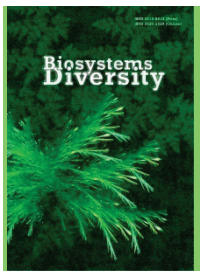

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Growth and morphological characteristics of some pyrophilous discomycetes in culture

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Three pyrophilous discomycetes species (*Anthracobia maurilabra* (Cooke) Boud., *Pyronema domesticum* (Sowerby) Sacc. and *Tricharina praecox* (P. Karst.) Dennis) have been cultivated and studied in vitro. Cultures were obtained from fresh apothecia collected in the Holosiivskyi National Nature Park (Kyiv, Ukraine). The culture growth and morphological characteristics of the studied fungi on different media (beer wort agar, Czapek Dox agar, potato-dextrose agar) were analyzed. All investigated species can quite easily grow under laboratory conditions on different tested nutrient media. Potato-dextrose agar was the most suitable medium for the enhancement of radial growth and the best expresses all the phenotypes of the colony of studied fungi. Macro- and micromorphological descriptions of all fungal colonies and illustrations are provided. The micromorphological analysis showed that common to the mycelium of all studied species of fungi was the presence of numerous drops of oil in the hyphae, anastomoses like *T. praecox*, a net- like structure of *A. maurilabra* similar to nematode capture hook. Beside this, *A. maurilabra* and *P. domesticum* formed the sexual stage under experimental conditions. Forming fruiting bodies of *A. maurilabra* in culture has not been reported before. Moreover, *P. domesticum* was found to form abundant dark brown sclerotia on potato-dextrose agar and Czapek Dox agar. Possible pigment composition in the *P. domesticum* sclerotia is discussed based on the Raman spectroscopy study, performed on this genus for the first time. The established cultural characteristics can be useful for taxonomic identification of fungal species and for pure quality control of mycelial cultures during their introduction, preservation and future potential applications in biotechnological areas.

Keywords: *Anthracobia*; *Pyronema*; *Tricharina*; growth parameters; culture description; Raman spectroscopy.

Introduction

Pyrophilous or postfire fungi are a highly specialized and distinctive group of organisms that occur mainly or exclusively in such an unstable and ephemeral habitat as burnt ground (Dix & Webster, 1995). Their appearance in post-fire environments is explained by decrease in competition and adaptation to the physicochemical environment after a fire, especially to higher pH levels (El-Abyad & Webster, 1968). Pyrophilous fungi include members within the Dykaria and Mucoromycota, but most belong to the Pezizales (Ascomycota). These operculate discomycetes are found mainly in families such as Pyronemataceae, Pezizaceae and Morchellaceae (Claridge et al., 2009).

Among the members of the family Pyronemataceae, a large number fruit amongst the ashes on recently burnt sites, often in large numbers immediately or in the first and second years following fire. Species of *Anthracobia*, *Pyronema*, *Tricharina*, etc. produce abundant fruit bodies, their mycelial mats may be important in minimising soil erosion after fire by aggregating soil particles (Claridge et al., 2009; Mcmullan-Fisher et al., 2011). Thus, pyrophilous fungi play an important role in soil stabilization. Some species, such as *Anthracobia* spp. are pivotal in early system recovery after the fire, helping minimize the movement of soil in the absence of plant roots. Another important value of postfire fungi might include nutrient acquisition, leading to the re-establishment of vegetation after disturbance (Claridge et al., 2009).

The role of pyrophilous fungi in the regeneration of surfaces destroyed by fires and their ability to form new mycorrhizal relationships with trees soon after fires have attracted attention of the mycologists around the world. These predominantly saprophytic fungi have been known for over a hundred years. Nevertheless, works on study of pyrophilous species in vitro are mainly devoted to such Pyronemataceae species as *Pyronema omphalodes* (Bull.) Fuckel (Seaver, 1909; Robinson, 1926), *P. domesticum* (Sowerby) Sacc. (Moore, 1963; Roxon & Batra, 1973; Moore-Landecker, 1981), *Anthracobia melaloma* (Alb. & Schwein.) Fuckel (Rosinski, 1956), *A. muelleri* (Berk.) Rifai and *Ascodesmis sphaerospora* W. Obrist (Roxon & Batra, 1973), *Trichophaea abundans* (P. Karst.) Boud. (Roxon & Batra, 1973; Turnau, 1983; Šimonovičová et al., 2014), *Pyropyxis rubra* (Peck) Egger (Filipova et al., 2016). Studies of morphogenesis of *P. domesticum* apothecia and sclerotia under the influence of various factors have also been conducted (Moore-Landecker, 1975; 1979a; 1987a; Filipova et al., 2016). In Ukraine, no studies of pyrophilous discomycetes in culture have been conducted until now.

As already noted, pyrophilic fungi are an extremely important group of organisms in post-fire successions. Therefore, obtaining their biomass for further introduction into ecosystems transformed by fire in order to accelerate their recovery is an important area of modern research. The present publication aims to describe the culture growth and micromorphological characteristics of the following saprophytic pyrophilous species: *Anthracobia maurilabra* (Cooke) Boud, *Pyronema domesticum* (Sowerby) Sacc., and *Tricharina praecox* (P. Karst.) Dennis (Synonym: *Ascorhizoctonia praecox* Chin S. Yang & Korf) (Van Vooren et al., 2017) on different media.

Materials and methods

Fungal species and their introduction in culture. Apothecia of *A. maurilabra*, *P. domesticum* and *T. praecox* were collected during our survey of burnt sites in the Holosiivskyi National Nature Park (Kyiv, Ukraine) between autumn of 2017 and spring of 2019 (Dzhagan et al., 2020). The introduction of fungi into the culture was performed according to conventional methods (Buchalo et al., 2009). Young intact fresh apothecia were selected as material for tissue culture isolation. Small parts of hymenium were immersed (for about 5 s) in a 3% solution of hydrogen peroxide for their disinfection. The prepared tissues of apothecia were transferred to the central part of the Petri dish (90 mm diameter) using a mycological loop on the solid surface of the potato-dextrose agar medium. Stock cultures of introduced fungi were maintained on beer-wort agar slants at 4 ºC.

