Iranian Biomedical Journal 5 (4): 121-126 (October 2001)

Identification of *Malassezia* Species in Patients with Pityriasis Versicolor Submitted to the Razi Hospital in Tehran

Masoomeh Shams[*], Mohammad Javad Rasaee[2], Marzieh Moosavi[1] and Mandi Razzaghi[3]

[1]Dept. of Mycology, Faculty of Medical Sciences, Tarbiat Modarres University; [2]Dept. of Biothchnology, Faculty of Medical Sciences, Tarbiat Modarres University; [3]Dept. of Mycology, Pasteur Institute of Iran, Tehran, Iran

ABSTRACT

Lipophilic yeast of the genus *Malassezia (Pityrosporum)* belongs to the normal flora of human skin and many warm-blooded animals. These fungi can produce a diverse range of diseases that the best known and most frequent of them is pityriasis versicolor, a chronic recurrent skin disease occurring primarily in tropical regions. The genus *Malassezia* has been recently enlarged to include seven distinct species. Very little information has been documented about identifying *Malassezia* species in Iran. This survey was undertaken to present a practical approach for differentiation of all Malassezia yeasts isolated from clinical materials of patients with pityriasis versicolor for the first time in Iran. The presence of the disease was confirmed on the basis of the observation of budding yeast cells and short curved hyphae in skin specimens by direct microscopy. *Malassezia* yeasts were isolated after culturing the samples on modified Dixon (mDixon) agar. A combination of different characteristics includes yeast cell morphology, ability to growth on sabouraud dextrose agar, catalase test and ability to utilize individual Tweens (20, 40, 60, 80) were used for identification of species. In general, 138 patients with pityriasis versicolor includes 52.2% male and 47.8% female were identified. Direct microscopy and culture results were positive in 94.4% and 63% of the patients, respectively. Totally, 91 isolates of *Malassezia* belonging to four different species, *M. globosa* (66 isolates), *M. furfur* (18 isolates), *M. obtusa* (5 isolates) and *M. sympodialis* (2 isolates) were identified. *Iran. Biomed. J. 5 (4): 121-126, 2001*

Keywords: Pityriasis versicolor, *Malassezia* species, Identification scheme

INTRODUCTION

Opportunistic yeast of the genus *Malassezia* have been recognized as members of the microbiological flora of human skin and many other warm-blooded vertebrates [1, 2]. These lipophilic fungi are associated with different types of diseases especially pityriasis versicolor, a chronic, benign and recurrent superficial infection which is generally occur in tropical and temperate regions [3-6]. This disease affects predominantly young adults of both sexes and characterized by circumscribed scaly maculae of various colors from ochre to brown especially in sebum-rich areas of the skin [7]. Demonstration of round to ovoid yeast cells and short filaments in scales from patients is considered to be the most diagnostic findings in direct microscopy [8]. Only slight inflammatory changes in cornified layer of affected area with no or weak cellular and humoral immune responses have been determined [9-11]. Although, pityriasis versicolor was first described at the beginning of the nineteen century, a great deal of confusion and controversy have surrounded the study of the etiologic yeast, because of their variable morphology and their fastidious requirements for growing *in vitro* [12, 13]. The list of synonyms is therefore long and the name *Malassezia furfur* was used for many years mycelial phase seen in pityriasis versicolor scales, while the names *Pityrosporum ovale* and *Pityrosporum orbiculare* were mainly used for description of the yeast phases. At the present, the genus *Malassezia* has been enlarged to include seven distinct species. The lipophilic yeast recovered from animals have been assigned to the unique non-lipophilic species *Malassezia pachydermatis (= Pityrosporum canis)* which was first isolated from cutaneous lesions of a rhinoceros in 1925 [14]. The genus was enlarged to include another lipid dependent species *M. sympodialis* in 1990, and a number of molecular, morphological and physiological studies led to description of four other species, *M. globosa, M. restricta, M. obtusa* and *M slooffiae* [12, 15]. The ecology and pathogenicity of all these new species are still a

*Corresponding author; Tel. (98-21) 8011001; Fax (98-21) 8006544; E-mail:shamsm@net I cs.modares.ac.ir

[DOR: 20.1001.1.1028852.2001.5.4.3.3]

matter of controversy and there are a few reported data in this regard. This communication was carried out to evaluation of the prevalence of pityriasis versicolor and isolation and identification of related ethiological agents *(Malassezia* species) in involved individuals submitted to the Razi Hospital for a 12-month period (1378-79). On the basis of our results, strains belonging to four different species include *M. globosa, M. furfur, M. obtusa* and *M. sympodialis* isolated from patients with pityriasis versicolor were identified using simple and reliable morphological and physiological methods. This is the first documented report on the isolation and identification of these *Malassezia* species in Iran.

