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Macro-microscopic and qualitative enzymatic characterization of mycelial strains obtained from basidiocarps of *Mycena* species (Agaricales) in Chile

Caracterización macro-microscópica y enzimática cualitativa de cepas miceliales obtenidas de basidiocarpos de especies de *Mycena* (Agaricales) en Chile

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ABSTRACT

A macro-microscopic and enzymatic characterization of six mycelial strains of *Mycena*, from pure cultures of the corresponding basidiocarps is given. The studied strains were UACHMMa-464, UACHMMc-495, UACHMMg-250, UACHMMm-302, UACHMMp-354 and UACHMMr-260. Their isolation in pure culture, their growth in the culture media PDA, MEA, CzA, and some hyphal structures are discussed. A marked presence of enzymes involved in the degradation of the ligno-cellulose complex was detected. This could be an indication of a certain specificity or specialization in the degradative processes of these fungi in nature.

Key words: Mycelial strains of *Mycena*, pure culture, morphology, enzymes, Chile.

RESUMEN

En el presente trabajo se entrega una caracterización macro-microscópica y enzimática de seis cepas miceliales de *Mycena* obtenidas en cultivo puro a partir de basidiocarpos. Las cepas estudiadas son: UACHMMa-464, UACHMMc-495, UACHMMg-250, UACHMMm-302, UACHMMp-354 y UACHMMr-260. Se discuten su aislamiento en cultivo puro, su crecimiento sobre los medios de cultivo PDA, AEM, CzA y algunas estructuras hifales. Se detectó una marcada presencia de enzimas implicadas en la degradación del complejo ligno-celulosa. Esto podría indicar cierta especificidad en los procesos degradativos desarrollados por estos hongos en la naturaleza.

Palabras clave: Cepas miceliales de *Mycena*, cultivo puro, morfología, enzimas, Chile.

INTRODUCTION

The Order Agaricales include fungi that develop putrefactive ephemeral fruit bodies, with sexual exogenous spores contained in basidia (Singer 1986). The Agarical genus *Mycena* (Pers.) Roussel includes about 200 species (Hawksworth et al. 1983), 62 of which together with 3 varieties have been cited for Chile (Garrido 1985, 1988). The taxonomy of the species of *Mycena* is based on macro-microscopic characteristics, chemical reactions and the ecology of the basidiocarps. Among the taxonomical studies that have used this traditional methodology in Chile, are Singer (1969) who described over 40 species, Garrido (1985) who authored the first Chilean taxonomical index, and more recently Valenzuela & Moreno (1995) who commented taxonomic and ecological aspects of 18 species of *Mycena* from Southern Chile.

The life cycle of Agaricales is dominated by its mycelial phase where morphological and biochemical processes develop. However, macro-microscopic and qualitative enzymatic characterization of the mycelial phase of the strains of *Mycena* are rather scarce.

This paper characterizes the morphology and biochemistry of six mycelial strains of

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Mycena, *M. albogrisea* Peck., *M. capillaripes Peck.*, *M. galericulata* (Scop.:Fr.) Gray, *M. metuloidifera* Sing., *M. patagonica* Sing. and *M. rubromarginata* (Fr. ex Fr.) Kummer, obtained from pure culture of basiodiocarps that fructified on different ligno-cellulosic substrates in central-south Chile.

MATERIAL AND METHODS

The mycelial strains UACHMMa-464, UACHMMc-495, UACHMMg-250, UACHMMm-302, UACHMMp-354 and UACHMMr-260 were obtained in pure culture from the plectenchyma of the basidiocarps which were superficially disinfected with ethanol 96%. Then, the basidiocarps were longitudinally cut between the pileus and stipe aseptically removing with pincers ten 0.3 cm^2 pseudotissue pieces. These pieces were cultivated in Petri dishes containing malt extract agar 2% (MEA) with penicillin (0.6 mg/ml) and streptomycin (1.0 mg/ml). The Petri dishes were incubated at 23°C for 7^{td} (Molina & Palmer 1982). The mycelia with microscopic structures similar to those described by Nobles (1948, 1958, 1965) and Stalpers (1978) were considered as pure culture. To determine the characteristics of the culture, pieces of agar and mycelium of 0.6 cm diameter were extracted with hollow punch from the different strains and were cultivated in Petri dishes containing PDA, MEA and CzA culture media at 2%, and incubated at 23°C. To characterize morphologically and morphometrically the culture obtained in the above mentioned media, fresh mycelium samples from different parts of the colony, mounted in water were examined on days 7^{th} and 14^{th}. Form and texture of the colonies were determined according to Nobles (1948, 1965) and Stalpers (1978). Qualitative detection of the enzymes was done on day 14^{th}. To detect the cytochrome-oxidase, esterase, phosphatase, laccase, peroxidase and tyrosinase enzymes the procedures by Taylor (1974) and Stalpers (1978) were followed. To detect amylolytic, cellulolytic and pectinolytic enzymes, the Pochon & Tardieux (1965) modified technique was used. The extracellular oxidase enzyme was detected following Nobles (1958). Urease and proteolytic enzymes were determined according to Mac Faddin (1976), DNAse and lipase according to Hankin & Anagnostakis (1975). Control strains of (Tv) *Trametes versicolor* Schlecht and (Fo) *Fusarium oxysporum* (L. ex Fr.) Pilát were used.

