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Comparison of the metabolic profile of the mycelia and fruiting bodies of artificially cultured *Cordyceps militaris*

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ABSTRACT: *Cordyceps militaris*, a well-known traditional Chinese medicine, has multiple health-promoting effects. It is used as a herbal remedy and health food in Asian countries. Cultured mycelia are often used as a substitute for natural *C. militaris*. In the present study, the mycelia and fruiting bodies of artificially cultured *C. militaris* were analyzed using a metabolomics approach. The protein and crude fat contents of the mycelia were substantially higher than those of the fruiting bodies. The top three abundant amino acids in the mycelia were proline (3.9 g/100 g), aspartic acid (2.9 g/100 g), and glutamic acid (2.7 g/100 g). The carbohydrate content was similar in the fruiting bodies and mycelia. Analysis revealed that both the fruiting bodies and mycelia are rich in phenolic compounds and exhibit antioxidant activity. Further, six metabolites were significantly different between the mycelia and fruiting bodies. The levels of Ca, glucose, Mg, and Se were higher in the mycelia than in the fruiting bodies. In contrast, mannitol and Zn were more abundant in the fruiting bodies. The current study provides a comprehensive metabolic profile of the mycelia and fruiting bodies of artificially cultured *C. militaris*. Such an exercise is potentially important for understanding the metabolism of *C. militaris* and facilitating the use of cultured mycelia as a supplement to *C. militaris* fruiting bodies in traditional Chinese medicine.

KEYWORDS: Constituent; *Cordyceps militaris*; Fruiting body; Metabolomics; Mycelia

INTRODUCTION

The medicinal caterpillar fungus *Cordyceps militaris* (L.) Link (Cordycipitaceae, Ascomycetes), known to the Chinese as Bei Dong Chong Xia Cao or Bei Chong Cao, is an entomopathogenic species that infects and grows parasitically on Lepidoptera larvae or pupae in temperate countries (Chen *et al*., 2020). The abundant active chemical components of *C. militaris* and their pharmacological effects are similar to those of *Ophiocordyceps sinensis*, and *C. militaris* can produce the anticancer compounds cordycepin and pentostatin, which have not been detected in *O. sinensis* (Zhang *et al*., 2021). Therefore, *C. militaris* research and product development have become increasingly popular in recent years (In-on *et al*., 2022). To date, fruiting bodies and mycelium derived from fungal strains isolated from natural *C. militaris* have been successfully artificially cultivated by fermentation on an industrial scale, and they are widely consumed as substitutes for natural *C. militaris* (Ji *et al*., 2020).

Previous studies have shown that the metabolite production of cultivated *C. militaris* could be affected by the fruiting body or mycelium production processes, and chemical changes would influence the pharmacological effects on health (Kontogiannatos *et al*., 2021). The structural properties of polysaccharides obtained from the fermented mycelium and cultivated fruiting bodies of *C. militaris* were investigated and compared by Liu *et al*. (2016), who reported that the mycelium and fruiting bodies exhibited different total polysaccharide content, molecular weight, and monosaccharide mole ratios. Huang *et al*. (2006) reported on the nonvolatile taste components of the fruiting bodies and mycelium of *C. militaris*. The total free amino acid content in fruiting

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bodies and mycelium were significantly different, at 48.15 and 67.63 mg/g, respectively. Liu *et al*. (2014) analyzed the composition of *C. militaris* mycelium from submerged culture, and compared it with that of fruiting bodies, to clarify the chemical differences between them. In detail, the mycelium had lower levels of cordycepin and mannitol than the fruiting body.

A chemical comparison of cultivated *C. militaris* and its mycelium is important to ensure its quality, safety, and effectiveness. However, to date, many researchers have focused on one or a few bioactive compounds, such as adenosine and cordycepin, for the quality assessment of *C. militaris* (Jin *et al*., 2018; Tang *et al*., 2018; Raethong *et al*., 2020). However, the beneficial effects of traditional medicines are generally attributed to the combination of multiple components, rather than only a few bioactive compounds. Therefore, the targeted analysis of one or a few active components is considered insufficient to provide global information on the chemical profiles of *C. militaris*.