Cultivated media and mycelial growth of fungi. Analysis of cultural and morphological characteristics of vegetative mycelia of the above-mentioned species was provided using the selected media:

– Beer wort agar (BWA): liquid beer wort, diluted with distilled water (8 degrees on the Balling scale for sugar content), 20 g agar, pH 5.8;

– Czapek-Dox agar (CDA): 2.0 g $NaNO_3$, 0.7 g KH_2PO_4, 0.3 g K_2HPO_4 x $3H_2O$, 0.5 g $MgSO_4$ x $7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$ x $7H_2O$, 30 g sucrose, 20 g agar, 1000 mL distilled water, pH 7.3;

– Potato dextrose agar (PDA) (Difco, USA): 39 g, 1000 mL distilled water, pH 6.0.

The pH of the used media was adjusted to certain values before the sterilization using solutions of 0.1 N KOH or 0.1 N HCl. All prepared media were autoclaved at 121 ºC for 20 min. The Petri dishes (three replicates) were inoculated with mycelial plugs (5 mm diameter), cut from actively growing mycelia on PDA. Inoculated cultures were incubated at temperature 26 ± 1 ºC in the dark.

The radial growth rate (Vr, mm/day) of the colony was calculated using the following equation (Bisko et al., 2012): $Vr = (R_1–R_0)/(t_1–t_0)$, where Vr – radial growth rate; R_1 – colony radius at the end of the linear growth phase, mm; R_0 – colony radius at the beginning of the linear growth phase, mm; $t_1–t_0$ – the duration of the linear growth phase, days.

Fungal colony morphological descriptions. Mycelia were observed daily after inoculation and monitored during 30 days. Fungal colony morphology descriptions were performed after full colonization of the Petri dishes (7–10 days after inoculation). The texture of most types of colonies was recorded according to the scale proposed by Stalpers (1978). Characteristics of mycelial colonies such as colour, peculiarities of the marginal zone, aerial mycelium, and possible changes in the morphology and in the reverse colony surface were also recorded. The mycelial density was evaluated as follows: very scanty, scanty, moderate, abundant, very abundant. Macrophotographs of the fungal colonies were taken with Sony Cybershot DSC-H3 8.1 MP (Sony, Japan, 2017) and Canon PowerShot SX40 HS 12.1 MP (Canon, Japan, 2015) digital cameras.

Microstructural characteristics of vegetative mycelium of fungal species. Mycelial fragments of fungi were mounted on a microscope slide and observed in tap water (H_2O), potassium hydroxide solution (3% KOH), Melzer's reagent (MLZ) (Microscience, UK), and Congo Red (CR) aqueous solution (Sigma-Aldrich, USA). Microscopic features of the vegetative mycelium were observed with a light microscope XY-B2T (Ulab, China, 2003) and a Zeiss Primo Star light microscope (Carl Zeiss, Germany, 2008). Dimensions of fungal microstructures were measured using AxioVision Rel. 4.8 (Carl Zeiss Imaging Solutions, Germany). Digital microphotographs were made with a Canon PC 1089 Power Shot G6 digital camera (Canon, Japan, 2008).

Determination of the carotenoids in sclerotia of Pyronema domesticum. To establish the nature of the lipid globules from sclerotia found in the obtained mycelial cultures, the method of Raman spectroscopy (Raman scattering) was used. Measurements were performed using a Raman spectrometer based on a single monochromator MDR-23 (LOMO) (Leningrad, USSR, 1973), equipped with a TE-cooled CCD detector (iDus 420, Andor) (Oxford Instruments, Abingdon, UK, 2010). The spectra were recorded in the spectral range encompassing the range of vibrations of organic molecules, 400–3500 cm^{-1}, with a spectral resolution of 2 cm^{-1}. Raman spectra were excited with a solid-state laser emitting 532 nm light. The laser power was adjusted to 1 mW, to avoid any thermal damage of the sample during the measurement.

Statistical analysis. The experiments were repeated three times for each variant. The obtained data were processed by generally accepted methods of variation statistics. The data in the histogram (Fig. 1) are presented as x ± SD (mean ± standard deviation). Differences between the fungi growth (calculated in the program Statistica 7.1, StatSoft Inc., USA) were identified using the Tukey test, where the differences were considered significant at $P < 0.05$ (adjusted for Bonferroni).

Results

Growth of fungi on different nutritional media. The successful isolation of the fungi allowed us to study the tested species in vitro. As shown in Figure 1, the growth speed was different depending on the cultivated medium used and the fungi investigated. All the media used supported the growth of pyrophilous discomycetes to various degrees from 6.1 mm/day (*T. praecox*) to 52.8 mm/day (*A. maurilabra*). However, two species, *A. maurilabra* and *P. domesticum*, were similar in terms of growth on tested media. PDA medium was the most favourable for *A. maurilabra* and *P. domesticum* with the best growth rate 52.8 and 48.9 mm/day, respectively. *T. praecox* had a significantly slower growth rate, almost at the same level (6.1–6.8 mm/day) on all cultivated media.

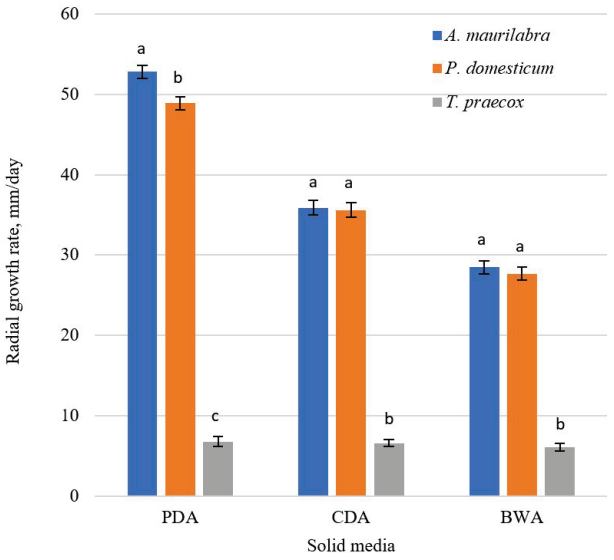

Fig. 1. Pyrophilous fungi growth on various solid media: potato-dextrose agar (PDA), Czapek-Dox agar (CDA), beer wort agar (BWA): the data were analysed with the Tukey test ($P < 0.05$, x ± SD, n = 3) with Bonferroni correction: letters *a–c* indicate statistically significant differences in the radial growth rate of fungi on a certain medium

The visual macroscopic changes in the morphology of mycelial colonies and macromorphological characteristics of vegetative mycelia of studied fungi were investigated on different media (Fig. 2).

