Isolation and Identification of *Malassezia* Species in Patients with Pityriasis Versicolor

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ABSTRACT:

BACKGROUND:

Malassezia are unipolar yeasts that comprised from eleven species and recognized as commensally skin flora that may be pathogenic under certain conditions.

OBJECTIVE:

To isolate and identify different species of *Malassezia* in Iraqi patients with pityriasis versicolor.

MATERIALS AND METHODS:

This case investigative study was done in Microbiology and Dermatology Departments, College of Medicine, University of Baghdad- Baghdad, Iraq during the period from April 2008 - October 2008.

One hundred patients had *pityriasis versicolor* were evaluated regarding all points related to the disease. Wood's light and skin scraping for mycological examinations were done. Methylene blue stained samples were examined for the presence of clusters of yeasts, budding cells, and hypha. Tween assimilation and splitting of esculin tests were carried out.

RESULTS:

The most common isolated species were *Malassezia globosa 40*(51%), followed by *Malassezia furfur 24*(30%), *Malassezia symbodialis 8*(10%), *Malassezia obtuse 5*(6%) and *Malassezia restricta*
2(3%).

CONCLUSION:

Malassezia globosa was the most predominant species involved in etiology of *pityriasis versicolor* lesions followed by *Malassezia furfur*.

KEY WORDS: *Malassezia spp, pityriasis versicolor*, lipophilic yeasts.

INTRODUCTION:

Yeasts of the genus *Malassezia* have been known to be a part of the normal flora of human skin and other warm-blooded animals [1,2]. Being lipid dependent, they are normally found in areas that are rich in sebaceous glands[3]. Once the lipophilic nature of these yeasts was recognized, culture made possible. However, apart from their lipid dependence little is known about the metabolism and nutritional requirements of *Malassezia* species [1-3].

Malassezia organism, a lipophilic non mycelial, unipolar budding yeast characterized by a thick cell wall that was detected by Eichsted in (1846) and by Sluter (1847) [4,5].

Cunningham *et al*.(1990)[6] differentiated three species of *Malassezia fufurr* A, B and C, which had culture and morphological differences that corresponded to serological differences determined by cell surface antigens[6].

The taxonomy of the genus *Malassezia* was still chaotic, with different group's tendency to favor their own classification scheme, resulting in an inability to compare work carried out by different groups. Chaos was finally resolved with a publication by Guillot *et al*(1995)[7].

They assembled 104 isolate of *Malassezia species* encompassing all different classification favored by different groups and carried out sequencing of the large –subunit rRNA and nuclear DNA complementary studies. On the basis of their results, they defined and later named seven species of *Malassezia:*

Malassezia furfur.*Malassezia sympodialis*. *Malassezia globosa*. *Malassezia obtusa*. *Malassezia restricta*. *Malassezia sloofiae*. *Malassezia pachydermatis* [8].

Malassezia species had been recently reclassified on the basis of morphology, genomic

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composition, and physiological characteristics of the yeast [9]. Currently, eleven species were identified which include ten lipid- dependent species: *Malassezia dermatis. Malassezia furfur. Malassezia globos. Malassezia japonica. Malassezia nana. Malassezia obtuse. Malassezia restricta. Malassezia slooffiae. Malassezia saympodialis. Malassezia patchydermatis* was the only non- lipid- dependent species [10], *Malassezia yamatoensis* was the new eleventh species identified.

So, the target of this work is to isolate and identify different types of *Malassezia* species in Iraqi patients with pityriasis versicolor.

MATERIALS AND METHODS:

This case investigative study was conducted in Microbiology and Dermatology &Venereology Departments, College of Medicine, University of Baghdad- Baghdad, Iraq; during the period from April 2008 - October 2008.

The samples were collected from 100 patients with *pityriasis versicolor* attending the Dermatological Out-patient Clinics in Baghdad Teaching Hospital, Medical City.

Detailed history, full clinical assessment, Wood's lamp examination were don. Also, mycological evaluation by skin scraping followed by dissolving of the scales in 10% KOH solution. Then microscopic examination was performed and considered positive when it revealed short stubby hyphae and yeast forms.

Apart from morphological criteria, *Malassezia* yeasts were primarily differentiated biochemically by their ability to assimilate various polyoxyetheylene sorbitan esters (Tween) following the methodology of Guillot *et al*. [8]

Culture:

Only samples which were KOH positive (n=100) were cultured. The scales were inoculated into modified Dixon's agar (mDixon's agar) as described by Guillot *et al* [8] and into Sabouraud's dextrose agar containing antibacterial agents (0.4g (400.000 IU) of penicillin and 1g of streptomycin). The plates were incubated at 32°C for 3-4 days [11].

