ELSEVIER

FEMS Yeast Research 5 (2005) 1101–1113

FEMS Yeast Research

www.fems-microbiology.org

MiniReview

Malassezia Baillon, emerging clinical yeasts

Roma Batra [a,1], Teun Boekhout [b,*], Eveline Guého [c], F. Javier Cabañes [d], Thomas L. Dawson Jr. [e], Aditya K. Gupta [a,f]

[a] *Mediprobe Research, London, Ont., Canada*
[b] *Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 85167 Utrecht, The Netherlands*
[c] *5 rue de la Huchette, F-61400 Mauves sur Huisne, France*
[d] *Departament de Sanitat i d' Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Barcelona E-08193, Spain*
[e] *Beauty Care Technology Division, Procter & Gamble Company, Cincinnati, USA*
[f] *Division of Dermatology, Department of Medicine, Sunnybrook and Women's College Health Science Center (Sunnybrook site) and the University of Toronto, Toronto, Ont., Canada*

Received 1 November 2004; received in revised form 11 May 2005; accepted 18 May 2005

First published online 12 July 2005

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.

Keywords: *Malassezia*; Yeast; Identification; Animals; Disease

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

* Corresponding author. Tel.: +31 30 212 2671; fax: +31 30 251 2097.
E-mail address: boekhout@cbs.knaw.nl (T. Boekhout).
[1] Present address: W281N4904, Theodores Cove, Pewaukee, WI, USA.

1567-1356/$22.00
doi:10.1016/j.femsyr.2005.05.006

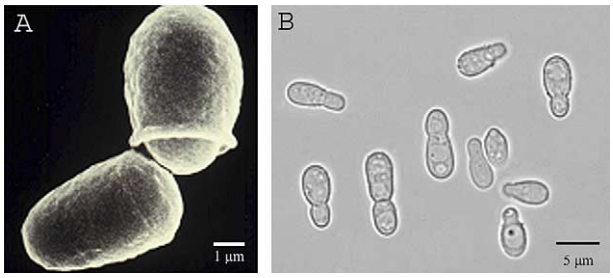

Fig. 1. Morphology of *Malassezia* cells. (A) Budding cells of *M. pachydermatis* viewed by scanning electron microscopy. (B) *M. furfur* under bright field microscopy.

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. Guého, unpublished data). It has also been isolated from animals [22–24]. *M. furfur* is the only *Malassezia* species

Table 1
Salient characteristics of *Malassezia* species [1,2,10,12,14–17,24,26–28,30–33,36,38–42,55,57,62,63,65,72,80,82–84,92,95,96,99–101]

Species	Occurrence	Cell morphology	Lipid dependency	Tween 20	Tween 40	Tween 60	Tween 80	Cremophor EL	Catalase	β-Glucosidase	Growth at 37 °C
M. dermatis	AD	Ellipsoidal, globose	+	+	+	+	+	W, (+)	+	?	+
M. furfur	AD, AN, HS, OE, PV low, S, SD low	Globose, ellipsoidal, cylindrical	+	+, (−)	+, (−)	+, (−)	+, (−)	+, (−)	+, (−)	−, (w)	+
M. globosa	AN, AD, P, PV high, SD high, SD/D high	Globose	+	−	−[2]	−[2]	−	−	+	−	−, (w)
M. japonica	AD, HS	Globose, ellipsoidal	+	−	w	+	−	?	+	?	+
M. nana	AN, OE	Ellipsoidal	+	v	+	+	w	?	+	?	+
M. obtusa	AN, HS, OE, SD	Ellipsoidal, cylindrical	+	−	−	−	−	−	+	+	−, (w)
M. pachydermatis	AN, OE, S, SD/AN	Ellipsoidal	−, (w)	+[1]	+	+	+	+[1]	+, w	+, (−)	+
M. restricta	AD, HS scalp, P, SD, SD/D high	Globose, ellipsoidal	+	−	−[3]	−[3]	−	?	−	−	v
M. slooffiae	AN, HS, P, PV, SD	Ellipsoidal, cylindrical	+	+, w, (−)	+	+	−, (w)	−	+	−	+
M. sympodialis	AD, AN, HS, P, PV, SD	Ellipsoidal	+	−, w[2]	+	+	+	−, (w)	+	+	+
M. yamatoensis	AD, HS, SD	Ellipsoidal	+	+	+	+	+	?	+	?	+

AD, atopic dermatitis; AN, non-human animals; HS, healthy human skin; OE, otitis externa; P, psoriasis; PV, pityriasis versicolor; S, sepsis; SD, seborrhoeic dermatitis; SD/AN, seborrhoeic dermatitis in animals; SD/D, seborrhoeic dermatitis/dandruff; SD; w, weak; v, variable; ( ) indicate rare deviations from main pattern; [1], growth may be inhibited near the well where the substrate is placed; [2], growth may occur at some distance from the well where the substrate is placed; [3], opaque zone may occur.