RESULTS

A. Macro-microscopic characterization of mycelial strains in pure culture

Strain UACHMMa-464 obtained from a basidiocarp of *Mycena albogrisea* Peck, collected from a stump of *Nothofagus obliqua* "hualle" Popoén, Osorno, 10-I-95.

PDA Media

Macroscopic: Colony 90 mm diameter, regular and zonate (same in MEA); center texture woolly-subfelty to cottony with crystalline drops on the aerial mycelium, towards the marginal zone subfelty-pellicular, whitish-greyish to whitish. Advancing zone appressed (same as in MEA and CzA). Reverse yellowish-lemon to creamy (same in MEA). "yeast" odour.

Microscopic: –Aerial hyphae in center of colony 1.4-5.6 µ diameter, anastomosed, hyaline to yellowish-brown with age, refringent (refracting) walls somewhat thick, little branching, clamped (same in MEA)-appressed (1.4) 2.8-4.2 (7) µ diameter hyphae, sometimes somewhat varicose, clamped (same in MEA). -spirally coiled hyphae in the aerial mycelium together with frequently septate and hyaline hyphae, clamps absent (same in MEA) (Fig. 1; a, b).

MEA Media

Macroscopic: Colony with a subfelty texture, crystalline drops in the aerial mycelium, felty-cottony towards the marginal zone, whitish to creamy-yellowish, odour absent (same in CzA).

Microscopic: –Hyphae 4.2-5.6 µ diameter, with citoplasmatic refringent material, somewhat brown-yellowish in the aerial mycelium. –waved hyphae. –hyphae with

scarce intercalary and terminal 9-14 x 5.6-12.6 (14) µ protuberances, concolour (same colour) to hyphae in the appressed mycelium and more frequently towards the marginal zone of the colony (Fig. 2; a, b).

CzA Media

Macroscopic: Colony 90 mm diameter, irregular; pellicular texture, distant hyaline hyphae.

Microscopic: –Hyphae 1.4-4.2 µ diameter sometimes corrugated, interlaced, waved, with refringent walls, clamped (Fig. 3; a, b).

Strain UACHMMc-495 obtained from a basidiocarp of *Mycena capillaripes* Peck. collected among remains of leaves and branches of *Sequoia semper virens* "redwood", Arboretum, Univ. Austral de Chile, Valdivia, 13-IV-95.

PDA Media

Macroscopic: Colony 24-34 mm diameter, irregular and zonate; texture subfelty to downy, whitish. Advancing zone appressed (same in MEA and CzA). Reverse yellowish-pale. Odour absent (same in MEA and CzA).

Microscopic: –Hyphae 1.4-2.8 (4.2) µ diameter, some with short, lateral ramifications, sometimes coiled, clamped. –anastomosed 2.8 µ diameter hyphae, scarcely branched (same in MEA). –hyphae with scarce intercalary and terminal (4.2) 7-14 x (4.2) 5.6-9.8 (12.6) µ chlamydospores, refringent and hyaline walls. –hyphae with intercalary and terminal 7-9.8 x (5.6) 7-8.4 µ protuberances, concolour to the hyphae or refringent (same in MEA and CzA). –Crystals in the culture media or incrusted in the hyphae, hexaedric, rectangular, refringent (same in CzA) (Fig. 1; c, d).

MEA Media

Macroscopic: Colony 21 mm diameter, regular and zonate; texture subfelty, creamy-whitish. Reverse whitish. After a month's culture, reverse whitish to yellowish pale.

