Mini Review

Malassezia Baillon, emerging clinical yeasts

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Abstract

The human and animal pathogenic yeast genus Malassezia has received considerable attention in recent years from dermatologists, other clinicians, veterinarians and mycologists. Some points highlighted in this review include recent advances in the technological developments related to detection, identification, and classification of Malassezia species. The clinical association of Malassezia species with a number of mammalian dermatological diseases including dandruff, seborrhoeic dermatitis, pityriasis versicolor, psoriasis, folliculitis and otitis is also discussed.

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1. Introduction

Members of the genus Malassezia are opportunistic yeasts of increasing importance, due in large part to advances in detection and culture methodology which have both allowed their investigation and revealed their importance in human and animal disease [1,2]. The genus Malassezia belongs to the basidiomycetous yeasts and is classified in the Malasseziales (Ustilaginomycetes, Basidiomycota) [3–5]. The cells show a multilayered cell wall, enteroblastic budding (Fig. 1), urease activity, and a positive staining reaction with Diazonium Blue B (DBB) [3]. The genus was named in 1889 by Baillon [6] with the species M. furfur, to accommodate the filamentous fungus observed in scales of the human skin disease pityriasis versicolor (PV). Pityrosporum [7] has been proposed as an alternative generic name, but because Malassezia had been published earlier this name has nomenclatural priority. The genus remained limited to M. furfur and M. pachydermatis for a long time. M. pachydermatis is lipophilic but not lipid-dependent, and usually occurs on animals [8]. For many years all pathologies caused by M. furfur sensu lato, particularly disorders of the skin such as dandruff, seborrhoeic dermatitis (D/SD), pityriasis versicolor (PV), and folliculitis, were ascribed to a single species [9]. Only the recent recognition of a number of new species and the development of methods to differentiate them has
changed this approach [2,10]. The 28S-rDNA sequences revealed seven distinct genetic entities [11], which are now widely accepted as species (M. furfur, M. obtusa, M. globosa, M. slooffiae, M. sympodialis, M. pachydermatis, and M. restricta) [10]. Since then, five new Malassezia species have been reported (M. dermatis [12], M. equi [13], M. japonica [14], M. nana [15], and M. yamatoensis [16]), but further biochemical and molecular characterization will be required for their acceptance as distinct species. Biochemical identification tests for Malassezia species include catalase and β-glucosidase activity, and evaluation of growth with cremophor EL and Tweens 20, 40, 60, 80, using the diffusion method in Sabouraud glucose agar (Table 1, Fig. 2) [1,4,10,17].

2. Malassezia species

M. furfur is morphologically heterogeneous with globose, oval or cylindrical yeast cells. This species can be identified by its ability to grow at 37 °C, strong catalase activity, absence or a very weak β-glucosidase activity, and equal growth in the presence of cremophor EL (=castor oil) and Tweens 20, 40, 60, 80 as sole lipid sources [4,17]. Strains of M. furfur showed two different karyotypes [18], but demonstrated high percentages of DNA/DNA reassociation and high ribosomal RNA similarity [10,11]. Some strains are able to produce filaments, either spontaneously or under particular culture conditions [19]. Strains of the species originate from various hosts, body sites and diseases. However, M. furfur was not observed in recent epidemiological surveys of healthy persons and patients with pityriasis versicolor (PV) and seborrhoeic dermatitis (SD) or with only PV [20,21]. This absence may, perhaps, be caused by the isolation protocols used, or may arise from competition between different skin-inhabiting species of Malassezia. Using the same isolation protocol, the species has been isolated from systemic and mucosal sites, such as urine, vagina and blood, or exposed sites such as nails (E. Gue´ho, unpublished data). It has also been isolated from animals [22–24]. M. furfur is the only Malassezia species
to be isolated from non-mammalian sources, in one case from a hospital room floor [25].

*M. slooffiae* may be misidentified as *M. furfur*, but it can be differentiated by its absence of growth with cremophor EL [4,10]. *M. slooffiae* is regularly isolated from human skin and is mostly found in association with *M. sympodialis* or *M. globosa*. It may be a weak human pathogen, and seems better adapted to animals, especially pigs [22,23].