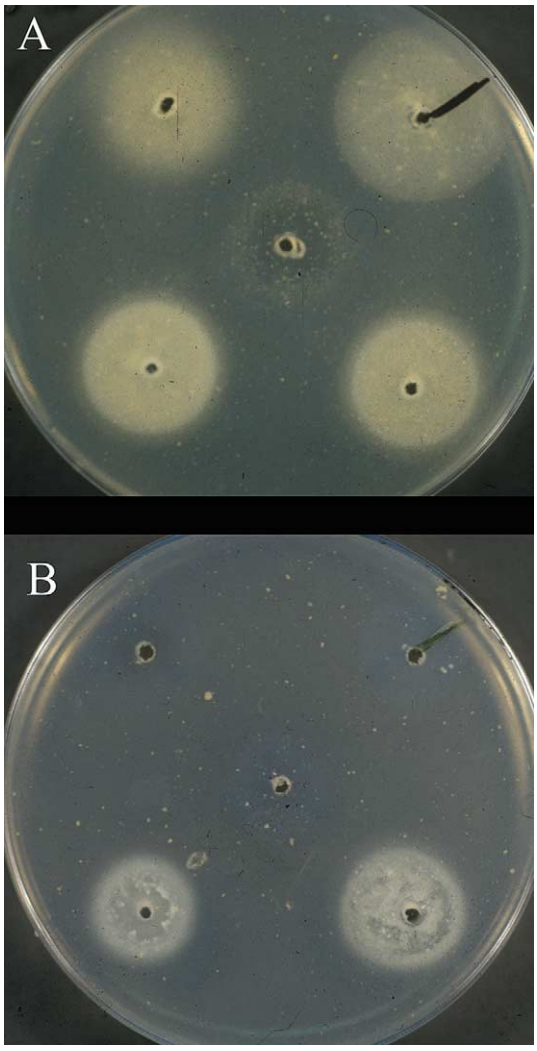

Fig. 2. Assimilation patterns of Tween 20, 40, 60 and 80 (Clockwise from bar) and Cremophor EL (center). (A) *M. sympodialis*; (B) *M. restricta*.

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 seborrhoeic 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

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 seborrhoeic 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. Cabañes, 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 seborrhoeic 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].

More studies are needed to better characterize and possibly validate the different lipid-dependent *Malassezia* spp., but data on fragment lengths of the ITS 1 and 2 (Table 2) suggest that different *Malassezia* spp. may be involved indeed.

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

Species	CBS Designation	ITS 1 (bp)[c]	ITS 2 (bp)[c]
M. furfur	7982	286	550
	1878[a]	286	550
	7981	286	550
	6000	286	550
	7019	286	550
	7860	286	550
	7865	286	550
	7984	286	550
	4171	286	550
	5332	286	550
	5333	286	550
	5334	286	550
	7970	286	550
M. globosa	7966[b]	331	469
	7874	314	457
	7990	344	472
M. obtusa	7968	290	546
	7876[b]	290	546
M. restricta	7991	339	455
	7877[b]	289	455
	8747	289	455
M. slooffiae	7875	274	492
	7956	273	488
	7971	273	488
M. sympodialis	7977[b]	239	412
	7979	239	413
	7222	239	413
M. japonica	9431	285	520
	9432[b]	285	520
M. nana	9557[b]	258	415
	9558	262	416
	9559	263	416
	9560	262	417
	9561	263	417
M. dermatis	9145	235	407
	9169[b]	236	407
	9170	236	408
M. pachydermatis	74522 (ATCC)	262	527

[a] Neotype.
[b] Type.
[c] +/−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 (*t*FLP) [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 (*t*FLP) [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 cm^2 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 fragments 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 *t*FLP 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 *t*FLP 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

