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BIOLOGICALLY ACTIVE SUBSTANCES FROM *LENTINULA EDODES* AND *PLEUROTUS OSTREATUS*

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ABSTRACT

The chemical compositions of fruiting bodies and mycelia after submerged cultivation of fourteen strains of *Lentinula edodes* (Berk.)Sing. and one strain of *Pleurotus ostreatus* (Jacq.:Fr.)Kumm. were studied. Palmitoleic (16:1) and heptadecenoic (17:1) fatty acids appeared upon submerged culture of the mycelia in both investigated species. *L. edodes* and *P. ostreatus* mycelia had higher indices of essential amino acids and nutrients, than did their fruiting bodies. The culture liquid contained 3-5 g/L of exopolysaccharides after submerged culture of *L. edodes* and *P. ostreatus*. The main component of these exopolysaccharides was glucose. The molecular masses of *L. edodes* and *P. ostreatus* exopolysaccharides were 180-200 kD and 200-220 kD respectively.

INTRODUCTION

Lentinula edodes (Berk.)Sing. and *Pleurotus ostreatus* (Jacq. :Fr.) Kumm. are popular cultivated edible mushrooms with high nutritional value and excellent medicinal properties (Chang 1996, Wasser and Weis 1997). Numerous articles and monographs contain detailed information on the physiology, chemistry, pharmacology, and medicinal value of these species (Crisan and Sands 1978, Bisko and Dudka 1987, Solomko *et al.* 1987, Hobbs 1995, Wasser and Weis 1997). However, *L. edodes* and *P. ostreatus* strains exhibit high phenotypical variability in their physical parameters for growth and fruiting, in their sources of carbon and nitrogen and in other biological activities.

Besides polysaccharides, a number of low molecular weight organic substances such as terpenoids, steroids, and phenols inhibit cancer cell growth (Mizuno 1996, Wasser and Weis 1999). The study involved identifying biologically active substances from mycelia prepared in liquid culture and fruiting bodies of different strains of *L. edodes* and *P. ostreatus*.

MATERIALS AND METHODS

Fourteen strains of *L. edodes* (Berk.)Sing. and one strain of *P. ostreatus* (Jacq. :Fr.) Kumm. were obtained from the culture collection of the N.G. Kholodny Institute of Botany, Kiev, and the Institute of Microbiology of the Academy of Sciences of Byelorussia, Minsk.

The mycelia of these species and strains were grown in submerged conditions, on a glucose-peptone nutrient medium, (g/l): glucose - 10, peptone - 3, K_2HPO_4 - 1, KH_2PO_4 - 1, $MgSO_4 \bullet 7H_2O$ - 0.25, corn extract - 20 ml, deionized water - 1000 ml, pH 5.5.

After preparation, the medium was sterilised by autoclaving for 20 minutes at 121°C. Mycelia were grown in 5 l flasks using the submerged cultivation technique. Inoculation material was produced in 0.5 l flasks containing 0.05 l of cultivation medium and homogenised mycelia from petri dishes. The biomass was ready to harvest after seven days of cultivation at 28°C.

Mycelia of the *L. edodes* and *P. ostreatus* strains were separated from the medium by means of filtration. The mycelia was rinsed in distilled water, dried to a constant weight at 60°C, and macerated.

L. edodes fruiting bodies were grown on a mixture of oak sawdust with wheat bran (4:1) (Bisko and Bilay 1996), and *P. ostreatus* fruiting bodies were cultivated on wheat straw (Zadrazil 1978). The *L. edodes* and *P.ostreatus* fruiting bodies were harvested, dried at 60°C and macerated.

The biological substances in *L. edodes* and *P. ostreatus* mycelia and fruiting bodies were estimated as follows: amino acids, using aminoacid analyzer AAA-881 "Microtechna" (Krischenko 1983); lipids, using Zalashko's method (Zalashko *et a.* 1983); fatty acids, with a gas liquid chromatograph "Crom-5" with 15% polyethylene glycol succinate as liquid (Column temperature was 160°C, and evaporation temperature was 210°C) (Vereschagin *et al.* 1963, Keits 1975).

Endo- and exopolysaccharides were measured in the *L. edodes* and *P. ostreatus* mycelia, fruiting bodies and culturing liquid after submerged cultivation (Chorlin 1975, Grushenko *et al.* 1978).

RESULTS AND DISCUSSION

In the mycelia of *L. edodes* and *P. ostreatus*, unsaturated fatty acids predominate over saturated ones (Table 1). The content of linoleic acid is highest in the lipids of *L. edodes* fruiting bodies and in *P. ostreatus* mycelia and the fruiting bodies (Table 1). The same regulatory mechanism was determined for the lipid composition of other species of medicinal mushrooms (Solomko *et al.* 1984, Wasser and Weis 1997).

