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# Biochemical Composition, Antifungal and Antioxidant Activities of Vegetative Mycelia of Ectomycorrhizal Fungi in Larch

**Peng Wang[1]*, Qiu-Feng Xu[1,2] and Feng-Wei Diao[1,2]**

[1]State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.
[2]University of Chinese Academy of Sciences, Beijing 100049, China.

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**The objective of the present study was to determine the biochemical composition, antifungal and antioxidant activities in vegetative mycelia of six vegetative mycelia of edible ectomycorrhizal mushrooms (EEMM). We found that the content of total amino acids ranged from 22.95 g 100g$^{-1}$ (*S. viscidus*) to 53.86 g 100 g$^{-1}$ (*B. pictus*). And four lipid biomarkers of 18:2ω6,9c, 18:0, 18:1ω9c and 16:0 were determined in all the EEMM mycelia, accounting for 85% of relative abundance. By multidimensional scaling analysis, the amino and fatty acid profile had clearly separated among the EEMM or between *S. grevillei*-1 and *S. grevillei*-2. Furthermore, *H. mesophaeum* inhibited all the root pathogenic fungi through exudation of allelochemicals. The methanol extracts of all the EEMM mycelia exhibited higher reducing power and DPPH scavenging capacities.**

**Key words**: Edible ectomycorrhizal mushroom (EEMM), vegetative mycelia, antioxidant activity, fungicidal activity.

Ectomycorrhizal (ECM) fungi are ubiquitous in temperate and boreal forests, where they represent key components of carbon, nutrients and biogeochemistry cycling processes. By extending the absorptive area of the root system, ECM extramatrical mycelia increase plant uptake of nutrients and fine root lifespan[1,2], and provide resistance to stresses of drought and soil pathogens[1,3,4]. More importantly, ectomycorrhizal fungi form fruiting bodies and produce edible mushrooms, which are pointed out as valuable sources of functional foods and their important nutritional value[5,6].

Fruiting bodies and mycelia of edible ectomycorrhizal mushrooms (EEMM) are low in fat and rich in essential amino acids, fatty acids, minerals, dietary fiber and vitamins[6,7]. About 200 EEMM [*Tuber melanosporum* Vittad., *Tuber magnatum* Pico, *Tricholoma matsutake* (S. Ito & S. Imai) Sing., *Cantharellus cibarius* Fr., *Boletus edulis* Bull., etc.] are regarded as highly prized edible mushrooms, some of which are among the world's most expensive foods[8]. It was estimated that the world production of *C. cibarius*, *T. matsutake* and *B. edulis* is 20000 t, 2000 t and 20000 ~100000 t, whose approximate in-season retail market could gain more than $ 1.6 billion, $ 500 million and $ 250 million, respectively[8].

EEMM fruiting bodies are also applied as a valuable resource for pharmaceutical compounds[9]. Many studies show that EEMM contain bioactive substances such as secondary metabolites (phenolics, metal chelating agents, terpenoids, alkaloids, lactones, sterols and vitamins), amino acids, proteins and polysaccharides. These bioactive compounds possess antimicrobial, antioxidant, antitumor and immunomodulatory properties[10,11]. For example,

* To whom all correspondence should be addressed. Tel.: +86-24-83970452 (O); Fax: +86-24-83970300; E-mail: wangpeng@iae.ac.cn

Antimitotic polysaccharides and terpenoids were isolated from fruiting bodies of *Boletus badius* and *Lactarius deliciosus*[12]. Some EEMM [*Entoloma sinuatum* (Bull.) P. Kumm., *Rhizopogon luteolus* Krombh., Tricholoma terreum (Schaeff.) P. Kumm.] produce alkaloids, others EEMM [*Tricholoma populinum* (Fr.) Bon and *Suillus luteus* (L.) Roussel] produce phenolics, which are contribute to their antioxidant properties[13,14]. Additionally, the EEMM [*Boletus aurantiacus* Bull., *Boletus chrysenteron* Bull., *C. cibarius*, *Lactarius deliciosus* (L.) Gray, *Leccinum scabrum* (Bull.) Gray, *Leccinum aurantiacum* (Bull.) Gray, *Suillus grevillei* (Klotzsch) Sing., and *T. portentosum*] are considered valuable sources of antioxidants[15]. Not only fruiting bodies, but also mycelia have plenty of bioactive metabolites[16] that could facilitate compound's extraction and purification, increasing their economic value. From ecological perspective, fruiting bodies are only produced in the field of forests and plantations so far, whereas in vitro mycelia production avoids the overexploitation of endangered species, representing a sustainable way to conservation and use of EEMM biodiversity.