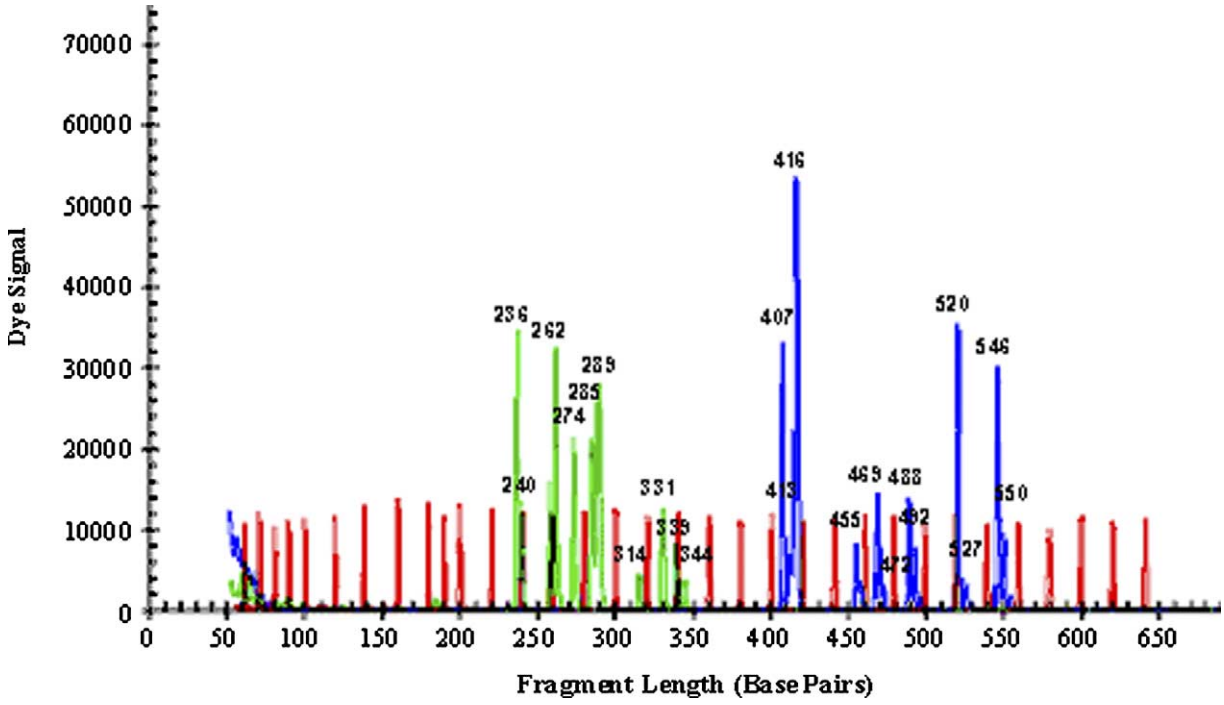

Fig. 3. Fragments of ITS 1 (green peaks) and ITS 2 (blue peaks) showing differences among all known *Malassezia* species. The different peaks correspond to the ITS 1 and 2 fragments given in Table 2.

early work in the 1960s and 1970s indicated that *M. furfur* (then referred to as *Pitrysporum 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].

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

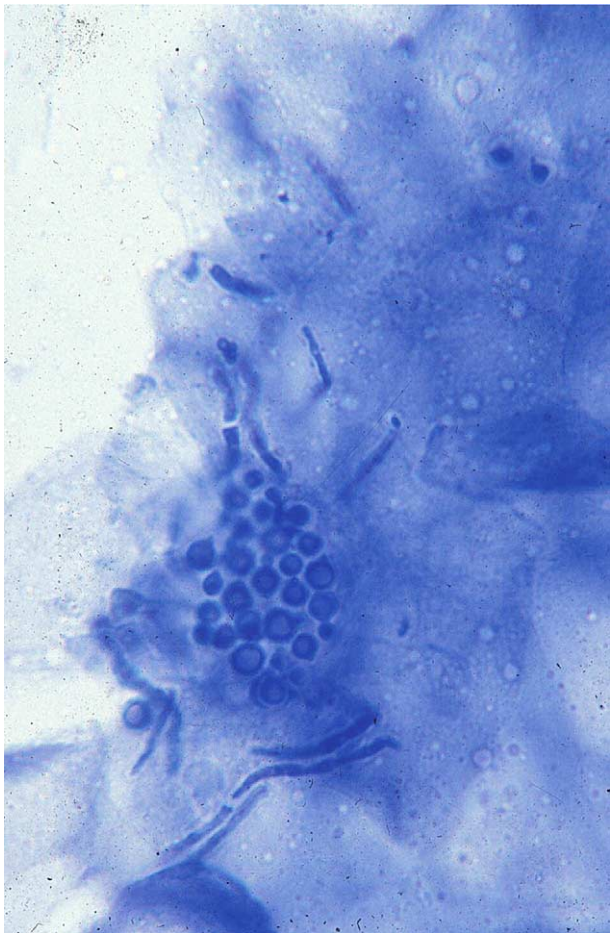

Fig. 4. Clinical image of Pityriasis versicolor showing yeast cells and filaments of *M. globosa*.

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, pinnepeds, 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].

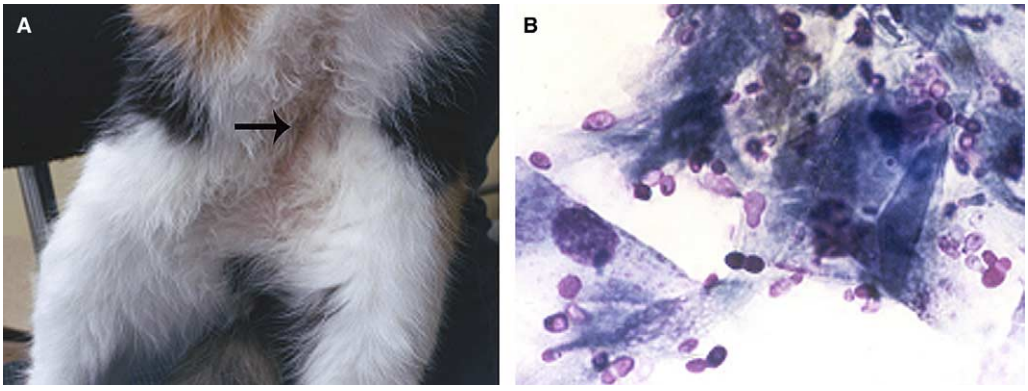

Fig. 5. Clinical images of *M. pachydermatis* infections in animals. (A) Dermatitis of a cat associated with *M. pachydermatis* (arrow). (B) Diff-Quick stain of a smear from an ear swab of a dog with otitis externa showing the typical monopolar budding on a broad base of *M. pachydermatis*.

