

Malassezia globosa as the causative agent of pityriasis versicolor

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Summary

Background The taxonomic revision carried out in 1996 on the genus *Malassezia*, which now comprises seven different species, made necessary a re-evaluation of the data concerning the ecology and pathogenicity of these lipophilic yeasts. Very little has been published since then. **Objectives** The aim of this work was to contribute to the knowledge of the aetiology of pityriasis versicolor (PV) with a mycological study made according to the new species.

Methods Ninety-six patients with PV completed the study. In all of them, samples were taken from the lesions for direct microscopy with KOH+ Parker ink and culture. Samples were also taken from normal skin of the trunk and the forehead of the same patients for culture. Cultures were made in modified Dixon medium in Petri dishes, incubated at 31 °C and the isolates were identified by morphological and physiological characteristics.

Results In the PV lesions, direct microscopy always showed the typical mixture of globose blastoconidia and pseudomycelium. Only one patient, having received previous topical antimycotic treatment, was negative. The spherical yeasts observed *in vivo* were morphologically identical to the globose yeasts characteristic of *M. globosa*. In culture, *M. globosa* was found in 97% of cases, alone in 60% of them and associated with *M. sympodialis* in 29% and *M. slooffiae* in 7%. These two species were also found in similar percentages on the clinically uninvolved skin of the trunk, whereas *M. globosa* was not isolated at these sites. However, on the forehead, a small number of colonies of *M. globosa* was recovered in 12% of cases.

Conclusions These results support the data previously reported and strongly suggest that *M. globosa* in its mycelial phase is the causative agent of PV.

Key words: *Malassezia* spp., pityriasis versicolor

Pityriasis versicolor (PV) received its name from Robert Willan¹ at the beginning of the nineteenth century. In 1846, just after the reports of David Gruby² on the aetiology of favus and tinea capitis, Eichstedt³ observed fungal elements in the scales of the skin lesions of this disease. This fungus received different names and finally *Malassezia furfur* prevailed, as suggested by Baillon⁴ in 1889. However, the presence of at least two morphological types of yeast cells was recognized: Bizzozero⁵ named them *Saccharomyces ovalis* and *S. sphaericus* in 1884. In 1904, Sabouraud⁶ related the oval form to pityriasis simplex capitis, and called it *Pityrosporon malassezii*. This name was changed to *Pityrosporum ovale* in 1913.⁷ The globose form received the name *P. orbiculare* after Gordon in 1951.⁸ These

two names coexisted with the old name *Malassezia*, which was considered as the parasitic form of *P. orbiculare*. On the other hand, both kinds of yeasts had been observed in healthy human skin, particularly in seborrhoeic areas. A recent review of the history of the genus *Malassezia* and its nomenclature has been given by Midgley *et al.*⁹

Once the lipophilic nature of these yeasts was recognized and cultures were made possible, different workers observed spontaneous changing from one morphological type to the other, which led to the conclusion that *P. orbiculare*, *P. ovale* and *M. furfur* were only variants of the same species.¹⁰ In the taxonomic revision of Yarrow and Ahearn¹¹ in 1984, *M. furfur* was considered as the unique valid name, as it had been described originally. At that time, the genus *Malassezia* also included another species, *M. pachydermatis*, found in the skin of different animals.

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Nevertheless, the names *P. ovale* and *P. orbiculare* were still widely used among clinicians and, through the following years, different workers^{12,13} described stable morphological and serological variants in the apparently unique species, *M. furfur*. During the last decade, the use of molecular techniques led to the description of a third lipid-dependent species, *M. sympodialis*, by Simmons and Guého,¹⁴ and more recently, together with morphological and physiological studies, to the taxonomic revision of the genus carried out by Guého *et al.*¹⁵ and the description of four additional species: *M. globosa*, *M. restricta*, *M. obtusa* and *M. slooffiae*.

These important changes made it necessary to reconsider the previously accepted ecology and pathogenicity of these yeasts. The role of the different species, as they are now recognized, has not been fully investigated.^{9,16} In our recent previous reports,^{17,18} the presence of the different species of *Malassezia* in clinical lesions of PV and seborrhoeic dermatitis (SD), as well as in normal skin of patients and healthy controls, was analysed, showing that *M. globosa* was the predominant species in the PV lesions, while *M. sympodialis* was found mainly in the normal skin of the trunk and *M. restricta* in the face and scalp lesions of SD.

