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Ergothioneine Contents of Shiitake (*Lentinula edodes*) Fruiting Bodies on Sawdust Media with Different Nitrogen Sources

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ABSTRACT : Ergothioneine is a natural compound with strong antioxidant properties. In this study, the effects of different nitrogen sources including ammonium nitrate, ammonium sulfate, sodium nitrate, and histidine in sawdust media were investigated to enhance ergothioneine contents in Shiitake (*Lentinula edodes*) fruit bodies. The addition of 0.2% ammonium sulfate in the growth media showed the highest enhancement of ergothioneine content in shiitake fruit bodies which was 1.7-fold higher than the control. On the other hand, histidine, a building block of ergothioneine decreased the concentration of ergothioneine significantly. Our results demonstrate that the cultivation of shiitake in sawdust media with suitable nutrients was effective to enhance its ergothioneine contents.

KEYWORDS : Edible mushroom, High performance liquid chromatography, Metabolite

Introduction

Ergothioneine (ERG, $C_9H_{15}N_3O_2S$) is a water-soluble amino acid which is first discovered from ergot of rye by Tanret [1]. ERG has strong antioxidant properties [2, 3] and may prevent mitochondrial DNA damage [4]. It also has other biological properties such as anti-inflammatory [5], radioprotective [6] and neuroprotective effects [7]. It is synthesized by certain groups of organisms such as fungi, Cyanobacteria, and Actinobacteria, but it is also found in plants and mammals including humans [8].

Mushrooms can be regarded as health foods due to their nutritional compositions such as high proteins, low fat relatively large amounts of carbohydrates, vitamins, and minerals [9] and it is reported that cultivated mushrooms such as *Agaricus bisporus*, *Grifola frondosa*, *Lentinula edodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus* have ERG [10]. Among them, *Lentinula edodes* (Berk.) Pegler is one of the most popular edible mushrooms in the world especially in Asian countries such as Korea, China, Japan, and Taiwan [11, 12], and it had the second-highest ERG content among them [10]. It is reported that amino acids such as methionine could have positive effects on ERG contents from mushrooms including *Lentinula edodes* [13, 14]. However, the effects of inorganic nitrogen sources have not been examined.

In this study, ERG contents of *Lentinula edodes* were investigated to understand the effects of different nitrogen sources including one amino acid, histidine which functions as a building block of ERG [15].

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Materials and Methods

Fungal strain and cultivation conditions

The shiitake variety NIFoS 554 developed by the National Institute of Forest Science was used for this study. The fungal mycelia were grown on potato dextrose agar (Difco, Detroit, MI, USA) at 23°C for 10 days and used as inoculum. Sawdust media were prepared with oak sawdust and wheat bran in ratios of 8:2 (w/w). In addition, four different nitrogen sources, ammonium nitrate [$(NH_4)(NO_3)$], ammonium sulfate [$(NH_4)_2SO_4$], sodium nitrate ($NaNO_3$), histidine ($C_6H_9N_3O_2$) were supplemented with the concentrations of 0, 0.1, 0.2, and 0.3% (w/w). The

moisture content of sawdust media was adjusted at 65% (w/w). After the media (650 g) were put into 1,000 mL bottles (Dongwoo, Daejeon, Korea), they were autoclaved at 121°C for 90 min. The two agar blocks (10 mm × 10 mm) were inoculated to the media and they were incubated at 25°C for 90 days in the dark and then, for 30 days in the light conditions in an incubation room. At the end of the cultivation period, the bottles were removed, and the media were placed in a production room (18°C, 90% relative humidity). The resulting fruiting bodies were harvested, cleaned, and dried in oven at 55°C for 3 days.

Determination of ERG content

ERG extraction was performed according to Lee et al. [14] with minor modifications. The dried mushrooms were ground to a fine powder with a mortar and pestle. One g of dried mushroom powder was then added to 20 mL cold ethanolic extraction solution [10 mM dithiotreitol (DTT) 100 μM betaine in ethanol, 100 μM 2-Mercapto-1-methyl-imidazole (MMI) in 70% ethanol]. Then, the sample was vortexed for 90s and sonicated for 3 min. After 4 mL of sodium dodecyl sulphate (SDS) was added, it was centrifuged for 15 min at 4,000 rpm at 25°C. 10 mL of the supernatant was evaporated on a rotary evaporator at 40°C. After removing the solvent, 10 mL of distilled water (pH 7.3) was added and centrifuged for 15 min at 4,000 rpm. The resulting supernatant was used for high-performance liquid chromatography (HPLC) analysis.

The ERG content was determined using Hitachi HPLC System (Hitachi, Tokyo, Japan) equipped with a C18 column (4.6 × 250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA). The eluting agent was 1% acetic acid, and the flow rate was 0.7 mL/min. The injection volume was 20 μL. Absorbance was measured at 254 nm. L-ergothioneine (Enzo Life Sciences, Farmingdale, NY, USA) was used to calculate the standard curve and ERG content was quantified by the curve.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Duncan's Test ($\alpha < 0.05$) was used to analyze the results. This analysis was performed using SPSS version 10.0 program (SPSS Inc., Chicago, IL, USA).