MATERIALS AND METHODS

Organisms and culture conditions. Skin samples were taken from 138 patients suspected to pityriasis versicolor, 72 men and 66 women, aged 9 to 65 years. They were mounted in KOH (20%) for direct microscopic examination. The specimens were also inoculated on mDixon agar (3.6% malt extract, 0.6% mycological peptone, 2% desiccated ox-bile, 0.2% glycerol, 1% Tween 40, 0.2% oleic acid, 1.2% agar, 0.005% chloramphenicol and 0.05% cycloheximide, pH 6.0) and incubated at 32-35°C for 7 days [2, 16].

Identifcation scheme. Growth of colonies was monitored carefully using a stereomicroscope. *Malassezia* species were identified based on the diagnostic scheme presented by Guillot *et al.* [12] (Fig. 1), including morphological characters, catalase test and growth in the presence of different types of individual Tween (20, 40, 60, 80) as unique lipid supplementation. The cultural morphology (colony diameter and texture) of all the isolates was examined on mDixon agar after incubation at 32°C for 7 days. The microscopical features were evaluated at the same conditions using a Zeitz microscope after staining by methylene blue [12]. The catalase reaction was determined by the application of a drop of H_20_2, onto a culture smear on a glass slide. The production of gas bubbles indicated a positive reaction. For each isolate, the ability to utilize individual Tweens was examined by the following procedure. About 16 ml of sterile sabouraud dextrose agar (SDA) supplemented with 0.05% cycloheximide and 0.005% chloramphenicol was melted and allowed to cool about 45°C. Two ml of identified yeast suspension was added to the melted medium and mixed. The suspension was prepared by inoculating 5 ml of sterile distilled water with a loopfull of actively growing yeasts to adjusted at about 10 cells/ml. After solidification of the medium, four holes were made by means of a 2-mm diameter punch and filled with 10 µl of Tweens 20, 40, 60 and 80, respectively and incubated at 32°C for 5-7 days. Utilization of Tweens was assessed by the degree of growth and precipitation of the lipophilic yeasts around individual wells.

RESULTS

Clinical data. Of 138 patients with pityriasis versicolor, 72 cases (52.2%) were male and 66 cases (47.8%) were female. No significant relationship observed between the type of *Malassezia* isolates and the sexuality. Involved individuals were in the age groups between 0-9 to >60 years old. The highest and the lowest frequency of disease was observed in 20-29 (48 cases) and 0-9 (2 cases) year age groups, respectively. Involvement of different parts of the patients' body was observed (Fig. 2). The neck had the highest frequency with 28% (76 cases) involvement, whereas each of the whole body or cruris areas with 1.84% (5 cases) had the lowest frequency in this regard. Relapses were determined in 59.2% (73 cases) of the patients. There was not any significant correlation between the type of isolated *Malassezia* species and the relapse phenomenon. Seventy-nine patients were involved for more than 12 months, 50 had between 6 to 12 months, and 9 had suffered for under 6 months. Concurrent diseases were present in 32.6% (45 cases) of patients with the highest frequency about 11.6% (16 cases) regarding to food allergy.

Morphological characteristics. Microscopic examination of the skin scales in 20%. KOH showed the typical ovoid to globose blastoconidia and short filaments in majority (94.9%) of the samples. The four *Malassezia* species isolated in this survey exhibited typical morphological features. All of isolates reproduced by unilateral budding with prominent scars on the mother cell. Bottle-shaped yeast cells might be globose *(M. globosa),* ovoid *(M. furfur* or *M. sympodialis)* or cylindrical *(M. obtusa).* The colonies of *M. globosa* on mDixon agar were raised, folded and roughed with average diameter about 5 mm and a coarse and brittle texture (Fig. 3). For *M furfur,* colonies were dull, smooth or slightly folded with convex elevation

[DOR: 20.1001.1.1028852.2001.5.4.3.3]