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 [103].

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 biochemisty 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

[1] Crespo-Erchiga, V. and Guého, E. (2005) Superficial diseases caused by *Malassezia* species. In: Topley and Wilson's Microbiology and Microbial Infections, Mycology (Hay, R. and Merz, W., Eds.), Vol. 5, 10th edn. Arnold, London, UK (in press).

[2] Midgley, G., Guého, E. and Guillot, J. (1998) Diseases caused by *Malassezia* In: Topley and Wilson's Microbiology and Microbial Infections (Ajello, L. and Hay, R.J., Eds.), Vol. 4, pp. 201–211. Arnold, London, UK.

[3] Kurtzman, C.P. and Fell, J.W., Eds., (1998). The Yeasts, A Taxonomic Study, 4th edn. Elsevier, Amsterdam, The Netherlands.

[4] Boekhout, T. and Guého, E. (2003) Basidiomycetous yeasts. In: Pathogenic Fungi in Humans and Animals (Howard, D.H., Ed.), 2nd edn, pp. 537–542. Marcel Dekker, Inc., New York, USA.

[5] Begerow, D., Bauer, R. and Boekhout, T. (2000) Phylogenetic placement of ustilaginomycetes anamorphs as deduced from nuclear LSU rDNA sequences. Mycol. Res. 104, 53–60.

[6] Baillon, H. (1889) Traité de botanique médicale cryptogamique. Octave Douin Paris, France..

[7] Sabouraud, R. (1904) Maladies du cuir chevelu II. Les maladies desquamatives. Masson, Paris, France..

[8] Ahearn, D. and Yarrow, D. (1984) *Malassezia* Baillon In: The Yeasts: A Taxonomic Study (Kreger-van Rij, N.J.W., Ed.), 3rd edn, pp. 882–885. Elsevier, Amsterdam, The Netherlands.

[9] Marcon, M.J. and Powell, D.A.. (1992) Human infections due to *Malassezia* spp. Clin. Microbiol. Rev. 5, 101–119.

[10] Guého, E., Midgley, G. and Guillot, J. (1996) The genus *Malassezia* with the description of four new species. Antonie van Leeuwenhoek 69, 337–355.

[11] Guillot, J. and Guého, E. (1995) The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. Antonie van Leeuwenhoek 67, 297–314.

[12] Sugita, T., Takashima, M., Shinoda, T., Suto, H., Unno, T., Tsuboi, R., Ogawa, H. and Nishikawa, A. (2002) New yeast species *Malassezia dermatis* isolated from patients with atopic dermatitis. J. Clin. Microbiol. 40, 1363–1367.

[13] Nell, A., James, S.A., Bond, C.J., Hunt, B. and Herrtage, M.E. (2002) Identification and distribution of a novel *Malassezia* yeast species on normal equine skin. Veter. Rec. 150, 395–398.

[14] Sugita, T., Takashima, M., Kodama, M., Tsuboi, R. and Nishikawa, A. (2003) Description of a new yeast species, *Malassezia japonica*, and its detection in patients with atopic dermatitis and healthy subjects. J. Clin. Microbiol. 41, 4695–4699.

[15] Hirai, A., Kano, R., Makimura, K., Duarte, E.R., Hamdan, J.S., Lachance, M.A., Yamaguchi, A. and Hasegawa, A. (2004) *Malassezia nana* sp. nov., a novel lipid-dependent yeast species isolated from animals. Int. J. Syst. Evol. Microbiol. 54, 623–627.

[16] Sugita, T., Tajima, M., Takashima, M., Amaya, M., Saito, M., Tsuboi, R. and Nishikawa, A. (2004) A new yeast, *Malassezia yamatoensis*, isolated from a patient with seborrheic dermatitis, and its distribution in patients and healthy subjects. Microbiol. Immunol. 48, 579–583.

[17] Guého, E., Boekhout, T., Ashbee, H.R., Guillot, J., van Belkum, A. and Faergemann, J. (1998) The role of *Malassezia* species in the ecology of human skin and as pathogens. Med. Mycol. 36, 220–229.

[18] Boekhout, T. and Bosboom, R.W. (1994) Karyotyping of *Malassezia* yeasts: taxonomic and epidemiological implications. Syst. Appl. Microbiol. 17, 146–153.

[19] Guillot, J., Guého, E. and Prevost, M.C. (1995) Ultrastructural features of the dimorphic yeast *Malassezia furfur*. J. Mycol. Med. 5, 86–91.