Larch (Pinaceae) is one of the main conifer tree species characterized by a wide natural range, covering north part of China between latitudes 30ºN and 53ºN under a wide variety of site conditions[17]. The broad latitudinal range of larch from temperate to boreal forests allows abundant diversity of ECM to colonize the fine root of larch. Of these ECM fungi species, the EEMM of *Boletinus pictus*, *Leccinum scabrum*, *Hebeloma mesophaeum*, *Suillus viscidus* and *Suillus grevillei* were collected in larch plantation, and vegetative mycelia were produced by submerged fermentation. However, little knows about the biochemical composition of these mycelia and their therapeutic effects. Accordingly, the objectives of this study were to characterize the amino and fatty acids composition in the tested vegetative mycelia, (2) to compare the biochemical difference among the vegetative mycelia of ECM fungi representing various host trees and various forest locations, (3) to evaluate the antifungal activities of mycelia methanol extracts on tested root pathogen and to examine antioxidant potential on capacity of DPPH free-radical scavenging and reducing power.

## MATERIALS AND METHODS

### Chemicals and biological materials

Solvents used in this study were HPLC grade, while other chemicals were analytical grade. The amino acid standards and authentic fatty acids including nonadecanoic acid methyl ester, 37 component FAME mixture and 26 component mixture of bacteria acid methyl esters (BAME) were purchased from Sigma-Aldrich Co., China. Their standard stock solutions were stored at 4 °C until use.

Six ECM fungal species in larch including *Boletinus pictus* (strain EL 88125), *Leccinum scabrum* (Bull.) Gray (strain EL 88134), *Hebeloma mesophaeum* (strain EL 870191), *Suillus viscidus* (L.) Roussel (strain EL 88131), *Suillus grevillei* (Klotzsch) Singer (*S. grevillei*-1, strain EL 8803), *Suillus grevillei* (Klotzsch) Singer (*S. grevillei*-2, strain EL 88289) were used in this study. Fresh sporocarps of these ectomycorrhizal fungi were collected at two mature forest stands in Northeast China (Table 1). One stand of *Larix gmellini* locates at Mohe County, Heilongjiang province, while another stand of *L. kaempferi* is at Kuandian County, Liaoning province.

Six fungal species of root pathogen *Rhizoctonia solani* (strain R198), *Fusarium oxysporum* (strain F182), *F. solani* (strain F390), *F. monlliforme* (strain F269), *F. vasinfectum* (strain F253) and *Cylindrocarpon destrctans* (strain C27) were obtained from the Herbarium of Northeast China (Shenyang, China).

### Fungal pure culture

Mycelia of ectomycorrhizal fungi described above were each cultured in Petri dishes on a specific medium with 1.8% agar, 10% potato juice, 50% wort, 2% glucose and 25mg $L^{-1}$ vitamin $B_1$ at pH 6.5 and an incubation temperature of 25±1 °C. Mycelia were incubated for 7~28 days, depending on fungal species, and were harvested and freeze-dried. The samples were stored separately based on the methodological requirements of the procedures for antifungal activity, biochemical composition and antioxidant activity as described below.

### Antifungal activity

The antifungal activity of the prepared extracts of the mycorrhizal mycelia was evaluated by agar diffusion bioassay-cup plate method with

slight modifications[18]. Briefly, each species hyphae were incubated in 50 ml the medium described above for 7~15 days. After centrifugation for 10 min at 2100 g, the supernatant was decanted to a vial and filtered by 0.22 µm membranes. Small wells were punched in the agar using a sterile metal cylinder (6 mm diameter) and the extract of EMM (100 µL) was placed into them. The plates were left 1 h at 4 °C to allow the diffusion of the extracts and then incubated under aerobic conditions at 25±1 °C. After an incubation period of 2~5 days for fungi, the diameters of the inhibition zones of microbial growth around the samples were measured in millimeters. Nystatin (10 µM $mL^{-1}$, Sigma) was used as positive reference standards to determine the sensitivity of each fungal strain tested, respectively. All the tests were performed in triplicate.

