Genus *Malassezia* species isolation and identification in healthy dogs

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**ABSTRACT.** The aim of the study was the isolation of genus *Malassezia* species in healthy dogs, and their identification since there are ten known species and their number increases every year. Dixon medium is considered to be the best for *Malassezia* isolation, and the results of the study proved to be so. Of all the 74 *Malassezia* strains isolated, 71 were *M. pachydermatis*, 2 *M. furfur* and one *M. globosa*. In conclusion *M. pachydermatis* is the most frequent isolated specie, with a percentage of 95.94 of all the *Malassezia* isolated strains.

**Keywords:** *Malassezia*, isolation, healthy dogs, *M. pachydermatis*.

**Introduction**

Genus *Malassezia* is part of *Deuteromocete* class, *Criptococcaceae* family and comprises ten species of lipodependent fungi (*furfur, obtusa, globosa, sloofiae, japonica, nana, etc.*) and *Malassezia pachydermatis* (*Pityrosporum canis*) which is lipophylic but not lipodependent. For the human beings the most relevant would be *Malassezia furfur*, responsible for the aetiology of pityriasis versicolor(3,6,8).

The microscopic appearance of *Malassezia pachydermatis* is oval to peanut-shaped and is approximately 2-3 µm in width and 4-5 µm in length. Colonies are medium sized, round and convex shaped, white-yellow coloured, developed on solid environments in 5-7 days. Reproduction is asexually achieved by unipolar or sympodial (*M. sympodialis*) budding (1,2,5). *Malassezia globosa* cells have spherical shape (2,5-8 µm), with daughter cell on a small area (2,8).

Unlike many bacteria or other fungi, *Malassezia* yeasts are rarely found in the environment. In healthy dogs, *M. pachydermatis* can be isolated from the ear channel, anus, rectum, oral cavity and, less commonly, the nose and vagina (4,7). On the normal canine skin, carriage of the yeast is most common in the interdigital areas and around the mouth but uncommon on the axilla or dorsum (2,3,6,7). In other species, *Malassezia* organisms have been recovered from the skin of healthy cats, ferrets, foxes, bears, pigs, horses, birds and rhinoceroses.

**Material and methods**

The researches took place during April 2005 – October 2006 within the Microbiology Laboratory of Veterinary Medicine Faculty, Cluj-Napoca. The samples consisted of sterile tampons were collected from the external ear channel and skin from 53 healthy dogs, all registered at Medical Care Labs or the Faculty of Veterinary Medicine Cluj-Napoca. Four anatomical sites were considered in our researches: external ear canal, perioral, interdigital and perianal area. The culture media that we utilized in our investigations was Dixon agar, the number of plates used in the researches being 212.

Genus *Malassezia* requires particular conditions regarding cultivation, so the plaques were incubated at a temperature of 32°C for 5 days, the optimum time in which the colonies reach their normal sizes. After the bacterioscopic examination of the samples, the embedment of the plates was made, following a square margin.
The colonies were examined using magnifying glass, and afterwards a microscopic exam was performed in order to certify their appertainence to the genus *Malassezia*. The colonies were sowed on Sabouraud agar in order to differentiate *M. pachydermatis* of *M. furfur*.

**Results and discussions**

A number of 53 dogs were examined, 212 plates were sowed, the number of *Malassezia* strains isolated being 74. After the collection, samples from external ear channel, perioral, interdigital and perianal regions were sowed on Dixon agar, the medium that isolates all known *Malassezia* species. A microscopic examination followed, and the *Malassezia* colonies were re-sowed on Sabouraud agar. *M. pachydermatis* is the only specie that develops on Sabouraud agar, due to its lipophilic features. A number of 72 species developed colonies on Sabouraud agar, 71 were *M. pachydermatis*, one specie being *M. globosa*. The identification was made by microscopic examination of the cells, round shaped, with daughter cell attached on a small area. Often daughter cells may appear elongated, maintaining although a narrow base, giving yeast a globular shape.

The other two species were identified using biochemical tests. The first test was the Tween assimilation, 2 ml fungi suspension is mixed with 16 ml Sabouraud agar, and after solidification we practiced cavities into the agar and added Tween 20, 40 and 80. *Malassezia furfur* is the only specie that assimilates all the three Tween solutions (as shown in image 5).

The images below are presenting cells and colonies aspects, but also the Tween assimilation test.

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**Figura 1**
*M. pachydermatis* cells (1000x)

**Figura 2**
*M. pachydermatis* colonies

**Figura 3**
*M. globosa* cells (1000x)

**Figura 4**
*M. globosa* colonies (4x)
The researches proved that *M. pachydermatis* is the most frequent isolated *Malassezia* specie, the carriage in healthy dogs being a raised one, 53 dogs providing 74 *Malassezia* strains. The data are according to the literature articles, the percent of isolation being around 30%.

**Conclusions**

1. *M. pachydermatis* is the most frequent *Malassezia* specie isolated in healthy dogs with a percentage of 95.94.

2. 74 *Malassezia* strains were isolated on 212 plates, the percentage of isolation being of 34.9.

3. There is a high level of *Malassezia* carriage in healthy dogs.

4. *M. furfur* and *M. globosa* are rarely isolated from healthy dogs, with a percentage of 2.7 respectively 1.35.

**References**


