

CONTROVERSIES IN DERMATOLOGY

Pityriasis Versicolor and the Yeasts of Genus *Malassezia*

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Abstract. Although pityriasis versicolor is the only human disease for which *Malassezia* yeasts have been fully established as pathogens, it is still not clear which species are implicated. Most studies carried out in recent years support our hypothesis—proposed in 1999—that *Malassezia globosa* is the predominant species in pityriasis versicolor lesions, at least in temperate climates. Confirmation of this hypothesis could help us understand the conditions, as yet unclear, that induce transformation of this yeast from the saprophytic form present in healthy skin to the parasitic form, characterized by the formation of pseudomycelium, and could also guide therapy. In addition, isolation of another species, *Malassezia furfur*, which seems to be predominant in the tropics, raises the possibility of a second etiologic agent confined to certain areas, as occurs with some other human mycoses.

Key words: pityriasis versicolor, *Malassezia* species.

LA PITIRIASIS VERSICOLOR Y LAS LEVADURAS DEL GÉNERO *MALASSEZIA*

Resumen. Aunque la pitiriasis versicolor (PV) es la única enfermedad humana en la que el rol etiológico de las levaduras *Malassezia* está plenamente establecido, permanece como objeto de controversia cuál (o cuáles) es la especie implicada. La mayoría de los estudios llevados a cabo en estos últimos años apoyan nuestra hipótesis, expuesta ya en 1999, indicando que *M. globosa* es la especie predominante en las lesiones de PV, al menos en los climas templados. La confirmación de esta hipótesis podría ayudar a comprender las condiciones, todavía poco claras, que inducen la transformación de esta levadura de su forma saprofita presente en la piel sana, a la parasitaria, caracterizada por la formación de pseudomicelio, y ayudarían también a establecer las mejores medidas terapéuticas. Por otro lado, el aislamiento de otra especie, *M. furfur*, que parece predominar en regiones tropicales, podría plantear la posibilidad de un segundo agente etiológico confinado a ciertas áreas geográficas, como acontece en algunas otras micosis humanas.

Palabras clave: pitiriasis versicolor, *Malassezia*.

Introduction

More than 150 years ago, Eichstedt¹ described a yeast-like fungus that is currently identified as belonging to the genus *Malassezia*, the causative agent of pityriasis versicolor (PV) and, to date, this is the only human disease in which these yeasts have been firmly established as pathogens. Nevertheless, debate remains over which species or indeed whether more than one species is involved. This article attempts to explore this issue more deeply, by analyzing the

association between *Malassezia* yeasts and PV in the light of recent studies conducted by different researchers, including those conducted by our group over the last 10 years.

The complexity of this issue has led us to include, first, a brief description of the main aspects relating to the incidence, epidemiology, pathogenesis, and diagnosis of the disease, followed by a description of the taxonomic, morphological, and biochemical aspects or characteristics of *Malassezia*, without neglecting its ecological role on normal human skin.

The Disease: Pityriasis Versicolor

Definition, Clinical Characteristics, Incidence

Pityriasis versicolor is a superficial fungal infection of the skin usually considered to be caused by saprophytes reflecting the null or almost null inflammatory skin response produced

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in this disease, as in so-called stones and tinea nigra palmaris. This disorder is common, widespread, and benign, although it often recurs. The name itself appropriately describes its main clinical characteristics: the appearance of macules and finely scaled plaques, with colors ranging from white (the alba or achromians variant) to pink, salmon, or brown. Although most patients present macules of the same color, sometimes the lesions are of 2 colors: those with a pink or brown tone on covered areas and white ones on exposed surfaces. Recently, a red variant (PV rubra) has been described and another macule which is blackish (PV nigra), as well as the possible transformation from one to the other or to the alba form.²

The lesions tend to present on the upper trunk, chest, back, and shoulders, and can extend toward the neck, face, and arms. Pityriasis versicolor predominates in young adults, without significant differences between the sexes. Its appearance in the facial region is rare in temperate countries, such as Spain, and is mainly seen in children, but is quite common in tropical and subtropical regions, where more than half of these patients are found.³ Other rare locations have also been described, such as the eyelids, armpits, penis, and perineum,⁴ and the disease is very difficult to distinguish from erythrasma (erythrasmoid PV)⁵ when it appears in the groin where both processes can coexist, although this is rare.⁶

