ACTA MYCOLOGICA
Vol. 47 (1): 57–64
2012

Analysis of indole compounds in fruiting bodies and in mycelia from *in vitro* cultures of *Calocera viscosa* (Basidiomycota)

BOŻENA MUSZYŃSKA and KATARZYNA SUŁKOWSKA-ZIAJA

Chair and Department of Pharmaceutical Botany, Collegium Medicum, Jagiellonian University
Medyczna 9, PL-30-688 Kraków, muchon@poczta.fm

Muszyńska B., Sułkowska-Ziaja K.: *Analysis of indole compounds in fruiting bodies and in mycelia from in vitro cultures of Calocera viscosa (Basidiomycota)*. Acta Mycol. 47 (1): 57–64, 2012.

Calocera viscosa (Pers.: Fr.) Fr. (Basidiomycota) from *Dacrymycetaceae* family is a widespread species of mushroom in Poland. The aim of this study was to investigate the content of indole compounds in fruiting bodies and in mycelium cultured *in vitro* on solid and liquid medium of this species.

Fruiting bodies of *Calocera viscosa* were collected in coniferous forests in south Poland and were used to derive *in vitro* cultures. The optimal medium composition for cultures was determined. Fresh material: fruiting bodies and mycelium from culture *in vitro* was frozen and then dried by lyophilization. The crushed dry biomass was extracted with petroleum ether to remove oil fraction, material was dried and extracted with methanol. Analysis of indole compounds was performed in methanol extracts using chromatographic methods: TLC, UV Vis, EIMS and HPLC. This analysis presented in all three extracts the following indole compounds: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, melatonin and indole (contents fluctuated in the range: 0.37 to 11.88 mg/100 g d.w.). 5-hydroxytryptophan contents in all extracts were significant and amounted to 11.88 mg/100 g d.w. in fruiting bodies, and 11.42 in mycelium from liquid cultures and and 10.59 in mycelium from solid cultures. In addition, the fruiting bodies and mycelium from cultures on liquid medium revealed the presence of serotonin (0.39 and 3.19 mg/100 g d.w. respectively).

Key words: β,β-carotene, 5-hydroxytryptophan, mushroom, mycelial culture, serotonin

INTRODUCTION

Since the beginning of human evolution fruiting bodies of mushrooms belonging to the taxon Basidiomycota are appreciated for flavor and texture but especially for their chemical and dietary properties. Mushrooms are a rich source of a variety of biologically active compounds belonging to both primary and secondary metabolites

(Yang et al. 2001; Wasser 2002; Muszyńska et al. 2010). More than 140,000 species of mushrooms exist in natural sites, but less than twenty five species are widely accepted as food (Barros et al. 2007). The mushroom glucans are well known for their immunomodulatory properties and are used for anticancer therapy (Sułkowska-Ziaja et al. 2005; Zaidman et al. 2005; Muszyńska et al. 2011a). Antitumor and cytostatic properties presented numerous terpenoids, especially sesquiterpenes and triterpenoids. A number of recent mycochemical papers have reported on the presence of many different terpene compounds (Barros et al. 2008; Liu 2005).

The antioxidants found in edible mushrooms were flawonoids, phenolic compounds, ascorbic acid, tocopherols and carotenoids. Recently, Muszyńska (Muszyńska et al. 2007; Muszyńska et al. 2009; Muszyńska et al. 2011b) have hinted at the occurrence of numerous non-hallucinogenic indole compounds in edible, conditionally edible and inedible Basidiomycota species. *Calocera viscosa* (Pers.:Fr.) Fr. (Yellow Stagshorn) is a species common in Polish, European and Asiatic coniferous forests, which develops intensely yellow-orange branched, bush-like fruiting bodies. This species occurs from June to November on dead conifer wood in mixed and coniferous forests, mainly in mossy spruce and pine stumpsas saprotrophs. *C. viscosa* is a good source of free exo- and endogenous amino acids, indole compounds, sterols (especially ergosterol) and unsaturated fatty acids (Muszyńska 1999). Although the carotenoids content in *Calocera viscosa* fruiting bodies growing in Poland were estimated spectrophotometrically at 2.46 μg/g f.w. by Czeczuga (1980) at the end of the seventies years of the 20th century. Barros (2008) demonstrated that of six Basidiomycota species collected from natural sites in the northwestern Portugal, *Cantharellus cibarius* (Chantarelle) contained the greatest amounts of β,β-carotene (13.56 μg/g d.w.) while the contents of this metabolite in the remaining species ranged from 1.95 - 12.77 μg/g d.w.

