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CULTURE DESCRIPTION OF SOME SPONTANEOUS LIGNICOLOUS MACROMYCETES SPECIES

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Abstract: 24 species of lignicolous macromycetes from 4 taxonomic families and 2 orders, Class Agaricomycetes, Phyllum Basidiomycota, have been analyzed. The cultural characters of these isolates had been observed, some of them being little studied till now. The dikaryotic mycelium from the trama of the sporoms was used for the isolation purpose. The fungal isolates were cultivated onto malt extract-agar media (malt extract 20g l^{-1}) and incubated at 25 °C, in the dark, for 6 weeks. The cultures were observed directly and using a Nikon stereomicroscope in order to measure the growth rhythm and to observe the changes of the colonies: edge, surface, reverse, shape, colour, smell, presence or absence of the exudates. After 6 weeks from the inoculation, microscopic slides were made in order to investigate the types of hyphae, the colour and the structure of the mycelium and to note the presence of particular elements: cuticle, chlamydospors, arthrospores, conidia, and basidia. We noticed that the analyzed species present similar characters but also significant differences between them.

Keywords: lignicolous macromycetes, fungal growth, cultural characters

Introduction

Lignicolous macromycetes represent a diversified group of fungi in terms of ecological and morphological aspect but it also represents a heterogeneous taxonomic group. The traditional methods of identifying the lignicolous macromycetes based on collecting and analyzing the fruit bodies present the disadvantage of not being able to identify the macromycetes that do not form fruit bodies (due to local ecological and climatic conditions) or those with degraded fruit bodies. The isolation of lignicolous macromycetes in pure cultures and the analysis of the macroscopical and microscopical features of the mycelium grown *in vitro* offer the possibility to identify species without using fruit bodies (when the fungi are isolated from substratum) and offer, also, valuable resources to taxonomic studies.

The cultural features of some isolated lignicolous macromycetes have been studied by different authors [NOBLES, 1948; STALPERS, 1978, 1993]. Different authors [BAKSHI & al. 1969, 1970; NIEMELÄ, 1975, 1977] have realized ample studies on genera *Fomes*, *Phellinus* and *Trametes*. The lignicolous macromycetes present different characters *in vitro*, they can sometimes form fruit bodies, specialised structures for asexual reproduction, exudates, but their presence is not prerequisite for all the species/ isolates. The basidiomycetes may present hyphae with clamp connection, formed from the

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dikaryotic cells and avoid the septum for connecting with the proximal cell [TĂNASE & ŞESAN, 2006]. The presence of the clamp connection is a distinctive feature.

Many lignicolous macromycetes present economical importance, not only as agents of depreciation of the wood but also as agents that can be used in mycoremediation strategies, due to the presence of ligninolytic enzyme system involved in xenobiotics degradation. Studying the macromycetes grown *in vitro* is very important to the elaboration of mycoremediation strategies and the optimization of culture conditions. Some of the analyzed species from this paper have been little studied until now.

Materials and methods

Fungal strains and isolation procedures

All the tested strains were isolated using fruit bodies collected from deciduous woods in different stages of decay found in forest habitats in north-eastern Romania. The isolation process occurred under sterile conditions using the context mycelium of sporoms and the pure isolates thus obtained have been maintained by sub culturing them onto malt-extract media and stored in the dark at a temperature of 4 °C. The identification of the selected species was performed using classical macroscopic and microscopic methods according to the literature [BERNICCHIA, 2005; ERIKSSON & RYVARDEN, 1976; HANSEN & KNUDSEN, 1992, 1997; RYVARDEN & GILBERTSON, 1993, 1994; SĂLĂGEANU & SĂLĂGEANU, 1985; JÜLICH & STALPERS, 1980], and the specimens were lyophilized (UniEquip lyophilizator, UNICRYO MIC 4 L model, Planegg, Germany) or dehydrated (using a dryer, Ezidri Ultra 1000 FD) and then deposited in the Faculty of Biology Herbarium [I], "Alexandru Ioan Cuza" University of Iaşi, Romania. All the tested strains and the corresponding herbarium number are listed in Tab. 1. The nomenclature used in this paper is according to the Species Fungorum database.

Culture conditions

In order to analyse the cultural characters it has been used the method established by STALPERS (1978). Consequently, the 9 cm diameter Petri dishes filled with 25 ml MEA (20 g malt extract, 15 g agar, distilled water – 1000 ml) have been employed for the purpose. All the media have been sterilized by autoclaving at 120 ºC in a 75 liters upright model autoclave (Raypas, Barcelona, Spain). Three replicates have been made for all the samples. The pH of media was adjusted with 0,1 M hydrochloric acid at the value 5.0. An electronic pH/ion-meter (model INOLAB, WTW, Weilheim, Germany) has been implied in the procedure. All the plates have been inoculated with small plugs of mycelium, placed at 1.5 cm from the edge of the plate and then incubated in the dark at 25 ºC, for 14 days, in an automated aeration incubator (Microbiotest, Gent, Belgium).