Pityriasis versicolor is one of the most common dermatomycosis, and is especially prevalent in regions with a warm humid climate, where up to 40% of the population may be affected. A recent study conducted in a Venezuelan fishing community reported a total prevalence of 15.52%.³ Prevalence seems to be far lower in temperate climates. Although there are few studies on the subject, a review conducted in the United States between 1971 and 1974 reported a figure of 0.8% in a population of 28 000 people.⁷ A study conducted in Sweden in 1979 found a prevalence of PV of 1.1% in a total of 20 296 patients in a large hospital,⁸ and a later study conducted in the same country in 1983, that included 3302 people, found PV in 0.5% of men and 0.3% of women.⁹

Recently, a study conducted with a representative sample of 1024 young Italian sailors demonstrated a prevalence of PV of 2.1%. No associations were found between disease and variables such as practicing sports, swimming, or hyperhidrosis, and the only significant association was with previous outbreaks of PV, an observation which supports the hypothesis of the determining role of constitutional factors in the pathogenesis of this process.¹⁰

Pityriasis versicolor accounts for an appreciable percentage of all other superficial mycoses, as demonstrated by studies conducted in Venezuela, where PV alone accounted for almost 30% of all dermatomycoses,¹¹ and is a very similar figure to that found in another contemporary study conducted in Libya.¹²

According to statistical data from the Mycology Department of the Dermatology Service of our hospital in Malaga, PV only represents around 10% of the group of cutaneous mycoses, although we believe that the real frequency of the disease is much higher, since it tends to be diagnosed and treated in a primary care setting, and thus only a limited number of patients are referred to the dermatologist.

Pathogenesis

Pityriasis versicolor tends to be asymptomatic, although some patients report moderate-to-severe pruritus, and it usually appears in healthy individuals. Some triggering or aggravating factors have been described, although what induces transformation of the saprophytic yeast form to the parasitic mycelial form remains subject to debate. Genetic factors seem have a certain role, since the disease is more common among first-degree family members.¹³ Other factors have also been studied, such as the use of oral anticoagulants¹⁴ or hyperhidrosis,¹⁵ although the role of latter has not been confirmed in recent studies.¹⁰ Other iatrogenic factors, such as treatment with systemic or immunosuppressive corticosteroids, could also be involved,¹⁶ and we have recently seen 2 cases of especially extensive PV in patients being treated with etanercept in whom no previous episode of this disease had been reported (unpublished data).

However, the role of immune response in PV is not well established, since studies by different authors have produced contrasting results.¹⁷ In general terms, local factors seem to predominate in the pathogenesis of this disease, such as high temperatures, the degree of humidity and occlusion produced by clothing, which combine with idiosyncrasies of the individual derived from small changes in sebum composition. These factors would induce the changes in the yeasts that, on the other hand, are already present on healthy skin, causing them to develop mycelium and transform to the parasitic form.

Diagnosis

For an experienced dermatologist PV is very easy to diagnose in most cases, but we consider that this should always be confirmed by direct examination with potassium hydroxide and Parker ink. This reagent consists of a mixture of equal parts of 20% potassium hydroxide and black Parker ink, which rapidly stains the yeasts and pseudomycelium blue, offering a typical image described as "spaghetti and meatballs" (Figure 1). Calcofluor can also be used with very good results, but this technique requires viewing under a fluorescence microscope. Wood light can be used to detect

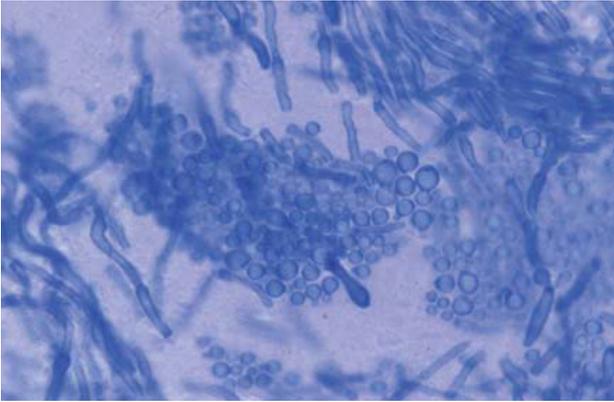


Figure 1. Direct examination of pityriasis versicolor with potassium hydroxide plus ink, $\times 1000$.

subclinical lesions, but it should be recalled that the yellowish fluorescence indicative of this disease only appears in approximately one-third of cases.¹⁸ On the other hand, biopsy is rarely proposed as a diagnostic procedure in PV although, like other fungi, the *Malassezia* yeasts stain well with the periodic acid-Schiff reaction and methenamine silver.

