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Characterization of vitamin B_{12} compounds in the fruiting bodies of shiitake mushroom (*Lentinula edodes*) and bed logs after fruiting of the mushroom☆

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ABSTRACT

This study determined the vitamin B_{12} content in commercially available dried fruiting bodies of shiitake mushroom, *Lentinula edodes*. The vitamin B_{12} contents in dried donko-type fruiting bodies with closed caps (5.61 ± 3.90 μg/100 g dry weight), did not significantly differ from those of dried koushin-type fruiting bodies with open caps (4.23 ± 2.42 μg/100 g dry weight). The bed logs after fruiting of the mushroom also contained the vitamin B_{12} levels similar to that in the dried shiitake fruiting bodies. To determine whether the dried shiitake fruiting bodies and their bed logs contained vitamin B_{12} or other corrinoid compounds that are inactive in humans, we purified corrinoid compounds using an immunoaffinity column and identified vitamin B_{12} using vitamin B_{12}-dependent *Escherichia coli* 215 bioautograms and liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) chromatograms. Dried shiitake fruiting bodies rarely contained an unnatural corrinoid vitamin B_{12}[c-lactone] that is inactive in humans. Given that shiitake mushroom lacks the ability to synthesize vitamin B_{12} *de novo*, the vitamin B_{12} found in dried shiitake fruiting bodies must have been derived from the bed logs.

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1. Introduction

Vitamin B_{12} (B_{12}) is synthesized only by certain bacteria (Scheider and Stroiński 1987). B_{12} synthesized by bacteria is primarily concentrated in the bodies of higher predatory organisms in the natural food chain system. Animal-derived foods (i.e., meat, milk, egg, fish, and shellfish) but not plant-derived foods are considered to be the major dietary sources of B_{12} (Watanabe 2007). Thus, the risk of developing B_{12} deficiency is greater in strict vegetarians than in nonvegetarians (Millet et al. 1989). The major symptoms of B_{12} deficiency are neuropathy and megaloblastic anemia (Scalabrino 2009). Thus, it is necessary to identify plant foods that contain high levels of B_{12} to prevent vegetarians from developing B_{12} deficiency.

Among the wild edible mushroom fruiting bodies that are consumed by European vegetarians, black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) mushroom fruiting bodies contain considerable levels (1.09–2.65 μg/100 g dry weight) of B_{12}, whereas the remaining mushroom fruiting bodies have zero or trace levels (Watanabe et al. 2012). In our preliminary study, we found that Japanese edible wild (*Lactarius laeticolorus*, *Suillus spectabilis*, *Ramaria botrytis*, *Cortinarius pseudosalor*, *Boletopsis leucomelas*, and *Sarcodon aspratus*) and cultivated (*Pleurotus eryngii*, *Grifola frondosa*, and *Hypsizygus marmoreus*) mushroom fruiting bodies contained trace amounts (approximately 1.0 μg/100 g dry weight), whereas high levels of B_{12} were detected in commercially available dried fruiting bodies of shiitake mushroom (*Lentinula edodes*). Shiitake mushrooms are cultivated and consumed throughout the world. In particular, fresh and dried shiitake mushroom fruiting bodies are also used in various vegetarian dishes. Two types of high grade dried shiitake fruiting bodies are available in Japan: donko-type fruiting bodies with closed caps (early fruiting stage) and koushin-type fruiting bodies with open caps (late fruiting stage). However, little information is available on the B_{12} content in shiitake mushroom fruiting bodies, particularly whether the mushroom fruiting bodies contain "true" (authentic) B_{12} or an inactive corrinoid such as "pseudo B_{12}" (Watanabe et al. 2013). If shiitake mushroom fruiting bodies generally contain high levels of B_{12}, they would be good sources of B_{12} for vegetarians.

In this study, we analyzed the B_{12} contents in various dried shiitake fruiting bodies that are commercially available in Japan and characterized the B_{12} compounds found in these fruiting bodies.

2. Materials and methods

2.1. Materials

B_{12} was obtained from Sigma (St Louis, MO, USA). A B_{12} assay medium based on *Lactobacillus delbrueckii* subspecies *lactis* (formerly *L. leichmannii*) ATCC7830 was obtained from Nissui (Tokyo, Japan). Silica gel 60 thin layer chromatography (TLC) aluminum sheets were obtained from Merck (Darmstadt, Germany). The raw and dried shiitake mushroom fruiting bodies were purchased in Japan. Raw mushrooms were lyophilized and then used in the experiments. Fruiting bodies of Shiitake mushroom (cultivar Kinko-702) were cultivated on bed logs (one year-old bed logs of konara oak, *Quercus serrata*), harvested at the indicated stages 1–5, and dried at 50 °C for 24 h. Bed logs (konara oak, *Quercus serrata*) after five times fruiting of Shiitake mushroom (cultivar Kinko-702) were used for the experiments. *Lentinula edodes* TUFC 100154 and 100177 were obtained from Fungus/Mushroom Resource and Research Center, Tottori University, Japan. They were cultured in a malt medium (Bacto™ Malt Extract: Becton, Dickinson and Company, Sparks, MD, USA) for 3 weeks at 25 °C and each mycelium was collected, washed with distilled water, lyophilized, and used in the experiments.

2.2. Extraction and assay of B_{12} in shiitake mushrooms

Each dried fruiting body (approximately 10 g) was homogenized in a mixer (TML160; Tescom & Co., Ltd, Tokyo, Japan). A portion (5.0 g) of the homogenate was used as the test sample. The total B_{12} compounds were extracted by boiling at pH 4.8 in the presence of 4.0×10^{-4}% KCN and assayed using a microbiological technique based on *L. delbrueckii* ATCC 7830, according to the method described in the Standard Tables of Food Composition in Japan (Resources Council, Science and Technology Agency 1995). *L. delbrueckii* ATCC 7830 can utilize deoxyribosides, deoxyribonucleotides (known as alkali-resistant factor), and B_{12}. Thus, the correct B_{12} values were calculated by subtracting the results of the alkali-resistant factor from those of the total B_{12}.

The bed logs after fruiting of shiitake mushroom and uninoculated logs were chopped into small particles, homogenized in the mixer, and B_{12} compounds were extracted under the same conditions described above.

2.3. Bioautogram of B_{12} compounds using B_{12}-dependent Escherichia coli 215

A bioautogram of B_{12} compounds was produced using a published method (Tanioka et al. 2008). The B_{12} extract (10 mL) prepared above was partially purified and concentrated using a Sep-Pak® Plus C18 cartridge (Waters Corp., Milford, MA, USA), which had been washed with 5 mL of 75% (v/v) ethanol and equilibrated with 5 mL of distilled water. The C18 cartridge was washed with 5 mL of distilled water and B_{12} compounds were eluted using 2 mL of 75% (v/v) ethanol. The eluate was evaporated in a centrifugal concentrator (Integrated Speed VacR System ISS110; Savant Instruments Inc., Hicksville, NY, USA). The residual fraction was dissolved in 1.0 mL of distilled water. The concentrated B_{12} extracts, as well as authentic B_{12} and pseudo B_{12}, were spotted onto the silica gel 60 TLC sheet and developed in the dark using 2-propanol/NH_4OH (28%)/water (7:1:2 v/v) at 25 °C. After drying the TLC sheet, it was overlaid with agar containing basal medium and precultured *E. coli* 215, then incubated at 37 °C for 20 h. The gel plate was then sprayed with a methanol solution containing 2,3,5-triphenyltetrazolium salt, and the B_{12} compounds were visualized as red, which indicated *E. coli* growth.