Cultural characters analysis

The cultures have been analyzed weekly in order to measure the growth rhythm and to observe the macroscopic changes of the colony, with the naked eye and with a stereomicroscope at 15-30 x magnification (stereomicroscope with phototube SZM2 Optika). After 6 weeks from the inoculation, microscopic observations have been made paying attention to the features / characters and type of the hyphal system from the advancing zone, the submerged and aerial mycelium. There have been studied: the type of

the hyphae, their colour and aspect; presence / absence of the crystals on the hyphae's surface; the diameters of hyphae; the presence, form and dimension of conidia, chlamydospores, cuticular cells; the formation *in vitro* of fruit bodies and their characters; the presence of other particular structures, of exudates etc.

The measuring of microscopic structures has been realized at magnification of 1000x with a trinocular microscope (Optika). For testing the amyloidal, dextrinoid or cyanophilic character of some microscopic structures there were used: Lugol solution, Melzer's reagent and Methylene blue. In order to verify whether some microscopic structures change the colour or swell, a solution of KOH 5% was used.

Results and discussion

The characters of some isolates of lignicolous basidiomycetes from four families and two orders (Tab. 1), included in Class Agaricomycetes, Subclass Agaricomycotina, Phyllum Basidiomycota have been analyzed in the present study. An accelerated growth rhythm was observed for most of the isolates and a moderate one for those from Hymenochaetaceae family. Some isolates presented a slow growth: *Merulius tremellosus*, *Postia caesia* and *P. stiptica.*

The fungal isolates studied presented in culture characters common to the genera or the families but also significant differences. Some cultures distinguished by the presence of asexual reproduction structures (arthrospores, chlamydospores) but also by forming some primordia of fruit bodies or even mature fruit bodies (*Skeletocutis alutacea*). The colour of the mycelium, the general aspect of the colony, the types of hyphae from the mycelium, the presence / absence of cuticle, the crystals in media or on the hyphae's surface and the presence / absence of the clamp connections have been factors of differentiation (Tab. 2).

The species from Hymenochaetaceae family dinstinguished by the presence of some strongly pigmented hyphae and the lack of the clamp connections whereas the species from Polyporaceae family distinguished by forming primordia and fruit bodies.

Macroscopic aspects and microscopic characters of mycelium grown on nutritive media

Bjerkandera adusta (Willd.) P. Karst. Mycelium is homogeneous, appressed, more lax in the centre, with white aerial hyphae, arranged radially. On the edge the mycelium forms a thick ring, felty, compact, cream, powdery, climbing on the wall plate. Some areas of the ring formed soft scabs, with shiny areas, sometimes confluent. Hyphal system is monomitic. Hyphae are thin, of 1.5-2.5 µm, sometimes with thickened segments of up to 5 µm, tangled and branched, with thick septa, hyaline. Presents clamp connections. The submerged mycelium and from the advancing zone have thin hyphae, sometimes thick, up to 7 µm diameter, with clamp connections. Other authors [BAKSHI & al. 1969] have observed the formation of chlamydospores in culture, but in our cultures chlamydospores were not found.

Bjerkandera fumosa (Pers.) P. Karst. Mycelium is irregular, loose in the centre, with rare aerial hyphae, appressed, translucent and slightly powdery. Mycelial mat becomes

dense and felty on the edge, forming a thick mycelial ring that continues on the plate wall, with compact. Aerial mycelium is white to cream. Primordia appear after four weeks, and they form mycelial cords to the edges. Mycelium presents: generative hyphae, hyaline, with septa and clamp connections, branched, both in the advancing zone and submerged or aerial mycelium; skeletal hyphae, of 5 mm diameter, with simple septa. The submerged mycelium has thin hyphae, with numerous lateral branches, finger-like.

Coriolopsis gallica (Fr.) Ryvarden. Colony shows different zones: an area with appressed mycelium, thin, translucent near the point of inoculation and an area with dense mycelium and interwoven aerial hyphae, powdery-white or cottony creamy, shaggy, matted later, yellow-brown with erect air cords, 20-40 mm high, on walls (Fig. 1A). The mycelium has generative hyphae, hyaline and skeletal hyphae, long, slightly pigmented (yellow-brown, beige) rarely branched or with septa, from 1.5 to 4 μm in diameter. Some hyphae are thicker than 6 μm in diameter (skeletal) but without septa, hyaline and branched, with narrow lumina. At the edge of the colony hyphae are long, sinuous, unbranched or rarely branched, without septa, cream, up to 4 μm in diameter, numerous, often grouped in bundles, sometimes with crystals, rarely dilated.