The metabolomics approach provides the potential means for the qualitative and quantitative analysis of all metabolites in an organism (Jacob *et al*., 2018). Metabolomics has been used to authenticate genuine *C. militaris* and compare the metabolite profiles of *C. militaris* and other Cordyceps species (Oh *et al*., 2019). However, the chemical differences between the cultivated fruiting bodies of *C. militaris* and its fermentation mycelium are still unclear.

In this study, metabolomics was used to investigate and compare the chemical profiles of the cultured fruiting bodies and cultured mycelium of *C. militaris*. We aimed to understand the metabolic changes in cultured fruiting bodies and cultured mycelium.

MATERIALS AND METHODS

Mycelium preparation

C. militaris was obtained from KCCM (deposit number: 60304, Seoul, South Korea). Stock cultures were maintained on potato dextrose agar plates. Plates were incubated at 25°C for 14 d and stored at 4°C for use as subcultures every two months. *C. militaris* was initially grown on PDA medium at 25°C, and the mycelium were harvested after 14 d.

Submerged culture

Five mycelial agar disks (5 mm × 5 mm) were obtained from mycelium plates incubated at 25°C for 14 d using a sterilized punching machine and transferred to 250 mL flasks containing 100 mL of Sabouraud dextrose broth (pH 4.5, glucose 20 g/L, peptone 10 g/L). The samples were illuminated by a combination of red and blue LEDs at ratios of 3:7 for 7 h/d (Ha *et al.*, 2020). The medium was cultured at 24°C and 100 rpm for 7 d in a shaking incubator, and all cultivations were performed in five replicates. The culture broth was centrifuged at 4000 rpm for 15 min, and the obtained mycelium sediment was washed twice with distilled water for mycelium analysis. The mycelium was lyophilized in a freeze dryer until a constant mass was obtained, and then stored at 4°C prior to further analysis.

Solid-state fermentation for fruiting body growth of *C. militaris*

The culture medium used for fruiting body cultivation was prepared in the same way as that for the mycelium. Thereafter, the cultured medium was homogenized at 4000 rpm for 5 min with a homogenizer (AM-11; Nihonseiki kaisha Ltd, Tokyo, Japan) and filtered through sterilized gauze to obtain the entangled hyphae for fruiting body production. Oat solid culture medium (20 g) containing 20 mL distilled water was prepared in a 300 mL cylindrical plastic bottle (diameter: 8 cm, height: 12 cm), sealed with a plastic cap, and autoclaved for 30 min at 121°C. The medium was cooled to room temperature, inoculated with 5 mL of seed culture, and incubated at 24°C for 21 d in the dark to promote vegetative growth. The solid culture medium containing hyphae was maintained at 20°C. The hyphae were exposed to a light intensity of 1,000 lux for 12 h and maintained in the dark for 12 h to obtain primordial fruiting bodies. The growth chamber for fruiting body formation was controlled at a relative humidity above 60%. The cultivation conditions were maintained until the top of the fruiting bodies became round and covered with spores. Fruiting body formation was considered to be complete when the fruiting body had stopped growing and when there was a head at its end. The fruiting bodies were then harvested, freeze-dried, ground into a fine powder until a constant mass was obtained, and then stored at 4°C prior to further analysis.

Proximate composition

Protein concentrations were determined using a UV-visible spectrophotometer (JENWAY 7205, Staffordshire,

UK) and bovine serum albumin as a standard according to the method of Lowry *et al*. (1951). Crude fat was measured by extraction using Soxhlet apparatus according to AOAC official methods (AOAC, 2005). Ten grams of the sample was placed in a cylindrical filter paper and transferred to the extraction tube of the Soxhlet extractor. Approximately 1/3 of the volume of anhydrous ether was placed in a round flask and the fat was extracted for 8 h at 60°C. After extraction, the cooler was removed and the cylindrical filter paper in the extraction tube was removed with tweezers. The ether extracted into the round-bottom flask was completely evaporated in a water bath. Then, the round-bottom flask was dried at 105°C until it reached a constant weight (approximately 1 h), and then weighed to determine the crude fat content.

