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Some Physiological Aspects of the Submerged Cultivation of Culinary–Medicinal Shiitake Mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae)

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ABSTRACT: Production of biomass, lipids, protein, exo- and endopolysaccharides in different cultivation conditions, the composition of mycelia and fruiting bodies, and infrared spectra of exo- and endopolysaccharides of *Lentinus edodes* were studied. It was demonstrated that an intensity of aeration of 1.5 L/L/min with an agitation speed of 100 rpm in a fermenter provided the most favorable conditions for the biosynthesis of exopolysaccharides. Infrared spectra of exo- and endopolysaccharides showed that the most pronounced absorption zones were at 3400 cm^{-1} and 1550 cm^{-1}.

KEY WORDS: mycelium, fruiting body, exopolysaccharides, cultivation conditions, composition, shiitake mushroom, *Lentinus edodes*

INTRODUCTION

Lentinus edodes (Berk.)Singer is one of most widely used medicinal mushrooms in the world (Wasser and Weis, 1997; Wasser et al., 2000). It was found that many medicinal properties of this species were influenced by the presence of polysaccharides (Mizuno, 1996). This research aims to study the influence of submerged cultivation conditions on the production of biomass, protein, lipids, and polysaccharides including the investigation of polysaccharide composition.

MATERIALS AND METHODS

Strain

The strain of *Lentinus edodes* (N1) was obtained from the culture collection of the Institute of Microbiology of the National Academy of Sciences of Byelorus, Minsk.

Cultures and Growth Conditions

Lentinus edodes fruiting bodies were grown on a mixture of oak sawdust and wheat bran (4:1) (Bisko and Bilay, 1996). The mycelium of this strain was grown in submerged conditions on beer wort medium (7°) in a fermenter (volume 10 liters) at a temperature of 23–25 °C with an intensity of aeration 0.5, 1, 1.5, and 2 L/L/min; the agitation of speed was 0, 100, 150 rotations/min (rpm).

Separation

Mycelium was separated from the medium by filtration and washed with distilled water.

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Protein and Amino Acid Determination

In mycelia of studied strain, protein content was estimated according to Lowry's method (Lowry et al., 1951), and amino acids were detected on an amino acid analyzer, AAA-881 Microtechna (Krischenko, 1983).

Lipid and Fatty Acid Determination

Lipids were extracted by Folch's method (Folch et al., 1957), fatty acids were estimated on chromatograph Chrom-5 with 15% polyethylenglycol succinate as liquid (temperature of the column was 160 °C with a temperature of evaporation at 210 °C) (Vereschagin et al., 1963; Keits, 1975).

Polyphenol Determination

Polyphenols were determined with reactive Tolin–Denis (Zaprometov, 1985) in the extract of mycelia. Extract of mycelia was obtained after freezing and extraction with 70% ethanol for 30 min.

Exopolysaccharide and Endopolysaccharide Determination

To obtain an estimation of endopolysaccharides, the mycelia was homogenized, mixed with distilled water (1:5), and boiled in a water bath for 12–18 hours. The extracts were centrifugated (3.000 g) for 15 minutes. The supernatant was treated with 96% ethanol at a volume ratio of 1:1 (at a temperature of 4 °C), and the sediment (endopolysaccharides) was then separated by centrifugation (Chihara et al., 1970; Goncharova et al., 1996). Exopolysaccharides were determined in the cultural liquid and beer wort media, also without mushroom mycelia (Babitskaya et al., 2000). Traces of exopolysaccharides were determined in beer wort medium. Infrared (IR) spectra of polysaccharides were estimated using the Spectrometer UR-20. The carbohydrate composition of polysaccharides after hydrolysis was determined by gas–liquid chromatography (Khorlin, 1975; Babitskaya et al., 2000).

Mineral Elements Determination

The mineral elements of mycelia were estimated using the absorptive spectrophotometer Saturn 3P-1 (Russia).

RESULTS AND DISCUSSION

The obtained results indicate that the conditions of media aeration in the absence of agitation influence the synthesis of biomass and endo- and exopolysaccharides of *Lentinus edodes*. The intensity of aeration at 0.5 L/L/min resulted in the maximal yield of biomass, which was noted for 72–84 hours of cultivation (6.8–7.0 g/L). The increase of aeration to 1–1.5 L/L/min led to a higher concentration of biomass, 9–10 g/L, during the same period. Higher aeration of media (2.0 L/L/min) was connected with abundant foam production, a decrease in growth rate, and the synthesis of exopolysaccharides.

Analogous regularities were characterized for the indices of the content of exopolysaccharides, endopolysaccharides, proteins, and lipids in biomass. Therefore, the maximal yield of exopolysaccharides was 3.0 g/L, the highest content of endopolysaccharides (4.5–5% adm), protein (23% adm), and lipids (7.3–8.7% adm) in conditions of aeration 1–1.5 L/L/min.

