BIOTECHNOLOGICAL ASPECTS OF MEDICINAL MUSHROOM *GANODERMA LUCIDUM (*CURT.:FR.) P.KARST. SUBMERGED CULTIVATION

N. A. BISKO[1], *V.G. BABITSKAYA*[2],
V.V. SCHERBA[2], *N.YU. MITROPOLSKAYA*[1]
[1]M.G.Kholodny Institute of Botany, National Academy of Sciences of Ukraine,
Tereschenkivska 2, Kiev, 01601- GSP, Ukraine
[2]Institute of Microbiology, National Academy of Sciences of Byelorussia, Minsk, Byelorussia

Ganoderma lucidum (Curt.:Fr.) P.Karst. is one of the most popular medicinal mushrooms [15]. It is known that *G. lucidum* has antioxidant and antitumor activity, sedative effect on the central nervous system, improves cardiocerebral circulation, stimulates the immunity and etc. [6, 9, 11, 12, 17]. The study of the biomass and biologically active substances production from *G. lucidum* strains on different nutrient media and sources of carbon was the aim of this work.

Materials and methods

The studied strains of *Ganoderma lucidum* was obtained from the culture collection of the Institute of Microbiology of the National Academy of Sciences of Byelorussia, Minsk (N1) and the culture collection of the N.G.Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kiev (333, 362, 357, 358).

Mycelium of these strains was grown in submerged conditions on glucose-peptone nutrient medium, (g/l): glucose - 10, peptone - 3, K_2HPO_4 - 1, KH_2PO_4 - 1, $MgSO_4 \cdot 7H_2O$ - 0.25, corn extract - 20 ml, deionized water - 1000 ml, pH 5.5 and beer wort medium (7°C). Mycelia were grown in 5l flasks with using a submerged cultivation technique. Inoculation material was produced in 0.5l flasks containing 0.05 l of cultivation medium and homogenized mycelia from Petri dishes. Effects of carbon sources on the synthesis of biomass and exopolysaccharides were studied on glucose-peptone nutrient medium in which glucose was replaced by arabinose, xylose, fructose, galactose, mannose, lactose, maltose, saccharose, mannit, sorbit, cellulose, starch. The biomass is ready to harvest after 5 days of cultivation at 28°C. Mycelium was separated from medium by filtration and washed with distilled water. The mycelium was homogenized, mixed with distilled water (1:5) and boiled on water bath during 12-18 hours. These extracts were centrifugated (3.000 rotations/min.) for 15 min. The supernatant sedimented with ethanol (1:1) (the temperature is 4°C) and sediment (endopolysaccharides) was separated by centrifugation [2, 5]. Exopolysaccharides were determined in the cultural liquid [1]. Exopolysaccharides were determined in glucose-peptone and beer wort media without mushroom mycelia also. Exopolysaccharides were absent in glucose-peptone medium. Traces of exopolysaccharides were determined in beer wort medium. In mycelia of studied strains the content of protein was estimated according to Lowry's method [10], amino acids – on amino acid analyzer AAA-881 "Microtechna" [8]. Lipids were extracted by Folch's method [4], fatty acids were estimated on chromatograph "Chrom-5" with 15% polyethylenglycol succinate as liquid (temperature of column is 160°C, temperature of evaporation - 210°C) [7, 14]. Polyphenols were determined with reactive Tolin-Denis [16].

Results and discussion

The data obtained in our work indicate, that the biomass of all investigated strains of *G. lucidum* was greater on beer wort medium than on glucose-peptone medium (Table 1). Similar regulatory was characterizied for the content of endo- and exopolysaccharides of all strains too. Maximal biomass and the content of endopolysaccharides were accumulated by strains 357, 333 and 1 on beer wort medium. At the same time the content of exopolysaccharides was maximal for the strains 357, 333 and 362 (Table 1). It was demonstrated, that the cultivation of the all studied strains, with the exception of strain 358, on the glucose-peptone medium resulted in the reduction of nutrient medium pH. It should be noted that on the beer wort medium the value of final pH after cultivation of *G.lucidum* strains was lower than on the glucose-peptone medium (Table 1). However

the value of final pH after the cultivation of strain 358 was highest in case of the cultivation as on glucose-peptone medium as beer wort medium.

Table 1

The production of biomass and polysaccharides of *G. lucidum* strains

Strain	The final pH		Biomass, g/l		Endopoly-saccharides, %		Exopolysaccharides, g/l	
	Beer wort medium	Glucose - peptone medium	Beer wort medium	Glucose - peptone medium	Beer wort medium	Glucose-peptone medium	Beer wort medium	Glucose-peptone medium
1	4.3	5.0	10.0	9.5	12.0	8.5	3.0	2.5
333	3.8	4.5	11.5	9.0	8.0	7.0	8.0	4.5
362	3.9	4.5	8.0	9.0	6.8	5.0	7.0	4.0
358	5.0	5.5	6.5	8.0	6.4	4.0	3.5	3.0
357	3.6	4.0	12.5	8.5	9.0	8.3	8.0	5.5

It was demonstrated that the beer wort medium more promoted biosynthesis of lipids in the mycelia of all investigated strains of *G. lucidum* as compared with the glucose-peptone medium (Table 2). The differences between the studied strains on investigated media in the content of protein were not considerable.