The contents of this very important antioxidant were analyzed in extracts from fruiting bodies of *C. viscosa* and in extracts from mycelium of *in vitro* cultures (Muszyńska et al. 2012). These contents are higher than those determined by Czeczuga (1980). The cultures were maintained under different conditions to optimize biomass growth and to establish the most beneficial conditions for β,β-carotene accumulation. β,β-Carotene content in biomass from solid cultures was comparable with that found in fruiting bodies (7.1 and 7.5 μg/g d.w., respectively). Mycelia from liquid cultures contained half of that β,β-carotene amount which equaled 3.5 μ/g d.w. Polysaccharides extracted from the mycelial culture of *C.viscosa* and administrated intraperitoneally into mice at dosage of 300 mg/kg inhibited the growth of Sarcoma 180 and Erlich solid cancers by 90% (Ohtsuka et al. 1973). The aim of the present study was to initiate *Calocera viscosa* culture *in vitro*, to determine optimal conditions for mycelia growth and to evaluate the indole compounds contents in extracts of fruiting bodies and mycelia from *Calocera viscosa in vitro* cultures. Indole compounds have attracted much interest recently because *in vitro* and *in vivo* studies suggest that they have a variety biological properties, which play important functions in the maintenance of human health. These compounds are antioxidants, antidepressants, anticancer, being tissue hormones and neurotransmitters. Serotonin is a long known compound playing the role of a regulator of sleep, body temperature, mood, maturation and regeneration and an inhibitor of cell aging, thereby contributing to general strengthening of the immune system. Its daily dose when used, for instance, as an antidepressant drug ranges from 100 to 200 mg (Mosovich

et al. 2008). Recent reports have revealed further important biological aspects of serotonin action, including its usefulness in prevention of Alzheimer's disease and the antioxidant action (Ouchi et al. 2009). Indole compounds and their derivatives play also important role as analgesic and anti-inflammatory medicines.

MATERIALS AND METHODS

Origin of fruiting bodies. The studies were conducted on fruiting bodies of *Calocera viscosa* (Pers.: Fr.) Fr. collected at natural sites in mixed and coniferous forests in southern Poland (Brodła near Kraków) (deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Kraków, Poland).

Initial culture. Initial cultures were derived from explants originating from the top parts of branched, bushlike fruiting bodies of *Calocera viscosa*. These pieces of fruiting bodies were sterilized with 70% ethyl alcohol and placed on Petri dishes with solid Oddoux medium (Oddoux 1957). Cultures were incubated at a temperature 25 +/- 2˚C under 12-h light (900 lx)/12 dark cycle and were subcultured every second week. For more details see Muszyńska (Muszyńska et al. 2009).

Experimental *in vitro* cultures. Solid culture was maintained on Petri dishes, on Oddoux medium (Oddoux 1957).

Stationary liquid culture was established from the solid culture by transferring 0.1 g of mycelium into an Erlenmayer flask (500 mL) containing 250 mL of liquid Oddoux medium. Both types of experimental cultures were maintained under the same conditions as initial culture and were subcultured every two weeks.

Extraction. Lyophilized and powdered fruiting bodies and mycelia from *Calocera viscosa in vitro* cultures on solid and liquid media were extracted in a percolator with petroleum ether to remove oil fraction according to the procedure developed in our laboratory (Muszyńska et al. 2007). After this extraction, biomass was dried and extracted by methanol in percolator for 24 h. Extracts were concentrated to 5 mL by distillation in a vacuum evaporator under reduced pressure. For the purification of the extracts we used TLC method on aluminum – baked silica 60 plates (Merck, Art. No 1.05554.0001), on which 1 mL of the extracts was loaded and chromatograms were developed in mobile faze: n-butanol / acetic acid / water (12 : 3: 5 v/v/v). Spots were identified at λ=254 nm. Obtained fractions were analyzed by UV-Vis, EIMS and HPLC method.

