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Monitoring of distribution of mineral elements during cultivation of *Auricularia auricula* in northeastern China

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The aim of this study was to establish differences in mineral element levels in the mycelium substrate, fungal residues and different tidal fruiting bodies of *Auricularia auricula*. The distribution of mineral elements in the mycelium matrix and fruiting body was determined during the cultivation process, which would enable the determination of the physiological characteristics of *A. auricula* mineral nutrition status. Inductive coupled plasma optical emission spectrometry (ICP-OES) was used to measure 14 mineral element levels in the hypha matrix of fungal residues grown in three cultivation pilot areas. Data showed that the amounts of 3 mineral elements, such as potassium, boron and iron decreased while those of 6 elements including calcium, magnesium, and manganese increased. There were differences depending upon the experimental scenario. Evidence indicates that the growing fruiting bodies of *A. auricula* exhibit varying concentrations of mineral elements, which may be important for use as medicines to treat various diseases.

Key words: *Auricularia auricula* cultivation, Mineral element content distribution

INTRODUCTION

Auricularia auricula, also known as black fungus, is utilized as an edible and medicinal fungus [1]. A variety of mineral elements are required in addition to carbon nutrition and nitrogen nutrition for cultivation of *A. auricula*. Some studies reported the metal ion content in black fungus and other mushrooms. Li *et al.* [2] identified the presence of Ca, Cu, Fe, K, Mg, Mn, Na, and Zn in fruiting bodies of the highly-prized matsutake mushroom (*Tricholoma matsutake*) from 12 separate sites in Southwest China. Wang and Wang [3] demonstrated that the amounts of Fe and Mn in the black fungus of Qinba mountain areas were higher，while the amounts of Ca and Mg in the Northeast were higher. Shi and Luo [4] found that the concentrations of Ca and Zn in wild black fungi in the Chayu region were significantly higher than in the Yadong and Lulang regions. Feng *et al.* [5] determined that *A. auricula* from northeast regions contained higher amounts of Ca, Co, Fe, P, Zn and Cr while *A. auricula* from Fengqing country of Yunnan contained higher amounts of Cu, K and Se. Li [6] and Wang [3] studied the content of inorganic nutrients in *A. auricula*.

In addition, there are many studies on the accumulation of heavy metals (Pb, Hg, Cd, As) in edible fungi, and the different kinds of edible fungi having different enrichment ability to metal ion were found out [7, 8].

The aim of this study was to measure the contents of 14 mineral elements in mycelia matrix, fungus bran and fruiting bodies of *A. auricula* in 3 cultivated areas at different harvest times. The contents of mineral elements were determined by ICP-OES.

EXPERIMENTAL

Materials and methods

Test strains

The strains of *A. auricula* used in the study were “Hei 29” and “Heiwei 10”, which were preserved and provided by the Institute of Microbiology, Heilongjiang Academy of Sciences.

Culture medium

The medium used for Shangzhi was: sawdust 88%, fine bran 8%, soybean meal 3%, lime 0.5%, gypsum 0.5%; cultivated strain: Hei 29.

The medium used for Mudanjiang was: sawdust 83%, rice husk 10%, soybean meal 2%, wheat bran 2%, corn flour 2%, gypsum 1%; cultivated strain: Heiwei 10.

The medium used for Baishan was: sawdust 80%, rice husk 16%, soybean meal 3%, lime 0.5%, gypsum 0.5%; cultivated strain: Hei 29.

Cultivation method

The configured medium was installed in a polyethylene plastic bag, then steam-sterilized and inoculated. The inoculated medium bags were cultivated at 25℃. After about 45 days, 180-220

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round holes were punched on the outside of the bag, then water was sprayed on them. The fruiting bodies grew out of the bags after 10-15 days. When growing to a diameter of 4-5 cm, these fruiting bodies were cut from the bags and denoted as Flush-1; on keeping watering, other fruiting bodies were cut from the bags after about 7-10 days, denoted as Flush-2, and so on.

100 bags were in each plot, random sampling of 30 bags, 3 replicates in each plot.

Sample collection method

Medium filled with hyphae (mycelial matrix), harvested culture medium (fungus bran) and *A. auricula* fruiting bodies were harvested at the 1st , 3rd and 6th flushes, respectively, dried in sun light, weighed and the average value was calculated and recorded as dry weight (DW).

Sample digestion

Mycelial matrix, fungus bran and fruiting bodies were ground into powder with a whirlwind. 0.2g samples were put in PTFE crucibles in which 10 mL nitric acid and 0.50 mL perchloric acid were added. The crucibles were covered overnight and then placed in a controlled constant temperature electric heating plate at 120 ℃ to dissolve completely. The solution was concentrated to about 1.00 ml, then cooled, diluted with distilled water to 25.00 mL and shaken up before determination. A blank test was carried out by the same method.