Measurement of Amino acids

Amino acids were measured using an automatic amino acid analyzer (Hitachi L-8900, Japan). Hydrolysis of the samples was performed in the presence of 6 mol/L HCl at 110°C for 24 h under a nitrogen atmosphere. The hydrolysate was evaporated and the residue was redissolved in 1 mL 0.02 mol/L HCl. The sample was filtered through a 0.45 μm membrane-filter prior to analysis.

Mineral and Trace Element Determinations

To measure the mineral elements (Ca and Mg) and trace elements (Cu, Fe, Co, and Zn), the samples were digested in concentrated HNO_3. Mineral elements were quantified by inductively coupled plasma mass spectrometry (Perkin-Elmer Elan 6000), and the trace elements were quantified by plasma atomic emission spectrometry (Thermo Jarrel Ash IRIS Advantage). Selenium was determined using two different electrothermal atomic absorption methods. To determine low concentrations of Se (<0.050 mg/kg), the dried samples were digested in a mixture of concentrated HNO_3, $HClO_4$, and H_2SO_4, which resulted in reduction to Se (IV), chelated with ammonium pyrrolidine dithiocarbamate, extracted in methyl isobutyl ketone, and measured using AAS (Varian Spectr AA 400).

Carbohydrate analysis

The carbohydrate content was determined based on the total monomer content, which was measured after a two-step acid hydrolysis procedure. The first step involved exposure to 72% H_2SO_4 at 30°C for 60 min. In the second step, the reaction mixture was diluted to a final H_2SO_4 concentration of 4% and subsequently autoclaved at 121°C for 1 h. The carbohydrate (glucose, fructose, mannose, and xylose) content of this hydrolysis liquid was then analyzed by gas chromatography using a YL6100 instrument (Young-lin ins. Co., Ltd., South Korea), after hydrolysis with sulfuric acid and conversion to alditol acetates (ASTM method E1821-96).

Measurement of Nucleoside and Mannitol

The cordycepin and adenosine samples were prepared according to the method published by Yu *et al*. (2007), with slight modifications. Briefly, the sample (0.5 g) was suspended in 20 mL distilled water and sonicated for 50 min at 50 kHz, 400 W, and 60°C (JAC ULTRASONIC 2010, JINWOO Inc., South Korea). The supernatant was obtained by centrifugation at 1,740 × g for 15 min and filtered through a 0.45 μm membrane filter. High-performance liquid chromatography (HPLC) analysis was performed using an HPLC system (YL9100 plus, Youngin Chromass, Gyeonggi-do, South Korea). An HPLC system with a vacuum degasser, quaternary pump, UV/Vis detector, and analytical software was used for the detection and analysis of cordycepin and adenosine. The HPLC conditions were as follows: column, Agilent Eclipse plus C18 (250 mm × 4.6 mm, 5 μm); mobile phase, methanol:water (20:80, v/v); flow rate, 1.0 mL/min; UV detection at 260 nm; and injection volume, 10 μL. For mannitol analysis, the samples were extracted in methanol:water (20:80, v/v) solution under ultrasonication for 30 min at 25°C. After standing for several minutes, the supernatant was filtered through a 0.45 μm membrane prior to analysis. Analysis was performed on an Agilent 1260 (Agilent technologies, USA) liquid chromatograph, equipped with a refractive index detector, an Inertsil NH_2 column (4.6 mm × 250 mm, GL science, Tokyo, Japan) and controlled by Agilent ChemiStation software. Separation was achieved by isocratic elution in acetonitrile and water (72:28, v/v) at a flow rate of 1.0 mL/min. The cordycepin, adenosine, and mannitol content was calculated using a calibration curve generated using various concentrations of the standard. The adenosine, cordycepin, and mannitol peaks in the samples were identified by their retention times.