Microscopial features:

The morphology of the yeast cells was studied by making Gram stained smears of the isolates from m Dixon's agar after one week incubation at 32°C.

***Identification of* Malassezia *species* :[12]**

According to physiological characteristic (Figure-2):

1. **Urease test:**

Benzalkonium chloride(1%) was added to the test medium to disassociate the cell wall of yeasts to allow endogenous urease to be released into the test medium. The presence of ammonia, which changes the indicator to pink because of the alkaline conditions produced. [12]

2. **Dizonium B blue:**

DBB test on solid media; cultures were incubated for 5-7 days at 30°C. DBB reagent was added onto the surface of each culture, cell of most known basidiomycetous yeasts produced pink to red or violet color reactions with DBB.[13]

3. **Catalase reaction:**

Presence of catalase is determined by using a drop of hydrogen peroxide (3% solution) and production of gas bubbles is considered as a positive reaction. Lack of catalase activity is a characteristic feature of *Malassezia restricta*.[12]

4. **Tween assimilation test:**

According to the method reported by *Guillot et al* (2005)[12], ability to utilize different Tween compounds as a unique lipid supplement by *Malassezia* species was evaluated. Briefly, yeast suspension (at least 10^7 cfu/ml) was made in 2 ml sterilized distilled water and poured into plate containing Sabouraud's dextrose agar at 45°C. The inoculum was then spread evenly.

After solidification of each plate, four wells were made and filled with 30 µl of a Tween compound, i.e. Tween 20, 40, 60 and 80, respectively. These plates were incubated for a week at 32°C and the growth was assessed around the individual wells after 2, 4 and 7 days.[12]

5. **Splitting of esculin:**

The β-glucosidase activity of different *Malassezia* species was assessed by using this method. [12] Loop of fresh yeast was inoculated deeply in the esculin agar tube and incubated for 5 days at 30°C.

The splitting of esculin was revealed by darkening of the medium. This test was used to distinguish *Malassezia furfur*, *Malassezia slooffiae* and *Malassezia sympodialis* from other *Malassezia* species.

The splitting of esculin into esculetin and glucose was showed by darkening of the medium, with liberation of soluble ferric salt incorporated in the medium. Significant brown staining of more than a third of the medium was considered demonstrative of *Malassezia sympodialis* and *Malassezia obtusa. Malassezia furfur* caused weak staining.

Malassezia pachydermatis had a variable reaction. The others of *Malassezia* species were negative [12]. In order to differentiate *Malassezia sympodialis* and *Malassezia obtus*a they were incubated at 40°C for one week. [14]

RESULTS:

All the one hundred skin scrapings showed pseudohyphae and spores exhibiting the characteristic "spaghetti and meatball" appearance in the *Malassezia globosa* had stable spherical cells; buds were form on the narrow base while KOH preparation (Figure-1). Growth of *Malassesia* species were obtained on mDixon's agar which represent 79 (79%) of the 100 skin scrapings.

The isolated species were as follow: 40(51%) belonged to *Malassezia globosa*, 24(30%) *Malassezia furfur*, 8(10%) *Malassezia sympodialis*, the fourth species *Malassezia obtusa* formed 5(6%) of isolates species and 2(3%) *Malassezia restricta* (Tables-1& 2).

Among *Malassezia* species there was no growth in Sabouruad's dextrose agar without overlying oil, ruling out the presence of *Malassezia pachydermatis*, the only lipid independent species (Table-3).

The urease test and diazonium B blue were positive for all *Malassezia* species, the catalase reaction was positive for all except *Malassezia restricta* which consistently lack catalase.

The isolated species did not ferment or assimilate glucose that had negative result in sugar fermentation test for all.

The Tween assimilation test allowed the differentiation of most *Malassezia* species in our study population. This phenomenon resulted in a characteristic ring of tiny colonies around the corresponding well.

Table 1: Species of the culture positive lesions.

Species of the M.O. in culture positive cases	Number	Percentage %
M.globosa	40	51
M.furfur	24	30
M.sympodialis	8	10
M.obtusa	5	6
M.restricta	2	3
Total	79	100

Table 2: Age groups distributions according to species of micro-organisms of total patients enrolled in the study.