[20] Ashbee, H.R., Ingham, E., Holland, K.T. and Cunliffe, W.J. (1993) The carriage of *Malassezia furfur* serovar A, B and C in patients with pityriasis versicolor, seborrheic dermatitis and controls. Br. J. Dermatol. 129, 533–540.

[21] Aspiroz, C., Moreno, L.A., Rezusta, A. and Rubio, C. (1999) Differentiation of three biotypes of *Malassezia* species on human normal skin. Correspondence with *M. globosa, M. sympodialis* and *M. restricta*. Mycopathologia 145, 69–74.

[22] Guillot, J., Guého, E., Mialot, M. and Chermette, R. (1998) Importance des levures du genre *Malassezia* en pratique vétérinaire. Point-Vétér. 29, 21–31.

[23] Guillot, J., Guého, E. and Chermette, R. (1999) Infections animales à *Malassezia*. Rev. Prat. 49, 1840–1843.

[24] Crespo, M.J., Abarca, M.L. and Cabanes, F.J. (1999) Isolation of *Malassezia furfur* from a cat. J. Clin. Microbiol. 37, 1573–1574.

[25] Tanaka, R., Nishimura, K., Kamei, K. and Murayama, S.Y. (2001) Assimilation test of *Malassezia furfur* isolated from the environment. Nippon Ishinkin Gakkai Zasshi 42, 123–126.
[26] Crespo, M.J., Abarca, M.L. and Cabañes, F.J. (2002) Occurrence of *Malassezia* spp. in the external ear canals of dogs and cats with and without otitis externa. Med. Mycol. 40, 115–121.
[27] Crespo, M.J., Abarca, M.L. and Cabañes, F.J. (2002) Occurrence of *Malassezia* spp. in horses and domestic ruminants. Mycoses 45, 333–337.
[28] Bond, R. and Anthony, R.M. (1995) Characterization of markedly lipid-dependent *Malassezia pachydermatis* isolates from healthy dogs. J. Appl. Bacteriol. 78, 537–542.
[29] Senczek, D., Siesenop, U. and Bohm, K. (1999) Characterization of *Malassezia* species by means of phenotypic characteristics and detection of electrophoretic karyotypes by pulsed field gel electrophoresis (PFGE). Mycoses 42, 409–414.
[30] Guého, E. and Guillot, J. (1999) Comments on *Malassezia* species from dogs and cats. Mycoses 42, 673–674.
[31] Van Belkum, A., Boekhout, T. and Bosboom, R. (1994) Monitoring spread of *Malassezia* infections in a neonatal intensive care unit by PCR-mediated genetic typing. J. Clin. Microbiol. 32, 2528–2532.
[32] Mickelsen, P.A., Viano-Paulson, M.C., Stevens, D.A. and Diaz, P.S. (1988) Clinical and microbiological features of infection with *Malassezia pachydermatis* in high-risk infants. J. Infect. Dis. 157, 1163–1168.
[33] Guillot, J. and Bond, R. (1999) *Malassezia pachydermatis*: a review. Med. Mycol. 37, 295–306.
[34] Cunningham, A.C., Leeming, J.P., Ingham, E. and Gowland, G. (1990) Differentiation of three serovars of *Malassezia furfur*. J. Appl. Bacteriol. 68, 439–446.
[35] Crespo-Erchiga, V., Ojeda Martos, A., Vera Casano, A., Crespo-Erchiga, A., Sanchez Fajardo, F. and Guého, E. (1999) Mycology of pityriasis versicolor. J. Mycol. Med. 9, 143–148.
[36] Bond, R., Howell, S.A., Haywood, P.J. and Lloyd, D.H. (1997) Isolation of *Malassezia sympodialis* and *Malassezia globosa* from healthy pet cats. Vet. Rec. 141, 200–201.
[37] Gordon, M.A. (1951) The lipophilic mycoflora of the skin. I. In vitro culture of *Pityrosporum orbiculare* n. sp. Mycologia 43, 524–535.
[38] Gemmer, C.M., DeAngelis, Y.M., Theelen, B., Boekhout, T. and Dawson, T.L. (2002) Fast, noninvasive method for molecular detection and differentiation of *Malassezia* yeast species on human skin and application of the method to dandruff microbiology. J. Clin. Microbiol. 40, 3350–3357.
[39] Duarte, E.R., Lachance, M.A. and Hamdan, J.S. (2002) Identification of atypical strains of *Malassezia* spp. from cattle and dog. Can. J. Microbiol. 48, 749–752.
[40] Cabañes, F.J., Hernández, J.J. and Castellá, G. (2005) Molecular analysis of *Malassezia sympodialis* related strains from domestic animals. J. Clin. Microbiol. 43, 277–283.
[41] Crespo, M.J., Abarca, M.L. and Cabañes, F.J. (2000) Otitis externa associated with *Malassezia sympodialis* in two cats. J. Clin. Microbiol. 38, 1263–1266.