Determination of polyphenol and flavonoid content

The polyphenol content was determined using a

modification of the Folin–Ciocalteu method (Delgado-Torre *et al*., 2012) with minor modifications. Aliquots of sample (0.5 mL, 1 mg/ mL in 50% ethanol) were added to 10 mL of distilled water and mixed with 1 mL Folin–Ciocalteu reagent. Then, 3 mL Na_2CO_3 (20%, w/ v) was added to the mixture, which was heated to 50°C for 5 min and then maintained at room temperature in darkness for 30 min. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (SpectraMax 190 Absorbance Microplate Reader, Molecular devices LLC, United States). The results are expressed as milligrams of gallic acid per gram of extract (mg gallic acid/g extract). The flavonoid content was measured according to the method described by Abeysinghe *et al*. (2007) with minor modifications. The samples (0.1 mL, 1 mg/mL in 50% ethanol) were incubated with 0.8 mL 90% diethylene glycol, reacted with 0.02 mL NaOH (4 M), and incubated at 40°C for 10 min. The absorbance was measured at 420 nm using a UV-visible spectrophotometer (SpectraMax 190 Absorbance Microplate Reader, Molecular devices LLC, United States). A standard curve was prepared at 0, 32, 64, 128, and 256 mg/ mL quercetin dissolved in DMSO. The flavonoid concentration was expressed as quercetin equivalents (mg quercetin/g extract).

Antioxidant activity assay

The DPPH radical scavenging activity of the extracts was measured according to the procedure described by Lee *et al*. (2008), with slight modifications. The reducing power of the extracts was determined using the method described by Chang *et al*. (2006). The ABTS radical cation ($ABTS^{\cdot+}$) scavenging activity of the sample was analyzed using a method reported by Sasipriya and Siddhuraju (2012) with some modifications. The IC50 (inhibitory concentration) value, which is the concentration required to scavenge 50% antioxidant activity, was calculated. Lower IC50 values indicate higher antioxidant activity.

Metabolomics

The processed data were then imported into R software (version 4.0.2, R foundation, Auckland, New Zealand) for metabolomics analysis. Clustering and heat-mapping were carried out using R software (Version 4.0.2) to determine the content differences of characteristic metabolites in mycelium and fruiting bodies.

Statistical analysis

All experiments were performed in triplicate. Data are presented as the mean ± SD. The differences in the metabolite contents were evaluated at a 5% significance level using the Statistical Analysis System software (SAS (version 9.4), Inc., 2000) with Duncan's multiple-range test.

RESULTS AND DISCUSSION

The protein and crude fat content

After incubation at 25°C in the dark for 14 d, the plates of the mycelium (Fig. 1(a)) were fully colonized with dense aerial mycelium. The fruiting bodies cultured on solid culture medium tested with oats appeared as orange–yellow clustered strips (Fig. 1(b)). The protein levels in the fruiting bodies and mycelium of *Cordyceps* have been reported to be approximately 5.6–31.6 g/100 g (Dong *et al*., 2008). The fat content reported previously was above 5 g/100 g in *Cordyceps* mycelium (Yan *et al*., 2014). In this study, the protein and crude fat content in the mycelium were significantly higher than that in fruiting bodies (31.1 and 5.7 g/100 g, respectively, Table 1). These results are consistent with those published by Liu *et al*. (2014), who reported that the fermented mycelium had a higher protein and fat content (30.2 and 4.6%, respectively) than did the fruiting body (25.4 and 3.4%).

Amino acid composition

The amino acid content and composition of mycelium and fruiting bodies are presented in Table 1. Six amino acids, aspartic acid, glycine, alanine, proline, histidine, and arginine, were significantly higher in the mycelium than in the fruiting bodies. The glutamic acid content in the mycelium was significantly lower than that in the

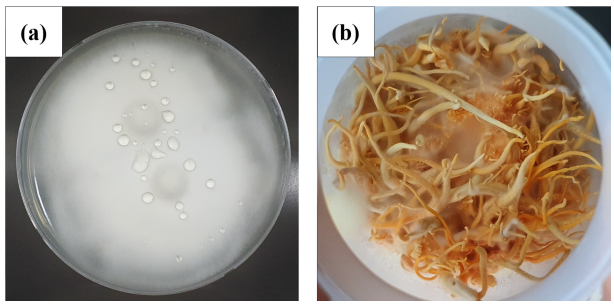

Fig. 1. Representative photos of (a) mycelia of *C. militaris* cultured under light condition, (b) fruit body grown from mycelia of *C. militaris* cultured under light condition.