Microscopic: –Hyphae (1.4) 2.8-4.2 µ diameter, clamped. –hyphae interlaced (Fig. 2; c, d).

CzA Media

Macroscopic: Colony 7 mm diameter, irregular; texture pellicular, grayish to concolour to culture media.

Microscopic: -Hyphae 1.4-2.8 µ diameter, vacuolized, sometimes corrugated, clamps scarce (Fig. 3; c, d).

Strain UACHMMg-250 obtained from a basidiocarp of *Mycena galericulata* (Scop.:Fr.) Gray, collected in serrated wood of *Nothofagus obliqua* "hualle", Rebellín, Valdivia, 30-V-94.

PDA Media

Macroscopic: Colony 33 mm diameter, regular; texture felty to subfelty, whitish to yellowish-pale. Advancing zone raised. Reverse brown-yellowish to yellowish-pale (same in MEA). Odour absent (same in MEA and CzA). After a month's culture, texture of colony crustose to felty, whitish to creamy with some whitish zones, towards the marginal zone downy to subfelty, whitish to mildy yellowish-olivaceous. Reverse brown-yellowish (same in MEA).

Microscopic: -Hyphae 1.4-5.6 µ diameter, appressed, sometimes interlaced, varicose, clamped. -hyphae with intercalary and terminal 5.6-11.2 x 4.2-8.4 µ protuberances, concolour to hyphae (Fig. 1; e, f).

MEA Media

Macroscopic: Colony 12-23 mm diameter, irregular (same in CzA); texture subfelty-felty to downy, creamy to whitish. Advancing zone appressed. The culture media becomes light brown. After a month's culture texture of colony, crustose-felty to downy, creamy-whitish to whitish-lemon.

Microscopic: –Hypha 1.4-7 µ diameter, branched, sometimes anastomosed, refringent walls, clamped (Fig. 2; e, f).

CzA Media

Macroscopic: Colony 10 mm diameter, texture floccose (scarce) to pellicular, whitish to grayish. Advancing zone appressed. Reverse whitish.

Microscopic: –Hyphae 1.4-2.8 (4.2) µ diameter, branching, sometimes incrusted with

crystals, others interlaced or corrugated. –hyphae with intercalary (4.2) 5.6-7 (8.4) x (2.8) µ protuberances concolour to hyphae, some others more vacuolizated. –Crystals big hexaedric and other forms (Fig. 3; e, f).

Strain UACHMMm-302 obtained from a basidiocarp of *Mycena metuloidifera* Sing. collected in stump of *Nothofagus obliqua* "hualle", Rebellín, Valdivia, 4-V-1994.

PDA Media

Macroscopic: Colony 84-90 mm diameter, regular and zonate (same in MEA); texture subfelty-farinaceous to felty, creamy. Advancing zone raised (same in MEA). Reverse cream. Odour sweet. After a month's culture texture is cottony, creamy-clear brown to whitish-pale yellowish.

Microscopic: –Hyphae 1.4-7 µ diameter, sometimes frequently septate, corrugated. Clamps scarce. –Arthroconidia 2.8-15.4 (24) x 1.4-9.8 µ, ovoid, cylindrical, periforms, rounded ends, somewhat refringent walls (same in MEA and CzA). Chlamydospores 10-15 x 6-10 µ, periforms, spherical (same in MEA). Crystals, puntiform, hexaedric, incrusted in the hyphae (Fig. 1; g, h).

MEA Media

Macroscopic: Colony woolly to scarcely floccose-farinaceous towards the advancing zone plumose, creamy to yellowish-pale. Reverse yellowish-pale. Odour absent (same in CzA). After month's culture marginal zone texture cottony. Reverse creamy.

Microscopic: –Hyphae 1.4-7 µ diameter, septate. –hyphae 1.4 µ diameter, without branching, refringent. –coiled hyphae in spiral, clamps scarce. –hyphae with intercalary and terminal 7-14 x 5.6-9.8 µ protuberances [in CzA they measure 4.2-7 (8.4) x 2.8-5.6 (7) µ], concolour to the hyphae (Fig. 2; g, h).

CzA Media

Macroscopic: Colony 90 mm diameter, regular; texture pellicular, hyaline-grayish. Advancing zone appressed. After a month's culture marginal zone floccose-farinaceous, whitish. Reverse grayish-opaque blueish.