*M. obtusa* resembles *M. furfur* morphologically but differs physiologically as it does not grow at 37 °C, and cannot utilize any of the five lipids used in the tests. However, it darkens esculin medium [4,10]. It is a rare species that was known only from healthy human skin. Recently it has also been isolated from goats, horses and dogs [26,27].

*M. pachydermatis*, a lipophilic species, is able to grow without supplementation of long-chain fatty acids or their esters. All isolates grow well at 37 °C, and some primary cultures show a certain lipid-dependence [28,29]. These weakly-lipid-dependent isolates may have smaller colonies than those growing on regular Sabouraud dextrose agar. Differences in catalase and β-glucosidase expression, that can be absent, weak or positive depending on the strain, and differences in reactivity to cremophor EL and Tweens 20, 40, 60, 80 occur in all rDNA genotypes [4,17]. These different compounds, particularly cremophor EL and Tween 20, may be more or less inhibitory. In this case, growth may occur only at some distance from the compound-containing well where the compound is more diluted, or it may occur within the inhibitory area as secondary growth [30].

*M. pachydermatis* occurs rarely on humans, although it has been found to cause septic epidemics, usually in neonates receiving intravenous lipid supplementation [31,32]. *M. pachydermatis* is well-known as a normal cutaneous inhabitant of numerous warm-blooded animals. Seborrhoeic dermatitis and otitis associated with this lipophilic yeast are now commonly recognized, especially in dogs [33].

*M. sympodialis* corresponds with the former serovar A of *M. furfur* [10,34] and is characterized by a strong β-glucosidase activity and growth at 37 °C, but cremophor EL as a lipid supplement does not allow good growth. The yeast cells are small and ovoid. The species is commonly isolated from healthy as well as diseased skin [20]. Its role as a pathogen has not yet been elucidated. Indeed, *M. sympodialis* is often present in skin lesions, but usually associated with the more abundantly occurring *M. globosa* [35]. *M. sympodialis* has also been isolated from healthy feline skin [36].

*M. globosa* corresponds with serovar B of *M. furfur* [10,34] but has spherical yeast cells only [10]. Buds are also spherical and emerge from the mother yeast through a narrow site, contrary to the patterns seen in other *Malassezia* spp. The species corresponds to the original description of *P. orbiculare* obtained from a PV case [37]. *M. globosa* is able to produce filaments, in particular in primary cultures. The yeast does not grow at 37 °C or does so very poorly, does not grow on the five lipid substrates, and does not split esculin. *M. globosa*, with *M restricta*, has been implicated as the causal organism in dandruff and seborrheic dermatitis [38]. *M. globosa* is also the most important species in PV, either alone or associated with other species, particularly *M. sympodialis* [35]. *M. globosa* occurs mainly on humans, but is also known from a cat [36].

*M. restricta* lacks catalase and β-glucosidase activity, does not grow at 37 °C, and is strongly lipid-dependent [10]. Growth of the colonies is very restricted. The species is isolated almost exclusively from the head, including scalp, neck and face [21], and it corresponds to serovar C [34]. *M. restricta* does not produce any filaments. Although *M. restricta* is very fastidious, new

Fig. 2. Assimilation patterns of Tween 20, 40, 60 and 80 (Clockwise from bar) and Cremophor EL (center). (A) *M. sympodialis*; (B) *M. restricta*. 
DNA-based methodologies will allow for more robust analysis in situ. More studies are needed to understand its implication in Malassezia-associated diseases, in particular dandruff and seborrheic dermatitis. This species is not known to occur in animals [22,23].

Recently a few other species have been reported. *M. dermatis* has been isolated from the skin of atopic dermatitis patients [12]. Analysis of the 26S ribosomal DNA (rDNA) and ITS sequences showed this species to be closely related to *M. sympodialis*. The two species differ by only 1.2% rDNA base divergence. *M. dermatis* and *M. sympodialis* have a strong catalase activity and assimilate the four Tweens ([10,12]; R. Batra and T. Boekhout, unpubl. observ.). These two species differ in growth on esculin and cremophor EL. In *M. sympodialis*, growth on esculin was positive, whereas in *M. dermatis* this was negative. Growth of *M. sympodialis* on cremophor EL was absent, but this was weakly positive for *M. dermatis* (R. Batra and T. Boekhout, unpubl. observ.).