Table 1. The most important constituents identified in mycelia and fruit body of *C. militaris*

	Mycelia	Fruit body
General constituents, g/100g		
Protein	31.1 ± 0.0 [a]	24.4 ± 0.0 [b]
Crude fat	5.7 ± 0.1 [a]	3.4 ± 0.0 [b]
Amino acid, g/100g		
Aspartic acid	2.9 ± 0.0 [a]	2.6 ± 0.1 [b]
Serine	1.2 ± 0.0 [a]	1.2 ± 0.1 [a]
Glutamic acid	2.7 ± 0.1 [b]	3.1 ± 0.1 [a]
Glycine	1.6 ± 0.0 [a]	1.3 ± 0.1 [b]
Alanine	2.2 ± 0.0 [a]	1.6 ± 0.0 [b]
Proline	3.9 ± 0.0 [a]	1.4 ± 0.1 [b]
Histidine	0.5 ± 0.0 [a]	0.3 ± 0.0 [b]
Arginine	1.6 ± 0.1 [a]	1.3 ± 0.1 [b]
Mineral, μg/g		
Cu	25.4 ± 0.2 [a]	16.1 ± 0.1 [b]
Fe	941.1 ± 1.1 [a]	172.5 ± 0.1 [b]
Mg	271.5 ± 1.1 [a]	200.8 ± 1.5 [b]
Zn	91.2 ± 0.8 [b]	111.5 ± 1.7 [a]
Ca	3485.1 ± 2.1 [a]	143.5 ± 1.1 [b]
Co	3.1 ± 0.7 [a]	0.7 ± 0.0 [b]
Se	0.5 ± 0.0	-
Carbohydrate, %		
Glucose	86.1 ± 0.1 [a]	85.2 ± 0.7 [a]
Fructose	6.7 ± 0.1 [a]	6.2 ± 0.2 [a]
Mannose	7.0 ± 0.0 [a]	7.2 ± 0.5 [a]
Xylose	-	-
Arabinose	-	-

[a, b] Means (n=5) with different letters in the same row (a comparison to mycelia and fruit body) are significantly different (5% probability level).

fruiting bodies. The top three abundant amino acids in the mycelium were proline (3.9 g/100 g), aspartic acid (2.9 g/100 g), and glutamic acid (2.7 g/100 g).

Minerals

The mineral content of the mycelium and fruiting bodies was significantly different (Table 1). Except for that of Zn, the content of the other six trace minerals in the fermented mycelium was significantly higher than that in the fruiting bodies. The Fe and Co content in the mycelium was 5.4 and 4.4 times higher, respectively, than that in the fruiting bodies and the Ca content was 24 times higher. In addition, Se, a trace mineral that plays an important role in human metabolism, was present at 0.5 μg/g in the mycelium, while not found in the fruiting bodies.

Carbohydrates

The carbohydrate profiles of the fruiting bodies and mycelium were similar (Table 1). In the mycelium, arabinose was not detected, and glucose had the highest content. In the fruiting bodies, the glucose content exceeded 85%, and the mannose content was the second highest. Chang *et al*. (2001) found that northern *Cordyceps* mycelium contained glucose (51.2 mg/g). The discrepancy in glucose content between the two studies might be due to the difference in strains and media used. In conclusion, the carbohydrate content was similar in the fruiting bodies and mycelium.

