

STUDY ON SELENIUM ACCUMULATION IN TURKEY TAIL FUNGUS (*TRAMETES VERSICOLOR*) BY USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

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1. INTRODUCTION

The selenium enrichment would be encouraged to improve the potentially active complexes of polysaccharides and peptides from fungi, particularly their antioxydative and antitumor properties. Se plays an essential nutritional role in many animals and humans as integral components of several enzymes [4]. Se would be an antioxidant and antimutagenic agent that can prevent the malignant transformation of normal cells and effectively inhibit the activation of oncogenes [16]. Selenium (Se) is now well established as an essential trace element for both human beings and many other forms of life.

Se-enrichment technologies have been developed with yeasts *Saccharomyces cerevisiae* for medical preparations as Se-rich yeast biomass, that is rich in proteins and SH-groups – a new anti-ageing medicaments with highly active scavengers (with SeH-groups) for toxic free radicals. The preparations of Se-enriched yeasts have been commercialized quite widely. However, the yeasts don't need Se as essential micronutritive, and also selenoproteins. In fact, the studies on Se-enrichments in higher fungi, particularly medicinal mushrooms, i.e. Button mushroom *Agaricus bisporus*, Lingzhi (Mannentake) *Ganoderma lucidum*, Oyster mushroom (Houbitake) *Pleurotus ostreatus*, Sitting hen mushroom (Maitake) *Grifola frondosa*,... have just been started recently [19, 14, 3] particularly for accumulation in mycelial biomass.

The accumulation of some bioactive minerals in fungal fermentation has been widely examined under nuclear method-aided researches and development. Anderson et al. (1978), Toepfer et al. (1977) using ^{51}Cr and biochemical analysis determined Cr-enrichment in fermented biomass of the yeast *Saccharomyces carlsbergensis* to obtain effectively anti-diabetic preparations. Many works dealing with Se accumulation in the yeast *S. cerevisiae* to produce antiaging biomass preparations with high activity for antioxydation (Se-enriched yeasts) have used tracer with INAA and conventional techniques [13, 5-8, 21]. Fermented products from *S. cerevisiae* enriched with Se have been developed and increasingly commercialized. We have developed ^{75}Se and INAA techniques to detect the translocation and accumulation of selenium in mycelial systems of Lingzhi fungus *Humphreya* sp. and *Ganoderma lucidum*, *Lentinula edodes* [18, 19]. The stationary fermentation for fruitful bioenrichments with vanadium and chromium in the yeast phase of Jelly fungi *Tremella* spp. have been conducted in Vietnam using INAA at Nuclear Reactor of Dalat [19, 9]. In the present study we determined Se bioenrichment in a polyporoid fungus commercialized for medicinal purposes, namely *Trametes versicolor* (Kawaratake in Japanese) by using INAA under similar procedures.

2. MATERIALS AND METHODS

2.1. Fungal strain

The germ of *Trametes versicolor* supplied from Chiba University, Japan was re-cloned (repurified) and collected from Tsukuba region isolated on PDA in Takasaki Radiation Chemistry Research Establishment, JAEA and fruitfully cultivated in Dalat, HoChiMinh City, Vietnam (Le Xuan Tham et al., 1999), and compared with some native strains collected in National Park of Cattien and Bidoup – Nui Ba, South Vietnam.

2.2. Fermentation

Stationary fermentation was maintained at room temperature (33-34°C) during 30 days in 250 ml flasks containing 150 ml of PG solution (without agar), supplemented yeast extract (2g/l) and selenium in NaSeO₃ at concentration of 10 ppm, prepared from Se source enriched in ⁷⁴Se as a target in nuclear reactor for ⁷⁵Se production.

2.3. Sampling

Biomass of fermented mycelia was collected at 5, 7, 9, 14, 20, 25 and 30 days after inoculation. Samples of mycelial biomass were gently washed with HCl 0.1 N and distilled water for 5 times to eliminated away free Se, then dried at 40°C until constant weight, ground finely and compressed to form pellets covered with thin PE film for irradiation.

2.4. Irradiation

In thermal column of the reactor under neutron flux appr. $10^{12} \text{ n.cm}^{-2} \cdot \text{s}^{-1}$ when reached 500 KW capacity, the samples of pellet were irradiated for 20 hs with standard and blank ones, and then stored for a week to eliminate some short-lived radionuclides.

2.5. Measurement and calculation

The irradiated samples were measured and analyzed for ⁷⁵Se by a Multichannel γ Spectrometer (Canberra) with HP Ge Detector. The calculations were based on areas of γ standard peaks of ⁷⁵Se activated (with specific softwares from IAEA).