Daedalea quercina (L.) Pers. Mycelium is white, cottony, with slightly different zones, radially arranged in bundles, fuzzy-felty, forms uneven clumps, rhizomorphic or cottony, white to cream, which rises on the walls forming hirsute areas with densely intertwined hyphae. The mycelium presents generative hyphae, branched, with frequent clamp connections of 1.5 to 4.5 μm in diameter, the aerial hyphae are up to 6 μm thick. Skeletal hyphae are present in the aerial mycelium, hyaline, rarely branched, without septa, 2-3 μm in diameter. It was reported the formation of mature fruit bodies with basidia and basidiospores in the case of other isolated [NOBLES, 1948].

Daedaleopsis tricolor (Bull.) Bondartsev & Singer. Mycelium is felty, attenuated, white in the centre, and then forms an irregular crust, thick, brown, cottony areas alternating with smooth areas with different shades from light to dark brown with bumps, appressed scabs, thin felty zones with shades of brown, gray and even white felty crusts (Fig. 1B). On the plate wall the mycelium forms pigmented primordia. The hyphal system is trimitic. The advancing zone and the submerged mycelium have generative hyphae, branched, thin to moderately thick, with thick septa and clamp connections, hyaline. Aerial mycelium present, also, brown skeletal hyphae and thick cuticular cells.

Fomes fomentarius (L.) J. Kickx f. The advancing zone is straight with the aerial mycelium uniform and then appressed. Mycelium is apressed in the proximity of the inoculation point, felty, later forms white crusts with appearance of elongated spots, arranged radially, confluent, powdery-soft, compact, cream-beige-yellow to cream-brown. Mycelium shows generative hyphae, hyaline, with swollen septa of 1.5-3.0 (4.0) μm diameter and thick-walled skeletal hyphae, refringent, hyaline to brown, with narrow lumina, sometimes branched without septa of 1.5 to 3.0 μm in diameter, closely intertwined with cuticular cells forming pseudoparenchimatic layers. Hyphae do not have clamp connections. Isolates tested produced rare chlamydospores (Tab. 2), unlike the isolates evaluated in other studies [NOBLES, 1948].

Fomitopsis pinicola (Sw.) P. Karst. Mycelium is felty and smooth in the peripheral zone, white. In the central area forms a white and cottony mat, with dense

agglomerations spot like. Near the point of inoculation forms a very thick mat, compact, smooth, white, with protrusions (primordia of fruit bodies). The hyphal system is trimitic. In the advancing and the submerged mycelium there are generative hyphae, branched, with numerous septa and clamp connections, hyaline, diameter 1.5 to 3 µm. The aerial mycelium presents generative hyphae; skeletal hyphae, unbranched, without septa and by 1.5 to 4 µm diameters, long, sinuous and fibber hyphae, hyaline, branched, without septa, up to 4 µm in diameter. Some authors [NOBLES, 1948; STALPERS, 1987] reported the presence of chlamydospors, but our studied isolates did not produced.

Inonotus hispidus (Bull.) P. Karst. Mycelium presents concentric zones, waxy crusts, smooth, coloured in various shades of yellow to brown and concentric rings with aerial hyphae (Fig. 1C). The crusts are dark brown and present few aerial hyphae. Aerial mycelium areas are powdery and present yellow or yellow-brown hyphae, erect, short, sometimes with felty or powdery areas. Colony edge is straight. Submerged mycelium and from the advancing zone presents generative hyphae, long, simple-septate, rarely branched, from 2.5 to 5 µm diameter, hyaline. Aerial mycelium present generative hyphae and skeletal hyphae, 4-5 µm thick, straight, frequently branched, with simple septa, yellow, sometimes thin and sinuous. Connection hyphae and cuticular cells are also present.

Irpex lacteus (Fr.) Fr. Mycelium is lax, appressed with radially arrangement, mostly translucent with veins and white cords, especially in the periphery. It can be observed felty-cottony crusts, white, sometimes with small clusters of hyphae. Hyphal system is monomitic. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with rare septa, 4.5 to 6 µm diameter, with clamp connections. The aerial mycelium presents generative hyphae and fibber hyphae, 1.5 to 2 µm diameter, hyaline, rarely branched, with simple septa. The tested isolate produced arthrospors unlike isolates tested in other experiments [NAKASONE, 1990].

Lenzites betulina (L.) Fr. Colony forms a very thick and dense mycelial mat, white, with smooth edges, cottony in the middle, ± smooth, silky at the edge (Fig. 1D). A soft cottony mycelial ring is formed on the walls, 0.5 cm thick, 1 cm high, with denticulate edge, sometimes cream. Hyphal system is dimitic. Submerged mycelium and from the advancing zone presents generative hyphae, branched, septate, with clamp connections, hyaline, of 2.2 to 4.5 µm in diameter. Aerial mycelium present generative hyphae and skeletal hyphae very numerous, hyaline, thick refringenţ walls and with narrow lumina, without septa, occasionally branched, of 1.5-3 (4) µm diameter.

