Malassezia and the Skin
It has been known for many years that the *Malassezia* yeasts are associated with a number of different human diseases ranging from pityriasis versicolor to seborrhoeic dermatitis. However the evolving history of their taxonomy and pathogenicity, and the management of the diseases that they cause has been a long, and often difficult, journey. Their fastidious growth requirements defied the initial attempts to culture these organisms on laboratory media and their true identification and the relationship between different species only became apparent with the application of modern molecular techniques. Likewise although recognised in the 19th century as potential causes of human infection, piecing together the complex and, in certain cases, still uncertain relationships to different human diseases has taken many years. Recognised initially as causes of infection of the skin, they are now known to be superficial commensals as well as potential causes of infections in domestic animals and more serious human conditions such as fungemia. They have also been implicated in the pathogenesis of allergic and other inflammatory diseases.

Given this complex, yet fascinating, history it seems appropriate to bring together current thought on these yeasts, their structure and function and their association with both human and animal disease states. This book provides such a view of the genus *Malassezia* and the diseases caused by its members. In accomplishing this task the book takes the reader through the history of the genus *Malassezia* and critically reviews its taxonomy, physiology and the diseases caused, both directly or indirectly. It is intended to provide an up-to-date account of these organisms that will appeal to the specialist scientist and student as well as the practising microbiologist or physician.

London, UK

Roderick J. Hay
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Contributors

H. Ruth Ashbee, PhD
Mycology Reference Center, Department of Microbiology, Leeds General Infirmary, Leeds, LS1 3EX, UK
h.r.ashbee@leeds.ac.uk

Dominik Begerow, PhD
Ruhr-Universität Bochum, Geobottanik, ND 03/174, Universitätsstr. 150, 44801 Bochum, Germany
dominik.begerow@rub.de

Teun Boekhout, PhD
Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
t.boekhout@cbs.knaw.nl

Ross Bond, DVM, PhD
Senior Lecturer in Veterinary Dermatology, Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK
rbond@rvc.ac.uk

F. Javier Cabañes, DVM
Veterinary Mycology Group, Department of Animal Health and Anatomy, Faculty of Veterinary, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
javier.cabanes@uab.es

Reto Crameri, PhD
Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, 7270 Davos, Switzerland
crameri@siaf.uzh.ch

Vicente Crespo Erchiga, MD
Department of Dermatology, Regional University Hospital Carlos Haya, Plaza del Hospital Civil s/n, 29009 Málaga, Spain
vicente.crespo.sspa@juntadeandalucia.es

Tom Dawson, PhD
The Procter & Gamble Co, Miami Valley Innovation Center, Cincinnati, OH 45253-8707, USA
saunders.cw@pg.com
Yvonne DeAngelis, BSc
The Procter & Gamble Co
Miami Valley Innovation Center
Cincinnati, OH 45253-8707, USA
saunders.cw@pg.com

George Gaitanis, DM, PhD
Department of Skin and Venereal Diseases,
University Hospital of Ioannina,
University of Ioannina Medical School,
S. Niarhos Avenue,
45500 Ioannina, Greece
ggaitanis@med.uoa.gr

Andreas Groll, DM, PhD
Infectious Disease Research Program,
Center for Bone Marrow Transplantation
and Department of Pediatric
Hematology/Oncology
Children’s University Hospital Muenster,
Albert-Schweitzer-Strasse 33,
48149 Muenster, Germany
grollan@ukmuenster.de

Eveline Guého-Kellermann, PhD
INSERM
5 rue de la Huchette
61400 Mauves sur Huisne, France
e.gueho@orange.fr

Jacques Guillot, DVM, PhD
Department of Parasitology-Mycology,
Joint Research Unit BIPAR (Biologie Moléculaire et Immunologie Parasitaires et Fongiques) AFSSA, ENVA, UPVM,
Ecole Nationale Vétérinaire d’Alfort,
94704 Maisons-Alfort, France
jguillot@vet-alfort.fr

Suzana Hadina, DVM, PhD
Department of Microbiology and Infectious Diseases with Clinic,
Faculty of Veterinary Medicine,
Heinzelova 55, 10 000 Zagreb, Croatia
suzana.hadina@vef.hr

Roderick J. Hay, MD
Professor of cutaneous infection,
Skin Infection Clinic,
Dermatology Department,
Kings College Hospital,
London SE5 9RS, UK
roderick.hay@ifd.org