Species of Micro Organisms	Age groups in years					Total
	<15	15-29	30-44	45-60	>60	
M.globosa	5	26	5	4	--	40
M.furfur	1	12	3	5	3	24
M.sympodialis	2	5	--	1	--	8
M.obtusa	--	3	2	--	--	5
M.restrica	--	1	--	--	1	2
Negative cultures	1	17	2	1	--	21
Total	9	64	12	11	4	100

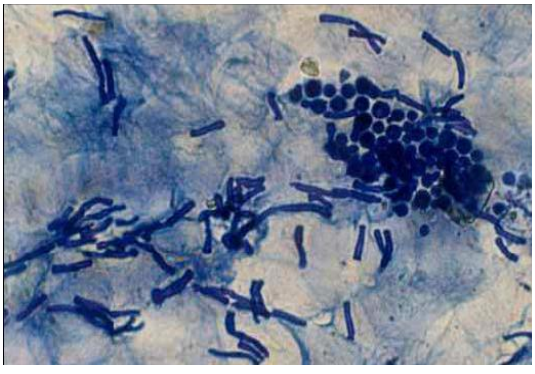

Figure 1: Short and curved hyphae with clusters of yeasts and budding cells of *Malassezia* methylene blue staining (×100).

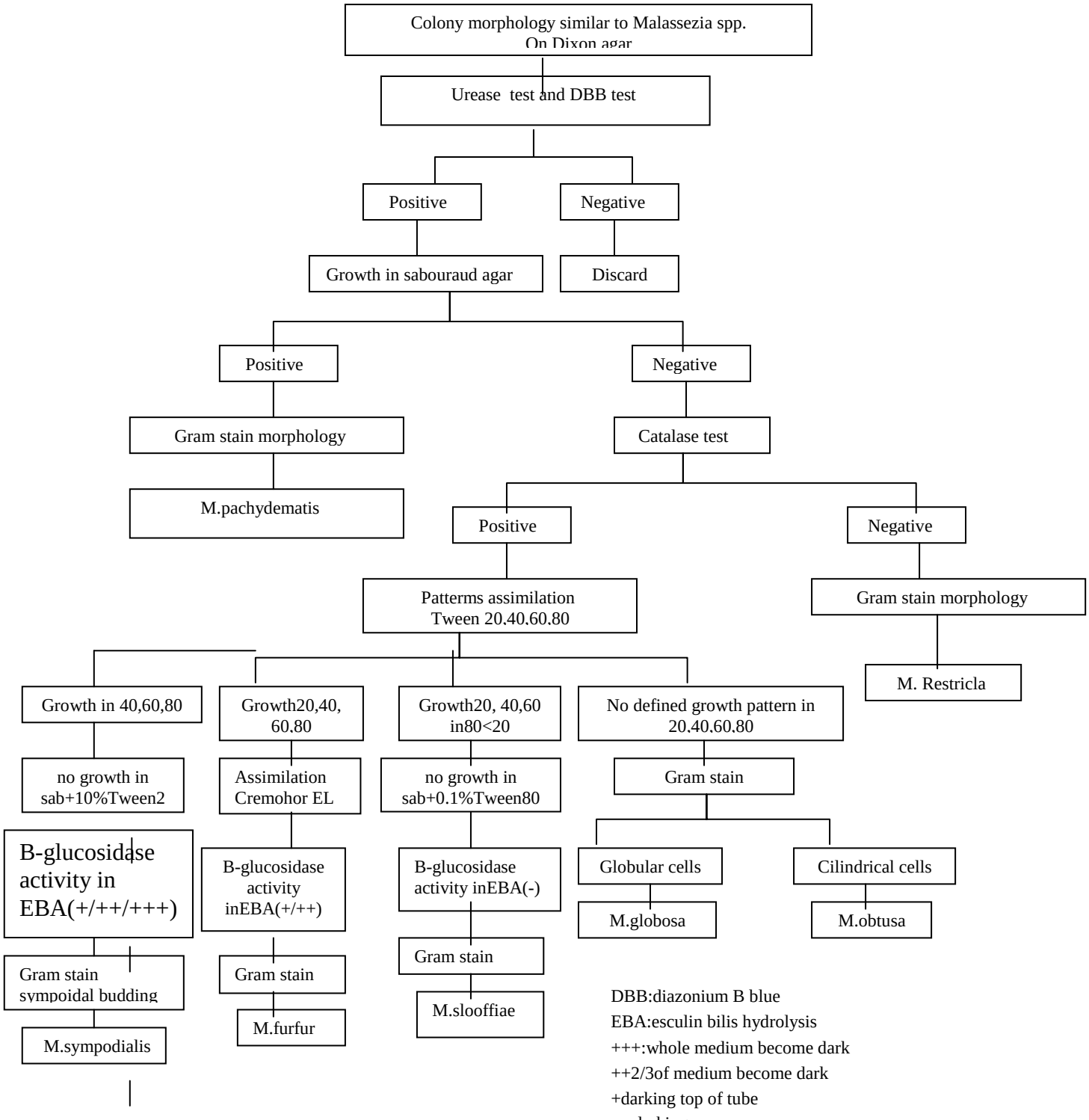

Figure 2: Identification of yeasts compatible with the Malassezia genus. [16]

Characteristic	M. dermatis	M. furfur	M. pachydermatis	M. sympodialis	M. globosa	M. obtusa	M. restricta	M. slooffiae
Morphological characteristics								
Colony morphology	Convex, butyrous, entire or lobed magin	Mat, dull, smooth, umbonate or slightly folded with convex elevation	Mat, convex, umbonate (sometimes)	Glistening, smooth, flat or with a slight central elevation	Raised, folded, rough	Smooth, flat	Dull, smooth to rough at the edges	Rough but usually with fine grooves
Cell shape (size [μm])	Spherical, oval, ellipsoidal (2.0-8.0 by 2.0-10.0)	Oval, cylindrical (1.5-3.0 by 2.5-8.0), spherical (2.5-5.0)	Oval (2.0-2.5 by 4.0-5.0)	Oval, globosal (1.5-2.5 by 2.5-6.0)	Spherical (2.5-8.0)	Cylindrical (1.5-2.0 by 4.0-6.0)	Spherical, oval (1.5-2.0 by 2.5-4.0)	Short cylindrical (1.0-2.0 by 1.5-4.0)
Physiological characteristics[b]								
Growth on Sa at 32°C	−	−	+	−	−	−	−	−
Growth on mDixon								
32°C	+	+	+	+	+	+	+	+
37°C	+	+	+	+	± or −	± or +	+	+
40°C	+	+	+	+	−	−	−	+
Catalase reaction	+	+	± or +	+	+	+	−	+
Utilization of:								
Tween 20	+	+	−	−	−	−	−	± or +
Tween 40	+	+	+	+	−	−	−	+
Tween 60	+	+	+	+	−	−	−	+
Tween 80	+	+	+	+	−	−	−	−

DISCUSSION:

Pityriasis versicolor is a major cosmetic health problem all over the world including the Iraq, the adverse cosmetic effects of the lesion may lead to significant emotional distress, particularly in adolescent [1,2,3,4,5].