**Amino acids and fatty acids analysis**

Amino acid compositions of squid samples were analysed using an amino acid analyzer (L-8900, Hitachi High-Technologies Corp.). Briefly, samples were hydrolysed in 6 M HCl solution and centrifuged at 6000g for 5 min, then the residual from 200 µL of supernatant was dissolved in 1.5 mL of 0.2 M HCl solution and filtered through a 0.22 µm membrane. The amino acids were identified and quantified by comparing peak profiles of the squid samples with standard amino acid profiles.

The fatty acid composition of ectomycorrhizal fungi were carried out with a four-step procedure according to KarliDski et al.[19] with minor modifications. Briefly, saponification: each sample (40 mg DW) was added in 1.0 ml of saponification reagent (3.75M NaOH in methanol, 1:1, v/v) in a glass vial and placed in a 100 °C water bath for 5 min. The glass vial was vortexed vigorously for 10 s and returned to the water bath for additional 25 min. (2) methylation: after cooling, samples were added 2 ml of methylation reagent (6N HCl in methanol, 3.25/2.75, v/v) and placed in a 80 °C water bath for 10 min. (3) Extraction: the fatty acid methyl esters were extracted by adding 1.25 ml extraction solvent (hexane+methyl tert-butyl ether, 1:1, v/v) and mixing the vials for 5 min in a rotary shaker followed by removal of the aqueous lower phase. (4) Wash: the resulting extract was washed with 3 ml of base wash solution (0.3M aqueous NaOH) to remove nonmethylated fatty acids and other residues. The organic phase was then transferred to another vial for analysis.

Separation and identification of FAMEs were performed using an Agilent GC-MS (7890A-5975C) equipped with a 30 m fused silica capillary column (DB-5MS). The injector temperature was 250 °C and the detector temperature was 300 °C. The temperature was programmed at 140 °C for 1 min, raised from 140 °C to 190 °C by a rate of 5 °C $min^{-1}$ and held constant at 190 °C for 2 min, then raised from 190 to 230 °C by a rate of 2 °C $min^{-1}$ and held constant at 230 °C for 2 min, then raised from 230 to 250 °C by a rate of 4 °C $min^{-1}$ and held at 250 °C for 1 min and finally raised from 250 to 300 °C by a rate of 10 °C $min^{-1}$ and held constant at 300 °C for 5 min. The duration of the analysis was 48.83 min. One µl of sample preparation was injected. The fatty acid peaks were identified according to retention time and mass spectrum information. Identification of fatty acids was done by comparing the retention time of FAME peaks with Supelco 37 FAME and 26 BAME mixture standards (Sigma).

**Assay for antioxidant activity**

In vitro antioxidant activity of these mycelia was evaluated in a concentration-dependent manner through DPPH free-radical scavenging capacity and reducing power. An authentic antioxidant vitamin C served as the control to confirm the usefulness of the assay. All measurements were done in triplicate. Effects were evaluated by comparison with the treated and control groups.

DPPH radical scavenging activity was evaluated according to the following method[20]. Briefly, 100 µl of methanol extracts or vitamin C was added to the freshly prepared 0.12 mM (47.3 mg $L^{-1}$) DPPH solution (1.9 ml), respectively. The blank containing methanol only served as the control. The mixture was kept at ambient temperature for 30 min prior to measurement of the absorbance at 517 nm.

The reducing power was determined as described by Wang et al.[20]. Samples (2.5 ml, 1.0 mg/ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50 ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture (5.0 ml) was mixed with distilled water (5.0 ml) and ferric chloride (1.0 ml, 0.1%), and then the absorbance was recorded at 700 nm.

**Data analysis**

The data were presented as means ± standard error from independent experiments for each determination. Interspecies differences of amino acids concentration, and fatty acids profile were analyzed using one-way ANOVA, followed by the Tukey honestly significantly different tests. Spearman's correlation coefficients (rs) were used to assess bivariate relationships between antioxidant activity and biochemical composition. The datasets of amino acids and fatty acids were subjected to nonmetric multidimensional scaling analysis (NMS). The scores of the first two components from the NMS were used to compare differences among the species of ectomycorrhizal fungi and biochemical composition. All graphs were performed by Statistica 7 (StatSoft Inc., USA).

## RESULTS AND DISCUSSION

**Biochemical composition of vegetative mycelia of ECM fungal species in larch**

Table 1 shows the amino acid composition of the vegetative mycelia of ECM fungi. Concentration of total amino acids ranged from 22.95 g/100g to 53.86 g/100g. Total essential amino acid and non-essential amino acid concentration of *B. pictus* (20.69 g/100g, 33.17 g/100g) was much higher than the concentrations of the other species. All the contents of amino acids showed significant differences between ectomycorrhizal fungal species (Table 1).