Nucleoside and mannitol

Cordycepin, adenosine, and mannitol were used as markers for the quality control of *Cordyceps* spp. Table 2 shows the cordycepin, adenosine, and mannitol content, and clearly indicates that the adenosine content in the mycelium was much higher than that in the fruiting bodies, in contrast to the trend exhibited by cordycepin and mannitol. In this study, relatively high cordycepin content, 4.2 mg/g, was detected in mycelium compared to that reported in other studies. Liu *et al*. (2014) reported that a trace quantity of cordycepin, 0.16 mg/g, was detected in *C. militaris* fermented mycelium. This difference is considered a result of the LED light condition used in this study. In fact, Yang *et al*. (2016) reported that illumination by LED light increased the cordycepin content of the *Cordyceps* mycelium. However, the mycelium still exhibited a lower cordycepin content than the fruiting body, which is consistent with the results of the previous study. Adenosine, isolated from Cordyceps, has been demonstrated to have biological activity. Adenosine is a cardioprotective and therapeutic agent for the treatment

Table 2. The nucleoside and mannitol identified in mycelia and fruit body of *C. militaris*

	Mycelia	Fruit body
Cordycepin, mg/g	4.2 ± 0.1 [b]	23.7 ± 0.5 [a]
Adenosine, mg/g	6.4 ± 0.0 [a]	0.9 ± 0.1 [b]
Mannitol, mg/g	88.7 ± 1.0 [b]	131.1 ± 3.8 [a]

Means (n=5) with different letters in the same row are significantly different (5% probability level)

of chronic heart failure. As shown in Table 2, adenosine was present at higher levels in mycelium than in the fruiting body. Stored glucose may be converted to mannitol in the mycelium of *C. militaris* during fruiting body formation. Therefore, the mannitol content is likely to be higher in the fruiting body than in the mycelium because mannitol is generated during development of the mycelium into fruiting bodies (Table 2).

Polyphenol and flavonoid content

As antioxidant activity appears to be related to the polyphenol content of mushrooms, the mycelium and fruiting body extracts were also evaluated for their total polyphenol and flavonoid content. The mycelium were much richer in total polyphenol content than the fruiting bodies (Table 3). When compared with the total phenolic content, the flavonoid content obtained for all extracts was relatively low. The evaluation of total polyphenol content and the identification of the main phenolics in mushrooms are important components of a nutritional and functional characterization study. Polyphenols are secondary metabolites commonly found in plants, mushrooms, and fungi and have been reported to exert multiple biological effects, including antioxidant activity. The values obtained for *C. militaris* extracts indicate that both the fruiting bodies and mycelium were rich in phenolic compounds. Ahn *et al*. (2012) showed that the total polyphenol compound content of *C. militaris* fruit body was 30.7 mg/g dry weight, which was higher than in this study. Meanwhile, the total polyphenol content of domestic mushrooms was 3.4 mg/g for shiitake mushroom, 2.0 mg/g for reishi mushroom, and 3.4 mg/g for oyster mushroom (Lie *et al*., 1995). Compared to Kim *et al*. (2002) reported that the total polyphenol content of the enoki mushroom extract was 3.17~3.50 mg/100 g, polyphenol content of *C. militaris* in this study was relatively high. Awang *et al*. (2021) reported that the flavonoid content of *C. militaris* fruiting body was 0.066 mg/g dry weight. The flavonoid content of the samples used in our study is significantly higher than in previous studies. This may vary depending on the growth environment and origin of the Cordyceps mushroom.

Antioxidant activity

Although flavonoids such as quercetin and myricetin have been putatively identified in mushrooms, including *C. militaris*, these findings require confirmation by more sensitive and specific methods. Because different antioxidant compounds may act through different mechanisms *in vivo*, no single method can be used to fully evaluate the total antioxidant capacity of materials. Therefore, in this study, three complementary test systems were used to evaluate the antioxidant activities of the extracts (Table 4). Awang *et al*. (2021) reported that DPPH antioxidant activity of *C. militaris* fruiting body was 0.60 mg/mL based on IC 50. In ABTS scavenging activity, *C. militaris*, showed an IC50 value of 0.702 to 1.138 mg/mL, and a similar value was also shown in the DPPH assay (Quy and Xuan, 2019). The DPPH radical scavenging activities of *C. militaris* exhibited the strong DPPH radical scavenging activity from 2.5 to 5 mg/mL (IC50) (Chakrabarti and Patra, 2015; Li *et al*., 2021). Our study also confirmed similar antioxidant activity as previously reported. The DPPH scavenging activity and reducing power results indicate that stronger antioxidant activity was produced by the mycelium than by the fruiting bodies. The ABTS scavenging activity results demonstrated the opposite trend. One reason for this discrepancy may be that the different extracts contained different types of polyphenolics with significantly different reactivities. In addition, phenolics are not solely responsible for the antioxidant activity of fungal extracts. The activity of several types of organic acids is also detectable using various antioxidant methods. The high quantities of