Nell et al. [13] have reported the presence of a new species from normal equine skin, which they tentatively named *M. equi*. This species has not formally been described. Like *M. dermatis* this species is also closely related to *M. sympodialis*. Unfortunately, data on growth on the various Tweens, esculin and cremophor EL are missing in the description of *M. equi*, as no strain has been preserved.

Hirai et al. [15] reported a new species from the ear discharge of animals and proposed the name *M. nana*. It is possible that this species is the same as that presented by Duarte et al. [39] as atypical strains of *M. sympodialis* [40]. Previously, Crespo et al. [41] described the presence of *M. sympodialis* from otitis externa in cats, which based on D1/D2 and ITS sequences turned out to be identical to *M. nana* [40] (F.J. Cabanes, unpubl. observ.). It is likely that more species are involved, as was suggested by a D1/D2 and ITS sequencing study of *M. sympodialis*-like isolates from animals [40].

Sugita et al. [14] recently have described another new species, *M. japonica*. This species can be distinguished from the seven known lipophilic species by the ability to assimilate Tween 40 and Tween 60, the inability to assimilate TWEEN 20 and Tween 80, and lack of growth at 40 °C. *M. japonica* seems to represent a separate species as the D1/D2 26S sequence is significantly divergent (4.6% with *M. furfur* and 6.9% with *M. obtusa*).

*M. yamatoensis* was described from a Japanese patient with seborrheic dermatitis (SD) [16]. The species is physiologically close to *M. furfur* and *M. dermatis*, and sequence analysis of the D1/D2 domain of the 26S rDNA placed the species in a cluster with *M. furfur*, *M. Obtusa* and *M. japonica* with 93% bootstrap support, and the ITS sequences were 40% different. Molecular detection using *M. yamatoensis*-specific primers detected the species in only 9.7% of the SD patients, approximately 14% of the atopic dermatitis patients and approximately 55% of the healthy people sampled. Therefore, the species was considered a minor component of the skin mycobiota [16].

Table 2
Fragment lengths of ITS 1 and ITS 2 of strains belonging to different Malassezia species as determined by terminal fragment length polymorphism (tFLP)

<table>
<thead>
<tr>
<th>Species</th>
<th>CBS Designation</th>
<th>ITS 1 (bp)</th>
<th>ITS 2 (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. furfur</em></td>
<td>7982</td>
<td>286</td>
<td>550</td>
</tr>
<tr>
<td></td>
<td>1878*</td>
<td>286</td>
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<td>7874</td>
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<td><em>M. obtusa</em></td>
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<tr>
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<td>7876*</td>
<td>290</td>
<td>546</td>
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<td>7877*</td>
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<td>8747</td>
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<td><em>M. slooffiae</em></td>
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</tr>
<tr>
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<tr>
<td><em>M. nana</em></td>
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<td>258</td>
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<tr>
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<td>9561</td>
<td>263</td>
<td>417</td>
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<tr>
<td><em>M. dermatis</em></td>
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<td>235</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td>9169*</td>
<td>236</td>
<td>407</td>
</tr>
<tr>
<td></td>
<td>9170</td>
<td>236</td>
<td>408</td>
</tr>
<tr>
<td><em>M. pachydermatis</em></td>
<td>74522 (ATCC)</td>
<td>262</td>
<td>527</td>
</tr>
</tbody>
</table>

* Neotype.
* Type.
* +/−1 base pair.
3. Detection and identification

Assigning the role of individual species in the clinical context has been hampered by the difficulties involved in the isolation, cultivation and identification of Malassezia spp. [1,2,41]. Cultivation requirements vary by species [10]. M. furfur is by far the most robust of the Malassezia spp. in culture, and therefore is the organism most frequently isolated when using culture-based techniques. M. restricta and M. obtusa are the most difficult to grow. Even under the most-favourable conditions, M. restricta particularly grows much more slowly than M. furfur and M. sympodialis and would be quickly overgrown by any of these two species even if initially present in much smaller numbers. Indeed M. globosa, as a primary culture on Dixon or mDixon agar at 30–35 °C, is growing very well, giving colonies smaller than those of M. sympodialis but having about the same diameter as those of M. furfur. However, M. furfur may outcompete M. globosa when the experiments are performed at 37 °C.