The aim of the present work was to contribute to the knowledge of the aetiology of PV, by studying a new group of patients, in which the clinically healthy skin of two seborrhoeic areas of the same patients was used as controls.

Materials and methods

The study examined 96 patients with PV, 44 male and 52 female, age range 8–78 years. In each case, the clinical form (pigmented or achromic), the severity or spreading of lesions and the number of episodes were assessed. Samples were taken from the PV lesions by means of Sellotape for direct microscopy with KOH + Parker ink, and scales were obtained by scraping for culture, and inoculated on modified Dixon agar¹⁹ plates. This medium comprises 3.6% malt extract, 0.6% mycological peptone, 2% ox bile, 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 1.2% agar, 0.5% chloramphenicol and 1 g L⁻¹ cycloheximide.

Samples were also taken from healthy skin of the forehead and of the shoulders, by rubbing with sterile swabs. On the trunk, we were careful to take the sample as far as possible from the lesions, and in two cases, due to the great extent of lesions, samples were obtained from the deltoid region or the upper arm. All these

samples were used for culture and inoculated on the medium, but were not examined by direct microscopy.

The cultures were made immediately in almost all subjects. In only seven, the samples were taken in the afternoon and cultures made the next morning. Nevertheless, no significant difference was found in the isolates from these subjects. The plates were incubated at 31 °C, in plastic bags to avoid desiccation, for 2 weeks. The growth of colonies was surveyed daily. The yeasts were identified by their morphology, catalase test and growth in the presence of Tween 20, 40, 60 and 80 as unique lipid supplementation, following the scheme established by Guillot *et al.*²⁰ completed by the use of Cremophor EL, as suggested by Mayser *et al.*²¹ As we reported previously, *M. globosa* is the only *Malassezia* species that can be identified by its morphology alone (Fig. 1). The identification of the species was confirmed on reference strains of the yeasts, kindly provided by Mme E. Guého from the Mycology Unit of the Pasteur Institute of Paris.

Results

All results are summarized in Table 1.

Pityriasis versicolor

Direct microscopy. In all but one case, direct microscopy of the PV scales showed the typical picture of a mixture of abundant globose blastoconidia, usually grouped, forming clusters and pseudomycelia (Fig. 2). In our patients, only globose forms were observed even in the few cases where the lesions spread to the face. The patient with negative direct microscopy had been under treatment with clotrimazole 1% cream during the

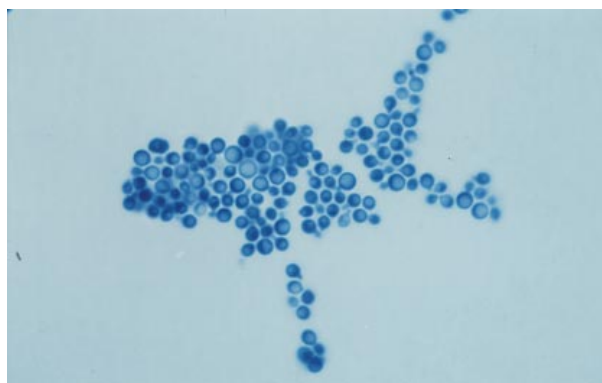


Figure 1. Typical globose yeast cells of *Malassezia globosa* in culture (lactophenol-cotton blue; original magnification $\times 1000$).

Table 1. Summary of results

Species	Direct microscopy	Culture, pityriasis	Culture, normal skin, shoulders	Culture, normal skin, forehead
<i>Malassezia globosa</i>	99%	97%	0	12%
<i>Malassezia sympodialis</i>	0	32%	42%	2%
<i>Malassezia slooffiae</i>	0	7%	5%	0

previous 2 weeks. In this patient only *M. sympodialis* was isolated from the PV scales, as well as from the clinically normal skin of the trunk, both with abundant colonies.