Culture is also unnecessary for routine diagnosis, but it is essential if one wishes to identify the species present in the lesions or those which may form part of the normal flora. The *Malassezia* yeasts, with the single exception of *Malassezia pachydermatis*, do not grow in standard mycological media, such as Sabouraud agar, and require quite complex media that, in addition, have to be prepared from their components, since these media are not commercially available. These media and the techniques used to identify *Malassezia* are discussed below.

The Yeasts: Introduction to the Genus *Malassezia*

The genus *Malassezia* includes a group of lipophilic yeasts whose natural habitat is the skin of humans and other warm-blooded animals. As indicated in the introduction, a long and confused history has accompanied the study of these yeasts ever since they were first described in patients with PV.¹ Among other factors, the impossibility of isolating and cultivating these yeasts, and their subtle morphological differences led to a prolonged debate that has continued to the present.

Briefly, we merely mention that Baillon¹⁹ identified the new genus *Malassezia* and the species *Malassezia furfur* as the causative agent of PV in 1889, although, due to the lack of effective culture methods, it was not even certain that the yeasts and mycelia that appeared intermingled under direct microscopic examination were the same

organism. Years later, Sabouraud²⁰ identified the genus *Pityrosporium* to describe similar yeasts, although lacking mycelium, that were present in *pityriasis capitis* scales, and that would subsequently be named *Pityrosporium ovale*.²¹ Subsequently, similar microorganisms were isolated from different animals, such as rhinoceroses (*Pityrosporium pachydermatis*)²² and dogs (*Pityrosporium canis*).²³ Finally, another species, characterized by having rounded cells, similar to those observed in PV scales, was isolated from humans and named *Pityrosporium orbiculare*.²⁴

The 2 species found on human skin require relatively complex culture media for their development, whereas those isolated from animals are less selective and grow in standard mycological media, such as Sabouraud agar dextrose, since, being lipophilic but not lipid dependent, they can take advantage of the simple lipidic substances present in the peptone contained in this medium. In any case, the fact these fungi are lipophilic made it difficult to use any of the identification systems applied to the known yeasts, and so in 1984 mycologists restricted the genus to just 2 valid species²⁵: a human one, *M furfur*, and an animal one, *M pachydermatis*. The old name *Pityrosporium* was rejected (*nomen rejiciendum*) because it was considered a synonym of *Malassezia*, a term already used and so given priority according to the rules of taxonomic nomenclature.

In 1990, Simmons and Guého²⁶ described a third species, *Malassezia sympodialis*, based on genomic differences and shortly afterwards the new molecular techniques, together with serological and ultrastructural studies, made it possible for Guého, Midgley, and Guillot to describe another 4 species in 1996.²⁷ Thus, the genus *Malassezia* included 7 species: *M furfur*, *M pachydermatis*, *M sympodialis*, *M globosa*, *M slooffiae*, *M restricta*, and *M obtusa*. In recent years, ribosomal DNA sequencing techniques have added another 6 species: *Malassezia dermatis* and *Malassezia japonica* (isolated from humans with atopic dermatitis), *Malassezia yamatoensis* (in seborrheic dermatitis) and *Malassezia equina*, *Malassezia caprae*, and *Malassezia nana* (in various animals). Nevertheless, these new species seem to be closely related to *M sympodialis* and *M furfur* from a phylogenetic perspective, which has led to the feeling among some authors that new studies are essential to definitively validate them²⁸ (Table 1).

In any case, all these species, including the most recent, can be identified and differentiated by their morphological characteristics and physiological tests that we describe below (E. Guého, personal communication). In case of doubt various molecular identification techniques can be employed.²⁹

The typical monopolar budding of *Malassezia* yeasts, which causes a prominent “scar” at the budding point of the mother cell, allows the genus to be identified in *in vitro* and *in vivo* skin samples stained with potassium hydroxide