Multiple approaches have been used to differentiate Malassezia species. Culture-based methods include mol% guanine + cytosine, DNA reassociation values, cell morphology, growth with different Tween non-ionic detergents (Fig. 2), the presence of catalase, temperature requirements, the presence of β-glucosidase as revealed by the splitting of esculin, and selective growth with cremophor EL [4,10,43,44]. These culture-based methods are effective but time consuming and technically demanding. Specific molecular methods have also been developed for the identification of Malassezia isolates, such as pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD), DNA sequence analysis, restriction analysis of PCR amplicons of ribosomal sequences, amplified fragment length polymorphism (AFLP™), denaturing gradient gel electrophoresis (DGGE), and terminal fragment length polymorphism (tFLP) [11,18,29,31,38,45–55]. Of the molecular methods, PFGE and DGGE have met with limited success due to the need for specialized equipment and training. AFLP, however, has been successfully applied to the identification of Malassezia isolates and yields highly-specific genotypic information about each strain. The detailed “fingerprint” achieved from careful AFLP analysis has revealed multiple genetic subgroups in each Malassezia species, and may lead to the differentiation of clinically-important subgroups or even new species. [46,47]. The numerous bands resolved in the AFLP fingerprints are providing detailed information relevant to the identification of strains, but the method requires clonal isolates from culture. AFLP is, therefore, the tool of choice for analyses where detailed information is necessary and many isolates are available, such as strain identification, epidemiology, phylogeny, and investigation of novel species. Multiple methods have been reported to identify species in complex Malassezia communities from skin without cultivation [38,48,50–52,55], but most of these methods require either separate amplification with specific primer sets or restriction digestion of the amplification products.

A new method, named terminal fragment length polymorphism (tFLP) [38], can be used on non-invasively acquired swab samples. This method uses only three primer sets, and therefore minimizes the potential bias related to amplification efficiency. It is sensitive enough to detect Malassezia with as few as 100 cells per sample, either as spiked directly onto the swab to control the extraction procedure, or from samples collected from 1 cm² of skin surface sample using a swab. As is the case with any amplification reaction, target species found at less than 1% of the total community may be under-represented in the final products. Therefore, care must be taken in the interpretation of results. This method involves isolation of fungal DNA, followed by nested PCR of the ribosomal gene cluster, followed by amplification with ITS 1- and ITS 2-specific fluorescently-labeled primers. The resulting terminally-labeled products are analyzed for fragment length on a fluorescent-DNA sequencer. The amplifications are carried out with universal-fungal primers [56], and therefore the methodology should be broadly applicable to other fungal species. The ITS 1 and ITS 2 amplifications are carried out individually and combined for final analysis. The primary advantage of this method is that the resultant sample contains only two labeled segments per species, allowing analysis of complex communities. All Malassezia species can be differentiated by length polymorphisms, including multiple genotypes per known species (Fig. 3, Table 2). Results obtained for standards and mixtures of standards show that all known Malassezia genotypes can be identified in a single amplification reaction based on unique fragment lengths, eliminating the need for restriction analysis [38]. Results from this study also have shown that tFLP is capable of reproducibly assessing the Malassezia species present in complex mixtures and clinical samples. Importantly, it is specific for fungi, and sufficiently sensitive to allow direct assessment of clinical samples without the need for prior cultivation. The tFLP method will become a powerful tool when used in conjunction with new fungal ITS region databases that are becoming available. These include a.o. databases by Chen et al. [57] and by Boekhout et al. [58], the latter of which is available as a CD-ROM.