Table 3. Comparison of bioactive components of Cordyceps mycelia and fruiting body

	Mycelia	Fruit body
Polyphenol content, mg gallic acid/g dry weight	15.55 ± 1.10[a]	7.66 ± 1.10[b]
Flavonoid content, mg quercetin/g dry weight	7.64 ± 0.21[a]	5.19 ± 0.51[b]

Means (n=5) with different letters in the same row are significantly different (5% probability level)

Table 4. Comparison of antioxidant activity of *Cordyceps* mycelia and fruiting body

Antioxidant activity, IC_{50} (mg/mL)	Mycelia	Fruit body	Ascorbic acid
DPPH	0.82 ± 0.21[b]	3.81 ± 0.70[a]	0.06 ± 0.00[c]
Reducing power	1.21 ± 0.02[b]	6.89 ± 0.37[a]	0.05 ± 0.00[c]
ABTS	1.15 ± 0.11[a]	1.13 ± 0.05[a]	0.08 ± 0.00[b]

Means (n=5) with different letters in the same row are significantly different (5% probability level)

amino acids, especially proline, found in the mycelium extracts suggest that these compounds could be at least partly responsible for the high DPPH scavenging activity and reducing power. Furthermore, possible synergistic effects involving phenolics and amino acids should not be ruled out and should be investigated in future studies.

Metabolic changes in mycelium and fruiting bodies

Principal component analysis (PCA), a type of multivariate statistical analysis, was applied to visualize major trends and classifications of mycelium and fruiting bodies based on the general constituents, amino acids, minerals, carbohydrates, nucleosides, and mannitol. PCA is an unsupervised statistical method that finds rules on its own in data with no pattern, which can reduce numerous correlated variables to a set of important variables, called principal components (PCs), for interpretation of data. As shown in Fig. 2, mycelium and fruiting bodies were separated, except for some into two groups. The first two PCs accounted for 100% (PC1 = 68.2% and PC2 = 31.8%) of the total variance. There was clear inter-group separation between the fruiting body and mycelium along PC1, indicating significant variations in the components. These results indicate that metabolic features may have a significant impact on the mycelium as it grows into a fruiting body. Hierarchical cluster analysis (HCA) was used to provide an overview of the metabolite characteristics of mycelium and fruiting bodies. The HCA results, based on 24 significantly different metabolites (Fig. 3), indicate that the mycelium and fruiting body were clearly separated and clustered in two categories: cluster I, consisting of the mycelium, and cluster II, comprising the fruiting bodies. These results are consistent with the clustering trends in the previous PCA score plot (Fig. 2). The variables were grouped into two classes based on the metabolite concentrations. One group consisted of compounds that were present at higher levels in the mycelium, such as Ca, glucose, Mg, and Se. The other group comprised mannitol and Zn, which occurred at higher levels in the fruiting body. This difference, which was verified using a significance test, is shown in Table 1 and Table 2. The results confirmed that the Ca, glucose, mannitol, Mg, Se, and Zn content of the mycelium was significantly different to that of the fruiting body. These results are consistent with the results of the multivariate statistical analysis shown in