Peter Mayser, MD
Department of Dermatology
and Andrology,
Justus Liebig University Giessen,
Gaffkystrasse 14, 35385 Giessen,
Germany
peter.mayser@derma.med.uni-giessen.de

Gillian Midgley, PhD
35 Lebanon Park,
Twickenham TW1 3DH, UK
mail@bryn-jones.net

Vincent A. Robert, PhD
Bioinformatics group leader,
Centraalbureau voor Schimmelcultures,
Uppsalalaan 8, 3584 CT Utrecht,
The Netherlands
v.robert@cbs.knaw.nl
www.cbs.knaw.nl

Charles D. Saunders, PhD
The Procter & Gamble Co,
Miami Valley Innovation Center,
Cincinnati, OH 45253-8707, USA
saunders.cw@pg.com

Annika Scheynius, MD, PhD
Department of Medicine Solna,
Clinical Allergy Research Unit,
L2:04, Karolinska Institutet and
University Hospital,
171 76, Stockholm, Sweden
annika.scheynius@ki.se
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takashi Sugita, PhD</td>
<td>Department of Microbiology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan</td>
</tr>
<tr>
<td>Athanasios Tragiannidis, MD, PhD</td>
<td>Second Department of Pediatrics, Hematology and Oncology Unit, Aristotle University Thessaloniki, AHEPA Hospital, S. Kiria kidi 1 str, 54636 Thessaloniki, Greece</td>
</tr>
<tr>
<td>Aristea Velegraki, PhD</td>
<td>Mycology Laboratory, Medical School, National and Kapodistrian University of Athens, Athens 11527, Greece</td>
</tr>
<tr>
<td>Jun Xu, PhD</td>
<td>The Procter &amp; Gamble Co, Miami Valley Innovation Center, Cincinnati, OH 45253-8707, USA</td>
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Core Messages

- The history of the recognition of the significance of the *Malassezia* yeasts and their potential role in causing disease in humans and animals is one of the more intriguing medical stories of the last 150 years. It contains all the elements of a classic mystery, from the early proposed association between an unusual looking organism seen in the skin diseases, seborrhoeic dermatitis and pityriasis versicolor, to its reclassification into a number of different species on the basis of molecular taxonomy. In the intervening period, other important discoveries were made. Its presence on normal skin indicated that it was a skin commensal, yet the argument over its true identity and nomenclature form a key part of this story. It was accepted at first as a cause of seborrhoeic dermatitis, but then rejected before the argument swung back in favour of a causal link in the late 1980s. While it has always been accepted as a cause of pityriasis versicolor, the new molecular work has allowed for the initiation of investigations to relate different clinical forms to individual species. The association of *Malassezia* species with animal disease has been an important development in veterinary medicine as skin disease caused by these organisms is very common in certain breeds. More recently, it has been reported that *Malassezia* species can be a cause of systemic infection, particularly in new born infants, and that it may also contribute to allergic disorders such as atopic dermatitis. As is often the case, the molecular work has outpaced the definition of clinical states, and it will be necessary to re-examine the status of individual diseases and reconsider whether they should be subdivided further. Early difficulty in the isolation of these lipophilic yeasts in the laboratory held back research, but the introduction of molecular methods has expedited work on the genus in recent years. This has contributed to our understanding of its pathogenesis, epidemiology and even its management. For instance, it has provided a more robust method of designing intervention strategies, such as the use of appropriate antifungals, as different species exhibit different drug sensitivity patterns.
1.1 Definition

*Malassezia* yeasts are lipophilic organisms, which have been recognised for over a century as members of the normal human cutaneous flora, and also as agents of certain skin diseases [1, 2]. In addition, since the 1980s, they have been reported as causing opportunistic systemic infections [3]. These yeasts have frequently been found on a diverse range of other warm-blooded animals (Chap. 3.3), and their wider distribution in nature is now being explored using molecular techniques [4–9]. Characteristic features of the genus *Malassezia* include a distinctive morphology and an affinity for lipids in culture. Electron microscopic observations have shown that these yeasts have a thick, multi-layered cell wall with buds formed successively from a single locus on the parent cell [10]. This leads to the formation of a prominent bud scar that gives the typical bottle shape to the parental cell and the bud, thus enabling the easy recognition of the yeasts in skin material (Chap. 2.3). They have the physiological property of being able to utilise lipids and, with *M. pachydermatis* as the exception, the species show an absolute requirement for lipids in culture media (Chap. 2.1). The genus is currently classified in the order Malasseziales among the Exobasidiomycetes (Basidiomycota) (Chap. 2.2).