Microscopic: –Hyphae 1.4-5.6 µ diameter, sometimes with short branches, interlaced, incrusted with crystals, corrugated, or waved, septate with clamps scarce (Fig. 3; g, h).

Strain UACHMMp-354 obtained from a basidiocarp of *Mycena patagonica*. Sing. collected on bark of *Salix babylonica* "sauce", Jardín Botánico, Universidad Austral de Chile, Valdivia, 5-VI-94.

PDA Media

Macroscopic: Colony 63 mm diameter, irregular and zonate; pellicular texture, toward marginal zone absent, whitish. Advancing zone submerged (same in MEA). Reverse creamy to pale-green (same in MEA). Odour absent (same in MEA). After a month's farinaceous zone extend all the colony.

Microscopic: –Generative hyphae 1.4-5.6 µ diameter vacuolizated or with refringent citoplasmatic content. –incrusted hyphae with crystals, filiform, refringent. –anastomosed 2.1-2.8 µ diameter hyphae, yellowish brown (same in MEA). Clamped. –skeletal 0.7-1.4 (2.1) µ diameter hyphae scarce, thick walls, refringent, hyaline to slightly-brown (same in MEA). –hyphal intercalary or terminal 5.6-11.2 (12.6) x (4.2) 5.6-9.8 µ protuberances concolour to hyphae or slightly more refringent (same in MEA). –hyphae with terminal (2.8) 5.6-7 (9.8) x (4.2) 5.6-9.8 µ inflation, thick refringent walls [in MEA measure (5.6) 8.4-9.8 (15.4) x (5.6) 7-8.4 (14) µ]. –Intercalary and terminal 5.6-11.2 (14) x (4.2) 5.6-9.8 (12.6) µ chlamydospores, spherical (same in MEA). –Crystals abundant, sometimes filiform, rectangular, incrusted in hyphae, sometimes yellowish-brown (same in MEA) (Fig. 1; i, j).

MEA Media

Macroscopic: Colony 41 mm diameter, texture subfelty (farinaceous) to absent, whitish to greenish-yellowish. After a month's culture crustose texture brown aerial hyphae, formed tufts, toward the marginal zone subfelty-farinaceaus to pellicular-ab-

sent, whitish with ochraceous tenuous to greenish-yellowish hyphae.

Microscopic: –Generative hyphae 1.4-5.6 µ diameter, sometimes brown-yellowish hyphae interlaced, with short branches (Fig. 2; j, k, l).

CzA Media: no growth.

Strain UACHMMr-260 obtained from a basidiocarp of *Mycena rubromarginata* (Fr. ex Fr.) Kummer. collected in vegetal remains under *Pseudotsuga menziesii* "pino oregón", Arboretum, Universidad Austral de Chile, Valdivia, 9-VI-94.

PDA Media

Macroscopic: Colony 36-40 mm diameter, irregular and zonate (same in MEA); texture subfelty-farinaceous to pellicular, center of colony with whitish-greyish striations, toward the marginal zone creamy to yellowish-lemon. Advancing zone appressed (same in MEA and CzA). Reverse greenish-yellowish tenuous. Fungic odour (same in MEA and CzA).

Microscopic: –Hyphae 1.4-4.2 µ diameter, sometimes with short fingered branches, ondulated, corrugated, interlaced refringent walls, clamped (the same characters in MEA, but they measure 1.4-5.6 µ diameter). –scarse hyphal intercalary and terminal 5.6-9.8 x 4.2-9.8 µ protuberances, concolour to hyphae. Measurements in CzA 5.6-12.6 (14) x 4.2-9.8 (11.2) µ. – Crystals hexaedric, puntiform (same in MEA) (Fig. 1; k).

MEA Media

Macroscopic: Colony 8-48 mm diameter, texture subfelty-downy (farinaceous) to pellicular, yellowish-lemon to greyish. Reverse dark brown, towards the marginal zone creamy to yellowish-lemon.

Microscopic: as in PDA (Fig. 2; m).

CzA Media

Macroscopic: Colony 8 mm diameter, irregular; subfelty texture, creamy. Reverse whitish.

Microscopic: –Hyphae 1.4-2.8 µ diameter, sometimes with short branches or well corrugated, clamped (Fig. 3; i, j).

