A Simple Quantitative Culture of *Malassezia* spp. in HIV-Positive Persons

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Key Word

Malassezia
Quantification
Method
HIV

Abstract

Background: Etiological role of *Malassezia* spp. remains controversial in certain skin diseases. **Objective:** To adapt a 'tape method' for quantitative culture of *Malassezia* spp. **Method:** Samples for culture were taken from clinically normal forehead skin of HIV-positive and negative persons by stripping with a tape that was then placed on Leeming & Notman medium. The number of colonies was counted after 14 days. **Results:** 74/78 (94.8%) cultures were positive, for a median count of 9 CFU/tape (range 0 to > 200). High skin density of *Malassezia* spp., defined as more than 100 CFU/tape, was found in 7/38 (18.4%) HIV-positive persons and was absent (0/40) in the HIV-negative group ($p < 0.01$). **Conclusion:** The method used is simple, unexpensive and reliable. High *Malassezia* spp. density was only found in HIV-positive patients.

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Introduction

Malassezia spp. are lipophilic yeasts resident on adult human skin [1]. The genus *Malassezia* comprises three species: *Malassezia furfur*, an obligatory lipophilic organism commonly found on human skin; *Malassezia pachydermatis*, a nonobligatory lipophilic species normally found on animals but occasionally isolated from humans, and *Malassezia sympodialis* exceptionally found in humans [2, 3]. The etiological role of *Malassezia* spp. has been proved in certain diseases like intravascular catheter-associated sepsis [4] but remains controversial in others such as seborrheic dermatitis and atopic dermatosis. Little is known about this microorganism despite its ubiquity. At the present time, micromorphological descriptions are the major method of taxonomic classification. More objective methods of classification based on genetic, biochemical and physiological properties were not easily available because the organism could not be easily cultivated.

In the last few years, new media have been described [5, 6] and quantitative methods have been developed using a contact plate [7–9], a tape method [10] or a scrubbing technique [6, 11]. We have adapted a tape method on Leeming & Notman medium for quantifying *Malassezia* spp. density on the skin.

Methods

Samples for culture were taken from clinically normal forehead skin by stripping with a tape measuring 6 cm² (Opsite IV3000, Smith & Nephew). Thereafter, the tapes were placed on a 'milk' medium. In a preliminary study, olive oil medium was used.

Milk medium was described by Leeming and Notman [5]; it contains (per liter of medium) 10 g bacteriological peptone (Oxoid), 5 g glucose (Sigma), 0.1 g yeast extract (Oxoid), 4 g desiccated ox bile (Oxoid), 1 ml glycerol (Sigma), 0.5 g glycerol monostearate (BDH), 0.5 ml Tween 60 (Sigma), 10 ml whole-fat cow's milk (average fat content 3.7%, homogenized, ultra-high-temperature treated), 50 µg/ml chloramphenicol, 200 µg/ml cycloheximide and 12 g Agar number 1 (Oxoid). Olive oil medium was Sabouraud-chloramphenicol medium (Bio-Mérieux, Lyon, France) with 5% of commercial olive oil.

Plates were incubated at 37 °C in a plastic bag for 14 days and the number of colonies (colony forming unit, CFU) was counted. When colonial morphologic analysis was atypical, *Malassezia* spp. was confirmed by gram staining and the absence of growth on Sabouraud and trypticase soy agar medium. *Malassezia* spp. density was classified into 3 categories: low density with < 50 CFU/tape, intermediate density with more than 50 but less than 100 CFU/tape and finally high-density with > 100 CFU/tape.

Samples were taken from the forehead of 78 workers or patients of the Geneva University Hospital; 38 were HIV positive and 40 had negative or presumed negative HIV serology.

Results

Sampling required less than 2 min/case and was always well tolerated. In a preliminary study, olive oil medium was used in 20 HIV-negative persons. Cultures were positive in 15/20 (75%) for a median count of 5 CFU/tape (range 0-24). With this high rate of negative culture (25%), the following experiments were done on milk medium. On

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Table 1. *Malassezia* spp. density in HIV

Negative	Low	Intermediate	High
0 CFU	< 50	50 < CFU < 100	> 100 CFU
HIV- 0	37		
(n = 40) "HIV+	4*	23	
(n = 38)			

Exact Fisher's test: *p > 0.05, **p < 0.01.

Leeming & Notman medium, 74/78 (94.8%) cultures were positive, for a median count of 9 CFU/tape (range 0 to > 200). Colonies appeared after 72 h and were maximal after 14 days.

Three kinds of colonies were found: (1) white-creamy in color, < 3 mm, flat, with undulated margin; (2) like (1) but larger (3-5 mm) and lighter in color and raised, and (3) white-creamy in color, < 2 mm with a 'crystalline' appearance. These different colony types could not be ascribed to different microscopic morphologies. Each of them seems obligatory lipophilic and could not grow on Sabouraud or on trypticase soy agar medium. *Candida* spp. were found in only 1 case.

In this case, colonies were white in color, > 3 mm, regular and grew easily on Sabouraud medium. *M. pachydermae*, described as 7 mm, domed, irregular colonies

in positive and negative persons

Intermediate	High
50 < CFU < 100	> 100 CFU
0	
7**	

[1], were not isolated in our series. The majority of volunteers (60/78, 77%) had a low *Malassezia* spp. density. Intermediate density was found in 7/78 cases (9%) and high density was found in 7/38 (18.4%) HIV-positive patients and were absent (0/40) in the HIV-negative group ($p < 0.01$). Table 1 shows the distribution of *Malassezia* spp. in normal skin in this series.

Discussion

We have adapted the 'milk' medium of Leeming & Notman for a quantitative culture of *Malassezia* spp. with a tape method. Milk medium appeared to have several advantages over oil medium because *Malassezia* spp. grew faster, more easily, and colonies were well delimited and easier to read. The superiority of this medium has been shown previously [6]. Of the 4 negative cultures, 2 came from HIV-positive patients who took flu-conazole, a drug which can inhibit *M. furfur* [12].

The choice of the tape is important. Under Opsite IV 3000, *Malassezia* spp. grew harmoniously over the whole surface. In preliminary experiments, we used Scotch brand 3M and observed that colonies preferentially grew on the edges of the tape.

This method offers many advantages over the 'contact plates method'. For example, it can be used for skin areas difficult to access like the nasolabial folds. The tape can be cut when analysis of a small lesion is required. The tape method is simpler and less time consuming than the scrubbing technique.

As described previously [1, 3], three cultural groups based on colonial morphology were identified; this indicates that *M. furfur* probably includes different obligatory lipophilic yeasts. In the future, genome analysis will probably help to better determine micro-bial taxonomy of species and strains of *Malassezia* [13].

High *Malassezia* spp. density was found only in HIV-positive patients without clinical evidence of dermatosis. Wilker et al. [14] found low *Malassezia* density in HIV-sero-positive patients. We can explain this difference because they studied a small group of 21 patients and used an olive oil medium which is less performant for the culture of *M. furfur*.

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