Merulius tremellosus Schrad. Mycelium is irregular, waxy, with dark green hyphae, radially arranged. Mycelium is appressed in the rest of the colony, waxy-levurian, translucent. Near the point of inoculation, mycelium is denser, forming a waxy broad crust, dark-green. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, moderately branched, with thin wall which thickens with time, 2-5 (6) µm diameter. Aerial mycelium presents generative hyphae of 1.5 to 6 µm diameter, hyaline, moderately branched, with simple septa, frequently encrusted with numerous irregularly shaped crystals, some hyphae were opaque yellow.

Phellinus conchatus (Pers.) Quél. Mycelium presents concentric zones, with abundant aerial hyphae, cottony, orange-gray to cream-brown. Forms brown crusts around the edges, and a brown ring with numerous aerial hyphae, high, tangled, white, yellow or

orange-cream to brown. Mycelium mat is felty, thin near inoculum, but very heavy, felty-cottony, orange on the edges. The crusts are powdery, sometimes with small clusters of aerial hyphae. Submerged mycelium and from the advancing zone presents generative hyphae, thin, branched, with simple septa and rare, 2.5 to 4 μm thick, long. Aerial mycelium presents: generative hyphae and thick skeletal hyphae, with few septa, pigmented, sometimes inlaid.

Phellinus igniarius (L.) Quél. Mycelium mat presents concentric zones, with aerial brown-yellow-orange hyphae, cottony, lighter in the middle, forming brown crusts in the opposite side and denser and thicker networks on the edge. Aerial hyphae are easily felty, shorter, more frequent and longer in the sides. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, from 1.5 to 6 μm in diameter. Aerial mycelium presents generative hyphae and also skeletal hyphae with walls thickened, yellow-gray, rarely with septa, 1-3 μm in diameter. Chlamydospores were observed, but other authors [NIEMELÄ, 1975; NOBLES, 1948] showed no chlamydospores production for this species.

Phellinus pomaceus (Pers.) Maire. Mycelium forms a dense mycelial network, very thick, felty-cottony, tangled, velvety-brown, yellow-brown to cream-gray-brown. Near the point of inoculation, mycelial network is thin and smooth, then forms higher areas, compact, irregular, cottony or felty, darker, sometimes with gray or cream hyphae. For edges, colony forms primordia (Fig. 1E). Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, thin-walled, frequently branched, of 1,5-4,5 (6) μm diameter. Aerial mycelium presents generative hyphae and pigmented skeletal hyphae, thick walled, brown, rarely branched, without septa, of 1.5-3.5 μm diameter.

Polyporus arcularius (Batsch) Fr. Mycelium forms a waxy crust, brown, with a concentric wall-like mat, brown and wrinkled surface on external side and white-cottony on the inner side. The central wall-like cord is the highest (Fig. 1F). In the centre, mycelium is white and cottony and at the edges is appressed or with hyphal clusters, felty, white or cream. Aerial mycelium is trimitic and consists of highly branched generative hyphae with clamp connection, 2-4 μm thick; skeletal hyphae, thin, simple branched, septa without clamp connections, about 2 μm thickness and cuticular cells, up to 7 μm thick, amyloid, numerous.

Postia caesia (Schrad.) P. Karst. Mycelium is willing radially, translucent and colony edge is straight. Mycelium mat is appressed, thin and has several hyphal clusters and mycelial cords, small, white. Medium is deepened in the area covered by the colony. At the periphery, the mycelium is cream, arranged in the form of fine cords, radially branched. Mycelium is monomitic and consists of the generative hyphae, tree-like branched, with clamp connection, hyaline, up to 5 mm thick. Some lateral or terminal branches have crystals on their surface. Terminal branches are thin of 1-2.5 μm. Submerged hyphae are hyaline, with swellings.

Postia stiptica (Pers.) Jülich. Mycelium is appressed, only with submerged hyphae at the edge of the colony, translucent. Aerial mat is white, thin, with short hyphae. The medium is strongly deepened near the point of inoculation and faded. In this area develops a compact hyphal cluster, white, cottony. Mycelium is monomitic with generative hyphae,

with large clamp connection, of 1.5 to 3.5 μm thick. Aerial mycelium consists of generative hyphae, with clamp connection and many short side branches. Some hyphae have swollen septa or numerous blisters on their surface. The submerged mycelium has numerous and large irregular crystals.

Royoporus badius (Pers.) A.B. De. Mycelium mat forms dense networks, velvety, cottony, and thick near the point of inoculation and near wall plate, cream-brown or brown. From the center to the periphery forms thick crusts, soft, brown, which become thinner and less pigmented to edge, with wrinkles and projections. They are bounded to the centre by a white cord, thin and felty. In the centre, mycelial mat is thin and translucent. Submerged mycelium and from the advancing zone presents generative hyphae, strongly branched, 2-4 μm thick, with simple septa. Terminal branching of the submerged hyphae are tree-like. In the aerial mycelium are also found skeletal hyphae, 4-4.5 μm thick, curved or straight.