Fatty acids profiles of six ECM fungal species mycelia were dominated by the fatty acids18:2ω6,9c (10.1~44.4%), 18:0 (3.9~42.2%), 18:1ω9c (7.5~36.5%) and 16:0 (9.7~27.7%), while i16:0, 18:2ω6,9t, 18:1ω5c and i19:0 were in minor quantities (Table 2). Interestingly, the presence of 10Me 16:0 was the characteristic feature of all species tested. There were highly distinctive fatty acid profiles between *S. grevillei*-1 and *S. grevillei*-2. In particular, *S. grevillei*-1 showed differences in the levels of predominant 18:1ω9c and 18:0, which was 36%, 27%, respectively (Table 2).

The shifts in the biochemical composition among ECM fungal species were indicated by the two-dimensional ordination plots using NMS of amino acids and fatty acids profile (Fig. 1). Within these plots, the closer the points, the more similar they are in biochemical composition. The species of *L. scabrum*, *H. mesophaeum* and *S. viscidus* had a dramatically different biochemical composition from the other species, while the longest distance (2.668) occurred between *B. pictus* and *S. grevillei*-1.The distance (0.873) between *H. mesophaeum* and *S. viscidus* was shorter than the distance between *S. greillei*-1 and *S. greillei*-2, whose value were 1.361 (Fig. 1). Particularly, signature fatty acid of *S. grevillei*-1 was separated from that of *S. grevillei*-2. *S. grevillei*-1 originated from the host tree of *L. gmelini* grew in higher latitude, lower temperature and precipitation than *S. grevillei*-2 from host tree *L. kaempferi* (Table 1, Fig. 1). Multivariate analysis may illustrate the effect of host trees on microbial community structure[21]. It is, however, relatively little known whether a nonmetric multidimensional scaling (NMS) could elucidate the difference among the pure culture hyphae of ECM fungal species. This analysis clearly indicated a key role for host trees and such environment factors as temperature and precipitation in shaping biochemical composition of ECM fungal species in larch.

**Antifungal and antioxidant activity**

The effect of exudation of ECM hyphae against fungal pathogens was given in Table 3. All the ECM fungi had fungicidal activity against pathogenic fungi *F. monlliforme* and *F. vasinfectum.* In particular, exudates from *H. mesophaeum* could inhibit all the fungal pathogens tested, while *S. grevillei*-1 and *S. grevillei*-2 had little inhibitory effect on *R. solani*, *F. oxysporum* and *C. destrctans* (Table 3). The results indicated that antifungal activity of exudates from the ECM hyphae varied with ECM fungal species and fungal pathogens tested. An increasing number of studies have showed that ectomycorrhizal fungi may interfere with other plants and microorganisms through the exudation of allelochemicals[22]. For example, mycorrhizal hyphae increase the effectiveness of allelochemicals by extending the bioactive zone of juglone and hence influence plant community structure[23]. The mycelia of ECM fungi could release antibiotics, phenolics and phytoalexins to provide host trees resistance to fungal diseases of plants and the pinewood nematode[22]. The ECM species of *Laccaria bicolor*, *Suillus bovines* and *Laccaria laccata* may suppress root pathogenic fungi such as *F. moniliforme*, *Rhizoctonia* sp. and

**Table 1.** Amino acid proûle (g 100 $g^{-1}$ DW) of six ECM fungal species in *Larix gmellini* and *L. kaempferi* in larch