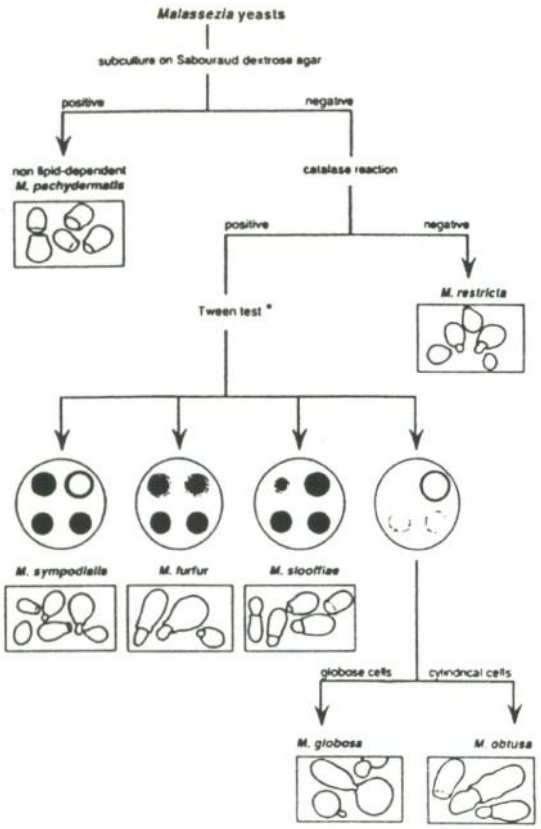

Fig. 1. Identification scheme for *Malassezia* species.

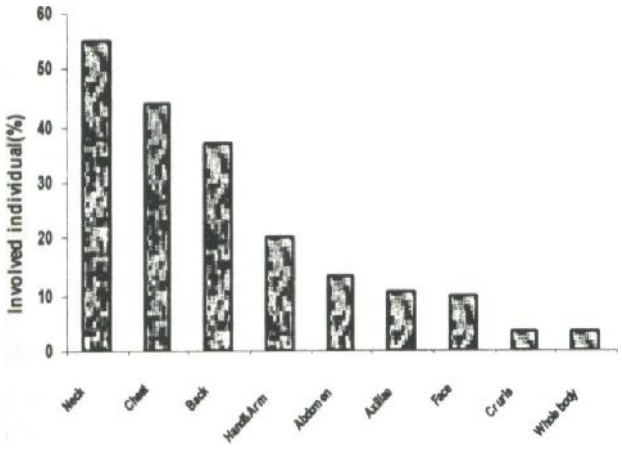

Fig. 2. The frequency of involved areas of the body in patients with pityriasis versicolor (Razi Hospita1,1378- 79).

and average diameter about 6 mm (Fig. 3). The colonies of *M. sympodialis* were smooth, with an elevated center and average diameter about 4-5 mm (Fig. 3).

M. obtusa colonies on mDixon agar were smooth with a sticky texture and 2-3 mm average diameter (Fig. 3).

Physiological features. An identification scheme for *Malassezia* yeasts was used in this survey which mainly based on physiological characteristics (Fig. 1). Because none of the examined isolates were able to grow on SDA at 32°C, we did not encounter with *M. pachydermatis*, an only non-lipid dependent *Malassezia* species in this survey. Also, we did not find any *M. restricta* isolate, because the catalase reaction was positive for all of the examined isolates. The Tween diffusion test allowed distinction of the most *Malassezia* species in this study. The lipid dependent species *M. furfur* and *M. sympodialis* utilized the four individual Tweens (Fig. 4A). Nevertheless, the growth of *M. sympodialis* was inhibited by high concentration of Tween 20 which resulted in a typical ring of small colonies around the pertaining well (Fig. 4B). The two species *M. globosa* and *M. obtusa* were unable to utilize any of the four Tweens as sole source of lipid. Thus, a ring of precipitation developed around the wells containing Tweens 40 and 60 without any visible growth as an inhibition phenomenon (Fig. 4C). This precipitation sometimes progressed towards the wells to form a complete opalescent disc (Fig. 4D). Totally, 91 isolates belonged to the genus *Malassezia* includes *M. globosa* (66 isolates, 72.53%), *M. furfur* (18 isolates, 19.78%), *M. obtusa* (5 isolates, 5.49%) and *M. sympodialis* (2 isolates, 2.20%) were identified based on the above mentioned experiments.

DISCUSSION

Pityriasis versicolor is a superficial fungal infection with a worldwide distribution. Several documented reports of the disease have been presented from different parts of Iran especially southern subtropical regions. In this survey, the highest prevalence of the disease was observed in 20-29 (34.8%) and 10-19 (30.4%) age groups. These data correlates with the results obtained from several other studies which indicate that the disease generally appears in the late teens with a peak in the 20 years, and rarely found in aged people [2, 17, 18]. Although, different parts of the body were involved in our understudied patients, the highest prevalence was related to regions e.g. neck, chest and back (Fig. 2). There are well-documented reports upon the usual body distribution