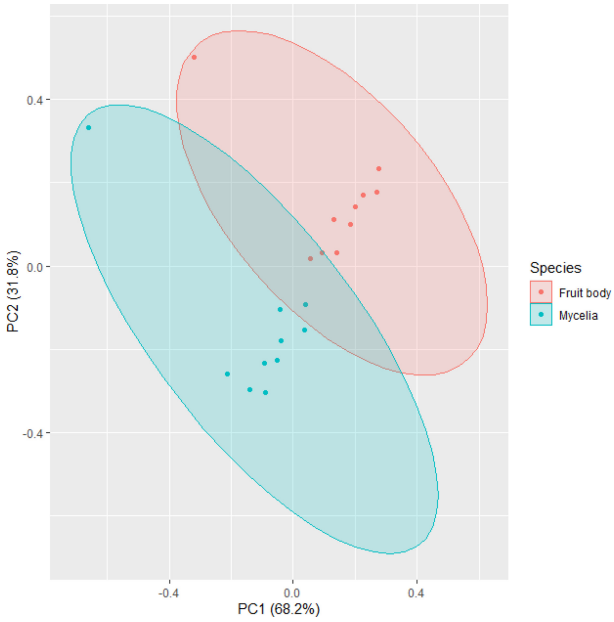

Fig. 2. Multivariate statistical analysis of cultured mycelia under light condition and fruit body grown from cultured mycelia under light condition.

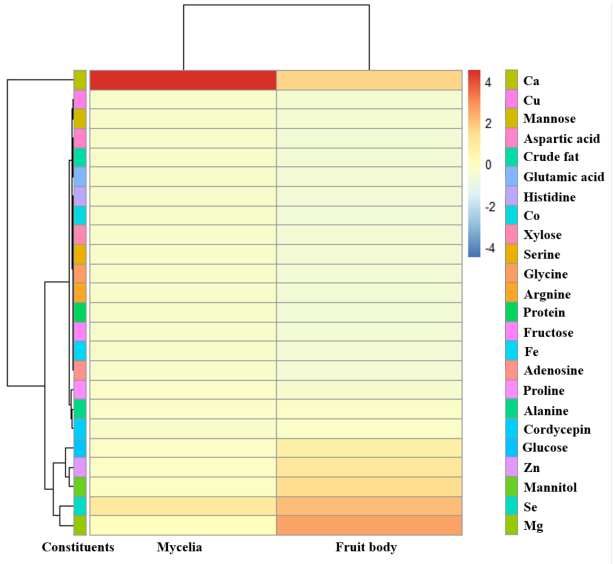

Fig. 3. Hierarchical clustering and heat map about constituents of cultured mycelia under light condition and fruit body grown from cultured mycelia under light condition.

Figs. 2 and 3. Previous studies have demonstrated that the major chemical substances in mycelium are similar to those of the fruiting body, including adenosine and polysaccharide. In this study, we found that the main metabolites that allow discrimination between the mycelium and fruiting bodies were related to glucose and minerals based on metabolic profiles. It is well

known that minerals are biologically important components for building blocks of proteins and maintaining optimal health. Similar to those reported previously, the relative quantities of observed minerals, such as Ca and Mg, were significantly higher in mycelium than in the fruiting body. Se was only found in mycelium, which is in good agreement with previously reported results. Se is an anti-inflammatory drug used to relieve symptoms such as pain and swelling associated with osteoarthritis. The Ca content was 24-fold higher in the mycelium than in the fruiting body. It has been reported that Ca participates in several physiological processes, including endocrine modulation, human reproduction, and stress response. Dietary Ca plays a relevant role in inhibiting chemically induced lung tumorigenesis and cadmium-induced kidney damage in mice. The present study reveals that mycelium is a good substitute for the fruiting body in terms of some major bioactive components, including Se and Ca.

In this study, the fruiting body and mycelium were characterized and compared based on the analysis of metabolomic data. The results indicate that the six metabolites can be used as potential markers for discriminating between the mycelium and fruiting bodies. The mycelium and fruiting bodies were demonstrated to have significantly different metabolic profiles, particularly with regard to glucose and minerals such as Ca, Mg, and Se. Our study also confirmed that the metabolites, which are intermediates or end products of cell metabolism, changed throughout the development of mycelium into fruiting bodies.

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