Cultures. *M. globosa* was isolated in 93 patients (97%), forming abundant colonies in most of them. This species was found alone in 58 cases (60%), and associated with *M. sympodialis* and with *M. slooffiae* in the remaining 35 (Fig. 3a). *M. sympodialis* was found in 31 patients (32%), associated with *M. globosa* in 28 and alone in only three cases. In these three patients, *M. sympodialis* was also cultured from the healthy surrounding skin; two of them had been treated with either terbinafine or clotrimazole cream during the previous weeks. *M. slooffiae* was isolated on seven (7%) occasions, always together with *M. globosa*. In all cases, *M. slooffiae* was also found on the healthy trunk skin.

Clinical pattern, severity and number of episodes. Fifty-six patients had the achromic form of the disease, 33 the pigmented form, and seven had lesions of both types. No significant differences were found in the species isolated according to the clinical form. Forty patients had PV for the first time, while the remaining 56 had had more than one episode. No statistical difference was found in the species isolated in these two groups. Three severity groups were determined according to

the extent of PV: those with lesions covering the equivalent of a palm were designated as 'mild' ($n = 26$); 'moderate' severity implied lesions covering the shoulders, the upper part of the trunk or an equivalent extension ($n = 36$); and the 'severe' group had lesions spreading to the whole trunk or even the neck and face ($n = 34$). No significant differences were found in the isolates from these groups.

Healthy skin samples

Forehead. *Malassezia* spp. were found in only 14 (15%) of the samples taken from the forehead. *M. globosa* was isolated in 12 cases (12%), always in small numbers (fewer than five colonies), and *M. sympodialis* was found only twice (2%) (Fig. 3b).

Trunk (shoulders). *Malassezia* spp. were isolated in nearly half of the patients (47%). *M. sympodialis* was

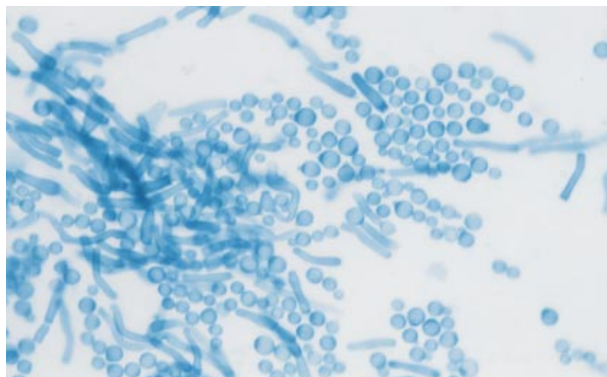
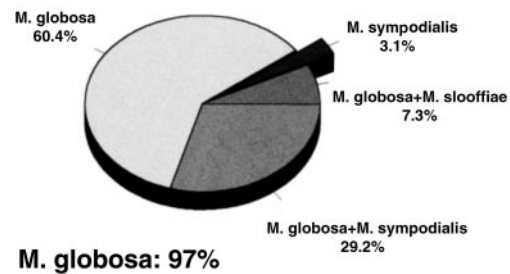


Figure 2. Direct microscopy of pityriasis versicolor scales, showing typical globose cells and mycelia (KOH+ Parker blue ink; original magnification $\times 1000$).



M. globosa: 97%

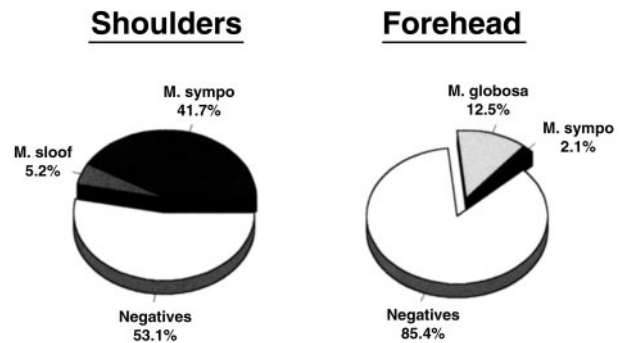


Figure 3. (a) *Malassezia* spp. isolated from pityriasis versicolor lesions. (b) *Malassezia* spp. isolated from healthy skin areas.

