AN ANTI-OXIDANT IN FRUITING BODIES AND IN MYCELIA FROM IN VITRO CULTURES OF CALOCERA VISCOSA (BASIDIOMYCOTA) – PRELIMINARY RESULTS

BOŻENA MUSZYŃSKA*, KATARZYNA SUŁKOWSKA-ZIAJA and HALINA EKIERT
Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 301-688 Kraków, Poland

Key words: antioxidant, Calocera viscosa, β,β-carotene, in vitro culture

Fruiting bodies of mushrooms belonging to the taxon Basidiomycota are a known source of a variety of biologically active compounds belonging to both primary and secondary metabolites (1, 2). The best known fungal polysaccharides are used for anticancer therapy (3, 4). Recently, Muszyńska (5–7) have hinted at the occurrence of numerous non-hallucinogenic indole compounds in edible, conditionally edible and inedible Basidiomycota species. A number of recent mycochemical papers have reported on the presence of many different terpene compounds (8). Studies of Barros (9, 10) indicated antioxidant activity of fruiting bodies extracts from several Basidiomycota species and attributed it to tetraterpenes, carotenoids. Under the influence of carotenoids occurring mainly in the intestinal wall and the hepatocytes, β-carotene breaks down to vitamin A (retinol). Retinol is a part of the rhodopsin and is responsible for proper vision twilight. It is necessary for the proper functioning of the epithelium, participates in the divisions of the epithelium and protects cells from damage caused by solar radiation and free radicals (11). Quantitative analysis of carotenoids in fruiting bodies of one of the species investigated by that team, Cantharellus cibarius, has demonstrated that fungal fruiting bodies can be a valuable rich source of those antioxidants.

The aim of the present study was to initiate Calocera viscosa culture in vitro, determine optimal conditions for mycelia growth and to evaluate the β,β-carotene contents in extracts of fruiting bodies and mycelia from Calocera viscosa (Pers.; Fr.) Fr. in vitro cultures. Calocera viscosa is a species common in Polish, European and Asiatic coniferous forests, which develops intensely yellow-orange branched, bush-like fruiting bodies. This species is a good source of free exo- and endogenous amino acids, indole compounds, sterols (especially ergosterol) and unsaturated fatty acids (12). The carotenoids content in Calocera viscosa fruiting bodies growing in Poland was investigated by Czeczuga (13) at the end of the seventies years of the 20th century.

In this study, for the first time we analyzed extracts from in vitro cultures that had been established earlier by Muszyńska (12). The cultures were maintained under different conditions to optimize biomass growth and to establish the most beneficial conditions for β,β-carotene accumulation.

EXPERIMENTAL

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 (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 (14). Cultures were incubated at a tempera-

* Corresponding author: e-mail: muchon@poczta.fm
ture 25±2°C under 12-h light (900 lx)/12 dark cycle and were subcultured every second week. For more details see Muszyńska (12).

**Experimental in vitro cultures**

Solid culture was maintained on Petri dishes, on Oddoux medium (14). Stationary liquid culture was established from the solid culture by transferring 0.1 g of mycelium into an Erlenmeyer 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 fruiting bodies and mycelia from *Calocera viscosa in vitro* cultures on solid and liquid media were extracted in a percolator with petroleum ether. Extracts were concentrated in a vacuum evaporator.

For the purification of the extracts, we used preparative PTLC method on aluminium silica 60 plates (Merck, Art. No 1.05554.0001), on which 2 mL of the extracts was loaded and chromatograms were developed in mobile faze: petroleum ether : ethyl acetate (20 : 1, v/v). The obtained fractions were concentrated to dry extracts and after dissolv-
An antioxidant in fruiting bodies and in mycelia from in vitro cultures... 137

Ultraviolet–Visible (UV-Vis) analysis of \( \beta \beta \)-carotene

Purified by PTLC method extracts of fruiting bodies and mycelia from cultures in vitro on solid and liquid medium were analyzed for the presence of \( \beta \beta \)-carotene by spectrophotometry using UV-Vis instrument – UV-Vis Cary-Varian Spectrophotometer. Absorption measurements were carried out at \( \lambda = 400–500 \text{ nm} \); solvent: chloroform AR; standard: \( \beta \beta \)-carotene (Sigma-Aldrich). In all three extracts was found an increase of the growth of absorption maxima at \( \lambda_{\text{max}} = 415 \) and \( \lambda_{\text{max}} = 440 \text{ nm} \) characteristic for \( \beta \beta \)-carotene.

Electron Impact Mass Spectrometry (EIMS) analysis of \( \beta \beta \)-carotene

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-MS, Finnigan MAT 95S. In the results of these studies, the spectra were obtained for the chloroform extracts from the fruiting bodies and mycelia from cultures in vitro on solid and liquid medium. The EIMS spectra for the chloroform extracts from the fruiting bodies and mycelia from in vitro cultures on solid and liquid medium containing peaks characteristic for \( \beta \beta \)-carotene are shown in Figures 1–3.