3. RESULTS AND DISCUSSION

3. 1. Liquid fermentation of mycelia of *Trametes versicolor* – Effective biomass production

Stationary fermentation at room temperature was conducted and continuously maintained for 30 days for regularly harvesting the mycelial biomass.

Table 1. Se content in mycelial biomass of Kawaratake in stationary fermentation supplemented with Se at 10 ppm

Time of incubation (days)	Mycelial biomass growth (g)	Se content (ppm)
7	0.110	1549
10	0.220	1395
15	0.460	868
20	0.625	823
25	0.639	680
30	0.635	678

At earlier stages we have not yet examined the accumulation of Se in so little biomass of mycelia just fermented.

Fruitful cultivation of Kawaratake *Trametes versicolor* was conducted effectively in Dalat, Hanoi and HoChiMinh City. However, recovery efficiency of polysaccharides, particularly bio-active fractions - their main components is not available in practice for materia medica supply.

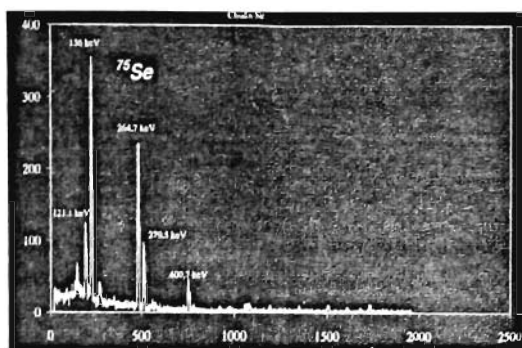


Fig. 1. Spectra of energy of ^{75}Se activated in standard

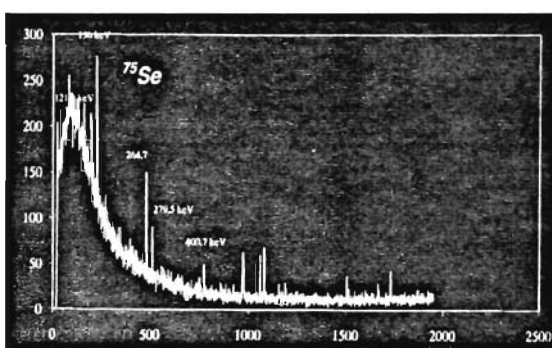


Fig. 2. Spectrum of gamma ray energy emitted Se activated in mycelial biomass of Kawaratake fermented for 10 days without Se supplement

The results have been obtained with *Trametes versicolor* showing high Se levels in mycelial biomass up to 600 - 1500 ppm, while in the control (without Se supplement), only trace of Se (1 ppm more or less) determined by using INAA (Instrumental Neutron Activation Analysis) in Nuclear Reactor, as shown in Table 1 and Figs. 1, 2, 3. This would be related to the researches on configurations of complex of polysaccharides and proteins in biomass with high bioactivities (Hobbs, 2005). Se would substitute S for some due structures and enhance their bioactivities.

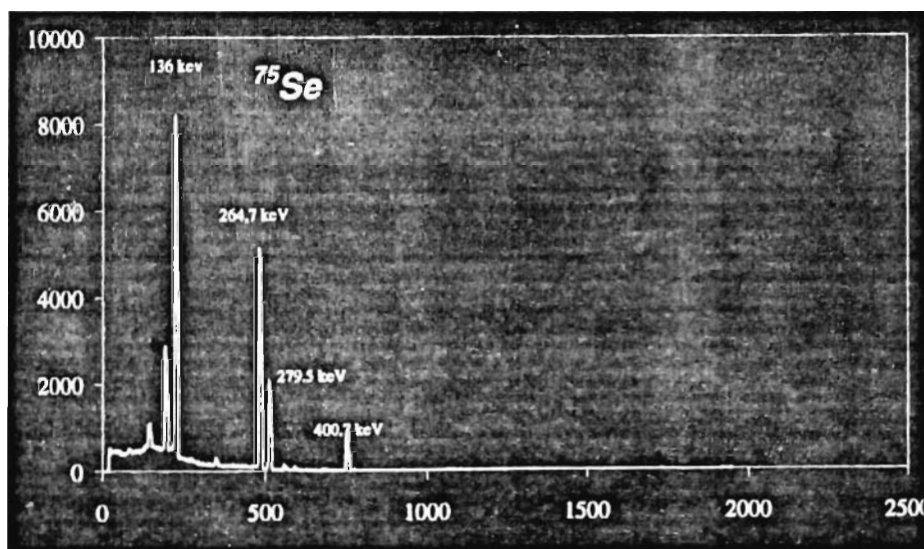


Fig. 3. Spectrum of gamma ray energy emitted from Se enriched and activated in mycelial biomass of Kawaratake fermented for 10 days with 10 ppm Se supplement

Perfectly consistent with its higher Se-content in *Ganoderma lucidum*, the protein exhibits approximately three times stronger activity of scavenging superoxide and hydroxyl radicals as

compared to the water-soluble protein extract, a finding demonstrating that the increasing antioxidant property of this protein depends quantitatively on its Se content [25, 10].