Anthracobia maurilabra (Cooke) Boud. – culture characteristics. On all media, the colony was circular with smooth margin, the mycelium was white, but the colour changed over time to light brown with an orange tinge on BWA and to light brown on CDA. On BWA and CDA colonies were felty (Fig. 2a, 2c), on PDA – wooly (Fig. 2b). The mycelial density was abundant on BWA and CDA, moderate on PDA and very scanty on CDA. The growing zone was appressed to raised. Agar (reverse) was brown on BWA, with dark brown pigmentation in the central part of the colony (around the inoculum) and along the edge of the colony on CDA but unchanged on PDA.

Vegetative mycelia from agar colonies were branched, of different thickness from 3 to 18 µm, hyphae hyaline to brownish, septate. Hyphae with numerous drops of oil were noticed (Fig. 3d, 3f, 3h, 3i). Pigmented thick-walled hyphae with gigantic lateral ellipsoid chlamydospore (Fig. 3e), arthrosporic "oidial" state consisting simply of mycelial fragmentation (Fig. 3a, 3c), hyphae transformed into intercalary globose

chlamydospores (Fig. 3i), and loops-producing hyphae (Fig. 3b, 3g) were observed on different media. We also noted the sexual sporulation in PDA. Apothecia (3 up to 5 mm in diameter) formation matured after 20th day of incubation (Fig. 4). Sclerotia, stromata, and pigments were not observed.

Pyronema domesticum (Sowerby) Sacc. – culture characteristics. On all media, the colonies were circular, felty, with smooth margin, the mycelium was white, but eventually acquired a light pink hue only on BWA (Fig. 2d, 2e, 2f). The mycelial density was very abundant on BWA, abundant on CDA and PDA. The growing zone considerably differed: umbonate on BWA and raised on CDA and PDA. Agar (reverse) was mainly unchanged but only on BWA turned light brown. On CDA culture with cottony clumps of light pink hyphae was observed on 7th day after inoculation.

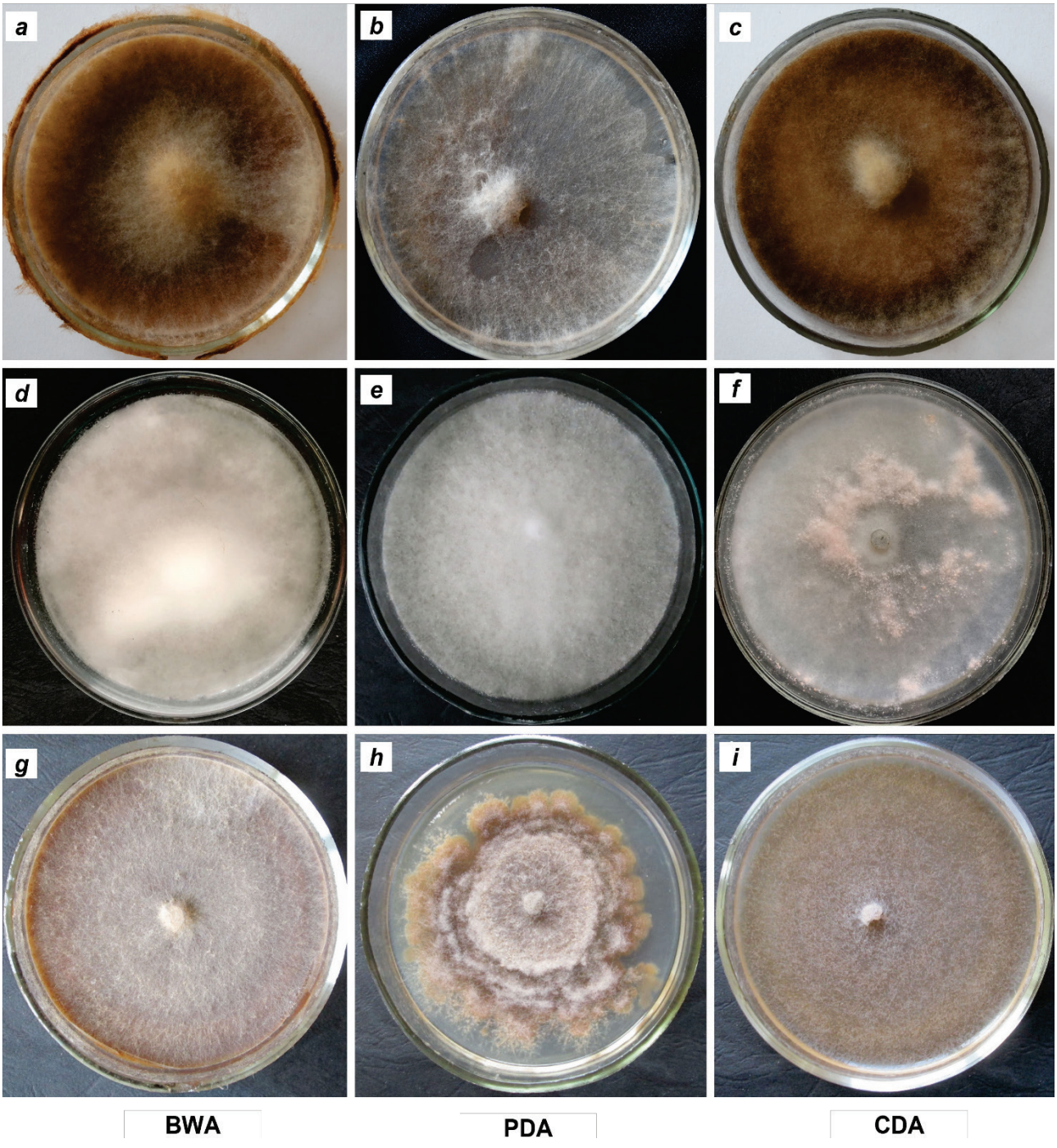

Fig. 2. Mycelial colonies of investigated fungi on 7–10th day of cultivation on three media:
BWA – beer wort agar; PDA – Potato dextrose agar; CDA – Czapek Dox agar; *a–c – A. maurilabra*; *d–f – P. domesticum*; *g–i – T. praecox*

Vegetative hyphae 7–12 µm in diameter, hyaline, branched, septate, some hyphae with thinner walls, 3–5 µm in diameter (Fig. 5f). Hyphae strongly vacuolated with numerous drops of oil (Fig. 5f, 5g). Sexual stage was found on the 5–7th day of incubation. *P. domesticum* formed apothecia abundantly on PDA. Numerous pink-orange apothecia (up to 1 mm) like confluent apothecial pads (Fig. 5a), mature asci and ascospores were also observed on the 10th day of cultivation (Fig. 5b, 5c). We also observed abundant dark brown sclerotia on PDA and CDA on the 7th day (Fig. 5d) with orange-brown drops of exudate, probably of a lipid nature, which were also visible under a light microscope (Fig. 5d, 5e).

