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# Biologically Active Substances from Mycelia of *Ganoderma lucidum* and *Lentinula edodes*

N. A. Bisko[1], V. T. Bilay[1], V. G. Babitskaya[2], V. V. Scherba[2], N. Y. Mitropolskaya[1] and T. A. Puchkova[2]

[1]*M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereschenkivska 2, Kiev, 01601-GSP, Ukraine (abuch@botan.kiev.ua)*
[2]*Institute of Microbiology, National Academy of Sciences of Byelorussia, Minsk, Byelorussia*

## ABSTRACT

The production of mycelial biomass, endo- and exo-polysaccharides, lipids, protein, polyphenols, amino and fatty acids was investigated for *Ganoderma lucidum* (five strains) and *Lentinula edodes* (14 strains). Mycelial biomass and levels of endo- and exo-polysaccharides of *G. lucidum* were higher on beer wort medium when compared to glucose-peptone medium. All essential amino acids were present in the proteins of all the fungal strains examined. Mycelial proteins of *G. lucidum* contained a high content of lysine and threonine. Linoleic acid dominated the lipid fraction of *G. lucidum* and *L. edodes* mycelia. Carbohydrate analysis of polysaccharides in the mycelia of *G. lucidum* and *L. edodes* revealed that glucose was the major constitutent.

## INTRODUCTION

*Ganoderma lucidum* and *Lentinula edodes* are among the most popular mushrooms in oriental medicine (Mizuno 1993; Hobbs 1995; Wasser & Weis 1997; Wasser et al. 2000). It is known that *G. lucidum* and *L. edodes* have antioxidant and antitumor activity, a sedative effect on the central nervous system and improve cardio-cerebral circulation (Liu 1999; Ooi & Liu 1999; Jones & Sanardhanan 2000; Reshetnikov et al. 2001; Zhow & Gao 2002). The objective of the present study was to investigate the biomass and chemical composition of vegetative mycelium produced by *G. lucidum* and *L. edodes* on various nutrient media.

## MATERIALS AND METHODS

The strains of *G. lucidum* (1, 333, 362, 357 and 358) and *L. edodes* (101, 182, 185 and 192) used in this study were obtained from the culture collections of the N. G. Kholodny Institute of Botany and the Institute of Microbiology of the National Academy of Sciences.

Mycelia of *G. lucidum* and *L. edodes* were grown in glucose-peptone nutrient medium (10 g glucose, 3 g peptone, 1 g $K_2HPO_4$, 1 g $KH_2PO_4$ and 0.25 g $MgSO_4$ $7H_2O$, 20 mL corn extract/L, pH 5.5) and beer wort medium (20 g carbohydrates/L, pH 5.5). The media were sterilized by autoclaving for 20 min at 121 C. Fungi were grown as submerged cultures in 5-L flasks. Inocula were produced in 500-mL flasks containing 50 mL of medium and homogenized mycelia prepared from Petri-dish cultures. Mycelial biomass was harvested after 5 to 7 days of growth at 28 C.

Mycelia were separated from the medium by filtration, washed with distilled water, dried to at 60° C and pounded. It was then mixed with distilled water (1:5, w/v) and boiled in a water bath for 12-18 h. The resultant extracts were centrifugated (3,000 x *g*) for 15 min. The supernatant was treated with an equal

vol. of cold 96% ethanol and the sediment (endo-polysaccharides) was collected by centrifugation (Chihara et al. 1970; Goncharova et al. 1996).

Exo-polysaccharides were assayed in the cultural broth (Babitskaya et al. 2000). Exo-polysaccharides were determined in glucose-peptone and beer wort media without mushroom mycelia as a control. Exo-polysaccharides were absent in the glucose-peptone medium, whereas only traces of exo-polysaccharides were detected in the beer wort medium.

Extracts of mycelia of *G. lucidum* and *L. edodes* were obtained after freezing in quartz sand and extraction with 70% ethanol for 30 min. The extracts were centrifuged at 8,000 x *g* for 15 min. Polyphenols were determined using reactive Tolin-Denis (Zaprometov 1985).

Protein content of mycelia was estimated according to the method of Lowry (Lowry et al. 1951), and amino acid profile using an amino acid analyzer AAA-881 "Microtechna" (Krischenko 1983).

Lipids were extracted after Folch et al. (1957), and fatty acids determined by chromatography with 15% polyethylenglycol succinate as the liquid (Vereschagin et al. 1963).

## RESULTS AND DISCUSSION

The data obtained from our investigation indicated that the biomass of all strains of *G. lucidum* examined was higher on the beer wort medium compared to the glucose-peptone medium (Table 1). At the same time, the content of endo- and exo-polysaccharides of all strains on beer wort medium was higher than on glucose-peptone medium. Biomass and endo-polysaccharides were produced at the highest levels by strains 1, 333 and 357 on beer wort medium. Further, the content of exo-polysaccharides was maximal for the strains 333, 357 and 362 on this medium as well (Table 1). We did not observe a relation between the content of polyphenols in mycelia of *G. lucidum* and the composition of the nutrient medium (Table 1).