The results obtained indicate that the mycelia of different strains *G. lucidum* had not high content of polyphenols (Table 2) as compared with the mycelia of *Pleurotus ostreatus* or *Lentinus edodes* strains which were cultivated in identical conditions [13]. We did not observed the relation between the content of polyphenols in mycelia of *G. lucidum* strains and the composition of used nutrient media (Table 2).

Table 2

The chemical composition of the mycelia of *G. lucidum* strains

Strain	Lipids, % a.d.m.		Protein, % a.d.m.		Polyphenols, mg %	
	Beer wort medium	Glucose-peptone medium	Beer wort medium	Glucose-peptone medium	Beer wort medium	Glucose-peptone medium
1	9.3	7.0	22.2	23.5	510	470
333	8.5	7.0	22.5	23.0	750	690
357	8.0	7.0	22.5	24.0	730	700
358	7.0	6.5	19.0	21.0	540	560
362	6.5	5.7	18.5	20.5	700	650

The content of amino acids and fatty acids were determinate in the mycelia of the strains 1, 333 and 357, which produced greatest biomass. Studies of amino the strains 1, 333 and 357, which produced greatest biomass. Studies of amino acids in *G. lucidum* mycelia showed small differences between strains in qualitative composition of individual constituents (Table 3). All essential amino acids were presented in the proteins of studied strains. It is interesting to note that the proteins of mycelia of *G. lucidum* strains contained a high content of lysine and threonine (Table 3). It is typical for other species of Higher Basidiomycetes [3].

Table 3

The content of amino acids in the protein of mycelia of *G .lucidum* strains (% of protein) on beer wort medium

Constituent	Strains		
	1	333	357
Lysine	6.8	7.0	7.5
Histidine	1.8	1.6	1.6
Argenine	5.6	5.0	4.5
Aspartic acid	8.9	9.3	10.0
Threonine	4.6	4.5	3.7
Serine	5.6	5.7	5.0
Glutamic acid	17.5	18.0	18.7
Proline	4.6	4.1	4.4
Glycine	5.8	5.3	5.0
Alanine	8.7	9.5	8.7
Cystine	2.4	2.0	2.7
Valine	4.8	5.2	5.0
Methionine	1.4	3.4	3.6
Isoleucine	3.2	3.2	4.0
Leucine	10.0	8.0	7.8
Tyrosine	3.2	3.0	3.5
Phenylalanine	4.7	4.9	4.4

It is found that in lipids of mycelia of investigated strains the unsaturated fatty acids predominated over the saturated ones as on beer wort medium as glucose-peptone medium (Table 4). Linoleic acids dominates in the lipid composition of *G. lucidum* mycelia apart from the composition of nutrient media and biological peculiarities of strains. The same regulatories was determined for the lipid composition of many species of medicinal mushrooms [3].

Table 4

The content of fatty acids in the mycelia of *G.lucidum* strains (% of total lipids)

	Strain 1		Strain 333		Strain 357	
	Beer wort medium	Glucose-peptone medium	Beer wort medium	Glucose-peptone medium	Beer wort medium	Glucose-peptone medium
$C_{14:0}$	0.2	0.91	0.15	0.56	0.44	1.56
$C_{15:0}$	0.71	2.15	0.76	1.02	1.70	1.02
$C_{16:0}$	18.40	28.88	18.00	20.13	17.00	29.13
$C_{16:1}$	0.64	0.99	1.04	1.50	1.35	1.50
$C_{17:0}$	trace	1.65	trace	0.63	0.30	0.60
$C_{17:1}$	trace	1.97	trace	0.88	0.42	1.72
$C_{18:0}$	0.45	1.07	0.35	1.69	2.84	0.76
$C_{18:1}$	8.99	4.40	6.09	4.51	4.70	4.63
$C_{18:2}$	70.61	57.98	73.40	67.58	70.08	67.60
$C_{18:3}$	trace	trace	0.21	1.51	1.17	1.48
Saturated fatty acids	19.36	34.66	19.26	24.03	22.28	23.07
Unsaturated fatty acids	80.24	65.34	80.74	75.97	77.72	76.93
Ratio unsaturated fatty acids to saturated fatty acids	1.54	1.17	1.54	1.47	1.50	1.48

The cultivation of *G .lucidum* strains on glucose-peptone medium leads to the increase in the level of saturated fatty acids in comparison with the beer wort medium (Table 4). Difference in content of individual fatty acids between strains on the same medium were not considerable.