Table 1. Members of the Genus *Malassezia*

First Studies (3 species)	1996 Review (7 species)	The Genus in 2007 (13 species)
<i>M furfur</i> 1889 <i>Pityrosporum ovale</i> 1913 <i>Pityrosporum orbiculare</i> 1951	<i>M furfur</i> 1889 <i>M pachydermatis</i> 1935 <i>M sympodialis</i> 1990	<i>M furfur</i> 1889 <i>M pachydermatis</i> 1935 <i>M sympodialis</i> 1990 <i>M globosa</i> 1996
<i>M pachydermatis</i> 1935 <i>Pityrosporum pachydermatis</i> 1925 <i>Pityrosporum canis</i> 1955	<i>M globosa</i> 1996 <i>M obtusa</i> 1996 <i>M restricta</i> 1996 <i>M slooffiae</i> 1996	<i>M obtusa</i> 1996 <i>M restricta</i> 1996 <i>M slooffiae</i> 1996
<i>M sympodialis</i> 1990		<i>M dermatis</i> 2002 <i>M japonica</i> 2003 <i>M nana</i> 2004 <i>M yamatoensis</i> 2004 <i>M caprae</i> 2007 <i>M equine</i> 2007

plus Parker ink, 1% methylene blue, or calcofluor, as mentioned regarding the diagnosis of PV. The identification of the species requires special culture media, except for *M pachydermatis* which grows in standard media (Sabouraud dextrose). The 2 media normally used to isolate *Malassezia* are Dixon agar, described by Van Abbe in 1964, or its modified formula (mDixon agar), and that described by Leeming and Notman (LN medium) in 1987. The mDixon medium permits better visualization and isolation of the colonies, which is very important when, as is common, 2 or more species are found in the same plaque, and for this reason we have chosen to use this instead of LN medium in our studies. Incubation temperature is also an important factor, since several species (among them *M globosa* and *M restricta*) do not grow above 36°C. Thus, cultures should be incubated in ovens between 30°C and 35°C, and the dishes wrapped in plastic bags to ensure suitable humidity and prevent the medium from drying out (Figure 2).

The in vitro identification of the different *Malassezia* species can be conducted in any microbiology laboratory using simple techniques based on morphological study of the colonies at the macroscopic and microscopic levels, catalase and β -glucosidase reactions (esculin test), and by studying their lipid usage and assimilation patterns with the following lipid substances: Tween 20, 40, 60, and 80 and cremophor EL (polyethoxylated castor oil). A detailed description of the complete identification system can be found in specialized texts.^{29,30}

The presence of *Malassezia* yeasts on healthy skin of humans had already been described in the second half of the 19th century, and it soon became evident that colonization density was associated with age and differences

in the activity of sebaceous glands in different areas of the body. Recent studies using the identification system mentioned have demonstrated several species on human skin.³⁰⁻³³ In particular, *M restricta* seems to predominate on the scalp, whereas *M sympodialis* predominates on the trunk and *M globosa* is widely and equally distributed in all seborrheic areas. On the other hand, other species, such as *M slooffiae* and *M furfur sensu stricto*, seem to be uncommon on human skin in healthy individuals and *M obtusa* is extremely rare.

Veterinarians have also investigated these yeasts on the skin of several animals, demonstrating the role of *M pachydermatis* (and to a lesser extent, *M furfur*, *M obtusa*, and *M sympodialis*) as the causative agents of otitis in cats and dogs.³⁴⁻³⁷ Other studies have shown that different species of *Malassezia* also colonize the skin of many other animals,

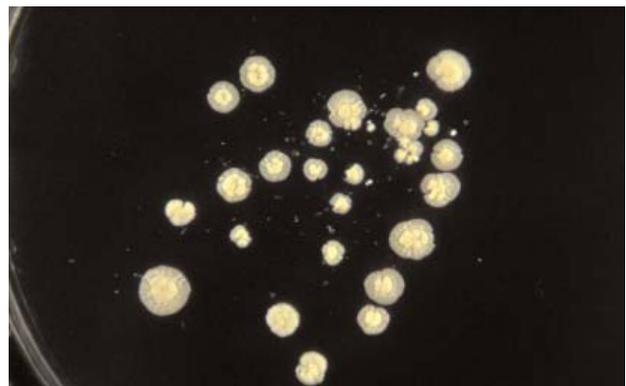
**Figure 2.** Typical colonies of *M globosa* in mDixon medium.

