IJMS
Vol 29, No.1, March 2004

Brief Report

Rapid Identification of *Malassezia furfur* from other *Malassezia* Species: A Major Causative Agent of Pityriasis Versicolor

M. Shams Ghahfarokhi*, M. Razzaghi Abyaneh**

Abstract

The present study is designed to evaluate the application of a simple method for rapid identification of *Malassezia furfur* among other *Malassezia* spp. based on production of a brownish-red pigment and its diffusion into the medium containing L-tryptophan as a sigle source of nitrogen. 91 strains of *Malassezia* (20% *M. furfur*, 2% *M. sympodialis*, 5% *M. obtosa* and 73% *M. globosa*) isolated from skin scales of 138 patients with pityriasis versicolor were examined. Reference *Malassezia* strains of all 7 species obtained from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, together with *Candida albicans* and *Rhodotorula* sp. were also studied. All of these strains were cultured on modified Dixon agar medium prepared by replacing pepton with equal amount (0.6%) of L-tryptophan. The pigment producing ability was evaluated after 7 days incubation of the cultures at 32 ºC. Out of yeast species tested, all *M. furfur* strains produced specific brown pigment, whereas strains belonging to all other known *Malassezia* spp. and also several other yeasts failed to produce pigment. The results obtained further substantiated that study of pigment producing ability on this specific medium can be used as a simple and reliable test for rapid differentiation of *M. furfur* from other closely related *Malassezia* spp..

Iran J Med Sci 2004; 29(1):36-39.

Keywords • *Malassezia spp* • *Malassezia furfur* • Pityriasis versicolor • L-tryptophan • identification

Introduction

Malassezia spp. are lipophilic yeasts and found to be a part of animal and human cutaneous microbiota. They have also been considered as medically important yeasts because of their involvement in the etiology of some important skin disorders including pityriasis versicolor, foliculitis, seborrhoeic dermatitis and dandruff.[1,2] They have also been reported with increasing frequency as causative agents of some life-threatening diseases.[3-5] The genus *Malassezia* has recently been revised on the basis of molecular data and lipid requirements and enlarged to include seven distinct species including *M. furfur*, *M. pachydermatis*, *M. sympodialis, M. globosa, M. obtusa, M. slooffiae,* and *M. restricta*.[2,6,7] All lipid-dependent species can be isolated from healthy and diseased human skin. *M. pachydermatis*, the only non-lipid-dependent species is often isolated from domestic and wild animals.[2]

*Department of Mycology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
**Department.of Mycology, Pasteur Institute of Iran, Tehran, Iran

Correspondence: M. Shams Ghahfarokhi Ph.D, Department of Mycology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
Tel: +98-21-8011001
Fax: +98-21-8006544
E-mail: shamsm@modares.ac.ir

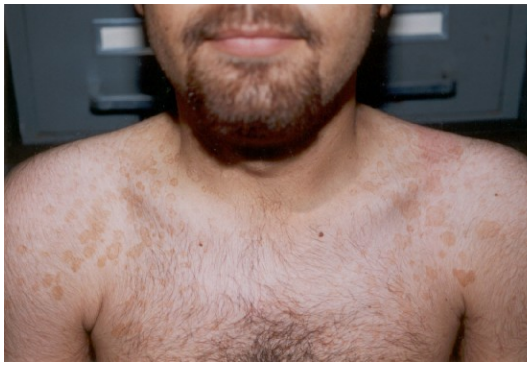

Fig 1: Brown colored maculae caused by an isolate of *Malassezia furfur* on the neck and upper trunk in a patient with pityriasis versicolor.

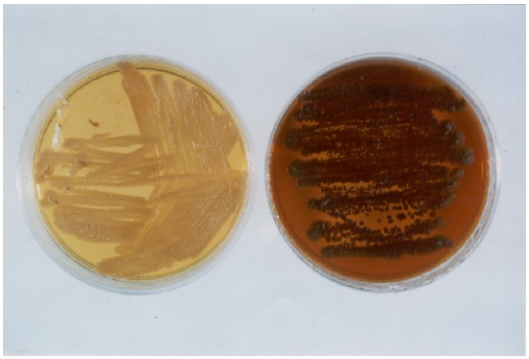

Fig 2: Colony appearance of *Malassezia furfur* (right) and *Malassezia sympodialis* (left) on modified Dixon agar containing L-tryptophan as a single source of nitrogen. Specific brownish pigment diffusion into the medium is observed only in colony of *M. furfur.*

