group.

Effect of selenium on the fruiting of *P. tuoliensis*

Table 2 summarized the development of *P. tuoliensis* cultivated on substrates containing different concentrations of selenite sodium. *P. tuoliensis* showed increased yield and good quality at a selenium concentration of 10 mg/kg, with longer stipe and shorter cap, which was one of characters of high quality fruiting body. However, a selenium concentration greater than 20 mg/kg in the substrates can negatively impact the growth and development of fruiting bodies for *P. tuoliensis*, with prolonged growth cycle, shortened fruiting body stipes, decreased cap size, and reduced yield. In addition, mycelia growth rate under selenium concentration of 150 mg/kg is faster compared with that of control group, but no fruiting bodies were formed at this concentration. In this study, fruiting bodies collected from the substrates containing sodium selenite of 0-100 mg/kg were used for further analysis on selenium speciation.

Selenium speciation in stipes and caps

The biological function of selenium is closely related to its chemical form and content. The organic form of selenium has a high absorption rate, high biological activity and little environmental impact in comparison with inorganic form of selenium. Therefore, conversion of inorganic selenium into organic forms is a popular area of research. Organic forms of selenium are produced via two processes: artificial synthesis and biotransformation (Funes-Collado et al., 2013). Organic

Table 1: the growth rate of *P. tuoliensis* mycelia inoculated in cultivation substrates with different concentrations of selenium[a] (mm d^{-1})

Concentrations (mg/kg)	Early stage	Middle and early stage	Middle and late stage	Late stage	Average
0	2.24±0.42^{a}	3.21±0.56^{abc}	2.07±0.48^{ab}	3.25±0.87^{ab}	2.66±0.44^{ab}
10	2.78±0.56^{c}	3.09±0.75^{ab}	2.61±0.36^{de}	3.30±0.36^{ab}	2.92±0.33^{c}
20	2.30±0.23^{a}	3.60±0.55^{cde}	2.32±0.27^{bcd}	3.27±0.45^{ab}	2.84±0.20^{bc}
40	2.64±0.47^{bc}	3.93±0.73^{e}	2.71±0.35^{e}	3.63±0.47^{b}	3.23±0.31^{d}
60	2.40±0.35^{ab}	3.02±0.36^{ab}	2.01±0.25^{a}	3.00±0.39^{a}	2.53±0.25^{a}
100	2.41±0.39^{ab}	3.42±0.79^{bcd}	2.48±0.45^{cde}	2.91±0.44^{a}	2.79±0.29^{bc}
150	2.79±0.45^{c}	3.78±0.63^{de}	2.42±0.37^{cde}	3.18±0.58^{ab}	3.01±0.29^{c}
200	2.13±0.28^{a}	2.79±0.48^{a}	2.21±0.31^{abc}	3.02±0.59^{a}	2.52±0.19^{a}

[a]Different lowercase letters mean significant differences in each column ($p<0.05$)

Table 2: Effect of selenium on the yield and production cycle of *P. tuoliensis*[a]

Concentra-tions (mg/kg)	Inoculation-bud formation (d)	Bud formation -picking (d)	Total yield (kg)	Average single weight (g)	Stipe length (cm)	Stipe diameter (cm)	Cap diameter (cm)	Deformity rate (%)
0	144	19	10.11	235.18±38.34^{a}	7.62	6.01	10.09	13.95
10	141	20	13.25	210.24±46.76^{a}	9.24	5.85	9.25	4.76
20	145	19	9.67	179.10±28.73^{b}	7.52	6.42	8.36	27.78
40	150	19	3.07	146.21±39.61^{c}	8.42	4.88	6.90	85.71
60	151	20	1.83	141.28±37.65^{c}	7.34	5.35	6.27	100
100	160	21	0.09	90.48	5.30	6.50	6.50	100
150	/	/	0	/	/	/	/	/
200	/	/	0	/	/	/	/	/

[a]Different lowercase letters mean significant differences in each column ($p<0.05$)

