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CAROTENOID COMPOSITION OF MYCELIUM AND APOTHECIA IN THE DISCOMYCETE *SCUTELLINIA UMBRARUM*

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Key Word Index—*Scutellinia umbrarum*; fungus; γ-carotene; phytoene; torulene; diphenylamine.

Abstract—Mycelium and apothecia from *Scutellinia umbrarum* (Discomycete) cultivated *in vitro*, have similar carotenoid compositions. Carotenoid contents correspond respectively to 0.16% dry wt in apothecia and 0.017% in mycelium when grown in the light. *trans*-γ-Carotene is the main pigment (*ca* 80%) together with minor amounts of *cis*-γ-carotene, torulene, lycopene and aleuriaxanthin. No pigment can be detected in dark-grown mycelium. *Cis*-Phytoene only accumulates in light-grown mycelium treated with diphenylamine which interfere with the desaturation steps from phytoene to lycopene.

INTRODUCTION

Carotenoid contents from fungi have been used as chemotaxonomic probes in fructifications of Basidiomycetes [1] and Acomycetes [2]. Carotenoid biosynthesis and function have been studied in the mycelium from several species cultivated *in vitro* [3, 4]. Carotenoid biosynthesis is photo-inducible in plants, and in fungi, blue light is the most effective [5]. Most of them accumulate only very small amounts of pigment in the dark, exceptions being *Phycomyces blakesleeanus* [6] and several *Mucor* species [7]. The physiological functions of carotenoids are not clearly known in fungi, although they are thought to be photoreceptors [5].

Carotenoids are generally dissolved in lipid globules, but in some genera such as *Scutellinia*, *Cheilymenia* and *Coprobia* crystallized pigment needles can be observed [8]. We have shown [9] that in *Ciliaria hirta* (Schum.) Boud. (= *Scutellinia hirta* (Schum.) Le Gal) these needles are derived from endoplasmic reticulum membranes, thickening during carotenoid synthesis.

In this work we have analysed the carotenoid content of mycelium and apothecia from *Scutellinia umbrarum* cultivated *in vitro*. This discomycete produces apothecia only when a *Pseudomonas* bacterium is present in the culture [10]. Both mycelium and apothecia accumulate reserve lipids and pigments [11]. Three carotenoids were identified by Arpin [2] in apothecia collected in the wild. These were mainly γ-carotene (84% of pigments), torulene (7%) and neurosporene (1%). On the other hand, the carotenoid composition of the mycelium from this fungus has never been studied, no culture of this fungus being available until now.

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RESULTS AND DISCUSSION

Mycelium and apothecia formed in the light, were strongly orange coloured. The pigments were dissolved in lipid globules. In apothecia these are found in paraphyses and in ascogenous hyphae. Crystallized needles were absent in this species. Dark-grown cultures were not coloured and did not produce apothecia.

TLC of extract from light-grown mycelium as well as of apothecia revealed a pronounced orange-coloured spot of γ-carotene and a faint pink spot of torulene. On the other hand, those of dark-grown mycelium did not show any spot either under visible or UV light. The lipid extract of mycelium grown in the light in the presence of diphenylamine showed a spot only detected under UV light and identified as phytoene.

HPLC analysis of pigments of light-grown mycelium and of apothecia revealed five carotenoids. The absorption spectra of the UV-absorbing products present in these crude extracts allowed their identificaton as sterols and sterol-esters and no carotenoid precursor could be detected. However, HPLC profiles of extracts from mycelium grown in the light in the presence of diphenylamine showed, besides sterols, one additional peak (λ_{max} 275–287–296) which corresponded to *cis*-phytoene [12]. Carotenoid contents are given in Table 1 in $\mu g\,g^{-1}$ dry weight. In apothecia, *cis*- and *trans*-γ-carotene represented 90% of total carotenoids. Three

Table 1. Carotenoid content ($\mu g\,g^{-1}$ dry wt) of apothecia and mycelium of *Scutellinia umbrarum* cultivated in various conditions (each level as a % of total is presented in parentheses)