UV VIS analysis of indole compounds. Purified by preparative TLC method extracts of fruiting bodies and mycelia from cultures *in vitro* on solid and liquid medium were analyzed for the presence of indole compounds by spectrophotometry using UV-Vis apart: UV-Vis Cary - Varian Spectrophotometer. Absorption measurements were carried out in the λ = 200-500 nm; solvent: methanol AR; standards: indole compounds - manufactured by Sigma-Aldrich. In all three extracts was found an increase of the growth of an absorption maximum (for example at λ_{max} = 227 and λ_{max} = 254) characteristic for indole compounds.

EIMS analysis of indole compounds. Electron Impact Mass Spectrometry Analysis (EIMS) was performed at the Regional Laboratory of Physicochemical Analyses and Structural Research. Apparatus: High Resolution Mass Spectrometer with

options: EI, ESI, GC-M, Finnigan MAT 95S. In the results of these studies were obtained the spectra for the methanol extracts from the fruiting bodies and mycelia from cultures on solid and liquid medium. The EIMS spectra for the methanol extracts from the fruiting bodies and mycelia from *in vitro* cultures on solid and liquid medium containing peaks characteristic for indole compounds (m/e=115, m/e=129, m/e=130, m/e=135, m/e=143, m/e=157).

Estimation of indole compounds by the HPLC method. Contents of indole compounds in extracts from fruiting bodies and in mycelia maintained in *in vitro* cultures on solid and liquid medium were determined after preliminary separation with preparative TLC method. Contents of L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, serotonin, melatonin, tryptamine, kynurenic acid, kynurenine sulfate, indoleacetic acid, β-indoleacetonitrile, indole and kynurenine were determined according to the procedure developed by Kysilka and Wurst (1985) with our modifications (Muszyńska et al. 2009). Briefly, the analytical conditions were as follows: HPLC apparatus: Hitachi; pump: L-7100; column: Purospher® RP-18 (4 x 200 mm, 5 μm) thermostated at 25°C. The solvent system used were: methanol/water/ammonium acetate (15:14:1 v/v/v); flow rate:1ml/min. Detection was carried out in a UV detector, using λ=280 nm; standards: manufactured by Sigma-Aldrich. Standards solutions were prepared in HPLC grade methanol.

The identification of the indole compounds was made by comparing the retention times of samples peaks with standards. The results are expressed in mg/100 g of dry weight, calculated by internal normalization of the of the chromatographic peak area.

RESULTS

We established that good mycelial mass growth of *Calocera viscosa* could be obtained in agitating liquid cultures and solid cultures on modified Oddoux (1957) medium at 25 +/- 2°C under 16 h photoperiod (900 lx/8 h dark). A 20-fold fresh biomass growth in cultures on solid medium and a 15-fold growth in liquid cultures were obtained within a 14-day growth cycle. The biomass growth in the initiated cultures averaged 8.3 g d.w./1 L of medium. The obtained biomass increments and dynamics of mycelium growth did not differ from the results that we obtained for *Sarcodon imbricatus* L. (Sułkowska-Ziaja 2010), *Xerocomus badius* (Fr.) Kühn. ex Gilb., *Tricholoma equestre* (L.: Fr.) Kumm. (Muszyńska et al. 2009) and *Cantharellus cibarius* Fr. cultures studied earlier. The *in vitro* cultures of these species were used for evaluation of qualitative and quantitative composition of non-hallucinogenic indole compounds and proved to be a valuable model for investigation of their metabolism. The present study is an extension of the previous studies on accumulation of indole compounds and is the first report about their presence in: fruiting bodies and in mycelia of *Calocera viscosa* cultured *in vitro.*