Table 1. Biomass (BM) and lipid (LP), protein (PRO), endo-polysaccharide (ENP), exo-polysaccharide (EXP) and polypenol (PP) content of mycelia of *G. lucidum* and *L. edodes* grown in submerged culture on beer wort (BW) and glucose-peptone (GP) media

| | ***G. lucidum* Strain** | | | | | | | | | | ***L. edodes* Strain** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BW Medium** | | | | | | **GP Medium** | | | | **GP Medium** | | | |
| | **1** | **333** | **362** | **358** | **357** | **1** | **333** | **362** | **358** | **357** | **101** | **182** | **185** | **192** |
| BM (g/L) | 10.1 | 11.5 | 8 | 6.5 | 12.5 | 9.5 | 9 | 9 | 8 | 8.5 | 5.7 | 8 | 7.8 | 6.2 |
| % LP | 9.3 | 8.5 | 6.5 | 7 | 8 | 7 | 7 | 5.7 | 6.5 | 7 | 7.5 | 9 | 8.7 | 6.9 |
| % PRO | 22.2 | 22.5 | 18.5 | 19 | 22.5 | 23.5 | 23 | 20.5 | 21 | 24 | 20 | 23 | 23 | 22.5 |
| % ENP | 12 | 8 | 6.8 | 6.4 | 9 | 8.5 | 7 | 5 | 4 | 8.3 | 3.2 | 3.5 | 3.2 | 2.8 |
| % EXP | 3 | 8 | 7 | 3.5 | 8 | 2.5 | 4.5 | 4 | 3 | 5.5 | 3.5 | 4.3 | 3.4 | 2.4 |
| PP (g) | 0.5 | 0.8 | 0.7 | 0.5 | 0.7 | 0.5 | 0.7 | 0.7 | 0.6 | 0.7 | 1.7 | 1.8 | 1.6 | 1.5 |

We observed that the beer wort medium supported the accumulation of lipids in all strains of *G. lucidum* to a greater extent than did the glucose-peptone medium (Table 1). There were no significant differences in protein content among the different strains on the two media.

The amino acid and fatty acid contents were determined for strains 1, 333 and 357, which produced the most biomass. Small differences in amino acid content existed among the three strains of *G. lucidum*, but all amino acids were represented (Table 2). It is interesting to note that the mycelial proteins of *G. lucidum* contained a high content of lysine and threonine (Table 2). This was shown to be typical for other species of higher Basidiomycetes as well (Crisan & Sands 1978).

On both nutrient media, the lipids in the mycelia were dominated by unsaturated fatty acids (Table 3). Linoleic acid ($C_{18:2}$) was the major fatty acid in *G. lucidum*, but its quantity varied with the medium and fungal strain. This same relationship was observed for other medicinal mushroom species (Crisan & Sands 1978).

Growth of *G. lucidum* on the glucose-peptone medium, as opposed to beer wort medium, led to an increased level of saturated fatty acids (Table 3). However, differences in the levels of individual fatty acids among the strains on the same medium were not significant.

An analysis of the mycelia of the *L. edodes* (Table 1) showed that on glucose-peptone medium, the amount of biomass and its chemical composition differed slightly among the strains. Of the four strains tested, strain 182 produced the most biomass and highest quantities of endo- and exo-polysaccharides, lipids, protein and polyphenols. For example, the polyphenol content of this strain was 16% higher than that of strain 192, which showed the lowest content (Table 1).

Table 2. The content of amino acids in the protein of *G. lucidum* and *L.edodes* strains on glucose-peptone medium (% of protein)

| Constituent | *L. edodes* Strain | | | | *G. lucidum* Strain | | |
|---|---|---|---|---|---|---|---|
| | 101 | 182 | 185 | 192 | 1 | 333 | 357 |
| Lysine | 2.1 | 4.9 | 2.0 | 2.5 | 6.8 | 7.0 | 7.5 |
| Histidine | 1.0 | 1.2 | 1.2 | 0.8 | 1.8 | 1,6 | 1.6 |
| Arginine | 8.5 | 4.2 | 4.1 | 4.7 | 5.6 | 5,0 | 4.5 |
| Aspartic acid | 10.7 | 10.0 | 9.9 | 11.5 | 8.9 | 9.3 | 10.0 |
| Threonine | 6.3 | 4.5 | 4.8 | 5.8 | 4.6 | 4.5 | 3.7 |
| Serine | 6.3 | 5.9 | 5.6 | 6.3 | 5.6 | 5.7 | 5.0 |
| Glutamic acid | 17.6 | 17.0 | 22.9 | 18.3 | 17.5 | 18.0 | 18.7 |
| Proline | 5.2 | 5.0 | 5.4 | 5.7 | 4.6 | 4.1 | 4.4 |
| Glycine | 6.0 | 5.2 | 5.3 | 6.1 | 5.8 | 5.3 | 5.0 |
| Alanine | 7.0 | 6.1 | 6.6 | 8.1 | 8.7 | 9.5 | 8.7 |
| Cysteine | 3.4 | 3.2 | 3.2 | 2.5 | 2.4 | 2.0 | 2.7 |
| Valine | 6.5 | 6.9 | 6.4 | 6.5 | 4.8 | 5.2 | 5.0 |
| Methionine | 1.2 | 2.2 | 1.2 | 1.2 | 1.4 | 3.4 | 3.6 |
| Isoleucine | 4.3 | 5.2 | 5.0 | 4.2 | 3.2 | 3.2 | 4.0 |
| Leucine | 9.3 | 10.0 | 8.4 | 7.5 | 10.0 | 8.0 | 7.8 |
| Tyrosine | 1.4 | 4.5 | 4.0 | 4.0 | 3.2 | 3.0 | 3.5 |
| Phenylalanine | 3.2 | 4.0 | 4.0 | 4.3 | 4.7 | 4.9 | 4.4 |