In order to obtain more information about these sclerotia content we measured Raman spectra in different parts of the sample of *P. domesticum*. The spectra measured on sclerotia exhibited distinct vibrational pattern dominated with three peaks – at about 1000, 1150 and 1500 cm^{-1} (Fig. 6) – characteristic of carotenoids (see Discussion section for more details).

Tricharina praecox (P. Karst.) Dennis. (Synonym: Ascorhizoctonia praecox Chin S. Yang & Korf) (Van Vooren et al., 2017) – culture characteristics. Colonies slow-growing on all cultivated media. On BWA and CDA the colony was circular with smooth margin, and irregular with undulate margin on PDA. Mycelium was white except PDA medium (light brown). On BWA, CDA media colonies were wooly (Fig. 2g, 2i), zonate on PDA (Fig. 2h). The mycelial density was abundant on BWA, PDA, moderate on CDA. Growing zone was appressed. Agar (reverse) was changed on all used media: yellow-brown with noticeable concentric rings on BWA, weakly pronounced concentric rings, the colour varied from pale brown in the center to pale grey at the edges of the colonies on CDA, and brown with clear concentric rings on PDA.

Vegetative hyphae were up to 25 µm in diameter with rare septa and numerous drops of oil (Fig. 7a), others branched almost at a right angle, hyaline, sparingly septate (Fig. 7e), 4 up to 11 µm in diameter. Terminal globose and lateral ovate nodules (Fig. 7b, 7c) were observed. Some hyphae were parallel and anastomosing forming bridges (Fig. 7d). On BWA medium small crystals were present (Fig. 7f), on CDA we also noted the chains of monilioid cells represented, as we suppose, asexual sporulation (Fig. 7g). However, sexual structures were not observed.

Discussion

The traditional studies of the *Anthracobia*, *Pyronema*, and *Tricharina* species focused on their taxonomic investigation, particularly based on molecular analysis, macro and microscopic studies, ecological characteristics. In general, the above-mentioned species, except *Pyronema*, were poorly studied. Despite the availability of data on the growth of such an important fungus for mycology as *P. domesticum* in culture (Moore, 1963; Moore & Korf, 1963; Roxon & Batra, 1973), studies of the influence of various factors such as cultural conditions (Moore-Landecker, 1975), culture age and a single photoperiod (Moore-Landecker, 1979b), light regimens and intensities (Moore-Landecker, 1979a; Moore-Landecker, 1981; Moore-Landecker, 1987), ultraviolet, radiation and inhibitory volatile substances (Moore-Landecker, 1984), aeration (Moore-Landecker & Shropshire, 1982), medium composition (Moore-Landecker, 1987) on apothecial and sclerotia morphogenesis in *P. domesticum*, the lack of modern information should be noted. Besides, there are limitations due to the short period of vegetation of fruit bodies and the fact that they are ephemeral. The isolation of mycelial strains in pure culture is an alternative method of comprehensive research. To the best of our knowledge, this study was the first report of successful isolation and detailed analysis of cultural, micro- and macromorphological characteristics of vegetative mycelium of *A. maurilabra* and *T. praecox* in pure culture.