Table 1. Contents of \( \beta \)-carotene (mg/g d.w.) in wild, commercial mushrooms and in mycelia from cultures in vitro.

<table>
<thead>
<tr>
<th>Species</th>
<th>Author</th>
<th>Contents of ( \beta )-carotene (µg/g d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>Barros, (9, 10)</td>
<td>1.95 ± 0.10</td>
</tr>
<tr>
<td>Agaricus silvaticus</td>
<td></td>
<td>5.42 ± 0.10</td>
</tr>
<tr>
<td>Agaricus silvicola</td>
<td></td>
<td>3.02 ± 0.12</td>
</tr>
<tr>
<td>Boletus edulis</td>
<td></td>
<td>2.73 ± 0.32</td>
</tr>
<tr>
<td>Calocybe gambosa</td>
<td></td>
<td>6.41 ± 1.27</td>
</tr>
<tr>
<td>Cantharellus cibarius</td>
<td></td>
<td>13.56 ± 0.51</td>
</tr>
<tr>
<td>Craterellus cornucopoides</td>
<td></td>
<td>12.77 ± 0.19</td>
</tr>
<tr>
<td>Marasmius oreades</td>
<td></td>
<td>1.99 ± 0.14</td>
</tr>
<tr>
<td>Calocera viscosa</td>
<td>Czezuga, (13)</td>
<td>2.46</td>
</tr>
<tr>
<td>Calocera viscosa (fruiting bodies)</td>
<td>Muszyńska (this paper)</td>
<td>7.5 ± 0.15</td>
</tr>
<tr>
<td>Calocera viscosa (mycelium from liquid cultures in vitro)</td>
<td></td>
<td>3.5 ± 0.10</td>
</tr>
<tr>
<td>Calocera viscosa (mycelium from solid cultures in vitro)</td>
<td></td>
<td>7.1 ± 0.32</td>
</tr>
</tbody>
</table>
Estimation of β,β-carotene by HPLC method

Contents of β,β-carotene in extracts from fruiting bodies and in mycelia maintained in in vitro cultures on solid and liquid medium were determined after preliminary separation with PTLC method. Qualitative and quantitative analyses were made by HPLC method according to the procedure described by Britton (15). Briefly, the analytical conditions were as follows:

- HPLC apparatus: Merck/Hitachi; pump-L-7100; column-Purospher RP–18 (4 × 200 mm, 5 µm); solvent system: acetonitrile/methanol/water (76:6:1, v/v/v); flow rate 1 mL/min; detector L–7400, λ = 450 nm; standard: β,β-carotene (Sigma-Aldrich).

RESULTS AND DISCUSSION

A 20-fold fresh biomass growth in PTLC, UV Vis, EIMS and HPLC cultures on solid medium and a 15-fold growth in liquid cultures were obtained within a 14-day growth cycle. The identity of β,β-carotene was confirmed on the basis of its parameters PTLC, UV-Vis, EIMS and HPLC methods. The identity of β,β-carotene by EIMS method was also made and described by Lutneas (16). The HPLC method was used for quantitation of β,β-carotene in extracts from fruiting bodies and in mycelia from in vitro cultures on solid and liquid medium.

β,β-Carotene content in biomass from solid cultures was comparable with that found in fruiting bodies (7.1 and 7.5 mg/g d.w., respectively). Mycelia from liquid cultures contained half of that β,β-carotene amount which equaled 3.5 mg/g d.w. (Tab. 1).

These contents are higher than those determined by Czezcuga (13), in Calocera viscosa fruiting bodies collected in Poland, which were estimated spectrophotometrically as 2.46 mg/g d.w. Barros 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 mg/g d.w.), whereas the contents of this metabolite in the remaining species ranged from 1.95 to 12.77 mg/g d.w. (9). β,β-Carotene from Cantharellus cibarius is used to color eggs and broilers skins. In recent years, there was a systematic decline of this species and their commercial cultures were not obtained till now. So, Calocera viscosa is the new, potential source of β,β-carotene. The similar contents of β,β-carotene in mycelia from in vitro culture on solid medium and fruiting bodies collected from natural conditions indicates that it may be possible to use this culture as a model for studies on the biosynthesis and accumulation of this compound. The contents of β,β-carotene obtained in the present study in Calocera viscosa mycelium indicate that after optimization of culture conditions to facilitate the antioxidant accumulation, our in vitro cultures may become its competitive source in the future. This is the first report of the quantification of β,β-carotene in mycelia of Calocera viscosa cultured in vitro.

REFERENCES


Received: 26. 10. 2010