4. General lipid requirements

As mentioned previously, all Malassezia species, except M. pachydermatis, are lipid-dependent. The complications with nomenclature are compounded in the study of Malassezia biochemistry and metabolism. Elegant
early work in the 1960s and 1970s indicated that *M. furfur* (then referred to as *Pityrosporum ovale*, CBS 1878) required saturated fatty acids greater than twelve but less than 20 carbons [59]. In this study, unsaturated fatty acids stimulated growth, but only when saturated fatty acids were also present. In a later study, it was reported that both saturated and unsaturated fatty acids could support growth of *P. ovale*, but it is unclear which current *Malassezia* species was investigated [60]. To further complicate interpretation of *Malassezia* lipid metabolic data, the concentrations of saturated fatty acids required to support growth are extremely low, in the nanomolar range. It is very difficult and expensive to obtain fatty acids of sufficient purity to fully elucidate their role in *Malassezia* metabolism. For example, food-grade oleic acid, which is frequently used in routine *Malassezia* culture [61,62], contains approximately 74% oleic and approximately 8% stearic acid, with the remaining being a complex mixture of primarily C14, C16, and C18 saturated and mono-unsaturated acids (T.L. Dawson, unpubl. observ.). Cremophor-EL presents the same difficulty, being a partially-purified plant extract containing primarily, but not exclusively, ricinoleic acid. Tween esters are likewise produced from enriched but not purified fatty acid sources. While complex fatty acid supplements are excellent tools for defining the species of *Malassezia* present in a culture, it will take a very detailed approach with analytically-pure standards to fully establish the fatty-acid requirements of each *Malassezia* species. This type of detailed biochemical information will be necessary to understand the individual role of each species in human pathogenesis, to unravel their phylogenic relationships, and to decide whether or not new genetic entities will become accepted species.

5. *Malassezia* species as human pathogens

*Malassezia* species have been associated with a number of diseases of the human skin, such as seborrhoeic dermatitis and dandruff, pityriasis versicolor, *Malassezia (Pityrosporum)* folliculitis, atopic dermatitis, psoriasis and, less commonly, with other dermatological disorders such as confluent and reticulated papillomatosis, onychomycosis, and transient acantholytic dermatosis [1,2,42,63–65]. Although *Malassezia* species are a part of the microbial community on normal skin, under certain conditions they can cause a superficial skin infection. In general, due to their dependence on lipids for survival, *Malassezia* species are most often found in sebum-rich areas of the skin, such as the trunk, back, face and scalp. The *Malassezia* species are well-adapted to the human skin, and possibly occupy well-defined niches on the human body. Studies have been carried out to answer the clinical question of whether there is a relationship between particular *Malassezia* species and various dermatological disorders [1,2,35,42,43,52,65].

Fig. 3. Fragments of ITS 1 (green peaks) and ITS 2 (blue peaks) showing differences among all known *Malassezia* species. The different peaks correspond to the ITS 1 and 2 fragments given in Table 2.
6. Seborrhoeic dermatitis and dandruff

Seborrhoeic dermatitis and dandruff (SD/D) are perhaps the most common diseases associated with Malassezia species, with SD occurring in 1–3% and dandruff in greater than 50% of the general population [20,66]. The incidence of SD is much higher in immunocompromised patients, especially AIDS patients, ranging from 30% to 33% [67,68]. The vast majority of more recent data supports a direct causal link between Malassezia and SD/D. The factors that support this hypothesis are: (i) antifungal treatment is found to be effective in treating these diseases, and (ii) improvement in SD/D is accompanied by a reduction in Malassezia levels on the scalp [69].

It has been reported that the proportion of Malassezia cells on the scalp is higher in patients with SD/D than in normal controls [70]. There are conflicting data regarding the number of yeasts in lesional versus non-lesional skin. Some have reported a decrease in the density of Malassezia cells recovered from lesional skin [43], while others have shown a greater number of yeasts in lesional skin [55] or a greater detectable incidence in affected subjects [38]. It has been suggested that seborrhoeic dermatitis is not caused by an overgrowth of Malassezia cells, but by an abnormal host response to the yeasts on the skin [71]. It is likely that SD/D is caused by M. restricta and M. globosa, in conjunction with increased host response to the fungi or their metabolites.