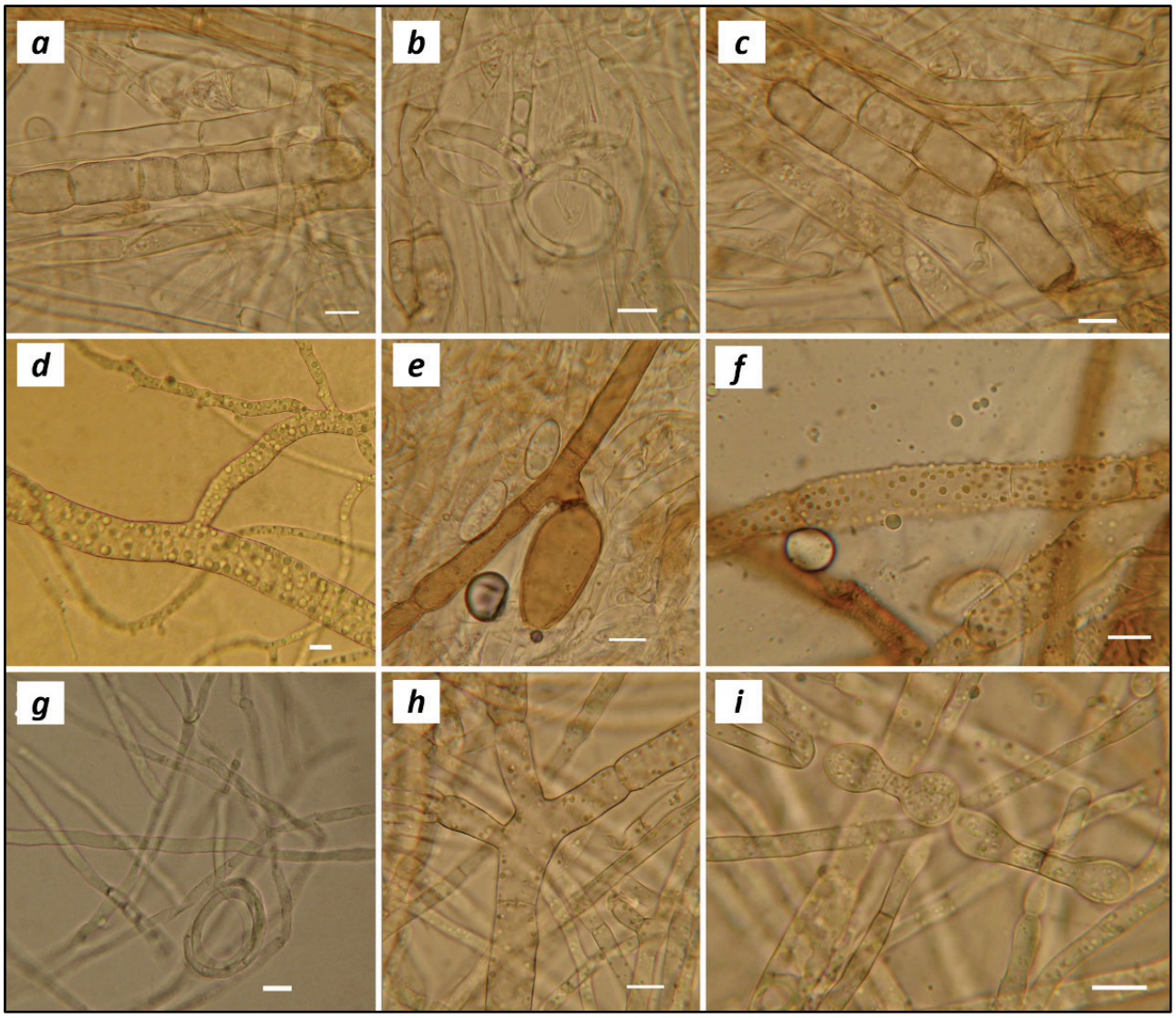

Fig. 3. Hyphal morphology of *A. maurilabra*: *a* – hyaline hyphae and arthrospores on CDA; *b* – hyphal loops on CDA; *c* – larger arthrospores on CDA; *d* – hyaline hyphae with oil drops on PDA; *e* – pigmented thick-walled hyphae with gigantic lateral ellipsoid chlamydospore and two ascospores on PDA; *f* – pigmented hyphae with oil drops on PDA; *g* – hyphal loops on PDA; *h* – branched hyphae with oil drops on BWA; *i* – hyphae turning into chlamydospores on BWA (*a–d*, *g–i*: tap water (H_2O), *e*,*f*: Congo Red aqueous solution (CR); the scale bar is 10 µm

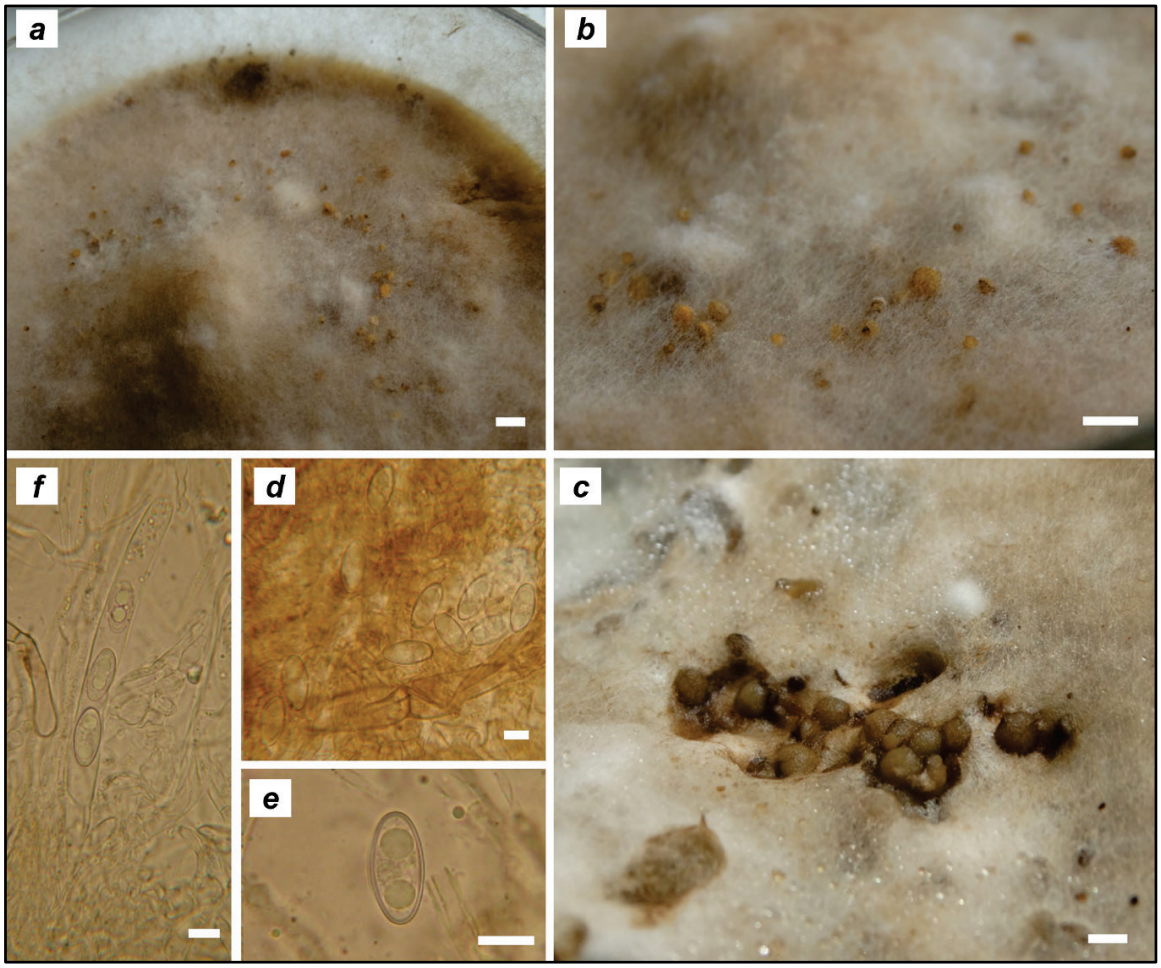

Fig. 4. Sexual stage of *A. maurilabra* on PDA: *a*, *b* – young apothecia, *c* – apothecia at later stages of culture development (20th day of cultivation); *d* – ascospores (Congo Red); *e* – separate ascospore (H_2O); *f* – ascus with ascospores (H_2O); the scale bar is 5 mm in *a*, *b*, *c* and 20 μm in *d*, *e*, *f*