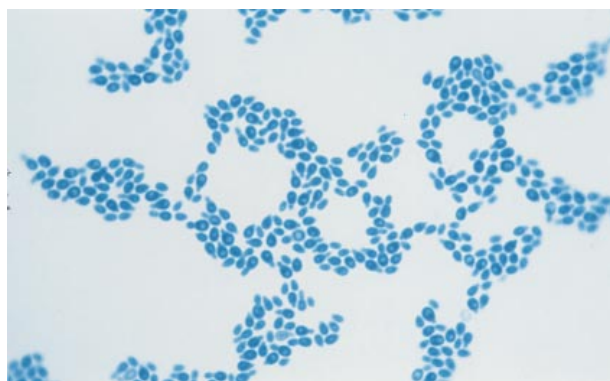


Figure 4. Ovoid yeasts of *Malassezia sympodialis* in culture (lactophenol-cotton blue; original magnification $\times 1000$).

by far the most common species, found in 40 cases (42%) (Figs 3b and 4); in half of which abundant colonies were observed. *M. slooffiae* was recovered on five occasions (5%). The number of isolates of these two species in healthy trunk skin was similar to that found in the clinical lesions of PV. *M. globosa* was not isolated from any of the healthy skin samples of the trunk.

Discussion

The taxonomic revision of the genus *Malassezia* carried out in 1996 and the description of four new species has made it necessary to review the data concerning the ecology and the pathogenicity of these yeasts, and to analyse the respective role of each of them. The few reports that have appeared since then^{9,15,16} suggested that *M. sympodialis* is the predominant species in human skin, healthy or diseased, and is found mainly on the trunk, while *M. globosa* is found in PV scales and in healthy skin, and *M. restricta* seems to be associated with pityriasis capitis. *M. pachydermatis*, known as an agent of external otitis in cats and dogs, has also been considered responsible for some cases of systemic infection, mainly in premature children.^{22,23}

In two recent reports,^{17,18} we have compared the *Malassezia* species isolated from clinical lesions of PV, SD and healthy skin. Our results suggested that *M. globosa* plays a predominant, if not exclusive, part in the aetiology of PV, while confirming that *M. sympodialis* and *M. slooffiae* are part of the normal skin flora and *M. restricta* is the most common *Malassezia* spp. in the scales of SD. *M. furfur sensu stricto* was only seldom found in human skin.

The results of the present study confirm and enlarge these data. We think that the small difference found in

the percentages of *M. globosa* isolates (86% in our previous studies, now increased to 97%) can be explained by improved experience in the study of *Malassezia* cultures. Colonies of *M. globosa* are the smallest and grow more slowly than the other species we have found in healthy skin. They also seem to be more fragile, not growing at higher temperatures and, in contrast to *M. sympodialis*, they do not survive preservation in Dixon broth for long periods. In cases where there is a considerable amount of *M. sympodialis* or *M. slooffiae*, which are more quickly developing species, and if the number of colonies of *M. globosa* is small, the latter may be obscured unless culture plates are examined daily.

The percentages of *M. sympodialis* and *M. slooffiae* in healthy skin found in this study are very similar to our previous data. Concerning *M. globosa*, the results are also very similar, although in one of our previous reports¹⁸ we had isolated this species from healthy skin of the trunk in three patients with SD. This species was also found in the forehead in three other cases only, from a total of 220 healthy skin samples.

Our results confirm the striking difference between the healthy trunk skin, where *Malassezia* colonies were recovered in nearly half of the cases (47%), and the forehead, where only 15% were positive. They also confirm that *M. sympodialis* is the predominant species on the healthy skin of the trunk and that, although to a much lesser extent, *M. slooffiae* also forms part of the normal skin flora.

In our patients, direct microscopy only showed spherical yeasts, arranged in typical clusters and associated with hyphae. This is the classic morphology observed in temperate countries, and also by us in almost all cases. In a previous report,¹⁸ a picture with ovoid yeasts mixed with the globose ones was found in only one patient (who had facial lesions) of 100.

Nevertheless, a distinct picture formed by ovoid or cylindrical yeasts seems to be common in tropical countries, and it has been suggested that it could indicate infection by a different species of *Malassezia*.²⁴ New studies are therefore necessary to identify the species observed *in vivo* in these cases.

Our results strongly support that *M. globosa*, in its mycelial phase, is the causative agent of PV. The presence of this species, in its yeast phase, in SD and even in healthy skin, indicates that local factors (humidity, sweat, heat, seborrhoea) together with some degree of idiosyncratic individual predisposition, are responsible for the transformation to the mycelial form and development of clinical lesions.