Pigment	Apothecia	Mycelium cultivated in the light	Mycelium cultivated in the dark	Mycelium cultivated with diphenylamine
trans-γ-Carotene	1360 (83.7%)	130 (76%)	—	—
cis-γ-Carotene	120 (7.4%)	24 (14%)	—	—
Torulene	100 (6.2%)	10 (5.8%)	—	—
Lycopene	24 (1.5%)	3 (1.8%)	—	—
Aleuriaxanthin	20 (1.2%)	4 (2.4%)	—	—
cis-Phytoene		—	—	110

other pigments were identified: torulene was the most abundant and was accompanied by small amounts of aleuriaxanthin and lycopene. The pigment composition of the mycelium was qualitatively similar to that of apothecia; the γ-carotene content, when compared with apothecia, was identical, but the *cis*/*trans* ratio was doubled (Table 1). The total carotenoid content was 0.16% of dry wt in apothecia and 0.017% in mycelium.

Our analysis shows that in *Scutellinia umbrarum* carotenogenesis is photodependent. Moreover this fungus, like *Aspergillus giganteus* mut *alba* [13], does not accumulate phytoene in the dark, unlike many organisms.

Phytoene produced in the light is rapidly transformed into carotenoids and thus is never detected in light-grown cultures. It only accumulates in light-grown cultures treated with diphenylamine which inhibits phytoene dehydrogenation [14]. The accumulation of uncoloured polyenes in the presence of diphenylamine, is lower than the carotenoid content on standard medium, as previously observed by Goodwin [15].

The light-grown mycelium and apothecia have similar pigment compositions but in apothecia the levels are 10 times greater. *Scutellinia umbrarum* is thus distinguishable from the majority of carotenogenic species in which the mycelia do not synthesize carotenoids [2]. The pigment composition we have observed is very close to that of apothecia collected in the wild by Arpin [2] (even if the amounts we have measured are lower). The major pigment is γ-carotene. The differences between our results and those of Arpin could be the consequence of environmental factors on carotenogenesis. Our samples were cultivated in the laboratory under conditions which are different from those which are encountered naturally. Aleuriaxanthin is the only oxygenated compound we found in *S. umbrarum*. This pigment is only present, in noticeable amounts, in the Aleuriaceae [16]. The fungi which possess it, are regarded as advanced. Similarly the lack of β-carotene, a common pigment in fungi, is also considered as an advanced characteristic.

The amount of pigments in ascogenous hyphae, before karyogamy and meiosis, is 10 times greater than that of the somatic mycelium before ascocarp formation. Because of such a difference, the involvement of carotenoids in reproduction of *Scutellinia umbrarum* might be expected. It would be interesting to study further the effects of light on carotenogenesis and on the sexual reproduction of this fungus which is photodependent (unpublished results), to determine if a single photoreceptor is involved in both phenomena.

EXPERIMENTAL

Culture conditions. Scutellinia umbrarum was grown as previously described [10]. Mycelia were collected after 3 weeks of growth either in the dark or under a 8 hr light period per day ($60\,\mu E\ m^{-2}s^{-1}$). Fully coloured apothecia were collected after 7–10 weeks growth. Some cultures were supplemented with the inhibitor diphenylamine ($5–10\ mg\,l^{-1}$) and the mycelium was collected 4 weeks after inoculation.

Pigment extraction. Mycelium and apothecia, washed with water, were homogeneized with a Ultra Turrax blender in Me_2CO–MeOH (4:1) mixture at 0°. After filtration, petrol (40–65°) and water were added to the pigment extract. The upper phase containing carotenoids and lipids was collected, washed and evaporated to dryness.

Pigment analysis. TLC separations were obtained on silica gel with hexane–toluene (4:1) as a solvent.

HPLC. Carotenoid pigments were separated by a non-aq. reverse phase method [17], with a du Pont Zorbax ODS column and a solvent gradient of 0.1–4.0% CH_2Cl_2 in MeCN–MeOH (7:3). A diode array detector permitted the on-line monitoring of the absorption spectra and integration of carotenoids. Quantification of pigments was achieved by calibration of the detector response with carotenoid standards using the coefficients published in ref. [18].

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