[42] Gupta, A.K., Batra, R., Bluhm, R., Boekhout, T. and Dawson, T.L. (2004) Skin diseases associated with *Malassezia* species. J. Am. Acad. Dermatol. 51, 785–798.
[43] Gupta, A.K., Kohli, Y., Summerbell, R.C. and Faergemann, J. (2001) Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. Med. Mycol. 39, 243–251.
[44] Mayser, P., Haze, P., Papavassilis, C., Pickel, M., Gruender, K. and Guého, E. (1997) Differentiation of *Malassezia* species: selectivity of cremophor EL, castor oil and ricinoleic acid for *M. furfur*. Br. J. Dermatol. 137, 208–213.
[45] Boekhout, T., Kamp, M. and Guého, E. (1998) Molecular typing of *Malassezia* species with PFGE and RAPD. Med. Mycol. 36, 365–372.
[46] Theelen, B., Silvestri, M., Guého, E., van Belkum, A. and Boekhout, T. (2001) Identification and typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE). FEMS Yeast Res. 1, 79–86.
[47] Gupta, A.K., Boekhout, T., Theelen, B., Summerbell, R.C. and Batra, R. (2004) Identification and typing of *Malassezia* species by amplified fragment length polymorphism (AFLP) and sequence analyses of the internal transcribed spacer (ITS) and large subunit (LSU) regions of ribosomal DNA. J. Clin. Microbiol. 42, 4253–4260.
[48] Gaitanis, G., Velegraki, A., Frangoulis, E., Mitroussia, A., Tsigonia, A., Tzimogianni, A., Katsambas, A. and Legakis, N.J. (2002) Identification of *Malassezia* species from patient skin scales by PCR-RFLP. Clin. Microbiol. Infect. 8, 162–173.
[49] Makimura, K., Tamura, Y., Kudo, M., Uchida, K., Saito, H. and Yamaguchi, H. (2000) Species identification and strain typing of *Malassezia* species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal trancribed spacer 1 regions. J. Med. Microbiol. 49, 29–35.
[50] Gupta, A.K., Kohli, Y. and Summerbell, R.C. (2000) Molecular differentiation of seven *Malassezia* species. J. Clin. Microbiol. 38, 1869–1875.
[51] Guillot, J., Deville, M., Berthelemy, F., Provost, F. and Guého, E. (2000) A single PCR-restriction endonuclease analysis for rapid identification of *Malassezia* species. Lett. Appl. Microbiol. 31, 400–403.
[52] Sugita, T., Suti, H., Unno, R., Tsuboi, H., Ogawa, T., Shinoda, T. and Nishikawa, A. (2001) Molecular analysis of *Malassezia* microflora on the skin of atopic dermatitis patients and healthy subjects. J. Clin. Microbiol. 39, 3486–3490.
[53] Sugita, T., Kodama, M., Saito, M., Tomonobu, I., Kato, Y., Tsuboi, R. and Nishikawa, A. (2003) Sequence diversity of the intergenic spacer region of the rRNA gene of *Malassezia globosa* colonizing the skin of patients with atopic dermatitis and healthy individuals. J. Clin. Microbiol. 41, 3022–3027.
[54] Yamada, Y., Osumi, M., Makimura, K. and Yamaguchi, H. (2002) Overview of lipophilic yeast *Malassezia*: the current status of the molecular diagnosis. Jpn. J. Antibiot. 55, 493–499.
[55] Sugita, T. and Nishikawa, A. (2003) Molecular and quantitative analysis of *Malassezia* microflora on the skin of atopic dermatitis and genotyping of *M. globosa* DNA. Jpn. J. Med. Mycol. 44, 61–64.
[56] White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: PCR Protocols: A Guide to Methods and Applications (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., Eds.), pp. 314–322. Academic Press, San Diego, USA.
[57] Chen, Y.-C., Eisner, J.D., Kattar, M.M., Rassoulian-Barret, S.L., LaFe, K., Yarfitz, S.L., Limaye, A.P. and Cookson, B.T. (2001) Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. J. Clin. Microbiol. 39, 4042–4051.
[58] Boekhout, T., Robert, V., Smith, M.Th., Stalpers, J., Yarrow, D., Boer, P., Gijswijt, G., Kurtzman, C.P., Fell, J.W., Guého, E., Guillot, J. and Roberts, I. (2002) Yeasts of the world 2.0. Expertcenter Taxonomic Identification. Amsterdam, The Netherlands.
[59] Wilde, P.F. and Stewart, P.S. (1968) A study of the fatty acid metabolism of the yeast *Pityrosporum ovale*. Biochem. J. 108, 225–231.