It was investigated that the glucose and the xylose are the best sources among the monosaccharides, lactose and maltose – among disaccharides for the production of biomass and endopolysaccharides. The content biomass of different strains on the medium, containing glucose was 8.5-10 g/l, endopolysaccharides – 8.0-8.5%, on the medium with xylose – 8.5-9.0 g/l and 8.0-9.5% accordingly. The accumulation of biomass on the medium with lactose was 10-11 g/l, endopolysaccharides – 7.5-9.5%. The investigated strains produced 7-8 g/l biomass and 7-8.7% endopolysaccharides on medium, containing maltose. It was demonstrated that the polysaccharides are the good sources for the biosynthesis of biomass and endopolysaccharides of *G.lucidum* strains too. So, the content of the biomass on the medium with starch was 10-12 g/l, endopolysaccharides – 7-8%, on the medium, containing cellulose – 9-9.8 g/l and 6-7% accordingly.

References

1. Babitskaya V.G., Scherba V.V., Mitropolskaya N.Yu., Bisko N.A. Exopolysaccharides of some medicinal mushrooms: production and composition // Intl. J. Med. Mushrooms. – 2000. – Vol. 2, N 1. – P. 51-54.

2. Chihara G., Hamuro J., Maeda Y.Y., Arai, Fucuoka F. Fractionation and purification of polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) Sing. (an edible mushrooms) // Cancer Res. – 1970. – Vol. 30. – P. 2776-2781.

3. Crisan E.V., Sands A. Nutritional value // The biology and cultivation of edible mushrooms / Eds. Chang S.T. and Hays W.A. – Orlando, FL: Academic Press, 1978. – P. 137-165.

4. Folich I., Lees M., Sloan-Staulet G.H.S. A simple method for isolation and purification of total lipids from animal tissues // J. Biol. Chem. – 1957. – Vol. 266, N 1. – P. 491-509.

5. Goncharova I.A., Scherba V.V., Babitskaya V.G. The polysaccharides of the cell wall of *Coriolus hirsutus* // Appl. Biochem. Mikrobiol. – 1996. – Vol. 32, N 4. – P. 434-437.

6. Jones S., Sanardhanan K.K. Antioxidant and antitumor activity of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. – Reishi *(Aphyllophoromycetideae)* from South India // Intl. J. Med. Mushrooms. – 2000. – Vol. 2, N 2. – P. 195-200.

7. Keits M. Methods of lipid analysis. – Moscow: Mir, 1975. – 322 p.

8. Krischenko V.P. Methods of the estimation of plant production quality. – Moscow: Kolos, 1983. – 112 p.

9. Liu G.T. Recent advances in research of Pharmacology and clinical applications of *Ganoderma* P.Karst species *(Aphyllophoromycetideae)* in China // Intl. J. Med. Mushrooms. – 1999. – Vol. 1, N 1. – P. 63-67.

10. Lowry O.M., Kosenbrough N.J., Farr A.L., Randall R.J. Protein measurement with the Folin phenol reagent // J. Biol. Chem. – 1951. – N 193. – P. 265-277.

11. Ooi V.E.C., Liu F. A review of pharmacological activities of mushroom polysaccharides // Intl. J. Med. Mushrooms. – 1999. – Vol. 1, N 4. – P. 195-206.

12. Reshetnikov S.V., Wasser S.P., Tan K.K. Higher basidiomycetes as a source of antitumor and immunostimulating polysaccharides (Review) // Intl. J. Med. Mushrooms. – 2001. – Vol. 3, N 4. – P. 361-394.

13. Scherba V. V., Babitskaya V. G., Truchonovec V. V., Fomina V. I., Bisko N.A., Mitropolskaja N.Yu. The Influence of the Cultivation Conditions on the Chemical Composition of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Lentinus edodes* (Berk.) Sing. // Intl. J. Med. Mushrooms. – 1999. – Vol. 1, N 2. – P. 181-185.

14. Vereschagin A.G., Scvorcov S.V., Ishakov N.I. The composition of threeglycerids of cotton oil // Biochemistry. – 1963. – N 5. – P. 868-878.

15. Wasser S.P., Nevo E., Sokolov D., Reshetnikov S., Timor-Tismenetsky M. Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations // Intl. J. Med. Mushrooms. – 2000. – Vol. 2, N 1. – P. 1-19.

16. Zaprometov M.N. The phenolic components of plants: Biosynthesis, transformation, function. – Moscow: Nauka, 1985. – 143 p.

17. Zhow S., Gao Y. The immunomodulating effects of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Ling Zhi, Reishi moshroom) (*Aphyllophoromycetideae*) // Intl. J. Med. Mushrooms. – 2002. – Vol. 4, N 1. – P. 1-12.

Biotechnological aspects of medicinal mushroom *Ganoderma lucidum* (Curt.:Fr.) P. Karst. submerged cultivation

Bisko N. A., Babitskaya V.G., Scherba V.V., Mitropolskaya N.Yu.

The production of biomass, endo- and exopolysaccharides, lipids, protein, polyphenols, amino and fatty acids of *Ganoderma lucidum* strains on the different nutrient media in submerged conditions was investigated. It was demonstrated, that the content of biomass, endo- and exopolysaccharides was higher on beer wort medium as compared with glucose-peptone medium. Lactose, maltose, glucose, xylose are the best carbon sources for the biosynthesis of biomass and endopolysaccharides of *G. lucidum* strains.