Table 2. Epidemiological Studies of Pityriasis Versicolor by *Malassezia* Species

Authors	Year	Patients	M Globosa	M Sympodialis	M Restricta	M slooffiae	M Furfur	M Obtusa	Culture Medium
Crespo-Erchiga	1999	75	87%	34%	3%	8%			Dixon
Nakabayashi	2000	22	55%	9%		5%	5%		Dixon
Crespo-Erchiga	2000	96	97%	32%		7%			Dixon
Gupta	2001	111	25%	59%	1%	1%	11%		LNM
Aspiroz	2002	79	90%	40.5%		1%			LNM
Dutta	2002	250	63.6%	4.8%			34%		Dixon
Gaitanis	2002	3	100%						PCR
Hernández	2003	11	47%	27%	13%		13%		Dixon
Tarazooie	2004	94	53.3%	9.3%		4%	25.3%	8.1%	Dixon
Razalakolona	2004	65	2.4%			26.8%	70.7%		?
Makni	2004	222	65%	5%			13%		Dixon PCR
De Quinzada	2005	150	5.3%	4.6%		5.3%	42%	0.6%	Dixon
Miranda	2006	95	2%	11.4%			77.8%	8.3%	Dixon
Crespo-Erchiga	2006	100	97%	34%	1%	3%	2%		Dixon
Gaitanis	2006	71	90%	3-4%		3-4%	3-4%		Dixon
Prohic	2007	90	63%	14%		4%	10%	8%	Dixon

such as monkeys, pigs, bears and birds,^{34,37} horses (*M equina*) and goats (*M caprae*),³⁸ and cattle (*M nana*).³⁹

Pityriasis Versicolor and *Malassezia* Yeasts: Etiological Considerations

In 1951, Gordon⁴⁰ was the first researcher to suggest that rounded yeasts, which he named *P orbiculare*, could be the etiologic agent of PV. Further progress was slow until, based on the extensive taxonomic revision by Guého, Midgley, and Guillot in 1996, an increasing number of studies were conducted based on the new classification of the genus and on the new identification techniques mentioned earlier (Table 2).

In 1999, Crespo Erchiga et al^{32,41} conducted 2 parallel studies in Málaga, Spain, on a small number of patients with PV, using the same number of seborrheic dermatitis samples and samples from healthy individuals, and showed for the first time that *M globosa* was, by far, the predominant species in PV lesions. These results were confirmed by the same authors in a study of 96 patients published 1 year later.⁴² Most studies published in those initial years, such as those of Nakabayashi et al⁴³ in Japan in 2000 and Aspiroz et al⁴⁴ in Zaragoza, Spain, in 2002, also supported these results. In particular, in the latter study, the percentages obtained for *M globosa* and *M*

sympodialis were practically identical to those reported by our group in the studies mentioned. Furthermore, Aspiroz et al demonstrated greater enzymatic activity, involving lipase and esterase production, in *M globosa* strains than in other species of the genus, a fact that may explain the greater level of pathogenicity associated with this species on human skin, and that may also support its hypothetical role in the etiology of PV. The only study conducted from that period with contrasting results was that of Gupta et al⁴⁵ in Canada, which found a predominance of *M sympodialis*, although there were important differences regarding both the culture medium used and the methodology followed compared to those of the other authors mentioned, including those of our group.

Later, Nakabayashi,⁴⁶ using a polymerase chain reaction (PCR) identification technique for *Malassezia* present in PV scales, also found that *M globosa* was the most commonly detected species (97%), although this was closely followed by *M restricta* (79%) and *M sympodialis* (68%). Another study conducted by Gaitanis et al⁴⁷ in Greece in 2002, using a PCR-restriction fragment length polymorphism technique applied to lesions caused by several dermatological processes, only detected and identified *M globosa* in PV scales.

In 2003, Hernández et al⁴⁸ in Mexico also found a predominance of *M globosa* in standard cultures of PV samples. These results were similar to those published 1

year later by 3 other authors: Dutta et al⁴⁹ in India, who isolated *M globosa* in 63.6% of cases; Tarazooie et al⁵⁰ in Iran, who detected *M globosa* in 53.3% of cases; and Makni et al⁵¹ in Tunisia who observed the predominance of *M globosa* in mDixon culture medium (65%), which they confirmed 1 year later using molecular techniques.⁵² In 2006, the Greek research group headed by Gaitanis⁵³ published a new study on the isolation of *Malassezia* in patients with PV and seborrheic dermatitis, using mDixon medium. *Malassezia globosa* was isolated from PV in 90% of cases; it was found in isolation in 77% and in combination with *M sympodialis*, *M furfur*, or *M slooffiae* in 13%. In view of the fact that these other species were found in isolation in less than 10% of cases, the authors did not specify these percentages. Finally, in 2007, Prohic et al⁵⁴ conducted a study in Bosnia-Herzegovina and again identified *M globosa* as the species most commonly isolated in PV lesions (63%).