In 2001 Sharquie's etal found that the pityrosporum orbiculare on normal skin of Iraqi healthy people was the common species [18].

In the present study, out of the one hundred specimens that were inoculated, 79% yielded growth of *Malassezia* in culture. Out of this, the most frequently isolated species was *Malassezia globosa* which represent 40(51%), followed by *Malassezia furfur* 24(30%) and *Malassezia sympodialis* 8(10%).

In the earlier study carried out by Crespo *et al* [7] who reported that *Malassezia globosa* was recovered from 97% of their patients, alone in 60% of them and associated with *Malassezia sympodialis* in 29%, *Malassezia slooffiae* in 7% [8].

Authors concluded that *Malassezia globosa* was in its mycelial phase which was the commonest causative agent of pityriasis versicolor [15].

So, the results of this work are compatible with the findings of other published literatures [7-14, 16-18].

The results suggest that *Malassezia globosa* in its mycelial phase is the most common *Malassezia* species associated with *ptyriasis versicolor* in the Iraqi population. The presence of this species, in its yeast phase in diseased and even in healthy skin, indicates that local factors (humidity, sweat, heat) together with some degree of idiosyncratic individual predisposition, are responsible for the transformation into the mycelial form and development of clinical lesions.

To the best of our knowledge this is the first study carried out in Iraq to identify *Malassezia* species in patients with pityriasis versicolor.

CONCLUSION:

Malassezia globosa is the most predominant species in the mycelial form of *Pityriasis versicolor* lesions and *Malassezia furfur* is the second agent being isolated.

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