[60] Porro, M.N., Passi, S., Caprill, F., Nazzaro, P. and Morpurgo, G. (1976) Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. J. Invest. Dermatol. 66, 178–182.
[61] Leeming, J.P. and Notman, F.H. (1987) Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. J. Clin. Microbiol. 25, 2017–2019.
[62] Guillot, J., Guého, E., Lesourd, M., Midgley, G., Chevrier, G. and Dupont, B. (1996) Identification of *Malassezia* species, a practical approach. J. Mycol. Med. 6, 103–110.
[63] Faergemann, J. (2002) Atopic dermatitis and fungi. Clin. Microbiol. Rev. 15, 545–563.
[64] Van Abbe, N.J. (1964) The investigation of dandruff. J. Soc. Cosmetic Chem. 15, 609–630.
[65] Gupta, A.K., Kohli, Y., Faergemann, J. and Summerbell, R.C. (2001) Epidemiology of *Malassezia* yeasts associated with pityriasis versicolor in Ontario, Canada. Med. Mycol. 39, 199–206.
[66] Warner, R.R., Schwartz, J.R., Boissy, Y. and Dawson Jr, T.L. (2001) Dandruff has an altered stratum corneum ultrastructure that is improved with zinc pyrithione shampoo. J. Am. Acad. Dermatol. 45, 897–903.
[67] Smith, K.J., Skelton, H.G., Yeager, J., Ledsky, R., McCarthy, W., Baxter, D., Turiansky, G.W., Wagner, K.F. and Turianski, G. (1994) Cutaneous findings in HIV-1-positive patients: a 42-month prospective study. Military Medical Consortium for the Advancement of Retroviral Research (MMCARR). J. Am. Acad. Dermatol. 31, 746–754.
[68] Farthing, C.F., Staughton, R.C. and Rowland Payne, C.M. (1985) Skin disease in homosexual patients with acquired immune deficiency syndrome (AIDS) and lesser forms of human T cell leukaemia virus (HTLV III) disease. Clin. Exp. Dermatol. 10, 3–12.
[69] Pierard, G.E., Arrese, J.E. and Pierard-Franchimont, C. (1997) Prolonged effects of antidandruff shampoos. Time to recurrence of *Malassezia ovalis* colonization of skin. Int. J. Cosm. Sci. 19, 111–117.
[70] McGinley, K.J., Leyden, J.J., Marples, R.R. and Kligman, A.M. (1975) Quantitative microbiology of the scalp in non-dandruff, dandruff, and seborrheic dermatitis. J. Invest. Dermatol. 64, 401–405.
[71] Bergbrant, I.M. and Faergemann, J. (1989) Seborrhoeic dermatitis and *Pityrosporum ovale*: a cultural and immunological study. Acta Derm. Venereol. 69, 332–335.
[72] Nakabayashi, A., Sei, Y. and Guillot, J. (2000) Identification of *Malassezia* species isolated from patients with seborrheic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. Med. Mycol. 38, 337–341.
[73] Broberg, A., Faergemann, J., Johansson, S., Johansson, S.G., Strannegard, I.L. and Svejgaard, E. (1992) *Pityrosporum ovale* and atopic dermatitis in children and young adults. Acta Derm. Venereol. 72, 187–192.
[74] Kieffer, M., Bergbrant, I.M., Faergemann, J., Jemec, G.B., Ottevanger, V., Stahl Skov, P. and Svejgaard, E. (1990) Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. J. Am. Acad. Dermatol. 22, 739–742.
[75] Zargari, A., Midgley, G., Back, O., Johansson, S.G. and Scheynius, A. (2003) IgE-reactivity to seven *Malassezia* species. Allergy 58, 306–311.
[76] Schmidt, M., Zargari, A., Holt, P., Lindbom, L., Hellman, U., Whitley, P., van der Ploeg, I., Harfast, B. and Scheynius, A. (1997) The complete cDNA sequence and expression of the first major allergenic protein of *Malassezia furfur*, Mal f 1. Eur. J. Biochem. 246, 181–185.
[77] Yasueda, H., Hashida-Okado, T., Saito, A., Uchida, K., Kuroda, M., Onishi, Y., Takahashi, K., Yamaguchi, H., Takesako, K. and Akiyama, K. (1998) Identification and cloning of two novel allergens from the lipophilic yeast, *Malassezia furfur*. Biochem. Biophys. Res. Commun. 248, 240–244.
[78] Andersson, A., Scheynius, A. and Rasool, O. (2003) Detection of Mala f and Mala s allergen sequences within the genus *Malassezia*. Med. Mycol. 41, 479–485.
[79] Ashbee, H.R. and Evans, E.G.V. (2002) Immunology of diseases associated with *Malassezia* species. Clin. Microbiol. Rev. 15, 21–57.
[80] Sandstrom, M.H., Bartosik, J., Back, O., Scheynius, A., Sarnhult, T. and Tengvall Linder, M. (2001) The prevalence of the *Malassezia* yeasts in patients with atopic dermatitis, seborrheic dermatitis and healthy controls. J. Eur. Acad. Dermatol. Venereol. 15, 104–274.
[81] McGinley, K.J., Lantis, L.R. and Marples, R.R. (1970) Microbiology of tinea versicolor. Arch. Dermatol. 102, 168–171.
[82] Aspiroz, C., Ara, M., Varea, M., Rezusta, A. and Rubio, C. (2002) Isolation of *Malassezia globosa* and *M. sympodialis* from patients with pityriasis versicolor in Spain. Mycopathologia 154, 111–117.
[83] Prohic, A. (2003) Identification of *Malassezia* species isolated from scalp skin of patients with psoriasis and healthy subjects. Acta Dermatovenerol. Croat. 11, 10–16.
[84] Back, O., Faergemann, J. and Hornqvist, R. (1985) *Pityrosporum* folliculitis: a common disease of the young and middle-aged. J. Am. Acad. Dermatol. 12, 56–61.
[85] Faergemann, J. (1999) *Pityrosporum* species as a cause of allergy and infection. Allergy 54, 413–419.
[86] Nordby, C.A. and Mitchell, A.J. (1986) Confluent and reticulated papillomatosis responsive to selenium sulfide. Int. J. Dermatol. 25, 194–199.
[87] Yesudian, P., Kamalam, S. and Razack, A. (1973) Confluent and reticulated papillomatosis (Gougerot-Carteaud). An abnormal host reaction to *Malassezia furfur*. Acta Derm. Venereol. 53, 381–384.
[88] Segal, R., Alteras, I. and Sandbank, M. (1987) Rapid response of transient acantholytic dermatosis to selenium sulfide treatment for pityriasis versicolor. Dermatologica 175, 205–207.
[89] Silva, V., Moreno, G.A., Zaror, L., de Oliveira, E. and Fischman, O. (1997) Isolation of *Malassezia furfur* from patients with onychomycosis. J. Med. Vet. Mycol. 35, 73–74.
[90] Crozier, W.J. and Wise, K.A. (1993) Onychomycosis due to *Pityrosporum*. Austral. J. Dermatol. 34, 109–112.
[91] Chang, H., Miller, H., Watkins, N., Arduino, M., Ashford, D., Midgley, G., Aguero, R., Pinto-Powell, R., von Reyn, C.F., Edwards, W., McNeil, M. and Jarvis, W. (1998) An epidemic of *Malassezia pachydermatis* in an intensive care nursery associated with colonization of health care workers' pet dogs. N. Engl. J. Med. 338, 706–711.
[92] Guého, E., Simmons, R., Pruitt, W., Meyer, S. and Ahearn, D. (1987) Association of *Malassezia pachydermatis* with systemic infections of humans. J. Clin. Microbiol. 25, 1789–1790.
[93] Jahagirdar, B.N. and Morrison, V.A. (2002) Emerging fungal pathogens in patients with hematologic malignancies and marrow/stem-cell transplant recipients. Semin. Respir. Infect. 17, 113–120.
[94] Dufait, R. (1985) Présence de *Malassezia pachydermatis* (syn. *P. canis*) sur les poils et les plumes d'animaux domestiques. Bull. Soc. Fr. Mycol. Med. 14, 19–22.
[95] Mansfield, P.D., Boosinger, T.R. and Attleberger, M.H. (1990) Infectivity of *Malassezia pachydermatis* in the external ear canal of dogs. J. Am. Anim. Hosp. Assoc. 26, 97–100.
[96] Uchida, Y., Mizutani, M., Kubo, T., Nakade, T. and Otomo, K. (1992) Otitis externa induced with *Malassezia pachydermatis* in dogs and the efficacy of pimaricin. J. Vet. Med. Sci. 54, 611–614.
[97] Pier, A.C., Cabañes, F.J., Chermette, R., Ferreiro, L., Guillot, J., Jensen, H.E. and Santurio, J.M. (2000) Prominent animal