Determination of the content of mineral elements

7000DV type ICP-OES analyzer of Perkin Elmer Company was used to determine the content of mineral elements (detection limit≤1ppb). Test conditions: delay time: 20 s; radio-frequency power: 1300 w; plasma flow: 15 L/min; sample flow: 1.5 L/min.

Data processing

On the basis of three replicates for each sample, mean values and standard errors were calculated. By EXCEL column diagram and broken line chart, the results were shown, and the significance of the difference between the two groups was analyzed by a t-test. $P>0.05$ indicates that the difference was not significant; $P<0.05$ shows a significant difference; $P<0.01$ shows an extremely significant difference.

RESULTS

Substrate consumption and fruiting body yield

Similar substrates and formula were selected for the cultivation test in three plots. Different cultivated varieties and harvesting methods of single flush (Shangzhi), three flushes (Baishan), and six flushes (Mudanjiang) were used. The harvest flushes, yield and substrate consumption are shown in Table 1. The results showed that the cultivation process of *A. auricula* was consistent with the utilization of substrate.

Contents of mineral elements in the mycelial matrix and the fungus bran

After determining the content of 14 mineral elements (K, Na, Ca, Mg, P, Cu, Fe, Mn, Al, B, Zn, Pb, As and Hg) which are common in *A. auricula*, it was found that the content of elements such as Ca, Mg, Mn, Na, Al, P in the fungus bran was higher than that in the mycelial matrix, among which the increasing range of Ca was the largest, up to more than 25%, as shown in Table 2. The content of elements such as K, B and Fe in the fungus bran was less than that in the original mycelial matrix, as shown in Table 3. The decrease of K was the largest, up to around 30%, which showed that much more quantity is demanded in the growth and development.

Table 1. Results of the consumption of substrate and the yield of fruiting body

Test area	Shangzhi	Baishan			Mudanjiang					
Harvesting flushes	1	1	2	3	1	2	3	4	5	6
DW of fruiting bodies A (g/bag)	37.0	4.8	4.6	20.7	5.9	6.4	4.8	6.2	26.0	6.2
DW of fungus bran B (g/bag)	240	280			290					
DW of mycelial matrix C (g/bag)	295	325			365					
Conversion rate of the substrate (%) A/(C-B)	67.6	67.4			74.0					

Table 2. Contents of mineral elements in the mycelial matrix and the fungus bran (Ⅰ)compared with the substrate mycelium in one group (by t-test) , $^{*}P<0.05$，$^{**}P<0.01$（n=3).

Element (mg/g)	Ca	Mg	Mn	Na	Al	P
Shangzhi mycelial matrix	15.8	1.71	0.126	0.242	0.884	0.766
Shangzhi fungus bran	20.5^{**}	2.14^{**}	0.192^{*}	0.252	0.933	0.747
Mudanjiang mycelial matrix	14.7	2.02	0.159	0.254	0.805	0.771
Mudanjiang fungus bran	18.9^{**}	2.07	0.182	0.263	0.851	1.11^{**}
Baishan mycelial matrix	20.3	1.382	0.172	0.267	0.729	0.695
Baishan fungus bran	25.1^{**}	1.51	0.229	0.341^{*}	0.819	0.763

Table 3. Contents of mineral elements in the mycelial matrix and the fungus bran (II) compared with the substrate mycelium in one group (by t-test), *P<0.05，**P<0.01（n=3).

Element (mg/g)	K	B	Fe
Shangzhi mycelial matrix	0.314	0.143	1.91
Shangzhi fungus bran	0.209**	0.081**	1.42**
Mudanjiang mycelial matrix	0.313	0.065	1.32
Mudanjiang fungus bran	0.195**	0.057	1.11*
Baishan mycelial matrix	0.358	0.035	1.26
Baishan fungus bran	0.291**	0.029	1.09

Table 4. Comparison of the contents of mineral elements between the mycelial matrix and the fungus bran (III)

Element(mg/g)	Cu	Zn	Pb	As
Shangzhi mycelial matrix	0.041	0.00303	0.00133	0.000233
Shangzhi fungus bran	0.039	0.00322	0.00114	0.000213
Mudanjiang mycelial matrix	0.041	0.00319	0.00131	0.000126
Mudanjiang fungus bran	0.039	0.00296	0.00128	0.000134
Baishan mycelial matrix	0.037	0.00298	0.00112	0.000106
Baishan fungus bran	0.038	0.00296	0.00121	0.000111

Table 5. Comparison of mineral element contents in fruiting bodies in Baishan area