Skeletocutis alutacea (J. Lowe) Jean Keller. Mycelium mat presents concentric zones, is thin near inoculum, translucent, or with very fine mycelial cords, radially arranged. In the opposite side mycelium is whitish and easily felty. The two areas are separated by a mycelial ring that shows fruit bodies, shaped like thick crusts, compact, pored, or even bumps up to 1 cm high, with irregular angular pores, 2-4 (5) / mm, tubes long by 0.5 to 0.7 mm (Fig. 2A-D). Fruit bodies are cream-yellow, with light gray or greenish colour and are surrounded by sterile areas, white, silky-cottony. Basidia are of 8-10-13 x 1-3 mm. Basidiospors are cylindrical to allantoid, thin, 4-5 x 1 μm, numerous, nonamyloid. Corresponding to the pores, below, on the medium surface, are formed stalactite-like structures, with thick hyphae, up to 10 μm, with simple septa or short clamp connections, branching, sometimes with swellings, hyaline in KOH, sometimes at the end with cylindrical hyphae, thick-tipped, straight, dextrinoid. Hyphal system is dimitic, with long generative hyphae, up to 5 μm thick, with many clamp connection of 2-3 μm thick, and thick skeletal hyphae, tree-like branched.

Trametes gibbosa (Pers.) Fr. Mycelium forms a dense network, white, felty, with tall aerial hyphae, strongly branched and with compact areas, velvety, powdery to glabrous, thick and globular, more numerous and larger next to walls, white-cream or cream-ochre. In these areas fruit bodies are formed. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, thin-walled, with frequent clamp connections, of 1.5 to 4 μm in diameter. Aerial mycelium presents generative hyphae and skeletal hyphae, thick-walled and with narrow lumina, unbranched, without septa, from 2.5 to 5 μm in diameter.

Trametes hirsuta (Wulfen) Lloyd. Mycelium is appressed near the point of inoculation, felty in the opposite side and forms a mycelial ring very thick and cottony near the plate wall, compact white to cream-coloured. The mat of the ring touches the upper plate and forms many large fruit bodies, compact, soft and cottony, uneven, often globular, with protuberances up to 2 x 1.5 x 1.2 cm and many hyphal clusters (Fig. 2E-F). The fruit bodies have not matured and have not developed basidia or basidiospores, unlike isolates analyzed by other authors [BAKSHI & al. 1969; Noble, 1948]. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with clamp connections, of 1.8-4.3 μm diameter. Aerial mycelium consists of branched generative hyphae, hyaline with numerous septa and clamp connections, and skeletal hyphae, thin, long, hyaline,

unbranched, of 1.5-3 μm thick. Fruit bodies are made up of fibber hyphae, short, thick, heavily branched and generative hyphae.

Trametes pubescens (Schumach.) Pilát. Aerial mycelium is soft-felty in the peripheral zone, matted. In the median zone is slightly appressed and thin. Near the inoculum the mycelial mat has long aerial hyphae, highly branched, cream-gray. At the periphery, soft and powdery crusts or very cottony areas are formed and high and soft fruit body with powdery surface, creamy white or cream to gray. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with septa, 1.5 to 4.5 μm in diameter. Aerial mycelium consists of generative hyphae, thick, strongly branched and twisted, with clamp connection, from 3.5 to 6 μm in diameter and skeletal hyphae, thin, long, with few and simple septa, of 1.8 to 2, 2 μm thick.

Trametes suaveolens (L.) Fr. Mycelium is dense, felty-soft, smooth, white, with a thick mycelial ring at the periphery. Fruit bodies of 1-1.5 x 0.7 to 0.9 x 5 cm are formed near the plate wall. The fruit bodies are irregular, with large protrusions, creamy white, sometimes yellow shades. Mycelial mat is powdery. Submerged mycelium and from the advancing zone presents generative hyphae, thin, with septa, branched, with frequent clamp connections, hyaline, from 2.2 to 4.5 μm in diameter. Aerial mycelium presents generative hyphae of 1.5-3 μm diameter and numerous skeletal hyphae, rarely branched, thick-walled.

Trametes versicolour (L.) Lloyd. Mycelium is sparse and thin in the opposite side of inoculum, but in the median side of plate forms a soft and powdery crust, thin, creamy-white or beige. Near the inoculum point, the crust becomes very thick, soft or cottony, white, compact and develops primordia. The hyphal system is trimitic. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with septa and clamp connections, 3.0 to 4.5 μm diameter. Aerial mycelium presents generative hyphae, long, hyaline; skeletal hyphae, thin, hyaline, long, unbranched, without septa, from 1.5 to 4 μm thickness and fibber hyphae, branched, without septa.