Results and Discussion

To determine the ERG contents from shiitake fruit bodies, different concentrations (0, 0.1, 0.2, and 0.3%) of nitrogen sources, ammonium nitrate, ammonium sulfate, sodium nitrate, and histidine were supplemented in the growth media. As shown in Fig. 1, the ERG contents from the resulting fruit bodies varied significantly from 12.04 mg/kg to 43.56 mg/kg. The highest ERG content (43.56 mg/kg) was detected from the fruit bodies grown in the media with the addition of 0.2% ammonium sulfate which is 1.7-fold increase over control (26.28 mg/kg), but more ammonium sulfate (0.3%) reduced the ERG content significantly (16.47 mg/kg) (Fig. 1B). ERG content increased with the addition of 0.3% ammonium nitrate (30.94 mg/kg) (Fig. 1A) and 0.3% sodium nitrate (32.84 mg/kg) (Fig. 1C). Compared with the control, no significant differences were observed at 0.2% ammonium nitrate (25.22 mg/kg), 0.1% and 0.2% sodium nitrate (29.72 and 28.73 mg/kg), and 0.2% sodium nitrate (28.73 mg/kg). Reduced ERG content was found from 0.1% ammonium nitrate (18.15 mg/kg). ERG contents were gradually decreased from 26.28 mg/kg to 12.04 mg/kg when histidine was added in the growth media and the lowest ERG content was detected from the fruit bodies from 0.3% histidine among the fruit bodies tested (Fig. 1D).

It is interesting to note that histidine was not a good source for enhancing ERG contents, since ERG is synthesized from directly histidine via hercynine [15]. In Lee et al. [13], mycelia of *Ganoderma neo-japonicum* produced 41% less ERG when histidine was supplemented in the growth media. On the other hand, no significant difference of ERG production was found from the fruit bodies of *Pleurotus eryngii* var. *eryngii* regardless of histidine addition in the growth media. Since histidine could be synthesized by fungi [16] and it has many roles inside the cells [17], the effect to ERG production might be different among fungal species and their growth conditions. Lee et al. [14] showed that the supplement of methionine had different effects on ERG contents from mycelia among different fungal species. Further study is needed to clarify the effect of histidine supplement on ERG in fungi.

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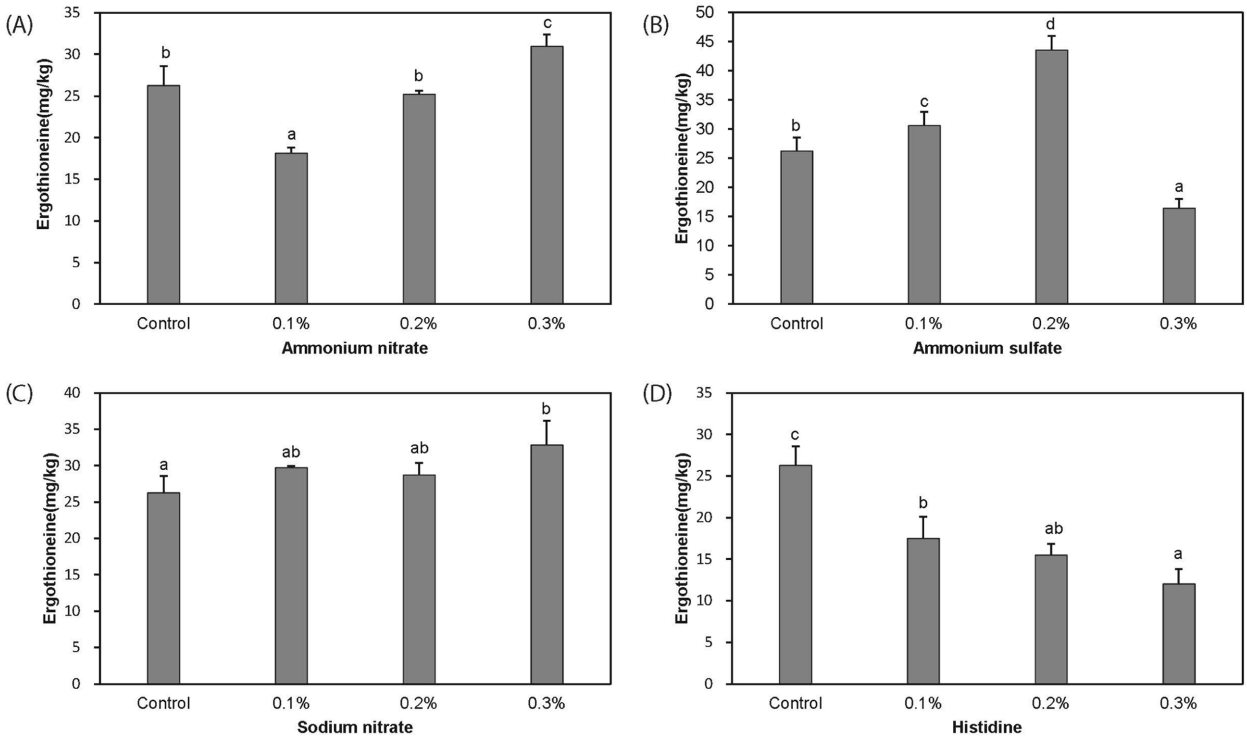

Fig. 1. Ergothioneine contents of shiitake depending on the nitrogen sources in the growth media. Nitrogen sources: A, Ammonium nitrate; B, Ammonium sulfate; C, Sodium nitrate; D, Histidine.

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