B. Qualitative enzymatic determination.

Table 1 shows the enzymes detected for each of the mycelial strains assayed. The cytochrome-oxidase, esterase, phosphatase, lacasse (1-naphtol and benzidine) and peroxidase enzymes were detected in all the mycelial strains. The amylolytic and oxidase extracellular enzymes were detected in five mycelial strains. Four strains were positive to cellulolytic and DNAse while proteolytic enzymes were detected in three strains. Furthermore, the pectinase enzyme was detected only in the strain UACHMMm-302. In the UACHMMp-354 strain a weak reaction to tyrosinase was observed. Finally, strain UACHMMm-302 presented the greatest number of enzymes detected (11), while the smallest number was detected in strain UACHMMp-354; only 8.

DISCUSSION

The classic taxonomic studies of the species of the genera *Mycena* are based on macro and macroscopic characteristics, chemical reactions and ecological characteristics of the basidiocarps (Singer 1986). However, there are limitations due to the putrid nature of the fruit bodies and because they are ephemeral (Lazo 1982). This determines short periods for their collection.

An alternative method for the taxonomic study of these fungi is the isolation of mycelial strains in pure culture, either from substrates colonized by its propagative or vegetative structures or from basidiocarps using common or selective culture media (Worrall 1991). The latter is one of the methods most widely used since there is a greater probability of isolating the mycelial phase as well as of finding out certain nutritional characteristics of the strain in isolation. A main factor limiting this method is obtaining the pseudotissue, since the extraction zone is very small and is normally influenced by environmental factors. Also influential is the content of moisture of the

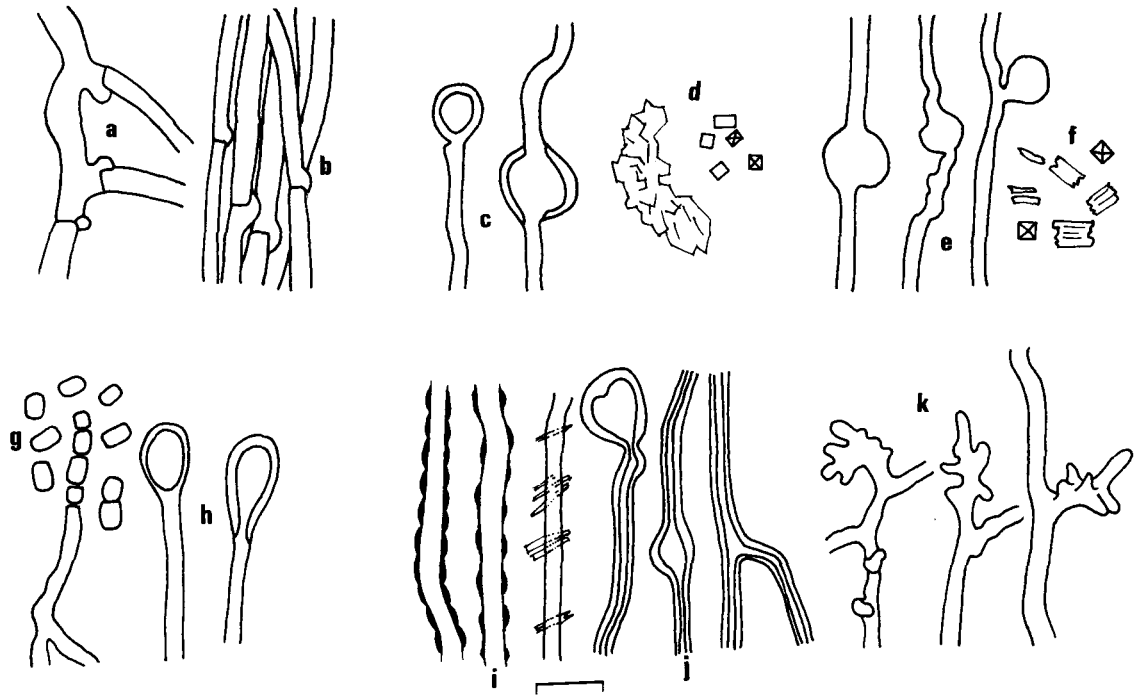

Fig. 1: Hyphal structures of mycelial strains of *Mycena* developed on PDA media

a = clamped hypha, b = anastomosed hyphae, c = chlamydospores, d = crystals, e = hyphal swellings, f = crystals, g = arthroconidia, h = chlamydospores, i = incrusted hyphae, j = skeletal hyphae, k = hyphae with fingered branches (Bar = 10 μ).