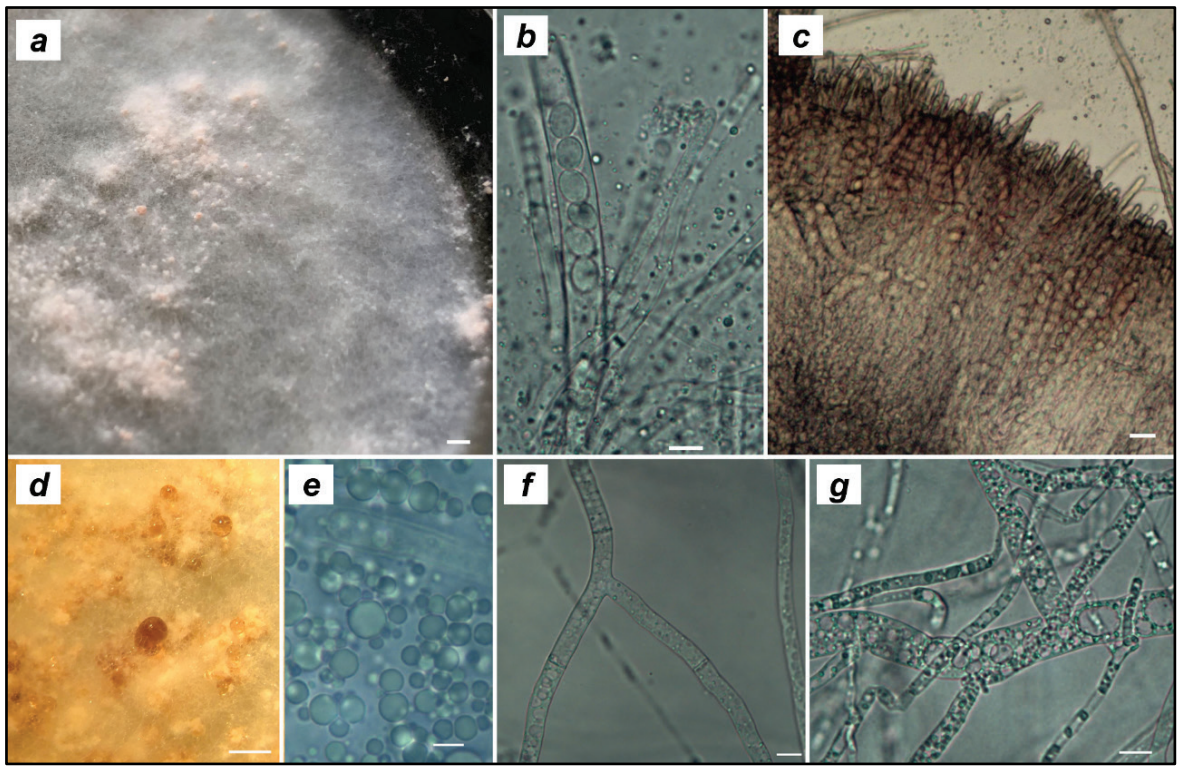

Fig. 5. Mycelial structures of *P. domesticum*: *a* – colony view (sexual stage), 7th day after inoculation with numerous apothecia on PDA; *b* – ascus and paraphyses (H_2O); *c* – ascohymenial layer (MLZ); *d* – sclerotia and lipid globules on PDA; *e* – sclerotia under optical microscope; *f* – branched hyaline hyphae with oil drops on BWA; *g* – vacuolated hyphae with oil drops on CDA; the scale bar is 3 mm in *a*, *d* and 10 μm in *b*, *c*, *e*, *f*, *g*

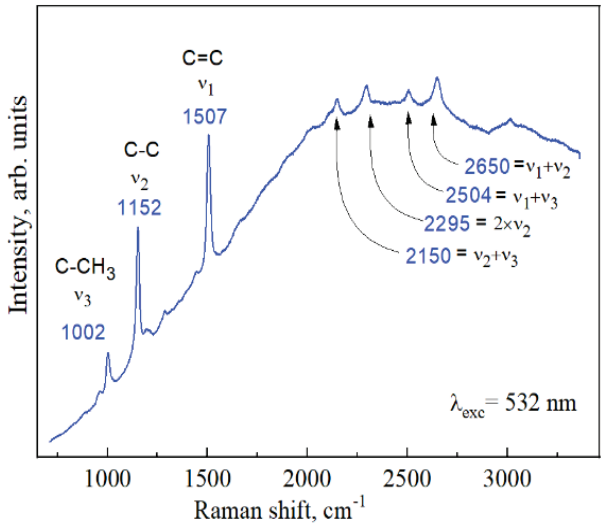

Fig. 6. Raman spectrum of the *P. domesticum* sclerotium content, recorded with 532 nm, with the frequency and assignments of the main vibrational peaks indicated above each peak (see Discussion for details)

The traditional micromorphological analysis is still the most common and reliable method to study fungi due to its low cost, technical availability for use in all basic laboratories, and the ability to obtain results fairly quickly (Senanayake et al., 2020). The basic characteristics that can be used to determine the purity of the studied pyrophilous discomycetes and represent obtained relatively stable characteristics of the cultures are type of hyphae, the presence or absence of anastomoses, hyphal loops (or coiled hypha), chlamydospores, hyphal with swelling, crystals, incrusted hypha and reproductive structures (anamorphic and teleomorphic). Common to the mycelium of all studied species of fungi was presence of numerous drops of oil in the hyphae, anastomoses like *T. praecox*, a net-like structure similar to nematode capture hook like *A. maurilabra*. It is typical that fungal mycelium usually consists of radially expanded hyphal filaments interconnected by bridges formed through anastomoses. Anastomoses and hyphal loops play an important role in the distribution of nutrients as well as water or signaling molecules within the colony. The lipids of the studied fungi are likely to be of paramount importance as energy stores.

The growth speed on different media is one of the important characteristics of fungi that can be useful to optimize the protocol of their introduction and further for their industrial fermentation processes. Three nutrient media were analyzed for the species growth. All investigated species can quite easily grow under laboratory conditions on different tested nutrient media.