mycoses from various regions of the world. Med. Mycol. 38 (1), 47–58.
[98] Scott, D.W., Miller, W.H. and Griffin, C.E. (1995) Muller's and Kirk's Small Animal Dermatology, 5th edn. W.B. Saunders, Philadelphia, USA.
[99] Raabe, P., Mayser, P. and Weiss, R. (1998) Demonstration of *Malassezia furfur* and *M. sympodialis* together with *M. pachydermatis* in veterinary specimens. Mycoses 41, 493–500.
[100] Crespo, M.J., Abarca, M.L. and Cabañes, F.J. (2000) Atypical lipid-dependent *Malassezia* species isolated from dogs with otitis externa. J. Clin. Microbiol. 38, 2383–2385.
[101] Duarte, E.R., Batista, R.D., Hahn, R.C. and Hamdan, J.S. (2003) Factors associated with the prevalence of *Malassezia* species in the external ears of cattle from the state of Minas Gerais, Brazil. Med. Mycol. 41, 137–142.
[102] Renker, C., Alphei, J. and Buscot, F. (2003) Soil nematodes associated with the mammal pathogenic fungal genus *Malassezia* (Basidiomycota: Ustilaginomycetes) in Central European forests. Biol. Fertil. Soils 37, 70–72.
[103] Crespo, M.J., Abarca, M.L. and Cabañes, F.J. (2000) Evaluation of different preservation and storage methods for *Malassezia* spp. J. Clin. Microbiol. 38, 3872–3875.