The identity of indole compounds was confirmed on the basis of their parameters TLC, UV-Vis, EIMS and HPLC methods. The HPLC method was used for quantitation of indole compounds and optimum conditions were established by this method for separation: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan,

Table 1
Contents of indole compounds (mg/100 g d.w.) in fruiting bodies of *Calocera viscosa* and in mycelia from cultures *in vitro*

	Fruiting bodies of *C.viscosa*	Mycelium of *C.viscosa* from solid medium	Mycelium of *C. viscosa* from liquid medium
Inndole compounds		mg/ 100 g d.w.	
melatonin	0.47 ± 0.02	0.39 ± 0.01	0.37 ± 0.01
L-tryptophan	1.26 ± 0.04	1.19 ± 0.01	1.79 ± 0.05
5-hydroxytryptophan	11.88 ± 0.19	10.59 ± 0.11	11.42 ± 0.20
5-methyltryptophan	3.39 ± 0.02	3.34 ± 0.03	3.36 ± 0.02
serotonin	0.39 ± 0.01	– [a]	3.19 ± 0.07
indole	1.26 ± 0.04	1.21 ± 0.02	1.25 ± 0.03

Data presented as mean of three series ± SE; a - content lower than 0.001 mg/100 g d. w.

serotonin, melatonin, tryptamine, kynurenic acid, kynurenine sulfate, indoleacetic acid, β-indoleacetonitrile, indole and kynurenine in extracts from fruiting bodies and in mycelia from *in vitro* cultures on solid and liquid medium. This analysis presented in all three extracts the following five indole compounds: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, melatonin and indole. In addition, the fruiting bodies and mycelium from cultures on liquid medium revealed the presence of serotonin (0.39 and 3.19 mg/100 g d.w. respectively). Results are presented in Table 1.

Contents of indole compounds in fruiting bodies ranged from 0.39 to 11.88 mg/100 g d.w., in mycelium from liquid cultures from 0.37 to 11.42 mg/100 g d.w., and in mycelium from solid cultures from 0.39 to 10.59 mg/100 g d.w. 5-hydroxytrypophan contents in all extracts were significant and amounted 11.88 mg/100 g d.w. in fruiting bodies, and 11.42 in mycelium from liquid cultures and 10.59 in mycelium from solid cultures. The second metabolites because of their amounts was 5-methyltryptophan and presented: 3.39 mg/100 g d.w. in fruiting bodies, 3.34 in mycelium from solid medium and 3.36 in mycelium from liquid medium. The contents of L-tryptophan and indole were very comparable and ranged from 1.19 mg/100 g d.w. to 1.79. Melatonin was found in comparable but small amounts in all three extracts: 0.47 mg/100 g d.w. in fruiting bodies, 0.39 in mycelium from solid medium and 0.37 in mycelium from liquid medium. In none of the extracts: tryptamine, kynurenic acid, kynurenine sulfate, kynurenine, indoleacetic acid, β-indoleacetonitrile and tryptamine were found.

DISCUSSION

Among indole compounds, indole alkaloids and hallucinogenic compounds originating from mushrooms have been the main focus of interest. Studies of nonhallucinogenic indole compounds have concentrated on tryptophan, among other compounds. Tryptophan was identified in many Basidiomycota species (Kohlmünzer et al. 2000). Tryptamine, serotonin, 5-hydroxytryptophan, indoleacetic acid, β –indoleacetonitrile, melatonin, kynurenic acid and kynurenine sulfate have also been quite frequently identified (Muszyńska et al. 2009).