References

- 1 Shelley WB, Crissey JT. Robert Willan. In: *Classics in Clinical Dermatology*. Springfield: Charles C Thomas, 1953: 5–18.
- 2 Ainsworth GC. *Introduction to the History of Medical and Veterinary Mycology*. Cambridge: Cambridge University Press, 1986: 15–18.
- 3 Eichstedt E. Pilzbildung in der Pityriasis versicolor. *Froriep Neue Notis A.D. Natur Heilk* 1846; **39**: 270.
- 4 Baillon H. *Traité de Botanique Médicale Cryptogamique*. Paris: Octave Douin, 1889: 234–9.
- 5 Bizzozzero J. Über die Mikrophyten der normalen Oberhaut der Menschen. *Arch Pathol Anat Physiol* 1884; **98**: 441–59.
- 6 Sabouraud R. Les maladies desquamatives. In: *Maladies Du Cuir Chevelu*. Paris: Masson et Cie, 1904.
- 7 Castellani A, Chalmers AJ. *Manual of Tropical Medicine*. London: Baillière Tindall, 1913.
- 8 Gordon MA. The lipophilic mycoflora of the skin I: *in vitro* culture of *Pityrosporum orbiculare* n.sp. *Mycologia* 1951; **43**: 524–35.
- 9 Midgley G, Guého E, Guillot J. Diseases caused by *Malassezia* species. In: *Medical Mycology* (Hay RJ, Ajello L, eds). *Topley & Wilson's Microbiology and Microbial Infections*, Vol. 4, Part IV (Superficial Basidiomycetous Yeasts). London: Arnold, 1997: 201–11.
- 10 Faergemann J. *Pityrosporum* infections. In: *Topics in Clinical Dermatology Cutaneous Fungal Infections*. New York: Igaku Shoin, 1992: 69–83.
- 11 Yarrow D, Ahearn DG. Genus 7: *Malassezia* Baillon. In: *The Yeasts, a Taxonomic Study* (van Kreger-Rij NYW, ed.), 3rd edn. Amsterdam: Elsevier, 1984: 882–5.
- 12 Midgley G. The diversity of *Pityrosporum* (*Malassezia*) yeasts *in vivo* and *in vitro*. *Mycopathologia* 1989; **106**: 143–53.
- 13 Cunningham AC, Leeming JP, Ingham E, Gowland G. Differentiation of three serovars of *Malassezia furfur*. *J Appl Bacteriol* 1990; **68**: 439–46.
- 14 Simmons RB, Guého E. A new species of *Malassezia*. *Mycol Res* 1990; **94**: 1146–9.
- 15 Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 1996; **69**: 337–55.
- 16 Guého 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** (Suppl. 1): 220–9.
- 17 Crespo V, Ojeda A, Vera A *et al.* Aislamiento e identificación de *Malassezia* spp. en pitiriasis versicolor, dermatitis seborreica y piel sana. *Rev Iberoam Micol* 1999; **16**: S16–21.
- 18 Crespo V, Ojeda A, Vera A *et al.* Mycology of pityriasis versicolor. *J Mycol Med* 1999; **9**: 143–8.
- 19 van Abbe NJ. The investigation of dandruff. *J Soc Cosmet Chem* 1964; **15**: 609–30.
- 20 Guillot J, Guého E, Lesourd M *et al.* Identification of *Malassezia* species. A practical approach. *J Mycol Med* 1996; **6**: 103–10.
- 21 Mayser P, Haze P, Papavassilis C *et al.* Differentiation of *Malassezia* species: selectivity of Cremophor EL, castor oil and ricinoleic acid for *M. furfur*. *Br J Dermatol* 1997; **137**: 208–13.
- 22 Guého E, Simmons RB, Pruitt WR *et al.* Association of *Malassezia pachydermatis* with systemic infections of humans. *J Clin Microbiol* 1987; **25**: 1789–90.
- 23 Chang HJ, Miller HL, Watkins N *et al.* An epidemic of *Malassezia pachydermatis* in an intensive care nursery associated with colonisation of health care workers' pet dogs. *N Engl J Med* 1998; **11**: 706–11.
- 24 Borelli D. Pitiriasis versicolor por *Malassezia ovalis*. *Mycopathologia* 1985; **89**: 147–53.