Selenium enrichments in fungal biomass would be promising for novel bioactive ingredients, due to Se substitutions in many S-containing compounds, particularly in amino acids containing S, such as cysteine, methionine,...; in peptides with disulphide bridges, that related to configures of many enzymes, proteins, polysaccharides-proteins complexes,... and free selenates, but at trace levels.

They would expect some novel potentials in antioxydative activity, antitumor activity,... with Se-rich Kowaratake, Mannentake, Maitake, Houbitake and Shiitake,... richest in Ergothionine! Selenium exerts its biological function in mammalian cell largely through selenoproteins and selenoenzymes, which contain selenocysteine (Se-Cys), the 21st amino acid, at the active site. These selenoproteins and selenoenzymes are an essential prerequisite for normal development and a long and healthy life. In all these selenoproteins, Se is incorporated into the protein molecule via the selenocysteinyl-tRNA, which recognizes the specific UGA codons in mRNA to insert selenocysteine into the primary structure of selenoproteins. In addition to incorporation as selenocysteine, selenium can replace sulfur in methionine of prokaryotic proteins forming selenomethionine (Se-Met), which can also be incorporated nonspecifically into proteins in place of methionine. The mammalian genome encodes 25 selenoproteins, several of which have antioxidant activities but the functions of most have not yet been determined. The fundamental importance of selenium to human health has received considerable attention. A deficiency of this element induces a variety of degenerative diseases including cancer, coronary heart, Keshan; and is also accompanied by loss of immunocompetence based on the fact that Se is normally rich in immune tissue such as liver, spleen, and lymph nodes. In contrast, supplementation with Se has pronounced immunostimulant effects including an increasing proliferation of activated T cells. Moreover, evidence is accumulating that there is an inverse correlation between selenium intake and cancer incidence at several sites such as prostate, colon, lung, and breast. Some reports in Japan suggested that 200 µg selenium per day is effective in reducing the incidence of prostate, lung, colon, and liver cancers and arthritis rheumatics, and in increasing immunomodulatory functions, as investigated just recently (in 2006-2007) by some groups of scientists from Cambridge (England), Maastricht (Netherlands), Arizona, Miami Universities (USA). Since selenium supplementation from natural food materials is considered to be safer than directly ingesting inorganic selenium, it is crucial for humans to find suitable dietary sources.

The food and medicinal mushrooms, incl. Maitake *Grifola frondosa*, Shiitake *Lentinula edodes*, Kowaratake *Trametes versicolor* and others should be advised for these purposes, because Se-enrichments in fungal biomass are feasible with considerably high efficiency.

The Turkey tail mushroom *Trametes versicolor* has been processed for medicinal utilizations during last 20 years, particularly in China and Japan (ca. 358 mill. USD obtained from PSK and PSP in Japan market in 1987, particularly for digestive tract and lung cancers). Recently, in Minnesota University, USA the scientists under the supervision of Prof. Joel Slaton have isolated and determined bioactivities, particularly antitumor ability of polysaccharides from *Trametes versicolor* for breast cancer. The group of scientists awarded 2.3 mill. USD for this promising achievement from NMI, and FDA has approved for further tests on human bodies (according to Science Daily, 7 January 2007). These results were supported by sophisticated reviews of researches on *Trametes versicolor* presented at 3rd International Medical Mushroom Conference, Seattle, USA (Hobbs, 2005).

Kawaratake uptake and accumulate Se so effectively during 10 days of fermentation. And we hope at determinations of Se-containing compounds with bioactivity in Kawaratake *Trametes versicolor* in further researches and development of Se-rich cultivations of these mushrooms and others.