| Parameter | *B. pictus* | *H. mesophaeum* | *L. scabrum* | *S. grevillei*-1 | *S. grevillei*-2 | *S. viscidus* |
|---|---|---|---|---|---|---|
| Isoleucine | 2.00 ± 0.25 ab | 1.16 ± 0.36 ab | 1.51 ± 0.27 b | 1.03± 0.13 ab | 0.70 ± 0.08 a | 0.71 ± 0.06 a |
| Leucine | 3.80 ± 0.46 ab | 2.10 ± 0.27 b | 2.71 ± 0.16 b | 1.88 ± 0.14 ab | 1.25 ± 0.15 a | 1.21 ± 0.18 a |
| Lysine | 3.43 ± 0.34 b | 2.04 ± 0.22 ab | 2.88 ± 0.29 b | 2.02 ± 0.35 ab | 1.67 ± 0.22 a | 1.48 ± 0.08 a |
| Methionine | 2.05 ± 0.31 b | 1.34 ± 0.21 a | 1.86 ± 0.23 ab | 1.28 ± 0.01 a | 1.18 ± 0.01 a | 0.99 ± 0.11 a |
| Phenylalanine | 2.79 ± 0.31 b | 1.84 ± 0.22 ab | 2.29 ± 0.44 b | 1.68 ± 0.22 a | 1.30 ± 0.32 a | 1.32 ± 0.22 a |
| Threonine | 2.51 ± 0.37 b | 1.55 ± 0.32 a | 2.01 ± 0.45 b | 1.28 ± 0.29 a | 1.33 ± 0.18 a | 0.99 ± 0.13 a |
| Valine | 4.11 ± 0.58 b | 2.90 ± 0.41 a | 4.72 ± 0.66 b | 2.91 ± 0.30 a | 2.13 ± 0.30 a | 2.54 ± 0.28 a |
| Total essential amino acids | 20.69 | 12.93 | 17.98 | 12.08 | 9.56 | 9.23 |
| Alanine | 3.49 ± 0.49 b | 2.13 ± 0.21 ab | 2.37 ± 0.16 ab | 2.16 ± 0.16 ab | 1.39 ± 0.10 a | 1.73 ± 0.08 a |
| Aspartate | 4.22 ± 0.60 c | 2.50 ± 0.35 ab | 3.33 ± 0.50 b | 2.45 ± 0.17 ab | 2.05 ± 0.14 a | 1.72 ± 0.08 a |
| Arginine | 3.40 ± 0.19 c | 1.79 ± 0.15 a | 2.37 ± 0.21 b | 1.64 ± 0.12 a | 1.23 ± 0.09 a | 1.1.10 ± 0.15 a |
| Cysteine | 1.16 ± 0.15 ab | 0.77 ± 0.05 a | 2.01 ± 0.15 b | 1.23 ± 0.13 ab | 1.14 ± 0.22 ab | 1.46 ± 0.21 ab |
| Glutamate | 7.86 ± 0.89 c | 3.69 ± 0.45 b | 3.66 ± 0.31 b | 2.81 ± 0.16 a | 2.08 ± 0.19 a | 2.03 ± 0.21 a |
| Glycine | 1.98 ± 0.18 b | 1.38 ± 0.15 a | 1.88 ± 0.19 b | 2.15 ± 0.34 b | 1.08 ± 0.14 a | 0.96 ± 0.10 a |
| Histidine | 1.35 ± 0.22 c | 1.05 ± 0.11 ab | 0.93 ± 0.13 d | 0.81 ± 0.06 b | 0.55 ± 0.02 a | 0.52 ± 0.03 b |
| Proline | 3.60 ± 0.42 c | 2.85 ± 0.21 b | 2.17 ± 0.13 b | 1.65 ± 0.11 a | 1.51 ± 0.12 a | 1.30 ± 0.07 a |
| Serine | 3.45 ± 0.27 c | 1.73 ± 0.24 a | 2.09 ± 0.33 b | 1.49 ± 0.12 a | 1.19 ± 0.09 a | 1.14 ± 0.07 a |
| Tyrosine | 2.68 ± 0.37 b | 2.07 ± 0.32 ab | 2.71 ± 0.08 b | 2.07 ± 0.02 a | 1.73 ± 0.23 a | 1.76 ± 0.13 a |
| Total non-essential amino acids | 33.17 | 19.96 | 23.52 | 18.47 | 13.97 | 13.72 |
| Total amino acids | 53.86 | 32.89 | 41.57 | 30.55 | 23.53 | 22.95 |

[a] The EEMM of *B. pictus*, *L. scabrum*, *H. mesophaeum*, *S. viscidus* and *S. grevillei*-1 were collected at the stand of *Larix gmellini*, locating at Mohe County, Heilongjiang province, China, while *S. grevillei*-2 was collected at the stand of *Larix kaempferi*, locating at Kuandian County, Liaoning province, China.

*Cylindrocarpon destructans*, respectively[24-26]. The data generated in this study implied that several ectomycorrhizal fungi such as *H. mesophaeum* could release some allelochemicals to inhibit the fungal pathogens.