Estructuras hifales de las cepas miceliales de *Mycena* desarrolladas sobre medio PDA

a = hifa fibulada, b = hifas anastomosadas, c = clamidosporas, d = cristales, e = protuberancia hifal, f = cristales, g = artroconidias, h = clamidosporas, i = hifas incrustadas, j = hifas esqueléticas, k = hifas con ramificaciones digitiformes (Barra = 10 μ).

basidiocarps. In the samples from very wet environments, a greater degree of pollution was detected while it was easier to obtain the corresponding mycelial strain from samples collected on dry days with little content of moisture (Garnica 1995).

Because of the difficulties in obtaining pure mycelial cultures from Agaricales with small basidiocarps and little flesh, data on the characteristics of mycelial strains in culture are scarce. This is also reflected in the small number of species that have been studied (Singer 1986). The species of *Mycena* (Valenzuela & Moreno 1995) present the same difficulties.

In the PDA and MEA culture media, all the mycelial strains showed good growth. However, in CzA all the strains showed poor development and in the UACHMMp-354 strain no development was observed. This difference could be attributed to the source of nitrogen, which in PDA and MEA is organic while in CzA it is inorganic. The inhability to use this last type of compound is common to all Basidiomycetes (Deacon 1988). Another factor that could have negatively influenced the development of the mycelial strains is the pH of the media which is about 7.3 in CzA (Stalpers 1978).

The principal characteristic used to determine the purity of the cultures obtained was the presence of clamp connections. In all the basidiocarps of the species studied the hypha presented clamp connections (Valenzuela & Moreno 1995); a characteristic that was also observed in the mycelial cultures. A tendency to lose this characteristic was observed in the strains UACHMMm-301 and UACHMMp-354. Lar-