Demonstration of round to ovoid yeast cells and short filaments in scales from patients is considered to be the most diagnostic finding in direct microscopy of all *Malassezia* infections.[8] Identification of *Malassezia spp*, specially unstable morphologic forms such as *M. furfur* using routine morphological and physiological techniques is laborious, time consuming and costly.[2,9-11] *M. furfur* is considered as one of the most prevalent species involved in the etiology of different *Malassezia* infections, especially pityriasis versicolor. Its high morphological variation in direct microscopy requires a rapid identification of this species among *Malassezia* isolates. This is of practical importance in epidemiological studies. In present study, a simple and reliable method was successfully used for differentiation of *M. furfur* from all other known *Malassezia* spp. based on the ability of pigment production.

Patients and Methods

Ninety-one strains of *Malassezia* from four species *M. furfur, M. sympodialis, M. globosa and M. obtusa* isolated from 138 patients (72 men and 66 women) with the diagnosis of pityriasis versicolor who aged 9–65 years, and whose demographic characteristics were reported previously,[9] were examined. These isolates were obtained from 87 clinical specimens; one isolate from each 83 specimens and two isolates from each 4 remaining ones. The clinical appearance of the disease is shown in Fig 1. Identification of these species was done based upon their macro- and micromorphologies and also on their key physiological and biochemical characteristics.[2,10,11] Reference *Malassezia* strains from all seven species obtained from Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, and some important yeast species, *Candida albicans* and *Rhodotorula* sp. were also included in our study.

The modified Dixon medium (mDixon) prepared by replacing peptone with equal amounts (0.6 %) of tryptophan was used for cultivation and pigment induction by all isolates. This medium consisted of 3.6% malt extract, 2.0% desiccated Ox-bile, 1.0% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.5% cycloheximide, and 1.2% agar. After sterilization and cooling to 50 ºC, filtered sterilized L-tryptophan, at a concentration of 0.6% was added to other ingredients. Formation of specific pigment was evaluated macroscopically after 1–7 days incubation at 32 ºC.

Results

Direct microscopic examination (DME) of KOH-treated specimens and identification of fungi on mDixon agar showed 95% (131 cases), and 63% (87 cases) of positive results, respectively. This accounts for an almost 5%, and 37% of false negative results obtained for DME and culture techniques, respectively. The species identification was carried out as described previously.[9] Four different *Malassezia* spp., *i.e., M. globosa*, *M. furfur*, *M. obtusa* and *M. sympodialis* were identified. The identity of these isolates was further confirmed by examination of their ability to produce pigment on mDixon agar medium.

All *M. furfur* strains (MFs. 1 to 18 and CBS 7710) produced a diffusible brown pigment, whereas strains of other species identified as *M. sympodialis* (MSs. 1 and 2 and CBS 7980), *M. globosa* (MGs. 1 to 75 and CBS 7705), *M. obtusa* (MOs. 1 to 5 and CBS 7968, GM 220), *M. restricta* CBS 7877, *M. pachydermatis* CBS 1891, and *M. slooffiae* CBS 7875 and also *C.*

albicans (CA. 1) and *Rhodotorula* sp. (Rh. 1) failed to produce pigment on this medium. The production of a brown pigment by *M. furfur* which started after 36–48 h, was completed by day 7 after incubation (Fig 2).

Discussion

Pityriasis versicolor is one of the most common microbial skin disorders which is caused by yeasts of the genus *Malassezia* (Fig 1). All *Malassezia spp*, but *M. furfur* were proved to have relatively stable morphological characteristics.[8,13] To the best of our knowledge, there is very little information on diverse clinical appearance and also pathogenesis of pityriasis versicolor. Mayser and coworkers[12,14-17] have evaluated the metabolism and nutritional requirements of *Malassezia* spp. in relation to fungal pathogenesis. They showed that mDixon agar allowed the growth of all *Malassezia* spp. tested (*M. furfur, M. sympodialis* and *M. pachydermatis*). The formation of a diffusible brown pigment in colonies, however, was restricted only to the *M. furfur* strains.[12] They concluded that the color change of the affected skin in pityriasis versicolor could reflect the exclusive ability of *M. furfur* to assimilate L-tryptophan as a nitrogen source. This ability was also confirmed by recent studies of Raabe *et al,*[18] on pigmentogenesis of all lipid dependent *Malassezia* spp. Based on their observations on lower pigment production by a non-lipid-dependent reference strain, *M. pachydermatis* CBS1892, they concluded that *Malassezia* isolates with different geographical origin should be further examined to confirm the validity and reliability of identification. In this context, we further studied Iranian *Malassezia* isolates, identified in our previous study,[9] for their ability to assimilate L-tryptophan as a single nitrogen source with the aim of introducing a simple method for rapid differentiation of *Malassezia* isolates. Only *M. furfur* strains produced diffusible brown pigment, whereas all other strains of *Malassezia* spp. tested failed to produce pigment on this medium.