selenium accumulated in mushrooms is mainly present in the forms of selenocysteine, selenomethionine and methyl selenocysteine (Turlo et al., 2007; Wu et al., 2012), and their proportions vary widely. Our study respectively explored selenium speciation in stipes and caps by HPLC-ICP-MS that was a conventional means for determining selenium speciation (Do et al., 2017; Supriatin et al., 2015). As shown in Fig. 1, inorganic selenium accumulated in stipes and caps primarily as sodium selenite (SeIV) and sodium selenate (SeVI). The primary organic selenium compounds were selenocysteine (SeCys) and selenomethionine (SeMet), while no methyl selenocysteine was detected, which is differs from that reported by Maseko et al., from a study only SeCys existed in *Agaricus bisporus* mushrooms grown on substrates supplemented with inorganic selenium (Maseko et al., 2013). This showed that the distribution of accumulated organic selenium varied among different mushroom species, but all organic forms were well absorbed and used for selenoprotein biosynthesis (Nunes et al., 2012). In our study, the contents of selenoamino acids determined in control stipes and caps were 0.64 and 0.65 mg SeCys/kg DW, 0.55 and 0.25 mg SeMet/kg DW, respectively. Selenium addition in cultivation substrates increased the concentrations of selenoamino acid in stipes and caps to the following levels: 42.19 and 31.49 mg SeCys/kg DW, 41.54 and 49.20 mg SeMet/kg DW at 20 mg/kg selenium addition; 50.02 and 84.79 mg SeCys/kg DW, 40.60 and 53.13 mg SeMet/kg DW at 40 mg/kg selenium addition; 43.88 and 54.64 mg SeCys/kg DW, 58.64 and 40.98 mg SeMet/kg DW at 60 mg/kg selenium addition; 41.64 and 22.76 mg SeCys/kg DW, 30.69 and 64.13 mg SeMet/kg DW at 100 mg/kg selenium addition; 36.70 and 26.69 mg SeCys/kg DW, 18.69 and 45.44 mg SeMet/kg DW at 150 mg/kg selenium addition. The average maximum fold increase of the individual selenoamino acids for stipes and caps was 78- and 130-fold for SeCys, 73- and 212-fold for SeMet at 40 mg/kg selenium irrigation compared to that of control stipes and caps. Thus it can be seen that *P. tuoliensis* fruiting bodies had extremely strong enrichment capacity on selenite sodium in the substrates. Moreover, the content of organic selenium was much higher than that of inorganic selenium in stipes and caps at all test selenium concentrations, indicating that the inorganic selenium in the substrates migrated to the fruiting bodies during the cultivation process, participated in the synthesis of amino acids, proteins and other biomolecules, and was transformed from inorganic selenium into organic selenium. Selenium-fortified mushrooms could be an interesting alternative to selenium-enriched yeast as a dietary selenium supplement (Thiry et al., 2013), but production must be carefully controlled and utilize appropriate substances in appropriate concentrations and forms, as shown in our study, although content of selenium enriched in fruiting bodies were higher when selenium concentration in the substrates was ≥20 mg/kg, the growth and development of fruiting bodies was damaged.

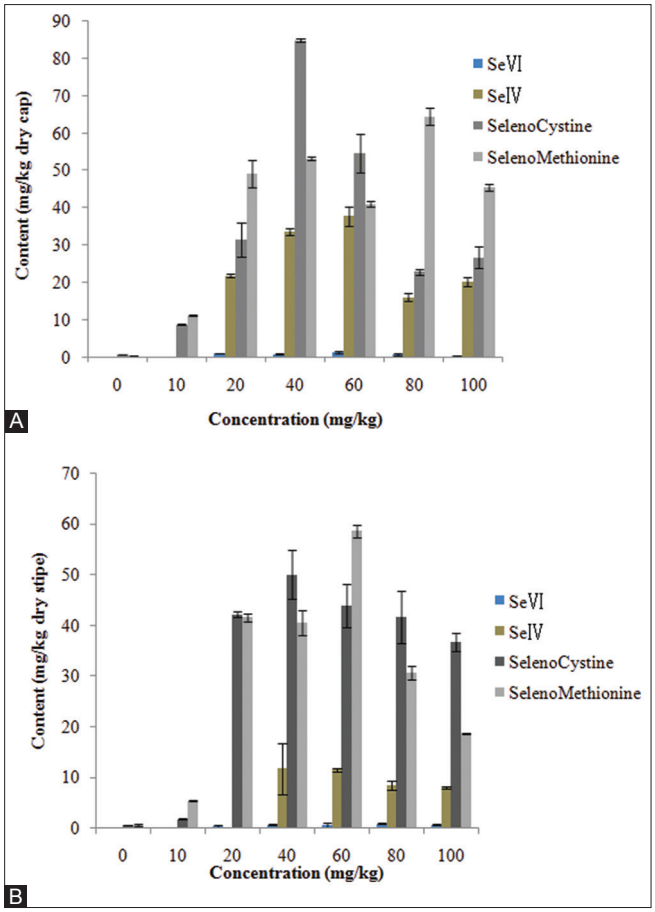

Fig 1. Selenium speciation in fruiting bodies collected from the substrates with 0, 10, 20, 40, 60, 80, and 100 mg/kg selenite sodium. A. The contents of organic selenium and inorganic selenium in caps. B. The contents of inorganic selenium and inorganic selenium in stipes. Notes: SeIV: sodium selenite. SeVI: sodium selenate.

CONCLUSIONS

The World Health Organization (WHO) and the International Food and Agriculture Organization (FAO) recommend daily selenium intake of 30 to 55 mg per person per day. The fruiting bodies of commercially cultivated mushrooms (such as *A. bisporus*) do not contain significant amounts of selenium, but the fruiting bodies of mushroom grown on substrates fortified with selenium can become enriched in inorganic and organic selenium. Therefore, selenium-rich mushrooms may be a novel method for producing a natural product that can be applied to address selenium deficiency. The present study showed that 10 mg/kg of selenium in the cultivation substrates is suitable for producing selenium-rich *P. tuoliensis* mushrooms.

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Authors' contributions

Yajie Zou performed all measurements and experiments and drafted the manuscript. Qingxiu Hu supervised and provided important intellectual content in the research design, coordination of the study and contributed in the drafting of the manuscript. Fang Du participated in the drafting and reviewing of the manuscript, and Haijun Zhang analyzed the data and contributed to the statistical analysis. All authors read and approved the final manuscript.

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