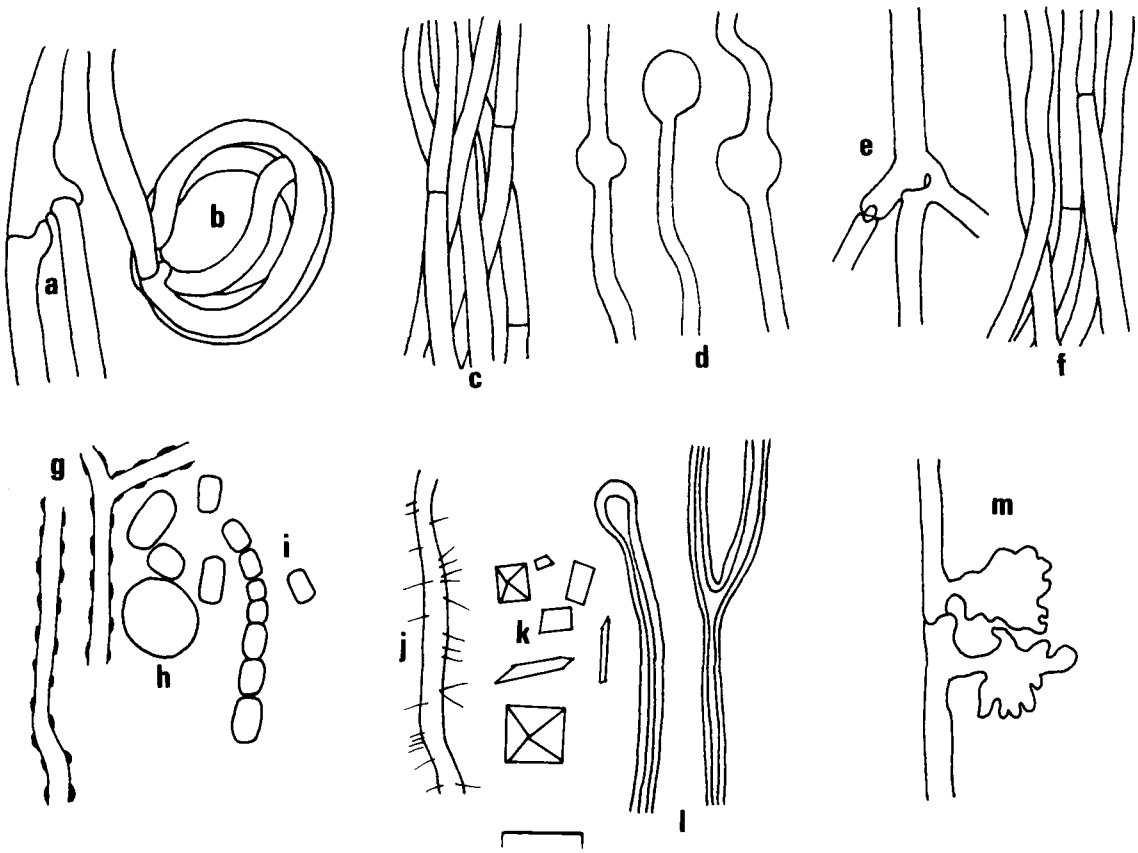

Fig. 2: Hyphal structures of mycelial strains of *Mycena* developed on MEA media
a = clamped hyphae, b = coiled hypha, c = anastomosed hyphae, d = hyphal swelling, e = branched hyphae, f = anastomosed hypha, g = incrusted hyphae, h = chlamydospores, i = arthroconidia, j = incrusted hypha, k = crystals, l = skeletal hyphae, m = hypha with fingered branches (Bar = 10 μ).

Estructuras hifales de las cepas miceliales de *Mycena* desarrolladas sobre medio AEM
a = hifas fibuladas, b = hifa en espiral, c = hifas anastomosadas, d = protuberancias hifal, e = hifa ramificada, f = hifas anastomosadas, g = hifas incrustadas, h = clamidosporas, i = artroconidia, j = hifa incrustada, k = cristales, l = hifas esqueléticas, m = hifa con ramificaciones digitiformes (Barra = 10 μ).

sen et al. (1992) indicate that the loss of the clamp can be attributed to the predominance of the diploid nature of the vegetative mycelia; a phenomenon which is observable in the species of *Armillaria* and which probably also occurs in the species of *Mycena.* For Cuevas & Herrera (1971) the loss of the clamp is genetically conditioned.

Noteworthy is the presence of skeletical hyphae in the strain UACHMMp-354. This type of hypha was not observed in the basidiocarps studied. As Cuevas & Herrera (1971) indicate, this formation is likely to be induced by some nutritional factor. It is important to point out that reproduction and resistance structures which are not found in basidiocarps were observed in some of the mycelial strains, being the arthroconidia the structure most commonly developed in the culture. This presupposes a genetic condition of the strain which develops this type of reproduction and dissemination. Thus, in strain UACHMMm-302 they were detected in very young cultures. This strain also developed chlamydospores, structures which have no apparent relationship with the adverse conditions of the culture media. The chlamydospores, were developed in PDA and MEA, media which have a pH similar to the substrates colonized by these fungi and where availability of nutrients should not be a limiting factor. The formation of

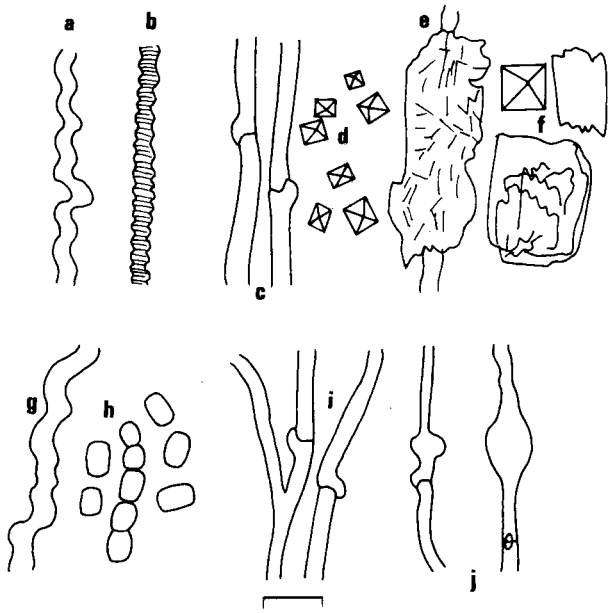

Fig. 3: Hyphal structures of mycelial strains of *Mycena* developed on CzA media
a = waved hypha, b = corrugated hypha, c = hypha with clamps, d = crystals, e = incrusted hypha, f = crystals, g = waved hypha, h = arthroconidia, i = clamped hyphae, j = hyphal swellings (Bar = 10 μ).

