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Spatial (cap & stipe) metabolomic variations affect functional components between brown and white beech mushrooms

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ABSTRACT

The beech mushrooms have customarily been revered by oriental societies for their nutritional and health benefits. We explored the mass spectrometry (MS) based spatial metabolomic variations between parts (cap and stipe) of two beech mushroom strains (brown and white). The principal component analysis (PCA) revealed their distinct primary (cap and stipe: PC1, 25.5%; strains: PC2, 12.5%) and secondary (cap and stipe: PC1, 10.3%; strains: PC2, 7.6%) metabolite patterns. The caps were rich in amino acids, fatty acids, and *N*-acetylglucosamine with higher protein and nitrogen contents. The stipes had abundant β-glucans, malic acid, and fructose. The discriminant secondary metabolites, especially, hypsiziprenols were higher in caps from brown strains. A fatty acid derivative, azelaic acid, was abundant in white strains (cap > stipe). We established a positive correlation for the cytotoxic activities of hypsiziprenols against ACHN cells. These spatial inter-strain metabolomic distinctions are potentially helpful for mushroom selection and improvement.

1. Introduction

A mushroom is a fungus with a distinctive fleshy fruiting body that has spatially metamorphosed into a cap, stipe, and spore-forming parts (Kalač, 2009). Based on the commercial uses, mushrooms are categorized into two main groups, *i.e.*, edible and non-edible varieties. The edible mushrooms have traditionally been important ingredients of gourmet foods in many parts of the world, owing to their unique textures, flavors, nutrient components, and functional aspects (Valverde, Hernández-Pérez, & Paredes-López, 2015). Although a large number of edible mushroom variants are employed for dietary and medicinal applications worldwide, beech mushrooms (*Hypsizygus marmoreus*) are especially delectable in East Asian countries. Intriguingly, the etymology of beech mushrooms is as diverse as the regions where they are been relished, for instance, shimeji in Japan, mangadak in Korea, and zhengjigu in China. In addition to their luscious taste and flavor, beech mushrooms also have nutraceutical functions owing to its gamut of antioxidant metabolites with potential anticancer activities (Kim et al., 2013; Xu et al., 2007). In particular, the abundance of hypsiziprenol compounds, the polyisoprenepolyols with vicinal hydroxyl groups and a terminal olefinic carbon, in beech mushrooms are reportedly associated with functional aspects (Akihisa et al., 2005; Sawabe et al., 1999; Sawabe, Morita, Ouchi, & Okamoto, 1996).

Metabolomics provide a quantitative and/or qualitative estimation of low molecular-weight metabolites (< 1800 Da) from biological systems, for instance, plants, animals, fungi, and prokaryotes with associated exo/endogenous perturbations (Crupi, Genghi, & Antonacci, 2014; Lee et al., 2014; Pinu, 2015). In recent years, metabolomics have emerged as an important methodology to determine the temporal and spatial correlations between metabolic cues and associated phenotypes in plant species (Han, Lee, Kim, & Lee, 2015; Shin et al., 2016; Suh et al., 2014). A large number of studies have elucidated the gamut of metabolites in beech mushrooms vital for their organoleptic properties, flavor, and nutritional as well as functional aspects, particularly anticancer potentials (Chauhan, Prasad, Rathore, & Sharma, 2017; Lee, Jian, & Mau, 2009; Xu et al., 2007). Though a majority of studies describes the characterization of hypsiziprenol class of secondary metabolites from beech mushrooms, the inter-strain spatial distribution of primary and secondary metabolomes in different mushroom parts is largely unknown with respect to the associated phenotypes.

Herein, we designed a non-targeted mass spectrometry (MS)-based metabolomic framework to explore the spatial metabolic differences in

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beech mushroom parts *i.e.*, the cap and the stipe, between its brown and white strain. Further, we established the correlation between the discriminant metabolite classes and the specific phenotypes as well as bioactivities.

2. Material and methods

2.1. Chemicals and reagents

Methanol, acetonitrile, water, and hexane were purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid, methoxyamine hydrochloride, pyridine, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA), acetic acid, sodium acetate, ethylenediaminetetraacetic acid (EDTA), and trition X-100 were obtained from Sigma Chemical Co. (St. Louis, USA). Hydrochloric acid, monopotassium phosphate, and dipotassium phosphate were purchased from Junsei Chemical Co. (Tokyo, Japan) and potassium hydroxide was obtained from Duksan Pharmaceutical Co. (Seoul, Korea). Glycerol was purchased from Calbiochem (La Jolla, USA) and Dithiothreitiol (DTT) was purchased from Roche Diagnostics (Mannheim, Germany).

2.2. Mushroom materials

Brown and white beech mushroom fruiting bodies were procured from a farm located in Yeoju-si, Gyeonggi-do, Korea. The samples were cultivated using bottle technology, with two bottles of each (brown and white) beech mushroom received three times from the farm on July 13, 20, and 27 in 2016. Fruiting body samples were separated into the cap and the stipe. Photographs of the samples used in this study are shown in Fig. 1A–B. Six biological replications of each brown and white beech mushroom were used in this study.

