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.

### Introduction

More than 150 years ago, Eichstedt\(^1\) 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.
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 to 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 the 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
subclinical lesions, but it should be recalled that the yellowish fluorescence indicative of this disease only appears in approximately one-third of cases. 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. 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. 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 them. (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. In case of doubt various molecular identification techniques can be employed. 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 vitro and in vivo skin samples stained with potassium hydroxide.
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 \( M_{\text{pachydermatis}} \) 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 \( M_{\text{globosa}} \) and \( M_{\text{restricta}} \)) 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 \( \beta \)-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, \( M_{\text{restricta}} \) seems to predominate on the scalp, whereas \( M_{\text{sympodialis}} \) predominates on the trunk and \( M_{\text{globosa}} \) is widely and equally distributed in all seborrheic areas. On the other hand, other species, such as \( M_{\text{slooffiae}} \) and \( M_{\text{furfur sensu stricto}} \), seem to be uncommon on human skin in healthy individuals and \( M_{\text{obtusa}} \) is extremely rare.

Veterinarians have also investigated these yeasts on the skin of several animals, demonstrating the role of \( M_{\text{pachydermatis}} \) (and to a lesser extent, \( M_{\text{furfur}}, M_{\text{obtusa}}, \) and \( M_{\text{sympodialis}} \)) 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,
such as monkeys, pigs, bears and birds,\textsuperscript{34,37} horses (\textit{M equina}) and goats (\textit{M caprae}),\textsuperscript{38} and cattle (\textit{M nana}).\textsuperscript{39}

\section*{Pityriasis Versicolor and Malassezia Yeasts: Etiological Considerations}

In 1951, Gordon\textsuperscript{40} was the first researcher to suggest that rounded yeasts, which he named \textit{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\textsuperscript{12,41} 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 \textit{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.\textsuperscript{42} Most studies published in those initial years, such as those of Nakabayashi et al\textsuperscript{43} in Japan in 2000 and Aspiroz et al\textsuperscript{44} in Zaragoza, Spain, in 2002, also supported these results. In particular, in the latter study, the percentages obtained for \textit{M globosa} and \textit{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 \textit{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 al\textsuperscript{45} in Canada, which found a predominance of \textit{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,\textsuperscript{46} using a polymerase chain reaction (PCR) identification technique for \textit{Malassezia} present in PV scales, also found that \textit{M globosa} was the most commonly detected species (97%), although this was closely followed by \textit{M restricta} (79%) and \textit{M sympodialis} (68%). Another study conducted by Gaitanis et al\textsuperscript{47} in Greece in 2002, using a PCR-restriction fragment length polymorphism technique applied to lesions caused by several dermatological processes, only detected and identified \textit{M globosa} in PV scales.

In 2003, Hernández et al\textsuperscript{48} in Mexico also found a predominance of \textit{M globosa} in standard cultures of PV samples. These results were similar to those published 1

\begin{table}[h]
\centering
\caption{Epidemiological Studies of Pityriasis Versicolor by \textit{Malassezia} Species}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Authors} & \textbf{Year} & \textbf{Patients} & \textbf{\textit{M Globosa}} & \textbf{\textit{M Sympodialis}} & \textbf{\textit{M Restricta}} & \textbf{\textit{M slooffiae}} & \textbf{\textit{M Furur}} & \textbf{\textit{M Obtusa}} & \textbf{Culture Medium} \\
\hline
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% & Dixon & \\
Dutta & 2002 & 250 & 63.6% & 4.8% & 34% & Dixon & \\
Gaitanis & 2002 & 3 & 100% & PC & \\
Hernández & 2003 & 11 & 47% & 27% & 13% & 13% & Dixon & \\
Tarazoolie & 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% & 53% & 42% & 0.6% & Dixon & \\
De Quinzada & 2005 & 150 & 5.3% & 4.6% & 11.4% & 77.8% & 8.3% & Dixon & \\
Miranda & 2006 & 95 & 2% & 34% & 1% & 3% & 2% & Dixon & \\
Crespo-Erchiga & 2006 & 100 & 97% & 3% & 3% & Dixon & \\
Gaitanis & 2006 & 71 & 90% & 3-4% & 3-4% & Dixon & \\
Prohic & 2007 & 90 & 63% & 14% & 4% & 10% & 8% & Dixon & \\
\hline
\end{tabular}
\end{table}
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

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

Finally, the fact that *M. farfur* 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.

**References**


