A new technique used in dermatophytes morphology description at *Microsporum gypseum* and *Microsporum canis* species

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Papers and books on electron microscopy preparation and observation for biological samples in romanian literature are a few [1]. The specialized literature describes just one review of dermatophytes in animals and humans [2]. Just a few cases of morphology examination in dermatophytes are described using electron microscopy [3], [4].

The main steps concerning SEM sample preparation include surface cleaning, stabilizing the sample with a fixative (most common glutaraldehyde and/or osmium acid), rinsing, dehydrating in acetone and drying at critical point, mounting the specimen on a metal holder, and coating the sample with a layer of a material that is electrically conductive (gold, cupper, silver, etc) [5]. Each of these steps are crucial and will affect the outcome of the final results.

In our experiments were examinated 4 strains of *Microsporum gypseum* from 4 patients with isolated cutaneous lesions of tinea corporis and 4 strains of *Microsporum canis* isolated from 6 animals. The obtained strains were examinated using an environmental scanning electron microscopy (ESEM) with cooling stage for morphological and structural particularities studies. Colony sample of *Microsporum gypseum* and *Microsporum canis* 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 novelty of our technique consists in fact that we use samples for study without coating and critical point dry applied before of examination. The parameters of ESEM examination were represented by the following items: the image mode, the working distance (WD), the beam conditions, stage temperature, 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; stage temperature 3°C; the pressure range: 910 to 1400 Pa and the relative humidity with a value of 100%.

In ESEM analysis, macroconidia of *Microsporum gypseum* has a thin - walled, verrucose surface. ellipsoidal and symmetrical in shape and rounded ends (fig.1). Microconidia are hyaline, single-celled, pyriform to clavate, smoothwalled. Macroconidia from *Microsporum canis* has a thickness greater than *M. gypseum*, ellipsoidal and symmetrical and the surface is verrucose. The verrucous processes from surface are more evident in M. canis. The ends are sharp (fig.2).

References

Figure 1. Macroconidia of *Microsporum gypseum*, symmetrical in shape with rounded ends.

Figure 2. Macroconidia of *Microsporum canis*, symmetrical in shape with sharp ends.