Conclusions

The macroscopic and microscopic characters of the 24 lignicolous basidiomycetes isolates were analyzed during *in vitro* cultivation of the mycelium. The malt-extract media were favourable for growth and the species from genus *Postia* used agar in their own metabolism.

There have been observed characters common to the families and genera but also significant differences between them, such as the growth rhythm, the presence of specialized reproduction structures and the mycelium colour. Some isolates formed fruit bodies or primordia, arthrospores or chlamydospores. Some isolates from the Hymenochaetaceae and Polyporaceae families formed cuticle on the surface of the nutritive media.

The isolates from Meruliaceae şi Polyporaceae families presented accelerated growth rhythm, and those from genus *Postia* had the slowest growth rhythm.

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Tab. 1. The tested fungal isolates, their taxonomic position and the number of the specimens in the Herbarium

ORDER	FAMILY	SPECIES	SPECIMEN
Hymenochaetales	Hymenochaetaceae	*Inonotus hispidus* (Bull.) P. Karst.	[I 137383]
		Phellinus conchatus (Pers.) Quél.	[I 137368]
		Phellinus igniarius (L.) Quél.	[I 137381]
		Phellinus pomaceus (Pers.) Maire	[I 137373]
Polyporales	Meruliaceae	*Bjerkandera adusta* (Willd.) P. Karst.	[I 137350]
		Bjerkandera fumosa (Pers.) P. Karst.	[I 137364]
		Irpex lacteus (Fr.) Fr.	[I 137351]
		Merulius tremellosus Schrad.	[I 137398]
	Polyporaceae	*Coriolopsis gallica* (Fr.) Ryvarden	[I 137358]
		Daedaleopsis tricolor (Bull.) Bond. & Sing.	[I 137365]
		Fomes fomentarius (L.) Fr.	[I 137376]
		Lenzites betulina (L.) Fr.	[I 137355]
		Polyporus arcularius (Batsch.) Fr.	[I 137362]
		Royoporus badius (Pers.) A.B. De	[I 137385]
		Skeletocutis alutacea (J. Lowe) J. Kell.	[I 137386]
		Trametes gibbosa (Pers.) Fr.	[I 137354]
		Trametes hirsuta (Wulfen) Lloyd	[I 137356]
		Trametes pubescens (Schumach.) Pilát	[I 137357]
		Trametes suaveolens (L.) Fr.	[I 137359]
		Trametes versicolour (L.) Lloyd	[I 137363]
	Fomitopsidaceae	*Daedalea quercina* (L.) Pers.	[I 137394]
		Fomitopsis pinicola (Sw.) P. Karst.	[I 137396]
		Postia caesia (Schrad.) P. Karst.	[I 137400]
		Postia stiptica (Pers.) Jülich	[I 137401]

Tab. 2. The principal macroscopic and microscopic characters of fungal isolates cultivated on synthetic nutritive media

SPECIES	GROWTH RHYTHM*	EXUDATES, PRIMORDIA AND FRUIT BODIES	SMELL	REVERSE OF COLONY**	REPRODUCTIVE STRUCTURES	PARTICULAR STRUCTURES AND CRYSTALS
Bjerkandera adusta	2	colourless exudates	decomposed vegetation	white	arthrospores, 3 x 3-10 µm, with thick walls	
Bjerkandera fumosa	2	primordia	anise like	white	arthrospores, 2 x 3-10 (13) µm; chlamydospors pear-shaped, of 4 x 6 µm	swollen septa; prismatic crystals
Coriolopsis gallica	2		hay like	yellow		swollen hyphae; small crystals, scarce
Daedalea quercina	3	primordia, scarce	mushroomy	unchanged	chlamydospors, spherical, numerous	numerous prismatic crystals
Daedaleopsis tricolor	2	brown exudates; primordia	indistinct	white	grouped chlamydospors, of 4-8 µm diameter	red-brown cuticular cells
Fomes fomentarius	3		indistinct	white	pink chlamydospors	swollen septa; numerous cuticular cells, 25 µm diameter, nonamyloid
Fomitopsis pinicola	2	primordia	rotten wood	unchanged		
Inonotus hispidus	6		indistinct	brown	yellow chlamydospors, 7-8 x 4-5 µm	cuticular cells
Irpex lacteus	2		indistinct	unchanged	arthrospores	swollen septa, scarce
Lenzites betulina	2	colourless exudates; primordia	mushroomy (*Agaricus*)	white		
Merulius tremellosus	6		scented	unchanged	arthrospores, 2-3 x 4-5 µm	crystals on hyphal surface