Our earlier studies analyzed nonhallucynogenic indole compounds in fruiting bodies of the following edible species from natural habitats: *Boletus edulis* Bull.: Fr , *Cantharellus cibarius* Fr., *Lactarius deliciosus* (L.: Fr.) Gray, *Leccinum rufum* (Schaeff.) Kreisel, *Suillus luteus* (L.: Fr.) Roussel, *Xerocomus badius* (Fr.:Fr.) Kühner ex Gillbert, and of commercial origin: *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm , and the species considered as conditionally edible: *Armillaria mellea* (Vahl:) P. Kumm. ss. lato., *Lactarius deterrimus* Gröger and *Tricholoma equestre* (L.: Fr.) P. Kumm. ss. lato. The contents of indole compounds in all these species were diverse and had a very wide variability range from 0.01 to 39.20 mg/100 g d. w. similar to that of *C. viscosa* (Muszyńska et al. 2007; Muszyńska et al. 2009; Muszyńska et al. 2011a, b). Mycelial cultures of three species: *Cantharellus cibarius, Tricholoma equestre, Xerocomus badius* were established in our laboratory and the contents of compounds under study range from 0.01 to 20.49 mg/100 g d. w. Found melatonin content in the extracts from fruiting bodies of *Calocera viscosa* was comparable to the content of these compounds in the previously studied species (ranged from 0.08 to 1.29 mg/100 g d.w.). In mycelia of cultures *in vitro* melatonin was established only in the extract from mycelium of *T. equestre* (0.60 mg/100 g d.w.) and was comparable with the contents of this compound in the mycelia of *C. viscosa* from solid and liquid medium (0.39 mg/100 g d.w. and 0.37, respectively). In the case of tryptophan, the highest content of this compound was found in the fruiting bodies of *A. mellea* (4.47 mg/100 g d. w.), while the other examined fruiting bodies and mycelia from cultures *in vitro* (*X. badius* - 0.83 mg/100 g d.w. and *T. equestre* - 1.04) content were identical for all extracts obtained from *C. viscosa*. The content of 5-hydroxytryptophan was previously the highest in the currently studied extracts from fruiting bodies and mycelium *C. viscosa.* In other species the highest content of this compound was in *P. ostreatus* (2.08 mg /100 g d.w.). In the present analysis for first time we established in extracts from fruiting bodies and mycelium from culture *in vitro* of *C. viscosa* 5-methyltryptophan and indole. Compared with previously studied species the content of serotonin in the fruiting bodies of *C. viscosa* was low - 0.39 mg/ 100 g d.w., while for example in the fruiting bodies of *S. luteus*, *L. rufum* and *C. cibarius* ranged from 29 to almost 38 mg/100 g d.w. However, in the mycelium of *C. viscosa* from cultures on liquid medium found content of serotonin was higher than had previously been detected in mycelium of *T. equestre* (3.19 mg/100 g d.w. and 0.59, respectively). Tryptamine was labeled compound in most of the earlier fruiting bodies and mycelium of *T. equestre* and *X. badius*, but it was not detected in any extracts from *C. viscosa*. Greater variety of indole compounds than in the fruiting bodies of *C. viscosa* was showed only in previously analyzed fruiting bodies of *S. luteus*, while mycelia from *in vitro* culture on solid and liquid medium of *C. viscosa* presented that amount of these metabolites was comparable with the amount indicated in the mycelia of *T. equestre* and *X. badius* (Muszyńska et al. 2009).

CONCLUSIONS

The obtained results indicate that *Calocera viscosa in vitro* cultures can be a good model for the studies on accumulation and metabolism of indole compounds in mushrooms. These similar contents indicates also that it is possible to use *in vitro*

cultures as a model for studies on the physiological activity of above compounds. The comparable quantity of indole compounds obtained in the present study in *C. viscosa* mycelium with fruiting bodies collected from natural condition indicate that *in vitro* cultures are a good source of these compounds. High serotonin precursor 5-hydroxytrypophan contents in this material prove also a potential for the use of the mycelium cultured *in vitro* as a source of this physiologically important compound for humans. *In vitro* studies demonstrated that serotonin, melatonin and their indole derivatives (N-acetylserotonin, 6-methoxytryptamine) dose-dependently decreased lipid peroxidation (Sewerynek et al. 2005).

REFERENCES

Barros L., Baptista P., Correira D.M., Casal S., Oliveira B., Ferreira C.F. R. I. 2007. Fatty acid and sugar compositions, and nutrional value of five wild edible mushrooms from Northeast Portugal. Food Chem. 105:140–145.