The presence of several biochemical constituents in fruiting body and mycelia of *Laetiporus sulphureus*, *Cordyceps sinensis* and *Ganoderma lucidum* plays an important role in antioxidant activity[27-29]. The data generated in this study showed that the methanol extracts of all the EEMM mycelia have clearly inhibitory effect on reducing power and 2, 2-diphenyl-1-picrylhydrazyl free-radical (DPPH) (Fig. 2).

The reducing power of ECM fungal mycelia also decreased with species when compared with the positive standard, vitamin C (Fig. 2). The methanol extracts of *H. mesophaeum* mycelia exhibited higher reducing power capacity when compared with that of the other species, which were statistically significant ($P < 0.05$). The reducing power of *S. grevillei*-2 was lower than that of *S. grevillei*-1 which was down 35.1% and 25.5% in comparison to control, respectively (Fig. 2).

The DPPH radical is a stable free radical to determine antioxidant capacity of natural compounds. The free radical scavenging capacity of methanol extracts of the EEMM mycelia and the one positive controls viz. vitamin C were compared in Figure 2. The rates of inhibition DPPH radical decreased in all the tested ECM fungal species. The two lowest rates of DPPH scavenging capacities in *S. viscidus* and *S. greillei*-1 were 69.0% and 73.0%, which reduced by 26.6% and 22.3% in comparison to the rate of vitamin C. Similarly, the

**Table 2**. Fatty acid profile from mycelia of six ECM fungal species in *Larix gmellini* and *L. kaempferi* in larch

| Fatty acids | *B. pictus* (n=5) | *L. Scabrum* (n=5) | *H. mesophaeum* (n=5) | *S. grevillei*-1 (n=5) | *S. grevillei*-2 (n=5) | *S. viscidus* (n=5) |
|---|---|---|---|---|---|---|
| 16:1ω7c | 1.24±0.22 | ND | ND | 2.15±0.33 | ND | ND |
| 16:0 | 17.67±2.76 | 14.13±2.01 | 16.32±2.21 | 27.67±4.21 | 16.22±2.99 | 9.69±1.66 |
| 10Me 16:0 | 0.62±0.11 | 0.44±0.20 | 0.55±0.04 | 0.46±0.09 | 0.60±0.08 | 0.39±0.02 |
| i16:0 | 2.08±0.11 | 1.96±0.31 | 1.85±0.28 | 3.90±0.21 | 2.11±0.31 | 5.17±0.42 |
| 18:2ω6,9c | 27.86±4.04 | 39.79±5.63 | 44.39±6.61 | 13.56±2.22 | 18.62±2.09 | 40.45±3.92 |
| 18:1ω9c | 7.50±1.28 | 18.64±2.84 | 19.78±2.88 | 36.49±6.87 | 23.41±3.45 | 30.84±4.85 |
| 18:0 | 35.20±5.32 | 12.73±2.26 | 6.08±1.91 | 3.90±0.38 | 26.47±4.56 | 6.97±1.41 |
| 18:2ω6,9t | 2.96±0.31 | 6.31±1.33 | 5.34±1.03 | 1.92±0.20 | 2.49±0.27 | ND |
| 18:1ω5c | ND | 2.52±0.38 | 3.09±0.62 | 5.39±0.72 | 2.78±0.16 | 3.14±0.51 |
| i19:0 | 4.66±0.83 | 1.50±0.42 | 0 | 0.28±0.03 | 2.97±0.18 | ND |
| 2OH 18:0 | ND | ND | ND | ND | 0.92±0.08 | ND |

ND: not determined
Data are presented as % relative abundance with means and standard errors. SE is for individual species

**Table 3**. Antifungal activity of exudation of ECM fungal species in *Larix gmellini* and *L. kaempferi* on pathogenic fungi

| ECM fungal Species | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *C. destrctans* | *F. monlliforme* | *F. solani* | *F. oxysporum* | *F. vasinfectum* | *R. solani* |
| *B. pictus* | 6±1 | 9±1 | 9±1 | - [a] | 12±2 | 10±2 |
| *H. mesophaeum* | 10±1 | 12±2 | 13±1 | 16±2 | 15±2 | 17±2 |
| *L. scabrum* | ND [b] | ND | ND | ND | ND | ND |
| *S. grevillei*-1 | - | 9±1 | 8±1 | - | 11±1 | - |
| *S. grevillei*-2 | - | 10±1 | 9±1 | - | 12±2 | - |
| *S. viscidus* | ND | ND | ND | ND | ND | ND |
| Nystatin | 11±1 | 13±1 | 16±1 | 20±2 | 17±2 | 18±2 |