Phellinus conchatus	3		indistinct	white or brown		prismatic crystals
Phellinus igniarius	4		indistinct	brown	yellow chlamydospors	brown cuticular cells
Phellinus pomaceus	3	colourless exudates	indistinct	brown		brown cuticular cells
Polyporus arcularius	2		green apple	white or pigmented		hyphae with swellings; cuticular cells, amiloyd
Postia caesia	> 6		indistinct	white		hyphae with spherical swellings, 10 µm diameter; octahedral crystals, sometimes irregular
Postia stiptica	> 6		indistinct	white		hyphae with lateral swellings and vesicles; swollen septa; octahedral crystals, 10 x 10 µm, numerous
Royoporus badius	5		scented	white	chlamydospors, 3 x 7-15 µm, numerous	finger-like lateral branches; pigmented cuticular cells, 25 x 15 µm; octahedral or irregular crystals, of 11 x 11 µm
Skeletocutis alutacea	2	fruit bodies; basidia, 8-13 x 3 µm; basidiospors allantoid to cylindrical, 4-5 x 1 µm, numerous	indistinct	unchanged		hyphae with swellings, 5-6 x 10-15 µm; large octahedral crystals

Trametes gibbosa	2	fruit bodies	rotten wood	white		
Trametes hirsuta	2	fruit bodies	indistinct	white		
Trametes pubescens	2	fruit bodies	indistinct	white		
Trametes suaveolens	2	colourless exudates; fruit bodies	anise like, strongly	white	chlamydospors, 7-15 x 4,5-6 µm, numerous, with thin walls	
Trametes versicolour	2	primordia	indistinct	white		cuticular cells, hyaline

* The needed time for covering the entire plate (in weeks)