1.2 Taxonomy

The earliest description of *Malassezia* is that by Eichstedt in 1846 [1] when he recognised the fungal nature of the disease, pityriasis versicolor (PV). He observed the presence of yeasts and filaments in material from patients, but the organism was not named until Robin identified it as *Microsporon furfur* [11] in 1853. This name was replaced by Baillon, when he created a new genus *Malassezia* in 1889, and the binomial *Malassezia furfur* was established [12].

Contemporary reports at this time revealed an interest in scaly conditions of the scalp including seborrhoeic dermatitis (SD), and dandruff (pityriasis capitis) where the presence of yeasts without filaments was seen not only in these patients but also in apparently healthy skin. Rivolta noted double contoured organisms in scales from psoriasis lesions in 1873 [2] placing them within the genus *Cryptococcus*, and in the following year, Malassez described both spherical and oval “spores” in scaly lesions of the scalp [13]. These spores were named as two *Saccharomyces* species, *S. ovalis* and *S. sphaericus*, by Bizzozero in 1884 [14] but other workers [15, 16] considered them to represent a single species and it was Sabouraud in 1904 who assigned them to a new genus *Pityrosporum*. Although he recognised the similarities between PV and pityriasis capitis, both in clinical signs and in the superficial site of colonisation by the yeasts, the presence of filaments in PV scales and the lack of them in material from other conditions led to his reluctance to place *M. furfur* and *Pityrosporum* in a single genus [17]. The yeasts observed in scaly scalps were therefore named by him as *Pityrosporum malassezii* but this was altered later to *P. ovale* by
Castellani and Chalmers in 1913 [18] who acknowledged the earlier specific name given by Bizzozero [14].

Subsequent studies by Panja however, recognised similarities between *Malassezia* and *Pityrosporum* and he suggested a single genus for these yeasts, naming them *M. furfur* and *M. ovalis*, with a third species, *M. tropica*, as a cause of a tropical variant of PV, pityriasis flava [19]. This concept was not accepted by workers at that time, and it was found convenient to reserve the name *M. furfur* to describe the diagnostic microscopic feature of yeasts together with filaments in PV scales, and to continue to use *Pityrosporum* to identify the yeasts found in other conditions and in culture studies. Although various combinations for these yeasts appeared in the literature up to the 1930s, as reviewed by Ingham and Cunningham [20], the majority of authors maintained the binomial *Pityrosporum ovale*. This species, under its various names continued to include yeasts with a diverse morphology, including spherical cells [15, 16, 21].

During the years 1925–1955, more species of *Pityrosporum* were described. In 1925, Weidman [22] isolated yeasts from the skin of a rhinoceros, which resembled *P. ovale* in morphology but could be distinguished by the lack of dependence on lipids for growth. He named it *P. pachydermatis*. A proposal by Dodge, in 1935 [23], that the designation, *Malassezia pachydermatis*, should be adopted for this yeast, was not accepted at the time. A similar yeast was described by Gustafson in 1955 from otitis externa in dogs, which he named *P. canis* [24] but this was later made a synonym with *P. pachydermatis* [17, 25].

This species is not a normal member of the human mycobiota but it has been found in a variety of animal hosts (Chap. 3.3). In the series of publications titled “The Yeasts, a taxonomic study”, the first edition of 1952 listed two species of *Pityrosporum*, *P. ovale* and *P. pachydermatis* [26]. A third species *P. orbiculare* [27] was added to the genus *Pityrosporum* in the second edition of 1970. This species was lipid-dependent and isolated from human skin and was distinguished by the spherical shape of the yeasts. In this 1970 publication, Slooff gave a very comprehensive historical review [17]. She acknowledged the similarity between *Pityrosporum* and *Malassezia*, but since the parasitic phase *Malassezia furfur* could not be obtained in culture, the genus *Pityrosporum* was maintained. The lipophilic yeasts were therefore included in two genera, *Malassezia* and *Pityrosporum*, which was not a satisfactory situation, but one that continued for more than a decade. Further evidence was forthcoming revealing similarities between *M. furfur*, the organism seen in PV, and the yeast *P. orbiculare* both in their morphology, such as ultra-structure of the cell wall [28–30], and in serological relationships [31, 32]. An additional factor was the demonstration of filament production by *P. orbiculare in vitro* [33–36]. Therefore in 1984, in the third edition of “The Yeasts” [37], and in 1986, at The International Commission on the Taxonomy of Fungi [38], a single generic name was proposed for these yeasts and *Malassezia* was accepted as correct. This name had precedence since it was created in 1889 [12], 15 years before Sabouraud’s *Pityrosporum* of 1904 [15]. Although this decision was recognised by taxonomists, “*Pityrosporum*” continued to be used, particularly in the dermatological literature [39–42].