Barros L., Cruz T., Baptista P., Estevinho L.M., Ferreira I. 2008. Wild and commercial mushrooms as source of utrients and nutraceuticals. Food Chem. Toxicol. 46: 2742–2747

Czeczuga B. 1980. Badania nad karotenoidami u grzybów IX *Dacrymycetaceae.* Acta Mycol. 16 (1): 115–120.

Kysilka R., Wurst M. 1985. High Performance Liquid Chromatographic Determination of Hallucinogenic Indoleamins with Simultaneous UV Photometric and Voltametric Detection. J. Chrom. 320: 414–420.

Liu J. 2005. N-Containing Compounds of Macromycetes. Chem. Rev. 105: 2723–2744.

Kohlmünzer S., Węgiel J., Muszyńska B., Sułkowska-Ziaja K, Gouillot J. 2000. Metabolity indolowe w hodowli mycelialnej grzybów wielkoowocnikowych. Herba Polonica 66: 98–104.

Mosovich S.A., Boone R.T., Reichenberg A., Bansilal S., Shaffer J., Dahlman K., Harvey P.D., Farkouh M.E. 2008. A new perspective on the relation between cardiovascular diseases and depression. Folia Cardiologica Excerpta 3: 296–307.

Muszyńska B. 1999. Chemical and biological studies of fruiting bodies and mycelium from solid and liquid cultures of *Calocera viscosa* (Pers.: Fr.) Fr. PhD Thesis. Jagiellonian University, Collegium Medicum, Kraków, 38–46.

Muszyńska B., Maślanka A., Sułkowska-Ziaja K., Krzek J. 2007. Analysis of indole compounds and nitric bases in fruiting bodies in *Lactarius deterrimus* by TLC-UV. J. Plan. Chrom. Modern TLC. 20: 55–58.

Muszyńska B., Sułkowska-Ziaja K., Ekiert H. 2009. Indole compounds in fruiting bodies of some selected *Macromycetes* species and their cultured *in vitro.* Pharmazie 64: 479–480.

Muszyńska B., Sułkowska-Ziaja K., Ekiert H. 2010. Główne grupy związków i pierwiastki z aktywnością biologiczną w wybranych gatunkach grzybów jadalnych z taksonu *Basidiomycota*. Farmacja Polska 66: 804–814.

Muszyńska B., Sułkowska-Ziaja K., Ekiert H. 2011a. Indole compounds in fruiting bodies of some edible Basidiomycota species. Food Chem. 125: 1306–1308.

Muszyńska B., Sułkowska-Ziaja K., Ekiert H. 2011b. Analysis of indole copounds in fruiting bodies of *Armillaria mellea.* Acta Polon. Pharm. Drug Res. 168: 93–97.

Muszyńska B., Sułkowska-Ziaja K., Ekiert H. 2012. An antioxidant in fruiting bodies and in mycelia from *in vitro* cultures of *Calocera viscosa* (*Basidiomycota*) – preliminary results. Acta Polon. Pharm. Drug Res. 169: 10.

Oddoux L. (ed.). 1957. Recherches sur les mycéliums secondaires des Homobasidiés en culture pure. Imprimerie de Trevoux, Lyon.

Ohstuka S., Ueno S., Yoshikumi C., Hirose F., Ohmura Y., Wada T., Fujii T., Takahashi E. 1973. Polysaccharides having an anticarcinogenic effect and a method of producing them from species of Basidiomycetes. UK Patent 1331513.

Ouchi Y., Yoshikawa E., Futatsubachi M., Yagi S., Ueki T., Nakamura K. 2009. Altered brain serotonin transporter and associated glucose metabolism in Alzheimere disease. J. Nucl. Med. 50: 1260–1266.

Sewerynek E , Stuss M., Oszczygieł K., Kułak J , Lewiński A. 2005. Protective effect of indole compounds on lipopolysccharide induced lipid peroxidation in *in vitro* conditions. Endokrynol. Polska 4: 508–511.