** Only the old part of the colony is considered. The recently covered medium remain, often, unchanged

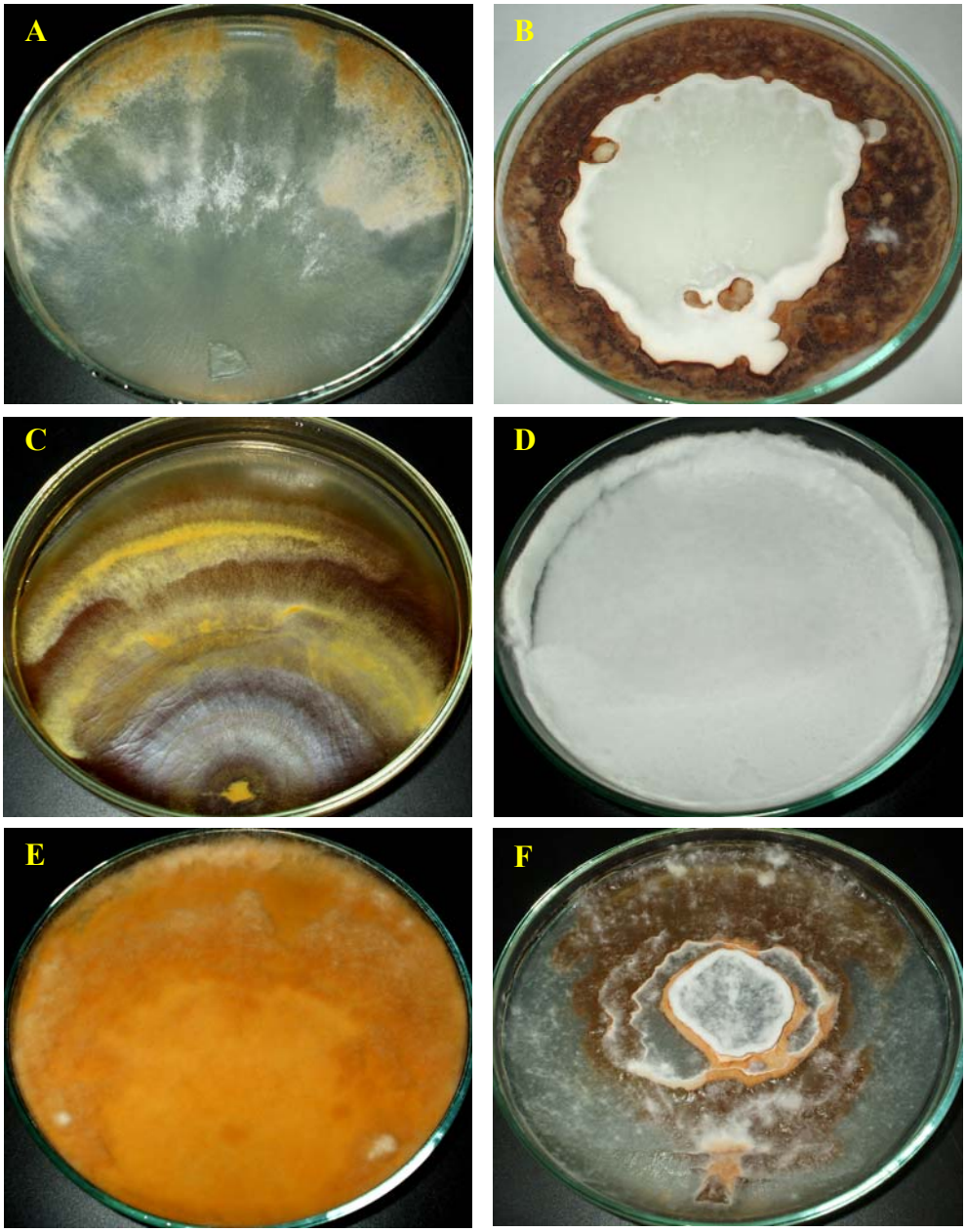

Fig. 1. General aspects of fungal colonies developed *in vitro*, in Petri dishes of 9 cm diameter: A - *Coriolopsis gallica*; B - *Daedaleopsis tricolour*, C - *Inonotus hispidus*; D - *Lenzites betulina*; E - *Phellinus pomaceus*; F - *Polyporus arcularius*.

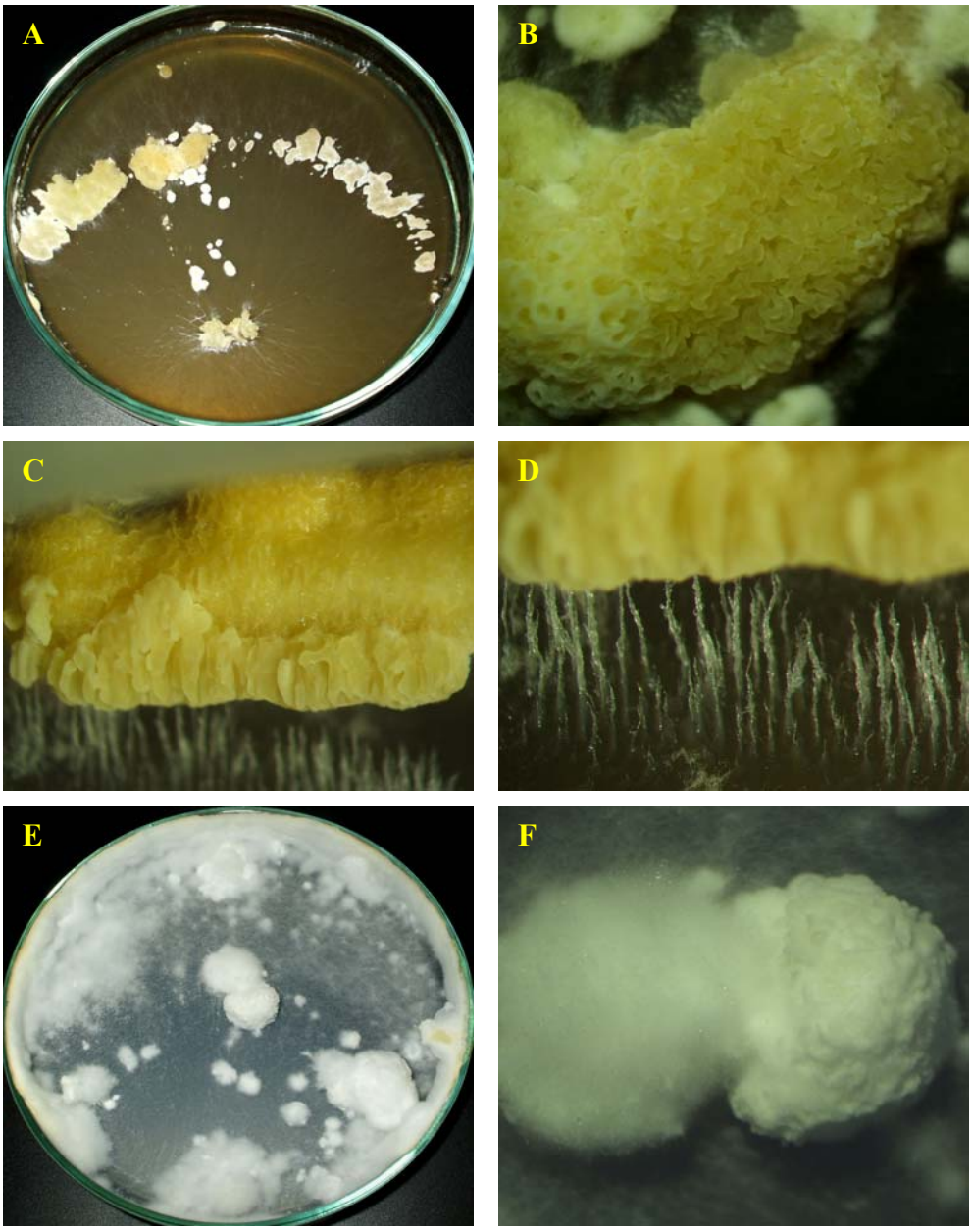

Fig. 2. *In vitro* development of fruit bodies, in Petri dishes of 9 cm diameter: A-D - *Skeletocutis alutacea*, general aspect (A) and details (B-D); E-F - *Trametes hirsuta*, general aspect (E) and detail (F).