2.3. Sample preparation for metabolite profiling

The samples were pulverized using liquid nitrogen and immediately placed in microfuge tubes. Ground samples (1 g) were extracted using 1 mL of 80% methanol by sonication for 5 min at 4 °C (Hettich Zentrifugen Universal 320, Tuttlingen, G ermany) and then homogenized using a Retsch MM400 Mixer Mill (Retsch GmbH & Co., Haan, Germany) at 30 Hz for 10 min. After centrifugation for 10 min at 50,000 × *g* and 4 °C (1730R, Gyrozen), the supernatants were filtered through a 0.2-μm polytetrafluoroethylene syringe filter. The filtered supernatants were completely dried using a speed-vacuum concentrator (Biotron, Seoul, Korea), and dried extracts were redissolved with 80% methanol to a final concentration of 50 mg/mL, then they were used to perform the antioxidant activity tests. For GC-TOF-MS, UHPLC-MS/MS, and UPLC-Q-TOF-MS analyses, the extracts were diluted to 10 mg/mL. The quality control (QC) samples were made by using the pooled mixtures (10 μL) samples of each sample analyte for ascertaining the operations or instrumental errors generated during the course of GC-MS and UHPLC-MS/MS analyses. The QC samples were run once every 10 samples to avoid any possible errors during the analyses (Fig. S1). The sample oximation for GC-TOF-MS analysis was performed through incubating the dried sample extracts with 50 μL of methoxyamine hydrochloride in pyridine (20 mg/mL) at 30 °C for 90 min. Subsequently, the silylation step was carried out by adding 50 μL of MSTFA to the reaction mixture with 30 min incubation at 37 °C.

2.4. GC-TOF-MS analysis

A GC-TOF-MS analysis was performed using an Agilent 7890A GC system coupled with an Agilent 7693 autosampler (Agilent, Santa Clara, CA, USA) and a Pegasus HT TOF-MS (Leco Corp., St. Joseph, MI, USA) system. An Rtx-5MS capillary column (30 m × 0.25 mm i.d.; 0.25-mm particle size; Restek Corp., Bellefonte, PA, USA) was used with helium gas and a flow rate of 1.5 mL/min. The front inlet and transfer line temperatures were 250 °C and 240 °C, respectively. The starting column temperature was maintained for 2 min at 75 °C and increased to 300 °C over 15 min at a rate of 15 °C/min. The detector voltage was 1600 V, and the scanning mass range was 50–800 *m/z*. One microliter of sample was injected onto the GC with a split ratio of 1:10. In the GC-TOF-MS analysis, six biological replicates were used for each sample, without analytical replicates.

2.5. UHPLC-MS/MS analysis

A Thermo Fisher Scientific LTQ XL is a linear ion trap mass spectrometer consisting of an electrospray interface (Thermo Fisher Scientific, San José, CA) coupled to a Dionex UltiMate 3000 RS Pump, RS Autosampler, RS Column Compartment, and RS Diode Array Detector (Dionex Corporation, Sunnyvale, USA). Samples were

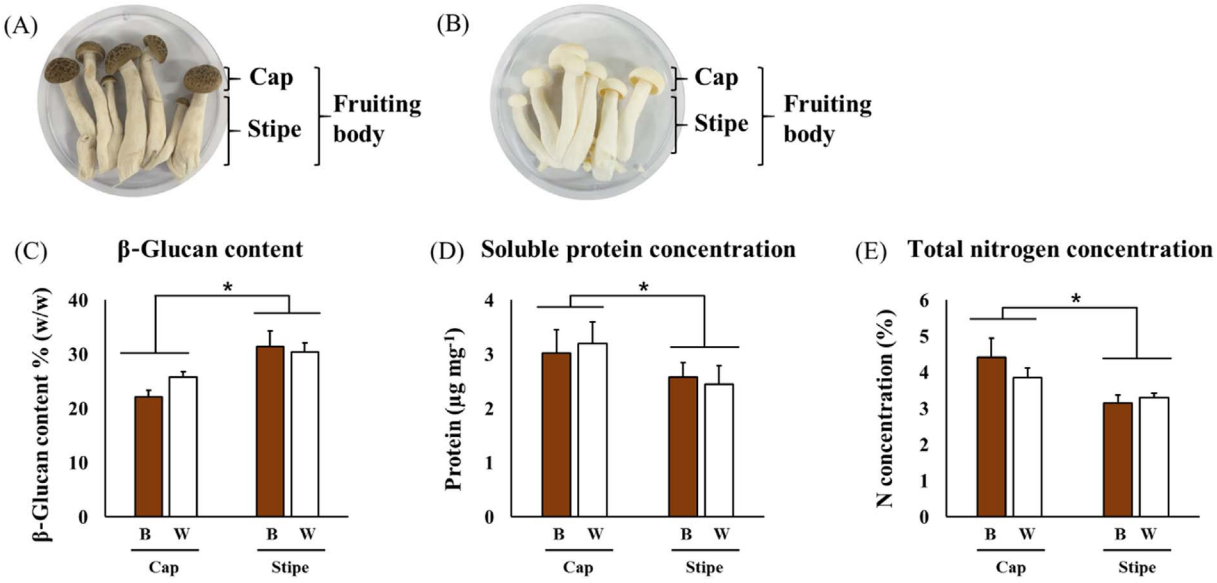

Fig. 1. Photographs depicting brown (A) and white (B) beech mushrooms (*Hypsizygus marmoreus*), with spatial components (cap and stipe) in respective basidiocarp. The graphs represent average β-glucan contents (C), soluble protein concentrations (D), and total nitrogen concentrations (E) in caps and stipe portions of brown and white beech mushrooms. (B: brown beech mushroom; W: white beech mushroom.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)