In contrast, oleic acid dominated the lipid composition of *L. edodes* mycelia (Table 1). *L. edodes* fruiting bodies and mycelia differed from *P. ostreatus* because of the presence of myristic acid. Palmitoleic and heptadecenoic acids appeared in *P. ostreatus* and *L. edodes* mycelia during submerged culturing (Table 1).

Submerged culture led to a decrease in the level of palmitic and linoleic acids in *P. ostreatus*, and to a decrease in the content of linoleic and linolenic acids, and to a considerable increase of oleic acid in *L. edodes* (Table 1).

Studies of amino acids in *L. edodes* and *P. ostreatus* mycelia and fruiting bodies showed qualitatively identical composition in both species (Table 2). All essential amino acids were present in the proteins of the studied strains (Table 2).

The difference in the content of individual amino acids between *L. edodes* mycelia and fruiting bodies, was greater than for *P. ostreatus* mycelia and fruiting bodies. For example, the quantity of glutamic acid in *L. edodes* fruiting bodies was approximately three times higher than in the mycelia, while the content of arginine and proline in the mycelia was approximately two times higher than in fruiting bodies. Interestingly, the proteins of the mycelia of the studied species contained a higher content of total essential amino acids than those of the fruiting bodies (Table 2).

Leucine and isoleucine, are the main sulphur-containing amino acids, which decreased the biological value of proteins in *L. edodes* and *P. ostreatus* mycelia and fruiting bodies. The proteins of the investigated species were also rich in the content of aromatic amino acids - phenylalanine and threonine (Table 2).

As for other species of edible mushrooms, *L. edodes* and *P. ostreatus* proteins differed from plant proteins, having a higher content of lysine and threonine (Crisan and Sands 1978).

The essential amino acid index of *L. edodes* and *P. ostreatus* mycelia and fruiting bodies was higher than that of a F.A.O. [Food and Agriculture Organization] reference protein. The nutritional index of *L. edodes* and *P. ostreatus* mycelia was approximately equal to the nutritional index of soya, and that of *L. edodes* and *P. ostreatus* fruiting bodies, to the index of beans.

After submerged cultivation of *L. edodes* and *P. ostreatus*, the culturing liquid contained 3-5g/ll exopolysaccharides. The main component of these exopolysaccharides was glucose (Table 3). Interestingly, the exopolysaccharides of *L. edodes* strains were considerably different in mannose content from its endopolysaccharides. Arabinose is absent in endo- and exopolysaccharides of *L. edodes* and *P. ostreatus* mycelia but was present in endo and exopolysaccharides of *L. edodes* fruiting bodies. The obtained results indicate that the higher content of galactose was typical for endopolysaccharides of *L. edodes* mycelia as compared to exopolysaccharides ones. The molecular masses of exopolysaccharides in *L. edodes* and *P. ostreatus* – were 180-200 kD, and 200-220 kD respectively.

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Table 1. The content of fatty acids in the lipids of *Lentinula edodes* and *Pleurotus ostreatus* (P.o.) strains (% of total lipids)

Strain	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Saturated fatty acids	Unsaturated fatty acids	Ratio of unsaturated fatty acids to saturated fatty acids
101 m	0.90	1.38	18.99	2.05	0.74	0.49	3.27	8.98	61.12	2.08	25.28	74.72	1.40
102 m	0.87	0.96	20.90	2.55	0.78	0.36	3.40	7.18	61.83	1.17	26.91	73.09	1.37
103 m	0.72	0.95	19.22	1.19	0.66	0.47	3.82	9.95	61.43	1.59	25.37	74.63	1.39
104 m	0.44	0.73	17.97	1.40	0.60	0.37	2.54	4.76	70.02	1.17	22.28	77.72	1.50
105 m	0.70	0.97	22.23	1.72	0.89	0.57	3.13	6.04	62.25	1.50	27.92	72.08	1.37
107 m	0.56	1.02	20.13	1.50	0.63	0.88	1.69	4.51	67.58	1.50	24.03	75.97	1.46
108 m	0.37	0.56	21.42	1.24	0.84	0.56	1.37	13.66	59.88	0.10	24.56	75.44	1.35
109 m	0.76	1.36	20.42	1.61	0.45	1.11	2.52	7.69	62.66	1.42	25.51	74.49	1.39
180 m	0.53	1.13	19.89	0.67	0.53	0.39	2.27	3.83	69.96	0.80	24.35	75.65	1.47
181 m	0.52	0.57	20.48	1.15	0.34	0.34	2.29	6.45	67.06	0.80	24.20	75.80	1.44
182 m	0.73	1.58	27.22	0.85	0.45	0.45	2.30	3.79	67.42	0.90	26.59	73.41	1.43
192 m	0.92	2.12	22.08	0.82	0.54	0.74	4.00	5.66	62.47	0.64	29.67	70.33	1.34
356 m	0.74	1.40	19.69	1.86	0.56	0.66	3.60	8.94	60.76	1.79	25.99	74.01	1.38
185 m	1.66	3.00	28.04	1.24	-	3.41	5.35	41.96	15.67	0.49	37.23	62.77	1.69
185 f	1.17	1.40	28.20	-	-	-	7.47	5.97	54.59	1.36	38.08	61.92	1.63
P.o.470 m	-	3.48	10.38	1.26	1.16	4.90	6.07	30.40	39.23	3.12	39.08	60.91	1.24
P.o.470 f	-	3.39	20.00	-	0.88	-	6.65	26.30	37.48	5.30	30.92	69.08	1.17