Based on our findings, evaluation of pigment production by all *Malassezia* spp. on mDixon medium further substantiated that only *M. furfur* can assimilate L-tryptophan as a single source of nitrogen. Thereby, the assay of pigment production on mDixon agar can be used as a simple and reliable screening test for differentiation of *M. furfur* from all other *Malassezia spp*, especially three confusing *M. sympodialis, M. slooffiae* and *M. pachydermatis* species.

References

1 Yarrow D, Ahearn DC: *Malassezia* Baillon. In: Kreger-van Rij NJW, eds: The Yeasts: A Taxonomic Study, 3rd. ed. Amsterdam: North Holand Publication, **1984:** 882–85.

2 Gueho E, Midgley G, Guillot J: The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek 1996*; **69:** 337–55.

3 Erchiga VC, Martos AO, Casano AV, et al: Mycology of pityriasis versicolor. *J Mycol Med 1999;***9:** 143–48.

4 Ashbee HR, Evans EGV: Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev 2002;***15:**21–57.

5 Erchiga VC, Florencio VD: *Malassezia* species in skin diseases. *Curr Opi Infect Dis 2002;***15:** 133 –42.

6 Guillot J, Gueho E: The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie Van Leeuwenhoek 1995*;**67*:*** 297–314.

7 Boekhout T, Kamp M, Gueho E: Molecular typing of *Malassezia* species With PFGE and RAPD. *Med Mycol 1998;***36:** 365–72.

8 Midgley G: Morphological variation in *Malassezia* and its significance in pityriasis versicolor. In: Bossche HV, eds. Dimorphic Fungi in Biology and Medicine, New York, Plenum Press, **1993:** 267–7.

9 Shams M, Rasaee MJ, Moosavi M, et al: Identification of *Malassezia* species in patients with pityriasis versicolor submitted to the Razi Hospital in Tehran. *Iranian Biomed J 2001;***5:**121–26.

10 Guillot J, Gueho E, Lesourd M, et al: Identification of *Malassezia* species: a practical approach. *J Mycol Med 1996;***6:** 103–10.

11 Gueho E, Boeckhout T, Ashbee HR, et al: The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med Mycol 1998;***36:** 220–29.

12 Mayser P, Wille G, Imkampe A, et al: Synthesis of fluorochromes and pigments in *Malassezia furfur* by use of tryptophan as the single nitrogen source. *Mycoses 1998*; **41:** 265–71.

13 Midgley G: The diversity of *Pityrosporum* (*Malassezia*) yeasts *in vivo* and *in vitro*. *Mycopathologia 1989;***106:**143–53.

14 Mayser P, Haze P, Papavassilis C, et al: Differentiation of *Malassezia* species: selectivity of cremophor E.L., castor oil and ricinoleic acid for *M. furfur. Br J Dermatol 1997;***137:**208–13.

15 Mayser P, Imkampe A, Winkeler M, et al: Growth requirements and nitrogen me-

tabolism of *M. furfur. Arch Dermatol Res 1998;***290:** 277–82.

16 Mayser P, Pickel M, Haze P, et al: Different utilization of neutral lipids by *Malassezia furfur* and *Malassezia sympodialis. Med Mycol 1998;***36(1):**7–14.

17 Mayser P, Schafer U, Kramer HJ, et al: Pityriacitrin–an ultraviolet-absorbing indole alkaloid from the yeast *Malassezia furfur. Arch Dermatol Res 2002;***294:**131–34.

18 Raabe P, Mayser P, Weiss R: Further comments on *Malassezia* species from dogs and cats. *Mycoses 2000;***43:** 437–38.