[a] -: no inhibition; [b] ND: not determined

lower values of DPPH scavenging capacities were 14.9%, 12.8%, 6.4% and 3.2% for *S. grevillei*-2, *L. scabrum*, *H. mesophaeum* and *B. pictus*, respectively (Fig. 2). Also, the DPPH radical scavenging capacities were higher in *S. grevillei*-2 than in *S. grevillei*-1. These results indicated that the vegetative mycelia of ECM fungi contained strong free radical scavenging capacity, and that the cultured mycelia of the ECM fungi could be used for the antioxidant activity to reduce the human demands on the natural resources of wild fungi species.

**Relationships between biochemical composition and antioxidant activity**

Bivariate correlations were analyzed by Spearman's Rank test. There is strong correlation between the antioxidant activities and biochemical composition of the methanol extract of the six ECM fungi species tested (Table 4). In particular, the negative correlation was observed between scavenging effects and the amino acids contents of leucine and phenylalanine, whereas the positive correlation occurred between scavenging effects and the amino acids contents of valine and arginine (Spearman's R < 0.05). The Spearman's R correlation coefficient between reducing power and arginine and lysine was 0.544 (p = 0.023) and -0.598 (p =

**Table 4**. Spearman rank order correlations between activity of scavenging DPPH radical and reducing power and amino acids and fatty acids. (n = 18)

| | DPPH | | Reducing power | |
|---|---|---|---|---|
| | Spearman R | *P* | Spearman R | *P* |
| Amino acids | | | | |
| Isoleucine | -0.333 | 0.175 | -0.061 | 0.815 |
| Leucine | -0.698 | 0.002 | -0.276 | 0.267 |
| Lysine | -0.447 | 0.063 | -0.598 | 0.008 |
| Methionine | -0.433 | 0.073 | -0.114 | 0.653 |
| Phenylalanine | -0.585 | 0.011 | -0.190 | 0.450 |
| Threonine | -0.327 | 0.185 | -0.435 | 0.071 |
| Valine | 0.548 | 0.022 | 0.359 | 0.143 |
| Total essential amino acids | 0.007 | 0.977 | -0.032 | 0.900 |
| Alanine | 0.409 | 0.092 | 0.336 | 0.173 |
| Aspartate | 0.023 | 0.929 | 0.302 | 0.224 |
| Arginine | 0.538 | 0.021 | 0.544 | 0.023 |
| Cysteine | 0.105 | 0.679 | 0.073 | 0.772 |
| Glutamate | 0.050 | 0.845 | 0.243 | 0.331 |
| Glycine | 0.358 | 0.145 | 0.266 | 0.287 |
| Histidine | 0.025 | 0.922 | 0.397 | 0.103 |
| Proline | 0.242 | 0.333 | 0.375 | 0.125 |
| Serine | -0.085 | 0.738 | -0.067 | 0.791 |
| Tyrosine | 0.059 | 0.817 | -0.006 | 0.981 |
| Total non-essential amino acids | 0.095 | 0.713 | 0.098 | 0.706 |
| Selected fatty acids | | | | |
| 16:1ω7c | -0.129 | 0.611 | 0.050 | 0.844 |
| 16:0 | 0.037 | 0.884 | 0.108 | 0.671 |
| 10Me 16:0 | 0.362 | 0.139 | 0.063 | 0.804 |
| i16:0 | -0.661 | 0.003 | -0.585 | 0.011 |
| 18:2ω6,9c | 0.115 | 0.651 | 0.319 | 0.198 |
| 18:1ω9c | -0.753 | <0.000 | -0.634 | 0.005 |
| 18:0 | 0.493 | 0.038 | 0.117 | 0.644 |
| 18:3ω6,9,12c | 0.767 | <0.000 | 0.673 | 0.002 |
| 18:1ω5c | -0.718 | 0.001 | -0.463 | 0.053 |
| i19:0 | 0.490 | 0.039 | 0.114 | 0.653 |

0.008), respectively. In addition, there were significant negative relationships between free radical scavenging activities and the specific fatty acids of i16:0, 18:1ω9c and 18:1ω5c, whereas the positive relationships occurred between free radical scavenging activities and the fatty acids of 18:0 and 18:3ω6,9,12c. Moreover, reducing power was significantly related to the fatty acids of i16:0 and 18:1ω9c (Spearman's R = -0.585, p = 0.011, Spearman's R = -0.634, p = 0.005, respectively), to the fatty acids of 18:3ω6,9,12c (Spearman's R = 0.673, p = 0.002, Table 4).

