MORPHOLOGICAL ASPECTS OF FRUITING BODIES IN MICROSPORUM GYPSEUM ON SABOUREAUD’S DEXTROSE AGAR MEDIUM

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Summary

Microsporum gypseum belongs to Arthrodermataceae family, order Onygenales of Ascomycota division from Fungi. The species teleomorphic phase is represented by Arthroderma gypseum and Arthroderma incurvatum. Soil is the natural environment of the species being which behaves as a dermatophyte, but it can also be isolated from animals fur once they had contact with soil. The diseases caused by fungi are represented by tinea corporis, tinea capitis and rarely by tinea barbae. The morphologic aspects of colony showed a plain dusty colony with a specific coffee milk colour, edged by a white dusty belt, grained cream-coloured aspect on one side and yellow-brownish on the other side.. The colony can easy detached from culture media and under microscope numerous macroconidia that contain 4-6 lodges can be observed. Modern investigations like ESEM (environmental scanning electron microscopy) can contribute to a more precise identification, the microscopic examination of colonies and macro/microconidia aspects are essential in species diagnosis in case of Microsporum genus.

Key-words: fruiting bodies; ESEM; morphology; Microsporum gypseum; species identification; ultrastructure.

Introduction

Microsporum gypseum is a soil saprophyte that can occasionally be pathogenic to humans causing tinea corporis or tinea capitis occasionally (Levine, 1982). It has also been isolated from animals like dogs, horses, rabbits, monkeys, cats, pigs, rats, and mice. M. gypseum is geophillic and has been found in soil all over the world.

The telomorph (the sexual form of a fungus is Arthroderma gypseum (Irene Weitzman, 1995). The typical dermatophyte skin infection is an irregular expanding ring with a raised serpiginous order thought to resemble a worm, hence the old term ringworm or tinea (Latin for worm). Infections are named according to the body location involved, e.g., tinea corporis (face, trunk, and arms and legs) (Irene Weitzman, 1995).

Colony morphology - Microsporum gypseum complex grows rapidly and matures within 6 days (Buiuc, 1999). On Sabouraud's dextrose agar, the surface of the colony is flat and spreading developing an irregular fringed border, the texture is powdery to granular, and the color is buff, sandy or beige at first turning to tan to cinnamon brown. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral boarder (Weitzman, 1986). A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains.

Microscopic morphology - Microsporum gypseum produces septate hyphae, macroconidia and microconidia. Macroconidia are abundant, measure 8-16 x 22-60 µm, contain 3-6 compartments and they are fusoid (tapered at both ends) and
symmetrical in shape with rounded ends (Note: *M. canis* has pointed ends). (Weitzman, 1988).

The walls of macroconidia are thin and rough. Microconidia are described as drop-shaped, pyriform (pear-shaped) or club-shaped, moderate in number, and located laterally along the hyphae (Coman, 2000).

Tests - Growth of *M. gypseum* on BCP (bromcresol purple agar) is profuse, with no change in pH; urea hydrolysis (urease is positive), hair perforation is positive, and vitamin growth factor tests are negative.

**Material and methods**

Were examined 4 strains of *Microsporum gypseum* from patients with isolated cutaneous lesions of tinea corporis. The obtained strains were examined using optical microscopy and environmental scanning electron microscopy (ESEM), for morphological and structural particularities studies (Glauert, 1974).

Light microscopy

1. Native samples was made using adhesive-tape method of sampling, the morphology studies was made using a BX 43 Olympus optical microscope with semi plan apochromate objectives 10X, 20X, 40X, images were captured using a digital CCD camera, CX3 Olympus.
2. The stain method used was with one drop of lactophenol cotton blue between the glass slide and adhesive-tape. The morphology studies was made using the same optical microscope and capture system, BX 43 Olympus optical microscope with plan chromate objectives at 10X, 20X, 40X, and a digital camera capture.

ESEM with cooling stage

Colony sample of Microsporum gypseum were excised and placed in Eppendorf tubes with Glutaraldehyde 2.7% for 24 hr at 4°C. After 24 hr, they were mounted into the Cooling stage which is a temperature controlled specimen holder of ESEM microscope. The morphology studies was made using Fei Quanta 250 ESEM, the parameters of ESEM examination were represented by the following items: the image mode, the working distance (WD), the beam conditions, chamber pressure and the relative humidity into sample. In the same order they were: the gaseous secondary electron detector detector (GSED); the working distance: 5 mm; the beam conditions: 15kV, spot 4,5; the pressure range: 910 to 1400 Pa and the relative humidity with a value of 100%.

**Results and discussion**

All four strains of *Microsporum gypseum* examined presents an abundance of fructification (Figs.1,2,3,10), characterised by numerous typical macroconidia. In native samples, „in situ” aspects are poor in details and they cannot be used for identification. The macroconidia are well represented but the structural details, (for instance – echinulatum septe) are difficult to observe. (fig. no.1, 2, 3). The hilar appendix isnt visible in macroconidia. There is a raw image observed with 10X, 20X, 40X objective as a consequence of different focused level, the surface details arent smooth. In the samples coloured with cotton blue stain the details are more obvious (Fig. 2, 3). The cultural aspects revealed the presence of cotton-like white colonies, with yellowish brown central area and radial ridges, with the reverse of the colonies being yellowish (Figs. 4,5,6,7,8,9). Macroconidia of *M. gypseum* present an ellipsoidal/ pyriform shape (from lateral view) (figs. 10,11,13) with rounded ends (fig.14). The wall surface of macroconidia is echinulate (fig. 15). Microconidia of *M. gypseum* was represented by a hyaline single-celled with a pyriforme to clavate shape and a smooth surface (fig.12).
Fig. 1 – „In situ” aspect of *M. gypseum* macroconidia (20X).

Fig. 2 – Micro (green arrow) and macroconidia (red arrow) of *M. gypseum* (100x).
**Fig. 3** - Micro and macroconidia (hilar flagella) of *M. gypseum* (100x).

**Fig. 4** - *M. gypseum*, cultural aspect.

**Fig. 5** - Surface of the colony is flat with an irregular fringed border.

**Fig. 6** - A reddish-brown pigment is present in some *M. gypseum* strains.

**Fig. 7** - Colony cultures develop a central white downy umbo (dome).

**Fig. 8** - The texture of *M. gypseum* colony is powdery to granular.

**Fig. 9** - *M. gypseum* grows rapidly and matures within 6 days.
Fig. 10 - General aspect *M. gypseum* colony.

Fig. 11 - *M. gypseum* ellipsoidal shape of macroconidia.
**Fig. 12** - *M. gypseum* macroconidia (M) and microconidia (m). Microconidia are hyaline, single-celled, pyriform to clavate, smooth-walled.

**Fig. 13** - *M. gypseum*, pyriform microconidia, detail.
Fig. 14 - *M. gypseum* macroconidia, symmetrical in shape and with rounded ends.

Fig. 15 - *M. gypseum* macroconidia, thin-walled, verrucose surface.
Conclusions

In all four strains of *Microsporum gypseum* in culture, using the optical microscope examination, the colonies have the following features: surface of the colony is flat with an irregular fringed border; colony cultures develop a central white downy umbo (dome).

In optical microscopy examinations it can be observed macroconidia and microconidia.

In ESEM analysis, macroconidia has a thin-walled, verrucose surface. Ellipsoidal and symmetrical in shape and rounded ends. Microconidia are hyaline, single-celled, pyriform to clavate, smooth-walled.

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References