The species that have been shown to be most closely associated with seborrhoeic dermatitis and dandruff to date are M. restricta and M. globosa [1,38,42], with the former occurring more frequently than the latter. However, some authors have also reported the presence of M. furfur, M. sympodialis, M. obtusa and M. slooffiae [72]. These authors found that M. globosa was isolated with the same frequency from both lesional and non-lesional skin. Gupta et al. [43] noted that significantly more Malassezia yeasts could be cultured from non-lesional skin. Two other species, M. sympodialis and M. obtusa, were often cultured from both lesional and non-lesional skin of seborrhoeic dermatitis patients. Gemmer et al. [38], using DNA-based detection, reported a significantly higher detection rate for both M. globosa and M. restricta in subjects with SD/D.

7. Atopic dermatitis

Malassezia species have been cultured from 83% of adult patients, in whom the disease was localized to the head and neck [73]. Because the yeasts are also frequently isolated from normal controls, it has been hypothesized that they act as allergens in susceptible patients, rather than as infectious agents [74,75]. Recently, molecular work has indeed elucidated the structure of some allergens derived from Malassezia species [76–78]. Three major allergen components have been identified, namely two protein components of 67 and 37 kDa each, and one carbohydrate component of 14 kDa [78]. These allergens are designated as Mala s 1, and Mala f 2 to Mala f 4 for the protein components, and Mala s 5 to Mala s 9 for the carbohydrate moieties. Genomic-DNA amplification by PCR and sequencing demonstrated that M. globosa, M. obtusa and M. sympodialis contained DNA sequences corresponding to all the allergens except Mala f 2 and Mala f 3. M. pachydermatis contained Mala s 1, Mala f 4, and Mala s 5 to Mala s 8. M. restricta and M. slooffiae possessed Mala f 4 and Mala s 6. M. furfur was seen to possess Mala f 2 to Mala f 4 as well as Mala s 5 to Mala s 7. Ashbee and Evans [79] and Faergemann [63] have elegantly described the immunological role of Malassezia in atopic dermatitis.

Some studies have examined the prevalence and the species composition of Malassezia strains in atopic dermatitis patients. Sandström et al. [80] sampled skin on the upper back, and found that M. sympodialis was the species most commonly isolated from both atopic dermatitis patients and healthy controls. These investigators were able to sample both lesional and non-lesional skin and found a significant difference, the yeasts being more common in non-lesional skin. Gupta et al. [43] demonstrated that the mean number of colony-forming units grown from samples taken from atopic dermatitis patients was significantly lower than that obtained from sampling healthy controls. In both groups, however, the dominant species was M. sympodialis. Sugita and Nishikawa [55] reported that M. restricta, M. globosa and M. furfur are present at significantly higher frequencies in atopic dermatitis patients than in healthy subjects. Nakabayashi et al. [72] found that M. furfur was isolated more frequently from lesional skin (21%) than from non-lesional skin (11%) of atopic dermatitis patients. Gupta et al. [43] sampled lesional skin and found that M. sympodialis was the species most commonly isolated from both atopic dermatitis patients and controls. Sandström et al. [80] found a difference in species distribution on lesional versus non-lesional skin in atopic dermatitis patients. Non-lesional skin was most frequently colonized by M. globosa, while M. sympodialis was most commonly found on lesional skin. The differences in sampling and identification methods used by various researchers may have contributed to the differences observed in the prevalence and species composition of Malassezia species.

8. Pityriasis versicolor

Pityriasis versicolor is a chronic superficial fungal disease that is characterized by the appearance of round to oval lesions, most commonly found on the trunk and upper arms. It is postulated that this disease occurs when the Malassezia species that normally colonize the
skin change from the round yeast form to a pathological mycelial form, which then invades the stratum corneum of the skin. Staining of the resident fungal elements will reveal a characteristic ‘spaghetti and meatballs’ appearance (Fig. 4), reflecting the presence of both hyphae and yeast cells. It has been reported by some authors that the number of yeast and hyphae in the lesions of pityriasis versicolor is greater than in normal skin [81], while others found that the difference is not statistically significant [43,72] (Fig. 4).