REFERENCES

1. Anderson, R. A., Polanski, M. M., Roginski, E. E., and Mertz M. - Factors affecting the retention and extraction of Yeast chromium. *J. Agric. Food Chem.* **4** (1978) 858-861.
2. Barceloux D. G. – Selenium, *Clinical Toxicology* **37** (1999) 45-172.
3. Beelman R. B. and D. J. Royse - Selenium enrichment of *Grifola frondosa* (Dicks.: Fr.) S.F. Gray (Maitake) mushrooms, *Intern. J. Med. Mush.* **7** (3) (2005) 340.
4. Birringer M., Pilawa S., Flohe' L. - Trends in Selenium chemistry. *Nat. Prod. Rep.* **19** (2002) 693-718.
5. Czauderna M., Makowska E., and Smolinski S. - Use of INAA to study the effect of selenium on uranium accumulation in cells of *Saccharomyces cerevisiae*, *J. Radioanal. Nucl. Chem.* **177** (1994) 393-401.
6. Czauderna M., Sierakowska S., and Sitowska B. - Use of INAA to study the determination of Se, Sb, Zn, and Co levels of yeast cells, *Appl. Radiat. Isotop.* **46** (1995) 1295-1298.
7. Czauderna M., Turska M., and Sitowska B. - Use of INAA to study the determination of Se, Th, Zn, Co, and Fe levels of yeast cells, *Appl. Radiat. Isotop.* **47** (1996a) 105-110.
8. Czauderna M., Turska M., Sierakowska S., and Smolinski S. - Use of INAA to study the interaction between Se and Te in cells of *Saccharomyces cerevisiae*, *Appl. Radiat. Isotop.* **47** (1996b) 153-157.
9. Dinh L. T., Toan N. V., Giang N., Tham L. X., and Kiet T. T. - Study of effect and bioaccumulation of V in the yeast phase of Jelly fungi *Tremella fuciformis* and *Tremella encephala* using nuclear techniques, *Vietnam J. Sci. Technol.* **39** (2) (2001) 20-26.
10. Du M., Zhao L., Li C., Zhao G-H., and Hu X-S. - Purification and characterization of a novel fungi Se-containing protein from Se-enriched *Ganoderma lucidum* mushroom and it's Se-dependent radical scavenging activity, *Eur. Food Res. Tech.* Springer-Verlag, 2006.
11. Duletic-Lausevic S., M. Stajic, I. Brceski, and J. Vukojevic - Media composition influences the ability of *Ganoderma lucidum* mycelium to absorb Selenium, *Proc. 5th Inter. Conf. on Mushroom Biol. Mushroom Prod.* 8-12th April, Shanghai, China, 2005.
12. Hobbs C. R. - Medicinal value of Turkey tail fungus *Trametes versicolor* (L.: Fr.) Pilaüt (Aphyllphoromycetidae), *Intern. J. Med. Mush.* **7** (3) (2005) 346-347.
13. Meng M., Ma C. L., Ding W. J., and Zhu X. L. - Determination of the content of selenium in selenium yeast by NAA, *J. Nucl. Radiochem* **12** (1990) 185-187.
14. Stajic M., I. Brceski, Duletic-Lausevic S., J. Vukojevic, S. P. Wasser, and E. Nevo - Effect of Selenium source on Selenium absorption by mycelia of nine *Pleurotus ostreatus* strains, *Proc. 5th Inter. Conf. on Mushr. Biol. Mushr. Prod.* 8-12th April, Shanghai, China, 2005.