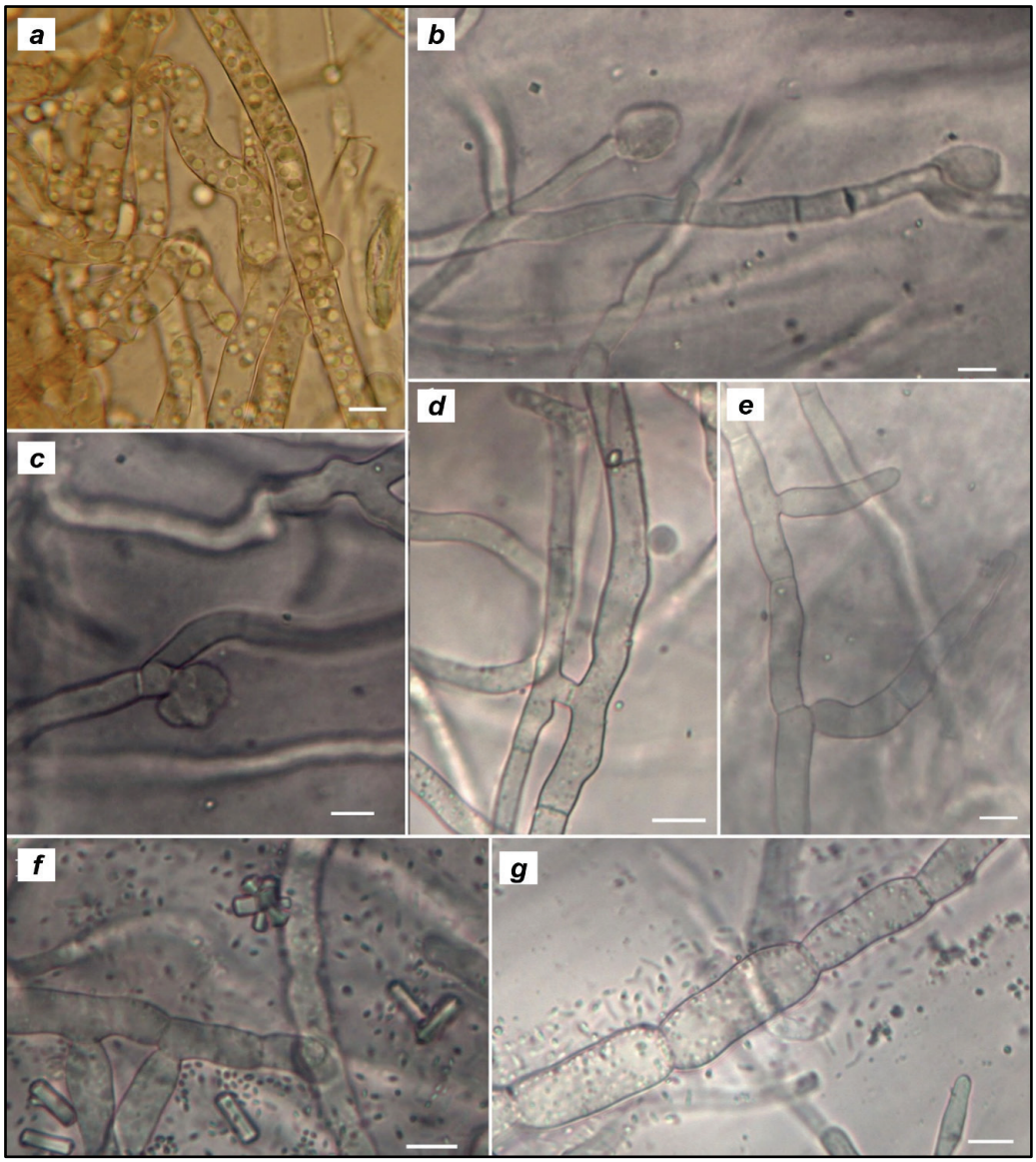

Fig. 7. Hyphal morphology of *T. praecox*: *a* – hyaline hyphae with oil drops on PDA (Congo Red); *b* – terminal nodules on PDA (H_2O), *c* – lateral nodules on PDA; *d* – anastomoses on PDA (H_2O), *e* – vegetative hyphae with right angled branching and septal positions on PDA (H_2O); *f* –crystallization on BWA (H_2O); *g* – chain of monilioid cells on CDA (H_2O); the scale bar is 20 μm in *a*, f and 10 μm in *b*, *c*, *d*, *e*, *g*

This fact indicates the adaptive nutrient abilities of these species despite their need to interact with post-fire soil chemistry in the life cycle in their natural habitat. The best growth of *A. maurilabra* and *P. domesticum* was obtained on PDA while *T. praecox* growth was much slower on all of the tested media. Thus, exactly *A. maurilabra* as well as *P. domesticum* could be of interest in terms of rapid growth for further biotechnological application. Our findings are in line with similar studies. PDA medium was also suitable for the growth of other *P. domesticum* strains (Roxon & Batra, 1973), and fungi closely related to our investigated fungus *A. muelleri* (Roxon & Batra, 1973), *T. mikolae* (Yang & Wilcox, 1984). However, other closely related species *T. tophiseda* and *T. japonica* were grown on Czapek yeast autolysate and on malt extract agar (Kušan et al., 2015). Of course, it is also necessary to take into account the type of medium that not only supported the growth of fungi but also represented all phenotypes of the colonies. So, an optimal nutrient medium for the growth of all fungi studied by us was PDA.

The growth of the studied species, as well as the morphology of their colonies, varied depending on the medium used, as the nutrient medium is one of the main factors that has such an impact. The media were differentially enriched by nutrients, such as inorganic or organic compounds, carbohydrates, nitrogen, C/N ratio, and metal compounds. Besides this, another key point that affected differences in the development of the mycelial growth and morphological characteristics of fungi colonies was the pH of the media, which was almost the same in BWA (pH 5.8) and PDA (pH 6.0), but higher in CDA (pH 7.3).

Different fungal species occupy various ecological niches of habitats, consequently they require diverse conditions for optimal growth. It is known that *A. maurilabra* occurs exclusively on burnt sites as early as 7 weeks after burning, but does not fruit later than 80 weeks after burning (Dix & Webster, 1995; Dougoud, 2001). *Pyronema* species occur in burnt areas worldwide (Petersen, 1970; Dougoud, 2001) and produce apothecia fairly early after fires (Moore & Korf, 1963; Bruns et al., 2020; Hughes et al., 2020). *T. praecox* occurs on limestone and burnt ground and fruits abundantly only after a fire event (Yang & Korf, 1985b; Dougoud, 2001; Raudabaugh et al., 2020) reported that this species always associates with burnt sites, probably mostly in the first or second year after a fire. *P. domesticum* was able to form sclerotia on CDA and apothecia on PDA quite quickly in our experimental conditions, likewise the asexual stage of *A. maurilabra* was noted on CDA and apothecia – only on PDA medium (after 20th day of cultivation). To the best of our knowledge, this is the first report of fruiting bodies of *A. maurilabra* in culture. However, development of apothecia was reported for closely related fungus *A. muelleri* in about 14 days of growth on rabbit food agar (Roxon & Batra, 1973). Other fungi from this genus such as *A. melaloma* can also grow and fruit readily in pure culture (Rosinski, 1956). As already mentioned above, detailed description of apothecia and sclerotia formation by *P. domesticum* has been previously performed in various in vitro studies. Our observation of confluent apothecial pads and sclerotia formation of *P. domesticum* on PDA medium is in line with other studies (Roxon & Batra, 1973; Moore-Landeckerand, 1987). Besides, development of *P. domesticum* sclerotia depending on nutrient media is also in agreement with a report by Moore-Landeckerand (1987). The production of fruiting bodies in a short time in culture is an important criterion for assessing the purity of fungal growth, and at the same time contributes to a faster search for potential ways to use them in biotechnology.

