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

**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 single 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 °C. 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.


**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, folliculitis, seborrheic dermatitis and dandruff.1,2 They have also been reported with increasing frequency as causative agents of some life-threatening diseases.3,4 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
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. Identification of Malassezia spp, specially unstable morphologic forms such as M. furfur using routine morphological and physiological techniques is laborious, time consuming and costly. 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, 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 isolates was further confirmed by examination of their ability to produce pigment on mDixon agar medium.

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

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 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 °C, 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 °C.

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. 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.

**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.
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. To the best of our knowledge, there is very little information on diverse clinical appearance and also pathogenesis of pityriasis versicolor. Mayser and coworkers 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. 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. on pigmentation 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, 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

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