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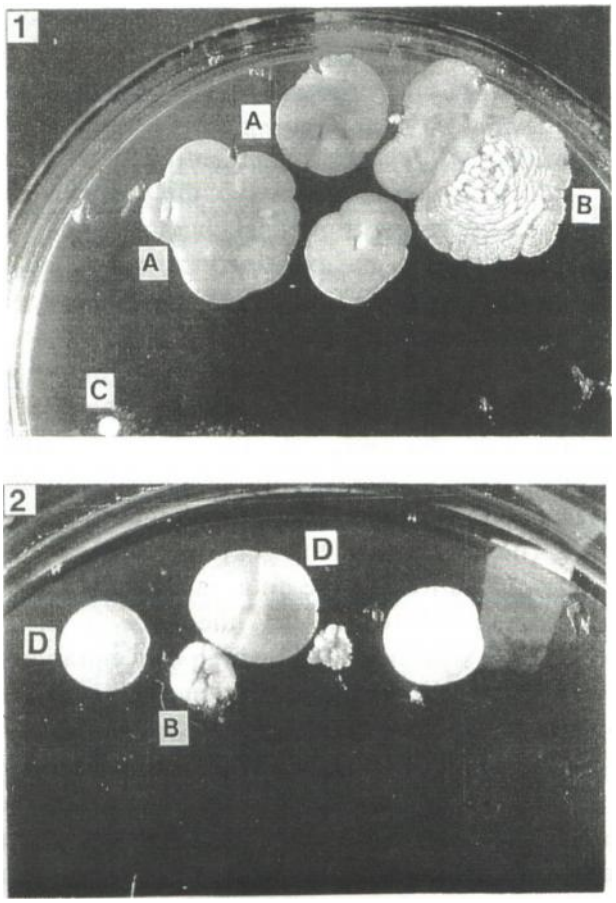

Fig. 3. Macroscopic morphology of *M. furfur* (1A), *M. globosa* (1B, 2B), *M. obtusa* (1C) and *M. sympodialis* (2D) on mDixon agar after 1 week incubation at 32°C.

with lesions over the upper trunk in patients with pityriasis versicolor especially in temperate and subtropical climates [2, 6, 9, 17, 19]. Direct microscopy with KOH and culture on mDixon agar were positive for 94.8% (131 cases) and 63% (87 cases) of patients, respectively. As the taxonomy of the genus *Malassezia* was clearly established a few years ago, there is very few data on the ecology and pathogenicity of these lipophilic yeasts. Practical identification scheme for *Malassezia* species is given in (Fig. 1). Direct examination of yeast colonies does not provide sufficient data for exact and specific identification of *Malassezia* species, because the presence of some variation occur with the culture medium and growth temperature [13, 15, 20]. However, differentiation of all *Malassezia* species could be completely performed using physiological tests includes growth on SDA at 32°C, catalase test, and ability to growth in presence of individual Tweens 20, 40, 60 or 80. Only non-lipid dependent species *M pachydermatis* can grow on SDA at 32°C. All lipid dependent species of the genus *Malassezia*, except *M. restricta*, have a positive catalase reaction. The lipophilic nature of *Malassezia* yeasts was documented in 1939 [21].

Since then, several investigations have tried to differentiate *Malassezia* isolates on the basis of their lipid requirements [15, 22, 23]. The Tween test described in this study seemed to be more convenient for routine identification. This test mainly used for differentiation of *M. furfur, M. sympodialis* and *M. slooffiae*. In this assay system, precipitate combined or not with obvious growth has been examined. If the organism cannot grow normally in the presence of different Tweens, hydrolysis of Tweens accompanied with precipitation of the related insoluble fatty acids (lauric, palmitic, stearic and oleic acids for Tweens 20, 40, 60 and 80, respectively) will be occurred. However, when the Tweens allow a growth, acidification of the medium resulted in the prevention of precipitate formation [24, 25]. In this study, 91 *Malassezia* isolates belonging to the four species including *M. globosa, M. furfur, M. obtusa* and *M. sympodialis* were identified based on aforementioned morphological and physiological characteristics. These species were isolated from 138 patients with pityriasis versicolor. Several investigators have been studied the ecology of different *Malassezia* species [2, 9, 26-29]. *M. globosa* has been recovered regularly from lesion of pityriasis versicolor. *M. furfur* survives in very hard conditions and therefore to be involved in different pathological conditions as pityriasis versicolor. *M. sympodialis* seems to be the commonest species on healthy skin; while, *M. slooffiae* is occasionally found on human skin. Other three species including *M. obtusa, M. pachydermatis* and *M. restricta* are involved in the small number of cases of pityriasis versicolor. Our results suggest that *M. globosa* especially in the mycelial form is the predominant species involved in etiology of pityriasis versicolor. It was isolated in cultures from 43.4% of the total examined cases, and its microscopic morphology was consisted of globose yeast cells mixed with short hyphae. *M furfur* was the second most frequent species with a frequency about 13.04%. The presence of other species, like *M. obtusa* and *M sympodialis* could be due to the fact that they are a part of the skin mycobiota. The frequency of these two species was 3.62% and 1.44%, respectively. There was no significant correlation between the species isolated from pityriasis versicolor lesions and the clinical picture or severity of the disease.