Element (mg/g)	K	Na	Ca	Mg	Fe	Mn	Al	B	Zn	P
Flush-1	9.49	0.81	5.53	2.31	0.54	0.07	0.37	0.03	0.001	3.49
Flush-2	10.80	0.61	5.68	2.12	0.83	0.08	0.50	0.02	0.002	3.87
Flush-3	4.71	0.302	3.73	1.18	0.18	0.04	0.10	0.01	0.001	2.13

Table 6. Comparison of mineral element contents in fruiting bodies in Mudanjiang area

Element(mg/g)	K	Ca	P	Mg	Na	Fe	Mn	Al	B	Zn
Flush-1	10.41	8.08	4.82	2.16	0.64	0.47	0.03	0.12	0.02	0.005
Flush-2	10.73	8.17	5.61	2.66	0.61	0.31	0.03	0.16	0.02	0.006
Flush-3	10.42	6.48	5.13	2.48	0.56	0.22	0.04	0.14	0.03	0.005
Flush-4	10.00	9.89	5.56	2.37	0.34	0.19	0.02	0.13	0.02	0.005
Flush-5	8.58	6.63	5.64	2.47	0.31	0.42	0.05	0.31	0.04	0.004
Flush-6	10.15	2.48	2.79	1.74	0.32	0.19	0.04	0.12	0.04	0.004

Mineral element contents in fruiting bodies

In the tests of Baishan and Mudanjiang, the fruiting bodies of *A. auricula* were collected in 3 flushes and 6 flushes, respectively. The content change of mineral elements in the fruiting bodies shows that most of mineral element contents decreased with the increase of flush, as shown in Tables 5 and 6.

Before the 3rd flush of Baishan and the 5th flush of Mudanjiang, the fruiting body yield obviously increased, while the content of mineral elements in the corresponding fruiting bodies significantly decreased. The results showed that the transfer efficiency of mineral elements was different from other nutrients.

In plants, researchers have laid a theoretical foundation for the regulation of plant resistance to mineral nutrients [9]. Meng *et al.* [10] studied the distribution of mineral elements in tobacco leaves of Guangxi base and analyzed the relationship between the supply of soil and the mineral composition of tobacco in different regions. Guo *et al.* [11] found that brown rice protein content significantly correlated with P, K, Cu, Mn and other mineral elements. Liu *et al.* [12] studied the content change of main mineral elements to explore the factors influencing the leaf senescence of raspberry fruit maturity. Yu *et al.* [13] studied the correlation between wild *Radix Salviae Miltiorrhizae* and its inorganic elements in different habitats. In the study of mineral elements in edible fungi, researchers sampled *Tricholoma matsutake* from 12 districts at different faraway distances in the Southwest of China to determine the contents of mineral elements such as Ca, Cu, Fe, K, Mg, Mn, Na and Zn by atomic absorption spectrometry [2]. Koyyalamudi *et al*. [14] determined the content of trace minerals in the fruiting bodies of *Agaricus bisporus*. Mleczek *et al*. [15] compared the content of six kinds of wild edible fungi such as *Boletus edulis*, *Cantharellus cibarius* collected in Poland and other six kinds of nutrient elements (Ca, Fe, K, Mg, Mn, Na), and respectively studied the biological concentration factor (BCF). Gramss and Voigt [16] determined the mineral content of matrix and fruiting body, and studied fungal mineral absorption rules from non-embedded beech wood

substrate. So far, there is no report on the migration of mineral elements in the cultivation of *A. auricula*.

In this study, although there were some differences in the matrix, cultivated varieties and management methods, the content changes of mineral elements were consistent. The results showed that the growth process of *A. auricula* was selective to mineral elements. Analysis of utilization of substrate showed that the selectivity of mineral elements is closely related to the physiological characteristics of *A. auricula*, the concentration of mineral elements in the substrate and the management methods of cultivation. The experimental data showed that the utilization of mineral elements (K, B and Fe) was higher than that of the whole nutrients. Therefore, it was suggested that the addition of these elements might improve the transformation efficiency.

The analysis showed that K, Ca, P, Mg and other elements have a higher content in fruiting bodies. It can be concluded that these four elements were important to the growth of *A. Auricula*, which was similar to the elements K and P in the growth of plants.

The change of mineral elements content in different flushes showed that, with the growth rate increasing and the growth time extension, the synthetic ability of proteins and carbohydrates of *A.auricula* gradually decreased, leading to a decrease in the content of relevant mineral elements, and this trend will decrease the yield till stoppage. It can be inferred that those mineral elements are the same as other nutrients whose transfer efficiency will affect the growth quality and yield of *A. auricula*.

CONCLUSION

In different experiments, 3 elements (K, B and Fe) and 6 elements (Ca, Mg, Mn, etc.) showed a consistent declining and rising tendency. The element content in the fruiting body gradually decreased along with the harvesting flushes. The results showed that the growth of *A. auricula* had different selectivity and transfer efficiency to mineral elements.

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