The growth of *L. edodes* in a fermenter, apart from the aeration of media, depended on the mixer rotation (agitation speed). It was demonstrated that the speed of 100 rpm appeared to be most favorable for the production of biomass, the reduction of the lag-phase, and the increase of the exopolysaccharide content (Fig. 1). The main growth indices of *L. edodes* with an agitation speed of 150 rpm were similar to values of those with an agitation speed of 100 rpm—the maximal biomass was noted for 72 hours of cultivation was 10.5 g/L, the maximal content of protein for 56 h of cultivation was 23.0%, and the maximal content of lipids for 96 hours was 7.2%. It was found that the quantity of endopolysaccharides and lipid content in biomass with an agitation speed of 100, and 150 rpm was smaller than in conditions of aeration only.

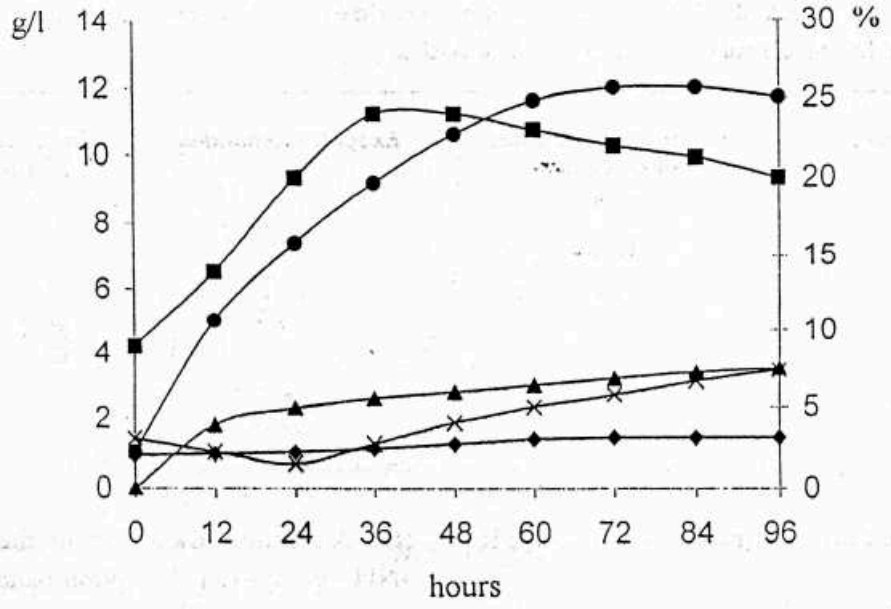

FIGURE 1. The growth of *Lentinus edodes* in the fermenter (the intensity of aeration 1.5 L/L/min, agitation of speed 100 rpm); • = biomass, g/L; ▲ = exopolysaccharides, g/L; ◆ = endopolysaccharides, %; × = lipids, %; ■ = protein, %.

Thus, the intensity of aeration 1.5 L/L/min and an agitation speed of 100 rpm in a fermenter were the most favorable conditions for the biosynthesis of exopolysaccharides of *L. edodes*.

The lipids of *L. edodes* mycelia after cultivation in a fermenter with an aeration of 1–1.5 L/L/min and an agitation speed of 100, and 150 rpm consisted mainly of linoleic acid at 54.81–59.85% and oleic acid at 15–20%. The content of unsaturated fatty acids (73.4–78.8%) was higher than saturated ones (21.2–26.6%). The ratio of unsaturated to saturated acids was 1.2–1.4. The same regularity was described for other species of mushrooms (Crisan and Sands, 1978; Wasser and Weis, 1997; Bisko et al., 2002).

The investigated strain of *L. edodes* produced 1800 mg % polyphenols. The benzoic acid, its derivatives (series of C_6-C_1) and flavons (series of C_3-C_6) were determined in polyphenols of *L. edodes*.

The data obtained in our work indicated that the content of protein in exopolysaccharides of *L. edodes* was 9.4 % and in endopolysaccharides 5.2%.

The chromatographic analysis of hydrolyzates of carbohydrates in exo- and endopolysaccharides of mycelium and fruiting bodies showed that they consist of glucose, galactose, mannose, and traces of xylose (Table 1). However, the composition of endosaccharides of mycelium and fruiting bodies, as well as exopolysaccharides, considerably differed in galactose content. It was demonstrated that the content of glucose in endopolysaccharides of fruiting bodies of *L. edodes* was higher when compared with endopolysaccharides of mycelium.

Our results indicate that the main component of exopolysaccharides of *L. edodes* strain 185 was glucose (Scherba et al., 1999). However, the change in composition of the media for cultivation of *L. edodes* mycelia resulted in the difference in quantitative composition of individual carbohydrates.