Sułkowska-Ziaja K., Muszyńska B., Końska G. 2005. Biologically Active Compounds of Fungal Origin Displaying Antitumor Activity. Acta Polon. Pharm. Drug Res. 6: 153–160.

Sułkowska-Ziaja K. 2010. Chemical analysis of *Sarcodon imbricatus* (L.) P. Karst fruiting bodies and mycelia from *in vitro* cultures and biological activity of their polysaccharide fractions. PhD Thesis. Jagiellonian University Collegium Medicum, Kraków, 97-107.

Wasser S.P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60: 258–274.

Yang J-H., Lin H-C., Mau J-L. 2001. Non-volatile taste components of several commercial mushrooms. Food Chem. 72: 465–471.

Zaidman B.Z., Yassin M., Mahajana J., Wasser S.P. 2005. Medicinal mushroom modulators of molecular targets as cancer therapeutics. Appl. Microbiol. Biotechnol. 67: 453–468.

Analiza zawartości związków indolowych w owocnikach i mycelium z kultur *in vitro Calocera viscosa* (Basidiomycota)

Streszczenie

Od tysięcy lat owocniki grzybów wyższych były wykorzystywane jako źródło pożywienia. Obecnie mogą być pozyskiwane nie tylko ze stanu naturalnego i hodowli komercyjnych lecz również z kultur *in vitro* prowadzonych w odpowiednich warunkach. Grzyby jadalne są coraz intensywniej badane ze względu na produkcję biologicznie aktywnych metabolitów wtórnych. Biologicznie i leczniczo aktywne metabolity grzybów są używane w terapii tak poważnych schorzeń jak np.: choroby krążenia, cukrzyca, miażdżyca oraz choroby nowotworowe. Niektóre z metabolitów wykazują działanie: przeciwwirusowe, przeciwbakteryjne, przeciwrobacze. W konwencjonalnej medycynie, w leczeniu onkologicznym najdłużej stosowane są polisacharydy grzybowe, stąd też są ich najlepiej poznanymi metabolitami. Innymi grupami szerzej badanych związków są również liczne związki fenolowe, terpenowe, indolowe, witaminy, biopierwiastki (np. selen) o działaniu antyoksydacyjnym.

Obiektem badań w ramach niniejszej pracy był gatunek grzyba wielkoowocnikowego *Calocera viscosa* (Pers.: Fr.) Fr. (Basidiomycota). Owocniki tego gatunku występują pospolicie w lasach iglastych Polski południowej. Materiał do badań stanowiły owocniki zebrane w Nowej Białej (powiat Nowy Targ) oraz mycelium otrzymane z kultur *in vitro*, które wyprowadzono z owocników tego gatunku. Kultury prowadzono na pożywce stałej i płynnej wytrząsanej wg Oddoux w temperaturze 25 ± 2°C przez 14 dni w warunkach sztucznego oświetlenia o intensywności 900 lx. Owocniki oraz zebrane biomasy z kultur *in vitro* wysuszono metodą liofilizacji, ekstrahowano eterem naftowym w celu usunięcia frakcji lipidowej, a następnie prowadzono ekstrakcję metanolem. Uzyskane wyciągi metanolowe po zagęszczeniu, oczyszczono metodą preparatywnej chromatografii TLC na płytkach (DC Alufolien Kiesel gel F-254), a następnie analizowano na zawartość związków indolowych metodą HPLC (identyfikacje związków indolowych dokonano metodami spektralnymi UV-VIS i EIMS). Na podstawie przeprowadzonych analiz stwierdzono obecność we wszystkich trzech ekstraktach następujących związków indolowych: tryptofanu, 5-hydroksytryptofanu, 5-metylotryptofanu, melatoniny i indolu (zawartości wahały się w przedziale: 0.37 do 11.88 mg/100g s.m.). Związkiem indolowym występującym w największych ilościach we wszystkich ekstraktach był 5-hydroksytryptofan. Dodatkowo w owocnikach i w mycelium z kultur na podłożu płynnym stwierdzono obecność serotoniny (odpowiednio: 0.39 i 3.19 mg/100g s.m).