m – mycelia
f – fruiting body
"-" – is absent

Table 2. The content of amino acids in proteins of *Lentinula edodes* (L.o.) and *Pleurotus ostreatus* (P.o.) strains (% of total amino acids)

Constituent	L.e. strain 101	L.e. strain 182	L.e. strain 192	L. e. strain 185		P. o. strain 470	
	Mycelia	Mycelia	Mycelia	Mycelia	Fruiting bodies	Mycelia	Fruiting bodies
Isoleucine	3.6	3.4	5.8	3.6	3.5	3.2	3.1
Leucine	7.1	8.0	10.5	6.2	6.5	5.5	5.3
Lysine	2.4	2.9	3.5	7.2	6.2	9.8	9.6
Methionine	3.3	1.6	1.6	1.7	1.1	1.2	1.4
Argenine	5.8	6.9	6.5	5.7	3.1	4.6	4.2
Cystine	2.8	0.7	0.5	0.1	0.2	1.8	1.2
Histidine	4.2	1.4	1.1	2.0	1.2	7.6	5.7
Phenylalanine	5.1	2.4	2.0	1.9	2.6	3.3	3.1
Threonine	6.0	6.8	6.1	6.5	6.0	6.6	6.9
Tyrosine	2.5	1.9	5.6	4.3	3.9	2.6	1.6
Valine	9.6	7.1	6.1	6.5	5.9	7.1	6.6
Alanine	6.5	6.8	6.3	6.8	5.8	6.1	6.0
Aspartic acid	9.8	14.9	12.1	13.2	9.2	5.4	8.7
Glutamic acid	11.0	10.8	10.6	10.9	31.4	15.2	14.1
Glycine	4.7	8.4	7.0	7.3	6.5	6.5	6.6
Proline	7.7	7.2	7.9	9.6	4.8	7.7	9.8
Serine	7.9	8.8	6.8	6.5	2.1	5.8	6.1

Table 3. The content of carbohydrates in polysaccharides of *Lentinula edodes* (L.e.) and *Pleurotus ostreatus* (P.o.) strains (% of total)

Carbo-hydrate	L.e. strain 101			L.e. strain 182			L.e. strain 185			L.e. strain 192			P.o. strain 470
	Mycelia		Fruiting bodies	Mycelia		Fruiting bodies	Mycelia		Fruiting bodies	Mycelia		Fruiting bodies	Mycelia
	Endo-poly-sacch arides	Exo poly-sacch arides		Endo-poly-sacch arides	Exo Poly-sacch arides		Endo-poly-saccha rides	Exo poly-saccha rides		Endo-poly-saccha rides	Exo poly-saccha rides		Exopoly-saccharides
Arabinose	-	-	0.10	-	-	0.10	-	-	2.40	-	-	0.20	-
Xylose	1.02	1.30	0.20	0.15	0.25	-	2.15	1.30	0.15	2.00	1.50	-	-
Mannose	8.16	-	5.50	10.10	16.40	4.68	10.65	0.20	6.72	10.60	14.10	6.20	2.18
Galactose	8.38	0.10	6.80	16.42	0.35	4.82	12.69	0.20	6.64	10.80	0.20	5.60	0.12
Glucose	82.44	98.60	87.40	73.33	83.00	90.40	74.51	98.30	84.09	76.60	84.20	88.00	97.70

"-" – is absent