In general, it seems that the most common Malassezia species cultured from lesions of pityriasis versicolor are M. globosa (Fig. 4) [34,35,72] and M. sympodialis [43,65,82]. Other species such as M. slooffiae and M. furfur are less common, but not completely absent.

9. Psoriasis

The role of Malassezia species in psoriasis is still undetermined, but several reports have associated these lipophilic yeasts with the development of skin lesions in psoriasis. In patients with psoriasis it has been demonstrated that these individuals have immunological responses to both Malassezia species and to proteins derived from them.

Gupta et al. [43] found that of the six Malassezia species they recovered from patients, M. globosa was most frequently isolated from patients with psoriasis and from those with seborrhoeic dermatitis. This species was isolated from the scalp, forehead and trunk with equal frequency. However, another study reported significant differences in the distribution of Malassezia species between psoriatic and healthy scalp skin, and in the distribution of Malassezia species according to the severity of the scalp involvement [83]. M. globosa in its yeast phase was the predominant species (55%) in psoriatic patients, followed by M. slooffiae (18%) and M. restricta (10%), the latter being the most common species isolated from healthy scalp skin.

10. Malassezia (Pityrosporum) folliculitis

Histological examination of patients with Malassezia folliculitis shows, as the name suggests, invasion of the hair follicles with large numbers of Malassezia yeasts [84]. In most cases of folliculitis, if the biopsy specimen is cut in serial sections, a typical dilated follicle containing abundant round budding yeast cells can be found and sometimes hyphae as well [85].

We could not find any report examining the possibility that one or more species of Malassezia might be more commonly involved in Malassezia folliculitis. This could be because the available studies have taken samples from the skin surface using techniques that might not reach the yeasts located in the deeper regions of the hair follicle.

11. Other disorders

There are a few scattered case reports in the literature associating Malassezia species with various other skin conditions. In particular, Malassezia has been shown to be involved in at least some cases of confluent and reticulated papillomatosis (CRP) [86,87]. A possible link between Malassezia and transient acantholytic dermatosis (TAD) has also been suggested [88], again on the basis of the response of the disorder to selenium sulfide. Finally, while up to 90% of cases of onychomycosis are caused by dermatophytes, there have been several reports [89,90] on patients with onychomycosis from whom Malassezia species were isolated. Isolates were identified afterwards as M. furfur ([89]; E. Guého, unpublished results). Yeasts do not normally colonize nails, as these are not a good source of lipids. However, presence of Malassezia yeasts in these cases may have
represented a secondary infection in patients with onychomycosis.

Malassezia species have been associated with deep-seated infections such as catheter-related fungemia, especially in neonates [31,32,91–93]. M. pachydermatis, earlier exclusively considered to be associated with animals, has been reported to cause intravascular catheter-acquired infections in humans [31,32]. The association of intravascular catheter-acquired nosocomial infection associated with colonization of health care workers’ pet dogs has also been mentioned [91].

12. Malassezia species on animals

Malassezia species inhabit the skin of a variety of mammals and birds [94]. So far, few studies have been published regarding the distribution of the different Malassezia spp. on animals following the last taxonomic revision of the genus [10]. Traditionally, the lipid-dependent species were thought to occur only on human skin, while M. pachydermatis was assumed to be restricted to animal skin and in particular to carnivores.

M. pachydermatis, the only species in the genus that does not require lipid supplementation for development in culture medium, has been traditionally considered to be zoophilic, and is frequently found on wild and domestic carnivores including dogs, cats, bears, pinnipeds, ferrets and foxes [22,26,28]. M. pachydermatis is usually associated with otitis externa and different kinds of dermatitis in domestic animals (Fig. 5), but especially in dogs [23]. This species is more frequently isolated from dogs than from cats and appears to be a relatively infrequent pathogen in other animals [23,26].