In a recent work by Fischer et al. (2021) *P. domesticum* metabolism and mineralization of aromatic compounds were investigated. The robust potential for *P. domesticum* to liberate carbon from pyrolyzed organic matter in post-fire ecosystems and return it to the bioavailable carbon pool was indicated. These results also confirm the importance and necessity for further research on *P. domesticum* biology *in vitro*.

Different natural pigments have important applications in various areas of modern industry, agriculture, ecology, medicine etc. (Sajid & Akbar, 2018). Pezizomycetes are a promising group of fungi capable of synthesizing acyclic and monocyclic structures, among which carotenoids occupy a special place. Thus, carotenoids have recently been studied as one of the classes of lipids considered in chemotaxonomy, since 60% of the studied fungi contain them (Verscheure et al., 2002). In particular, the production of carotenoids is a synapomorphic trait characterizing a monophyletic group Pyronemataceae (Hansen et al., 2013) and can be an additional and useful criterion for species identification, since classical morphological studies of Pyronemataceae fungi have not always been satisfactory for this group, as many genera are heterogeneous. The accumulation of carotenoids in the lipid globules of sclerotia of *P. domesticum* was found, which corresponds to the data for other fungi of the Pyronemataceae family (Hansen et al., 2013). In addition, the content of γ-carotene and its derivatives – neurosporaxanthin and its methyl ester is considered as an important taxonomic parameter in the taxonomy of the coprophilous fungus *Iodophanus carneus* (Pers.) Korf (Valadon et al., 1980).

Additional information about sclerotia content was obtained from Raman spectra measured in different parts of the sample of *P. domesticum*. The spectra exhibited a distinct vibrational pattern dominated by three peaks – at about 1000, 1150 and 1500 cm^{-1} (Fig. 6) – characteristic of carotenoids (Reszczynska et al., 2015; Osterrothová et al., 2019; Dzhagan et al., 2021). No Raman study of Pyronemataceae has been reported so far. The only work that reports carotenoid composition of *Pyronema confluens* Tul. & C. Tul. is the early short communication by Carlile & Friend (1956). The authors concluded from chromatographic analysis that β- and γ-carotene, torulene and its cis-isomer, probably neotorulene, as well as the fifth unidentified carotenoid are contained in the apothecia of *P. confluens*. The exact position of the main peaks in our Raman spectrum of *P. domesticum*, particularly $\nu_1 = 1507\ cm^{-1}$, $\nu_2 = 1152\ cm^{-1}$, and $\nu_3 = 1002\ cm^{-1}$, do not match the set of values typical for β- and γ-carotene. In particular, the ν_1 mode, which is most sensitive to the length of the carbon double bonds (C=C) length, usually is observed in the range of 1516 to 1532 cm^{-1} (Oliveira et al., 2010). Therefore, the spectrum we observed can be explained in two ways: (i) it belongs to another carotenoid than the most known carotene family, and/or (ii) it is the spectrum of β-/γ-carotene but the ν_1 vibration frequency is affected by a (strong) coupling to other (bio)molecules in the fungus. Although the Raman spectrum of torulene, the second sort of carotenoids suggested to exist in *P. confluens* (Carlile & Friend, 1956), is not known yet, the larger number of C=C bonds in its chain (13) compared to that of the β-/γ-carotene (11) could be a reason for the observed lower ν_1 value (Merlin, 1985). In the case (ii), a strong bonding of the carotene molecule to peptide, for instance, can be assumed. A good result of such effect is binding of astaxanthin to protein in β-crustacyanin, which shifts this mode from 1523 to 1492 cm^{-1} (Weesie et al., 1999; Reszczynska et al., 2015). It is unlikely that (i) and (ii) occur simultaneously, because the width of the ν_1 peak is not larger than that of the other key modes, ν_2 and ν_3, and thus does not assume more than one vibration to contribute to it. In view of the scarcity of the data on carotenoid composition in *P. confluens* and in the whole Pyronemaceae family, a more detailed investigation is to be undertaken by means of Raman spectroscopy.

Conclusion

The current database of nutritional requirements of pyrophilous discomycetes *in vitro* is a limiting factor in their culturing. It was with the view to a better understanding of particulars of cultural, micro- and macromorphological characteristics of vegetative mycelium of *Anthracobia maurilabra*, *Pyronema domesticum* and *Tricharina praecox* that the present study was undertaken. Radial growth of studied fungal species (from 6.1 to 52.8 mm/day) as well as their colony morphology varied depending on the used media: beer wort agar (BWA), potato-dextrose agar (PDA) and Czapek-Dok agar (CDA). PDA was the most suitable medium for the enhancement of radial growth and the best expresses all the phenotypes of the colony. Macro- and micromorphological characteristics of the mycelium described here can be useful for solving issues of taxonomic affiliation of fungal species as well as for pure quality control of mycelial cultures during their introduction, preservation, and fermentation. *A. maurilabra* and *P. domesticum* could be of interest in terms of rapid growth for further biotechnological application. Our results demonstrate that Raman spectroscopy can be a useful approach to the determination of some pigments. The frequency position of the main Raman peaks does not match those typical for β- and γ-carotene, therefore, the frequency is affected by the binding of carotene with other molecules in the fungus or these peaks belong to other carotenoids; these issues need further investigation.

The established species-specific peculiarities of *A. maurilabra*, *P. domesticum* and *T. praecox* in culture are helpful for better understanding of the pyrophilous discomycetes as a whole.

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