The high content of Ca and K was noted in the biomass of *L. edodes* (Table 2). A comparison of the mineral composition of fruiting bodies showed that the content of all investigated elements in fruiting bodies was lower than in the mycelium. The high variability of mineral composition of mushrooms, as with many vegetables and fruits,

TABLE 1. Content of Carbohydrates in Endopolysaccharides of Mycelium and Fruiting Bodies, Exopolysaccharides of Culture Liquid *Lentinus edodes**

Carbohydrate	Endopolysaccharides of mycelium	Exopolysaccharides	Endopolysaccharides of fruiting bodies
Glucose	73.33	88.18	90.4
Galactose	16.42	1.0	4.82
Mannose	10.25	10.82	4.78

* % of total

depended on the substrate (medium) on which it grew (Hobbs, 1996).

IR spectra of endo- and exopolysaccharides show that the most pronounced absorption zones were at 3400 and 1550 cm^{-1} (Fig. 2). The absorption band of 3400 cm^{-1} corresponds to the –OH– group. The displacement of maximum absorption to the zone of smaller waves (3625 cm^{-1}) were typical for endopolysaccharides and exopolysaccharides. It was connected with the inclusion of OH groups into inter- and innermolecular hydrogen bands of 2940 cm^{-1} that is characteristic for the presence of the CH_2 group in endo- and exopolysaccharides of *L. edodes*. The IR spectra of endo- and exopolysaccharides demonstrate that in their composition =NH– groups (an absorption band of 1550 cm^{-1}) are present (Fig. 2). The degree of amination in endopolysaccharides was higher than in exopolysaccharides. The presence of an absorption band of 1415 cm^{-1} indicates that both polysaccharides have –COOH groups.

It should be noted that the zone 1200–800 cm^{-1} (zone of carbohydrate fingerprints) is the main zone. Endo- and exopolysaccharides had absorption bands characteristic for deformative vibrations –C–C–, –C–O–, CH–, and OH– groups (Fig. 2).

The IR spectrum of the endopolysaccharides has an intensive absorption band of 1150 cm^{-1}. It was conditioned by the vibration of the connection –C–O– at the second carbon atom of ring C_2–O– (Fig. 2). The most intensive absorption band in this diapason is 1020 cm^{-1}, which shows up on uronic acid remains. The IR spectrum of the exopolysaccharides demonstrates the most pronounced absorption band of 1075–1074 cm^{-1} (the zone 1200–800 cm^{-1}), which is inherent in the biopolymers chitin and chitosan (Fig. 2).

The zone 800–900 cm^{-1} shows type and direction of connections and demonstrates the clear structural differences between endo- and exopolysaccharide. The absorption bands of 940–945 cm^{-1} and 850–860 cm^{-1} indicate the α-type connections, and 890–900 cm^{-1} indicate the presence of β connections in the endopolysaccharides. The exopolysaccharides were found to only have absorption band of 898–900 cm^{-1}, which is characteristic for the β-type connections.

Table 2. Content of Minerals in Mycelium and Fruiting Bodies of *Lentinus edodes**

Mineral	Mycelium	Fruiting bodies
Ca	750	110
Fe	65	30
Al	60	50
Mg	85.5	35
P	350	60
Na	270	90
K	600	150
S	130	65

* (mg/100 g)

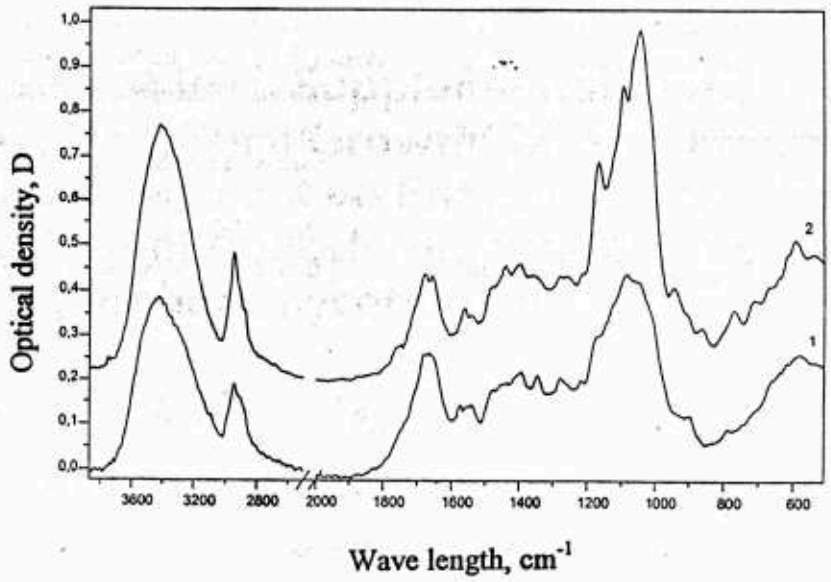

FIGURE 2. IR spectra of exo-(1) and endopolysaccharides (2) of *Lentinus edodes*.

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