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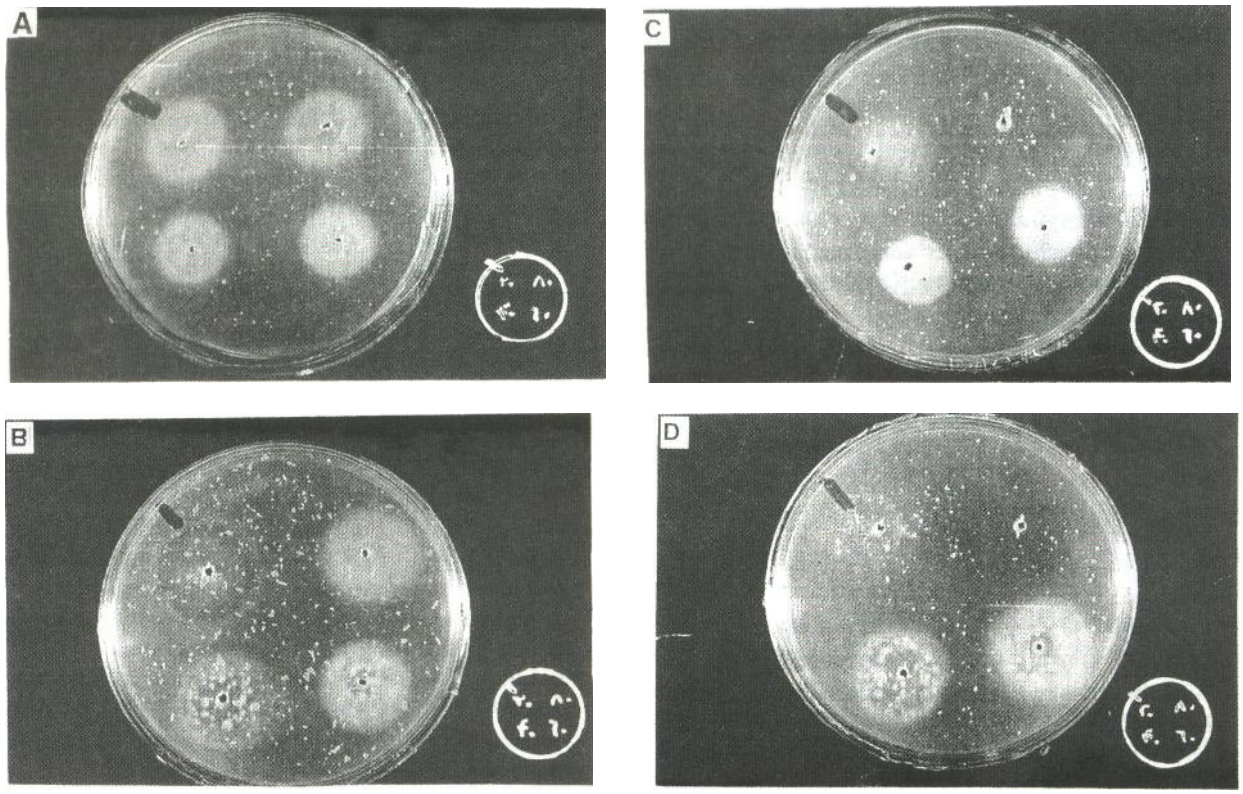

Fig. 4. Tween assimilation patterns of *Malassezia* yeast. A, *M. furfitr* and *M. sympodialis;* B, *M. sympodialis;* C & D, *M. globosa* and *M obtuse.*

As mentioned above, *M. globosa* was recovered from the most culture positive cases (75.86%). Similar results have been obtained by other workers. For examples, Erchiga *et al.* [5] showed that this species is involved in about 87% of the cases of pityriasis versicolor. It is appear that *M. globosa* is probably more pathogenic than other members of the genus *Malassezia*, at least on the skin. In general, the key characteristics described above represent an initial attempt to separate *Malassezia* species by conventional methods and the development of molecular techniques such as pulsed field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) has provided the foundation for establishing new species within the genus *Malassezia* [30-32].

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[DOR: 20.1001.1.1028852.2001.5.4.3.3]

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