Yarrow and Ahearn [37] placed both of the former lipid-dependent *Pityrosporum* yeasts in a single species, *M. furfur*. This was consistent with the view held by a number of workers that morphological differences in the *Pityrosporum* species were due to the instability of the cell shape [17, 43–45] and also to common antigenic properties [32, 46, 47]. This
opinion was not universally accepted, and it presented a problem for authors wishing to
distinguish yeasts according to differences in their morphology, revealed by variation in
the size and shape of the cell, the ability to produce filaments and colonial features.
Publications, therefore either referred to *Pityrosporum* spp., or divided *M. furfur* into sev-
eral varieties [48, 49]. These authors were able to demonstrate additional factors such as
physiological and serological properties of these organisms, which supported their pro-
posed divisions within the genus.

Once molecular characteristics were established, it became apparent that the varieties
reported in the literature did in fact, represent a diversity of species within the *Malassezia*
genus. A new species, *M. sympodialis*, was described in 1990 [50], being differentiated by
DNA/DNA reassociation experiments and values of the G+C content of nuclear DNA. This
species was incorporated within the genus in the fourth edition of “The Yeasts” [51]. Later,
a number of karyotypes, determined by pulsed field gel electrophoresis (PFGE), were
reported which could be related to phenotypic variants [52–54]. The analysis of large sub-
unit (LSU) rRNA sequencing published in 1995 by Guillot and Guého confirmed the divi-
sions already suspected within the genus and indicated further possible taxa [55].
Subsequently, four new species were established in 1996 where the molecular data could be
correlated with physiological properties, such as the ability to utilise different lipid supple-
ments, differences in the catalase reaction, and variation in temperature tolerance [56].

Schemes were developed which enabled the identification of species using phenotypic
characters [57, 58] and this has led to publications on ecological studies showing the dis-
tribution of species according to pathological conditions and geographical areas [59–61].
Additional molecular methods were subsequently introduced which not only allowed iden-
tification of species *in situ* [62–64], but also led to the revelation of further *Malassezia*
species. These have been from human skin in Japan, *M. dermatis* [65], *M. japonica* [66],
*M. yamatoensis* [67], and from animal sources, *M. nana* [68], *M. equina* and *M. caprae*
[69]. Currently, thirteen species of *Malassezia*, are recognised and have been included in
Chap. 2.1 and in the fifth edition of “The Yeasts: a taxonomic study” [70].

### 1.3 Culture Studies

Lipids have been incorporated in culture media for the isolation of *Malassezia/Pityrosporum*,
since the earliest studies on their recovery, as formulae in use at that time included natural
products such as milk, eggs, lanolin, meat infusion and butter, [19, 21, 71–73] all of which
would have supplied some lipid content. The validity of cultures isolated by Oudemans
and Pekelharing in 1885 [72], van Hoorn in 1896 [73] and Kraus in 1913 [71] is now a
matter of opinion but those of Castellani in 1925 [74] and Panja in 1927 [19] were studied
further by other laboratories and are therefore credited with being confirmed isolates of
*Malassezia/Pityrosporum* [17, 21, 27]. The work of Ota and Huang [21] and Benham [75]
stressed that lipid was, in fact, essential for the growth of *P. ovale*. Benham preferred the
inclusion of butter but it was olive oil, or its component oleic acid that was used by
the majority of subsequent authors [26, 27, 76, 77]. Even when cultures were successful,
the optimum conditions for the maintenance of isolates were not established and many of these were often short lived so that exchange of cultures and comparison of isolates between laboratories were slow to develop. Investigations were performed by several authors into the precise supplements needed for the culture of lipophilic yeasts. Shifrine and Marr [78], Meinhof and Braun-Falco [79] and Wilde and Stewart [77] demonstrated the effect of individual fatty acids, the critical factor being the number of carbon atoms in the molecule. Further to this, Martin Scott in 1952 [80] had demonstrated the positive effect of the inclusion of a bile salt in the medium and these studies led to the development of various formulae for the isolation and maintenance of these lipid-dependent yeasts, which remain in use to the present day [49, 81–83].