15. Stajic M., I. Brceski, S. P. Wasser, E. Nevo, J. Vukojevic, Duletic-Lausevic S. - Ability of selenium absorption by mycelia of *Pleurotus eryngii* (DC.: Fr.) Queùl., depending on selenium source in medium, *J. Med. Mush.* **7** (3) (2005) 469.
16. Stapleton R. S. - Introduction: the Selenium conundrum, *CMLS Cell. Mol. Life Sci.* **57** (2000) 1823-1824.
17. Le Xuan Tham, S. Matsuhashi, and T. Kume - Growth and fruitbody formation of *Ganoderma lucidum* on substrates supplemented with selenium, vanadium and germanium, *Mycoscience* **40** (1999) 87-92.
18. Tham L. X., Linh H. T. M., Do T. H. - Study of growth and uptake of selenium in Lingzhi fungus *Humphreya* sp. by using ^{75}Se techniques, *Vietnam J. Sci. Technol.* **35** (1997) 7-9.
19. Tham L. X., Huong P. T. T., and Dinh L. T. - Study of action and biological accumulation of chromium in the yeast phase of *Tremella* spp. by nuclear technique, *Pharmaceut. J.* **4** (2001) 13-17.
20. Tham L. X., Nguyen Giang, Le Viet Ngoc - Selenium enrichment in Shiitake *Lentinula edodes*. Proc. 3rd Inter. Conf. Med. Mus. (IMMC 3), 12-17th Oct. 2005. Port Townsend. Seattle, USA, 2005.
21. Thuong N. Q., Bao D. T., Muravieva D. A., Ogursob I. U. A., and Strapuchko P. A. - Etùude d'une levure rich en seùleùnium. *Rev. Pharmaceut.* **1** (1995) 21-26.
22. Toepfer E. W., Mertz W., Polanski M. M., Roginski E. E., and Wolf W. R. - Preparation of chromium-containing material of glucose tolerance factor activity from Brewer's Yeast extracts and by synthesis. *J. Agric. Food Chem.* **1** (1977) 162-166.
23. Yang X-T., Yang Q-Y., Mi K., Feng H-Q, Jones B., and Jennifer W. - The HPLC separation and bioactivity evaluation of the Polysaccharopeptide (PSP) of *Coriolus Trametes* *versicolor* fermentative mycelia. *Intern. J. Med. Mush.* **7** (3) (2005) 482.
24. Zhao L., Zhao G., Zhao Zh-D., Chen P., Tong J. and Hu X-S. - Selenium distribution in a Se-enrichment mushroom species of the genus, *J. Agric. Food Chem.* **52** (2004) 3954-3959.
25. Zhao L., Zhao Zh-D., Tong J-Y, Wang Sh-H., Sun J-Zh., Hu X-S. - Effect of Selenium on the nutritional components of *Ganoderma lucidum* (W. Curt.: Fr.) Lloyd, *Intern. J. Med. Mush.* **7** (3) (2005) 428.

ABSTRACT

Medicinal mushroom Kawaratake *Trametes versicolor* strain obtained from Chiba University, Japan, was fermented at stationary Erlenmayer flasks 250 ml, containing PG media with Se (10 ppm as selenate) and without Se (control) supplement, incubated at 32-34°C (room temperature in HoChiMinh City). Selenium was added as dissolve selenate Na_2SeO_4 , in which Se was enriched with ^{74}Se . Under neutron fluxes in nuclear reactor ^{74}Se will be activated into ^{75}Se emitted gamma rays (n,γ reaction), recorded for calculations of Se contents in the samples. Cultivation of Kawaratake on mixed substrates based sawdusts supplemented with Se by injecting directly into center region of the substrate based on rubber tree sawdust. Analysis of Se contents in fungal biomass harvested by using INAA with neutron flux at $10^{12}.\text{cm}^{-2}.\text{s}^{-1}$ in Nuclear Reactor at Dalat City, Vietnam.

The results obtained with *Trametes versicolor* showed Se levels in mycelial biomass up to 600-1500 ppm, while in the control (without Se supplement), only trace of Se (1 ppm more or

less) was found. It would be related to the researches on configurations of complex of polysaccharides and proteins in biomass with high bioactivities. Se would substitute S for some due structures and enhance their antioxydant activities.

Key words - Medicinal mushroom *Trametes versicolor*, Se-enrichment, INAA

TÓM TẮT

NGHIÊN CỨU TÍCH TỤ SELENIUM Ở NẤM VÂN CHI *TRAMETES VERSICOLOR* BẰNG PHÂN TÍCH KÍCH HOẠT NEUTRON

Nấm dược liệu Vân chi (*Kawaratake*) *Trametes versicolor*, chủng nhận được từ Đại học Chiba, Nhật bản được lên men tĩnh trong bình nón 250 ml, chứa môi trường PG có bổ sung Se (10 ppm dạng selenate Na_2SeO_4 , làm giàu với ^{74}Se) và đối chứng không bổ sung Se, nuôi cấy ở nhiệt độ phòng tại Tp. Hồ Chí Minh (32-34°C). Chùm neutron Lò phản ứng hạt nhân tại Dalat, Việt Nam, cỡ $\sim 10^{12} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ kích hoạt ^{74}Se thành ^{75}Se , phát xạ tia gamma (qua phân ứng n, γ), được ghi nhận cho các tính toán hàm lượng Se trong các mẫu sinh khối nấm.

Kết quả thu được cho thấy đối với nấm Vân chi *Trametes versicolor* trên môi trường có Se bổ sung, mức tích tụ Se trong sinh khối hệ sợi lên men đạt tới 600 - 1500 ppm, trong khi ở đối chứng không có Se bổ sung, chỉ thấy mức vết Se (~ 1 ppm). Sự tích tụ này có quan hệ với nghiên cứu về cấu hình các phức hợp polysaccharide và protein có hoạt tính sinh học cao. Se có thể thay thế S trong các cấu trúc tương ứng và gia tăng hoạt tính chống oxy hóa của chúng.



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