Several authors have mentioned the high incidence of this yeast in canine otitis externa, especially in chronic otitis. Although the pathogenic role of M. pachydermatis in otitis externa has been a matter of controversy, it was demonstrated that the yeast could induce inflammatory changes in the normal canine external ear canal in the presence of moisture [95], and the disease was experimentally induced with M. pachydermatis when a large inoculum was used [96]. This yeast seems to have an opportunistic nature and may become pathogenic with any alteration in the microclimate of the skin surface or in the host defense. In some canine breeds, hypersensitivity conditions such as flea allergy dermatitis, food hypersensitivity or atopy, and antimicrobial or corticosteroid therapy may be factors favouring proliferation of these yeasts [97]. Primary diseases that cause inflammation and increased sebum production provide a cutaneous microenvironment that encourages overgrowth of this yeast. M. pachydermatis is the most common yeast that contributes to otitis externa as a perpetuating factor in dogs [98].

Recently lipid-dependent species have been isolated from the skin of different animals, such as cats, dogs, cows and horses (e.g. [13,15,26,27]). Carnivores can be colonised by lipid-dependent species such as M. furfur, M. obtusa, M. sympodialis and M. globosa, in addition to M. pachydermatis [24,26,36,99]. Lipid-dependent species seem to be more frequent in cats than in dogs [26]. However, very little is known about their pathogenic role in animal skin. M. sympodialis was isolated from cats with otitis externa [41] and M. furfur and M. obtusa from dogs with otitis externa [100]. It is possible that these species are common in this microenvironment and play a similar role as M. pachydermatis in canine otitis externa.

On the other hand, the lipid-dependent species seem to constitute the major population of the lipophilic microbiota in herbivores such as horses, goats, sheep and cows [27], and they have been associated with otitis externa in bovines [101]. Otitis in cattle, among other factors, is commonly attributed to infestation caused by rhabditiform nematodes [102]. Recently, soil nematodes have been associated with Malassezia spp. by PCR techniques, and their role as possible vectors for species of Malassezia has been suggested [102]. M. sympodialis has also been isolated from affected skin of a horse [29].

Fig. 5. Clinical images of M. pachydermatis infections in animals. (A) Dermatitis of a cat associated with M. pachydermatis (arrow). (B) Diff-Quick stain of a smear from an ear swab of a dog with otitis externa showing the typical monopolar budding on a broad base of M. pachydermatis.
13. Conclusion

Recent research and technological developments have contributed greatly to elucidating the role of *Malassezia* spp. in skin diseases. It is now much easier to detect and identify individual *Malassezia* spp. from complex clinical samples, and specific information can be gleaned from detailed genetic analyses. These, coupled with the increasing interest and, therefore, increasing research commitment of the medical and scientific communities, will undoubtedly lead to rapid and significant advances in the field. However, difficulties remain in obtaining a high level of certainty in the identification of some lipid-dependent strains by means of physiological tests and further comparison of recent work to older data will remain difficult, due to changes in nomenclature and growth conditions. In addition, isolation and identification of these strains continues to be difficult due to the low viability associated with some isolate types and lack of suitable methods for their isolation and preservation [105].

New molecular approaches to the identification of different species will certainly contribute to improved management of diseases associated with *Malassezia* spp. As further detailed DNA-based identification techniques and biochemical analyses will be more broadly applied to the genus, almost certainly more species will be identified. The elaboration of molecular genetic differences and detailed biochemistry will also be necessary to define true species differences and to assess genetic variation between strains within species.

Future work will hopefully answer many of the outstanding questions associated with *Malassezia* spp. and their role in human and animal pathophysiology. The identification of specific metabolic requirements and by-products in situ will also be necessary to understand how the *Malassezia* spp. interact with human and animal skin. Furthermore, the organisms occupy an important and poorly-defined section of the phylogenetic “tree of life”. Application of whole-genome sequencing would allow a deeper understanding of the physiology, phylogeny, and medical importance of the *Malasseziales*.

References