Mycelial proteins of all strains of *L. edodes* included 17 amino acids, with aspartic and glutamic acids predominating (Table 2). Crisan and Sands (1978) reported similar findings for other mushroom species. Differences in the profile of amino acids among strain of *L. edodes* were quite considerable. The content of lysine, methionine and tyrosine was 2.3-, 1.8- and 3.2-fold higher, respectively, in strain 182 than strain 101. Further, the content of arginine in strain 101 was twice that of strain 182 (Table 2).

Lipids in the mycelia of all strains of *L. edodes* consisted of ten fatty acids (Table 3). The major acids were linoleic ($C_{18:2}$) and palmitic ($C_{16:0}$). In contrast to *P. ostreatus* grown on the same medium, the lipids of *L. edodes* contained myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$) and heptadecenoic ($C_{17:1}$) acids (Babitskay et al. 1999).

On the glucose-peptone medium, all strains of *G. lucidum* produced more biomass than all strains of *L. edodes* (Table 1). The highest accumulation of mycelium was observed for *L. edodes* strain 182, which only equaled the minimum produced by *G. lucidum* strain 358.

The largest difference between *G. lucidum* and *L. edodes* was noted for the synthesis of endo-polysaccharides. Here, the levels of endo-polysaccharides in mycelia of some strains of *G. lucidum* were three-fold higher than in *L. edodes*. Differences in all other constituents between the two fungal species were negligible.

Table 3. Fatty acids (% of total) comprising lipids of *G. lucidum* and *L. edodes* grown on glucose-peptone medium

| Constituent | *G. lucidum* Strain | | | *L. edodes* Strain | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 333 | 357 | 101 | 182 | 185 | 192 |
| $C_{14:0}$ | 0.91 | 0.56 | 1.56 | 0.90 | 0.73 | 0.66 | 0.92 |
| $C_{15:0}$ | 2.15 | 1.02 | 1.02 | 1.38 | 1.58 | 1.32 | 2.12 |
| $C_{16:0}$ | 28.88 | 20.13 | 29.13 | 18.99 | 21.53 | 19.72 | 22.08 |
| $C_{16:1}$ | 0.99 | 1.50 | 1.50 | 2.05 | 0.85 | 0.88 | 0.82 |
| $C_{17:0}$ | 1.65 | 0.63 | 0.60 | 0.74 | 0.45 | 0.55 | 0.54 |
| $C_{17:1}$ | 1.97 | 0.88 | 1.72 | 0.49 | 0.45 | 0.88 | 0.74 |
| $C_{18:0}$ | 1.07 | 1.69 | 0.76 | 3.27 | 2.30 | 2.74 | 4.0 |
| $C_{18:1}$ | 4.40 | 4.51 | 4.63 | 8.98 | 3.79 | 7.07 | 5.66 |
| $C_{18:2}$ | 57.98 | 67.58 | 67.60 | 61.12 | 67.42 | 66.18 | 62.47 |
| $C_{18:3}$ | Trace | 1.51 | 1.48 | 2.08 | 0.90 | Trace | 0.64 |
| Saturated fatty acids | 34.66 | 24.03 | 23.07 | 25.28 | 26.59 | 24.99 | 29.67 |
| Unsaturated fatty acids | 65.34 | 75.97 | 76.93 | 74.72 | 73.41 | 75.01 | 70.33 |
| Unsaturated/ saturated | 1.17 | 1.47 | 1.48 | 1.40 | 1.43 | 1.41 | 1.34 |

Strains of *G. lucidum* did not contain a high content of polyphenols when compared to *L. edodes* (Table 1) or *P. ostreatus* cultivated under identical conditions (Scherba et al. 1999).

Regarding lysine content, the greatest differences between *G. lucidum* and *L. edodes* were observed on the glucose-peptone medium. The amount of lysine in all strains of *G. lucidum* was 1.4- to 3.8-fold higher than in *L. edodes* strains.

Considering fatty acid content, *G. lucidum* had a higher content of heptadecenoic acid ($C_{17:1}$) compared to that of *L. edodes*. The level of this acid in *G. lucidum* strain 1 was 4.4–fold higher than in *L. edodes*

strain 182 (Table 3). Also, *L. edodes* strain 192 had 5.2 times more stearic acid ($C_{18:0}$) than was found in *G. lucidum* strain 357.

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