Our most recent study on the topic, published in 2006,⁵⁵ analyzed 100 samples from PV patients, of whom 20% had been referred from other Andalusian hospitals (Cádiz and Granada, Spain), and showed very clear results in this regard, *M globosa* being isolated in 97% of patients, followed by *M sympodialis* (34%), *M slooffiae* (3%), *M furfur* (2%), and *M restricta* (1%). Furthermore, we observed yeasts that were morphologically identical to those of *M globosa*, together with pseudomycelium, by direct examination in 98% of patients (Figure 3).

However, some studies conducted mainly in areas with tropical or subtropical climates show a clear predominance of *M furfur* in PV lesions. One was conducted in Madagascar by Razanakolona et al⁵⁶ in 2004, one by De Quinzada⁵⁷ in Panama in 2005, and another by Miranda et al in 2006 in Brazil.⁵⁸ These 3 studies isolated *M furfur* as the predominant species in 70.7%, 42%, and 77.8%, respectively. These results may lend credence to the old hypothesis made 80 years ago by Castellani and Panja, and recently defended by Midgley³⁴ and our group,¹⁷ whereby a second species other than *M globosa* observed in our temperate climates could be predominant in warmer and more humid climates. Although, in our view, larger and more reliable studies would be needed in these regions, it should be recalled that there are mycoses caused by more than one etiological species, as in the case of chromoblastomycosis. In this disease, the same skin lesions can be caused by at least 3 different fungi: *Fonsecaea pedrosoi*, *Phialophora verrucosa*, and *Cladosporium carrionii*, with distinct geographic distribution.

Conclusions

In our opinion, *M globosa* seems to be the predominant species, if not the only one, in the etiology of PV, at least in temperate climates. Our arguments in defense of this

hypothesis, which was proposed for the first time by our group in 1999, are as follows:

1. By direct examination, a large number of yeasts morphologically identical to those of *M globosa* are practically the only ones observed in 98% of cases of PV lesion scales. Such yeasts are the source of the pseudomycelia, also abundant, that form the other characteristic in vivo microscopic image of this disease, as can be verified by repeated and careful observation of the preparations.
2. The predominant species isolated in culture by most studies, exceeding 90% in some cases, is also *M globosa*. In order of frequency, the second most commonly isolated species is *M sympodialis*, observed in approximately 40% of cases and almost always associated with *M globosa*. However, it has been demonstrated that *M globosa* is able to form germination tubes in vitro, an event that has never been observed in *M sympodialis*.
3. *Malassezia sympodialis*, on the other hand, predominates on the healthy skin of the trunk, both in perilesional areas in PV patients and in healthy controls, which reinforces the idea of the exclusively saprophytic role of this species on human skin. In turn, *M globosa* can also be isolated on healthy skin in different locations in percentages that seem to depend on the isolation technique used.

These arguments suggest that *M globosa*, present in its yeast form on the skin of healthy adults, causes PV lesions after transforming into its mycelial form. The factors that induce this transformation remain subject to debate, although the available data, rather than pointing to immunological alterations (although this is also possible in some cases), suggest subtle changes in the environment, such as higher temperatures or humidity, or idiosyncratic changes in the sebum composition. Furthermore, the

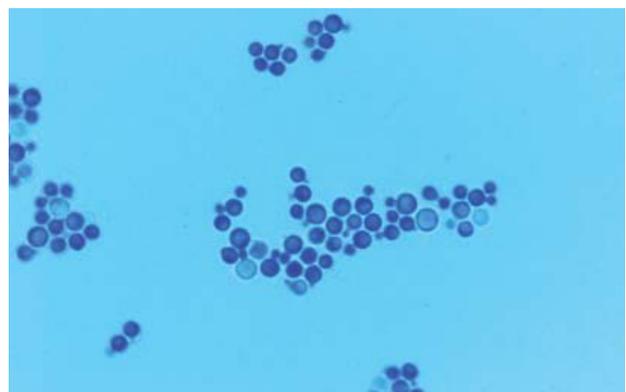


Figure 3. Yeasts of *M globosa* in culture. Lactophenol blue, ×1000.

potential existence of especially virulent strains of *M. globosa* should be assessed.

Finally, the fact that *M. furfur* is predominantly isolated in some studies conducted in tropical areas and that it is the only species of the genus able to produce pseudomycelium in vitro raises the intriguing possibility that PV is one of the rare mycoses that can be caused by more than one pathogen, depending on geographic area.

Conflicts of Interest

The authors declare no conflicts of interest.

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