Estructuras hifales de las cepas miceliales de *Mycena* desarrolladas sobre medio CzA
a = hifa ondulada, b = hifa corrugada, c = hifa con fíbulas, d = cristales, e = hifa incrustada, f = cristales, g = hifa ondulada, h = artroconidia, i = hifas fibuladas, j = protuberancia hifal (Barra = 10 μ).

arthroconidia and chlamydospores would have no relation with the age of the cultures. With regards to this, Wathling (1979) and Kendrick & Wathling (1979) have stated that chlamydospores and arthroconidia have been observed both in the hypogeium mycelium as well as being obtained in pure culture of some Agaricales.

On the other hand, the presence of crystals in the strains was more abundant when they were cultivated in the CzA media. They possibly correspond to secreted metabolits and could be related to antibiotic mechanisms or still yet, to other unknown factors. Lombard et al. (1975) cited the presence of crystals in the cultures of *Phlebia chrysocrea* and *P. livida*. Furthermore, Barraza et al. (1992) reported the presence of calcium oxalate crystals in decomposed wood of *Eucryphia cordifolia* by the fungi *Ganoderma australe*. However, it is necessary to characterize its composition and clarify its role in future studies.

The enzymes capable of degrading compounds such as cellulose and lignin have acquired special importance. The detection of the fenoloxidase type enzymes in the Agaricales has been done directly on the basidiocarps (Harkin et al. 1974) and to a lesser degree on mycelial strains obtained in pure culture, contrary to what occurs with the fungi of the Aphyllophorales (Stalpers 1978, Adaskaveg & Gilbertson 1989, Adaskaveg et al. 1991). The enzymes cytochrome-oxidase, esterase, phosphatase, peroxidase and laccase were detected in all the mycelial strains studied. These enzymes are important in the degradation of lignocellulosic materials (Stalpers 1978), overall on those rotting white fungi (Redhead & Ginns 1985). The other enzymes implied in simpler degradation compounds were detected in different degrees for each strain (Table 1). This proves the capacity and specificity that the strains have to grow and use these compounds. In spite of the fact that strains UACHMMg-250 and UACHMMm-302 came from the same substrate (*Nothofagus obliqua),* the enzymatic behaviour that they presented was different. This could be due to different stages of degradation of the substrate. The same occurred with the mycelial strains UACHMMc-495 and UACHMMr-260 obtained from basidiocarps growing on coniferous. It is important to point out that that qualitative enzymatic detection in this study was done under controlled laboratory conditions. Under natural conditions, the presence or absence of a given enzyme and its activity is determined by multiple factors. In spite of this, Stalpers (1978) reported a close relationship between the physiology and the ecological role of mycelial strain Aphyllophorales.

Since there are no mycelial strains of reference for these species and papers on this respect could not be found the comparison or corroboration of these results could not be carried out. Nevertheless, this study poses many new problems to be solved and leads to the need of carrying out further studies on the morphological and biochemical characteristics of mycelia in the future.

TABLE 1

Enzymes produced by mycelial strains on solid media.

Producción de enzimas por cepas miceliales sobre medio sólido.

Enzymes/Strains	464	495	250	302	354	260	Tv	Fo
Amylolytic	+	-	+	+	+	+	-	+
Cellulolytic	+	+	+	+	-	-	+	+
Cytochrome-oxidase	+	+	+	+	+	+	+	+
DNAse	+	+	+	-	-	+	-	+
Esterase	+	+	+	+	+	+	+	-
Laccase (1-naphthol)	+	+	+	+	+	+	+	-
Laccase (benzidine)	+	+	+	+	+	+	+	-
Lipase	-	+	-	+	-	-	+	-
Oxidase extracellular	-	+	+	+	+	+	+	-
Pectinase	-	-	-	+	-	-	+	-
Peroxidase	+	+	+	+	+	+	-	+
Phosphatase	+	+	+	+	+	+	+	-
Proteolytics	+	-	-	+	-	+	-	+
Tyrosinase (p-cresol)	-	-	-	-	d	-	-	-
Tyrosinase (tirosine)	-	-	-	-	d	-	-	-
Urease	+	+	-	-	-	-	-	+

(+) = positive reaction
(d) = weak positive reaction
(-) = negative reaction

(+) = reacción positiva
(d) = reacción positiva débil
(-) = reacción negativa

The taxonomic determination for the *Mycena* genus species could be carried out from their mycelial strains. It would be not necessary the previous basidiocarps development. Furthermore, the mycelial strains could be used in studies related with biodegradation, antibiosis and metabolite production.

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