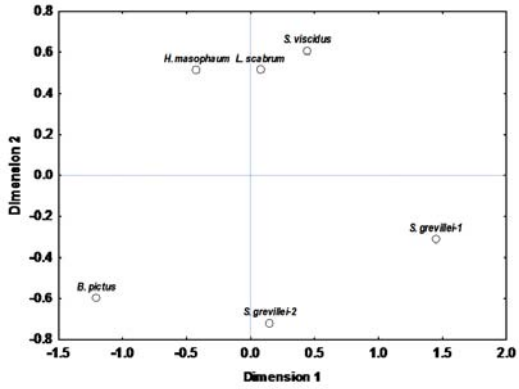


**Fig. 1.** Non-metric multidimensional scaling plots of the species of ectomycorrhizal fungi in larch and biochemical composition of different vegetative mycelia of ectomycorrhizal fungi

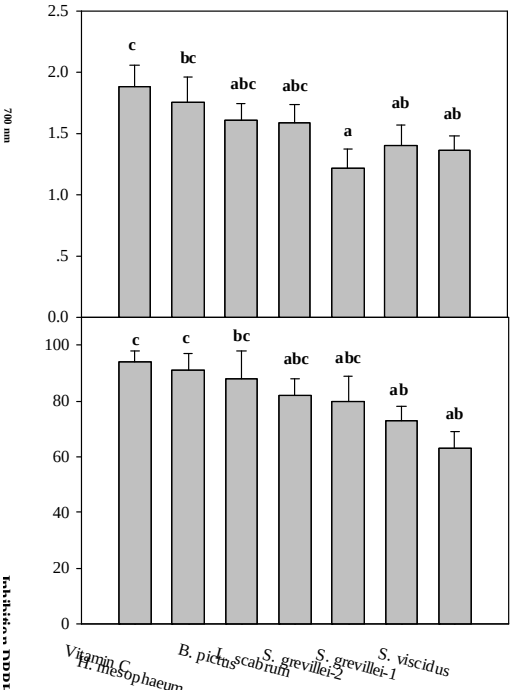


**Fig. 2.** Antioxidant activities of the vegetative mycelia of ectomycorrhizal fungi through reducing power and free radical scavenging activity (DPPH). Vitamin C served as the control. Columns with the same letter are not significantly different at P<0.05, one-way ANOVA, followed by the Tukey honestly significant difference tests

The biochemical constituents in EEMM and their ecological functions and health-related implications have been extensively investigated[6,9]. A few chemicals in EEMM mycelia may offer both ecological functions and health-related benefits. Such as total polyphenol and flavonoids, serves as antioxidant and putative cancer chemopreventive agents[30]. However, some other active constituents also contribute in part to the antioxidant properties of mushrooms. For example, it was reported that amino acids and fatty acids in the test solutions may produce higher antioxidant capacity[31,32]. Our data showed that chemicals in mycelia may serve as antioxidant agents providing beneficial health effects in humans. However, potential mechanisms remain obscure. Further clarification of ecological functions and mechanisms of chemicals in EEMM mycelia as functional foods may offer many potential applications in food industries.

## CONCLUSION

The biochemical parameters, antifungal and antioxidant properties of six tested ECM mycelia in larch were comparatively studied for the first time. It was shown that the contents of amino acids and fatty acids diffed with the species. The species of *B. pictus* and *S. viscidus* contained the higher concentration of total amino acid and relative abundance of unsaturated fatty acids than in the other species, which were 53.9% and 73.14%, respectively. Moreover, the host tree species and environmental factors were of importance in shaping amino and fatty acids composition among the EEMM through multidimensional scale analysis. Furthermore, *H. mesophaeum* showed better antifungal activities than the other species, while the methanol extracts of all the EEMM mycelia have efficient DPPH radical scavenging capacities and reducing power. There is clear

correlation between the contents of amino and fatty acids and antioxidant activities. These findings suggest that biochemical composition have a chemotaxonomic potential in EEMM species, and be useful for the application of EEMM mycelia and related products in the food industry.

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