Further physiological conditions found to be important for success in the isolation and maintenance of *Malassezia* cultures, were the use of freshly prepared media, humid conditions and a temperature between 30 and 35°C [49, 83]. Although some of the currently recognised species can survive even at temperatures above 37°C, many isolates from human skin, at least in temperate zones, belong to species such as *M. globosa* and *M. restricta*, which are more limited in their temperature tolerance (Chap. 2.1). It was also shown that frequent transfers onto fresh media and the avoidance of low temperatures for storage (e.g. refrigeration at 0–4°C) are both necessary to maintain the viability of cultures in vitro an exception being the use of liquid nitrogen, where even the most susceptible species were found to survive [49].

Descriptions of the colony characteristics of *Malassezia* cultures were very limited until media were developed to provide substantial growth. Formulae with an emulsion of lipid in the agar rather than an oily overlay allowed the formation of discrete colonies with reproducible features. Illustrations by Panja in 1927 [19] and Martin-Scott in 1952 [80] showed cultures with a rough surface and the various colony forms reported later by van Abbe in 1964 [83] on Dixon agar can be readily identified with those in later publications using a similar medium [44, 49, 56]. These features are now included in the standard species descriptions (Chap. 2.1).

From the earliest records of the lipophilic yeasts in tissue, and in cultures, a diversity in the shape of the cells was recognised, with descriptions of oval, globose, bottle shaped and elongated yeasts [1, 2, 13–16, 73]. Although in the illustrations in Benham’s publication [75] and in the first edition of “The Yeasts, a taxonomic study” in 1952 [26], *P. ovale* was shown with fairly uniform oval cells, with the acceptance of Gordon’s *P. orbiculare* in 1970 [27], spherical cells were again included in the genus description [17]. The characteristic feature of repetitive budding from a thick walled cell, which gives the typical bottle shape with a pronounced bud scar was seen in historical publications but was described more clearly in later years with electron microscopy (EM) studies. The cell wall of *Malassezia* was seen to be composed of several layers and a remarkable feature is the appearance of spiralling ridges on the inner surface. This can be visualised by the light microscope and was first noted, as long ago as 1899 by Matakieff [84] but later shown with precision by illustrations of the ultra-structure (Chap. 2.3). The production of filaments in *Malassezia* cultures was reported infrequently in the past [33, 35, 36, 71, 85, 86], and is found to be a typical feature of only two species, namely *M. furfur* and *M. globosa*. Saadatzadeh in 2001 obtained atypical tortuous filaments in *M. sympodialis* [87], but they have never been observed in vivo associated with this species.
The property of differences in fatty acid requirements of variants of *Malassezia* reported by Shifrine and Marr [78] and Meinhof and Braun-Falco [79] has been employed more recently in species identification. Using the assimilation patterns of Tweens (which have different fatty acids as major components), along with catalase activity and the tolerance of temperatures above 37°C, schemes have been devised to confirm the identity of the species by cultural methods [56, 58]. Two additional reactions, the growth with Cremophor EL (castor oil) and the presence of β-glucosidase activity, were found useful to aid in species identification [57, 70, 88].

### 1.4 Ecology

Numerous surveys to determine the extent of *Malassezia* colonisation on human skin have been undertaken using both direct microscopy of tissue and by culture [89, 90]. They revealed that these species are universally present in the population, with colonisation occurring in infancy, and reaching highest concentrations after puberty and in early adulthood [81, 91–94]. Many have focused on the scalp in relation to SD and dandruff [80, 83, 95] and investigations covering multiple sites have shown the highest concentrations of these yeasts to be in areas rich in sebaceous glands [76, 94]. In these reports, the individual species of *Malassezia* were often identified as far as the current knowledge allowed. However, after the newer species of *Malassezia* were established in 1996 [56], and molecular methods expanded to identify the species in tissue as well as in culture, the numerous studies reviewed by Ashbee in 2007 [89] have indicated the distribution of each species from healthy skin and their relationship with dermatological conditions.

Surveys on animals have focused on the recovery of *M. pachydermatis*, which shows a wide range of animal hosts (Chap. 3.3). Several of the remaining *Malassezia* species have been reported from animals, but the extent of the spectrum of the thirteen species in both human and animal skin, as well as the non-living environment, is still being determined (Chaps. 2.1 and 3.3).

### 1.5 Associated Diseases

*Malassezia* species have been associated with a number of different diseases in both humans and animals [96]. These range from “true” infections, where there is invasion of tissue, such as PV, or the much rarer cases of systemic *Malassezia* infection, to conditions where *Malassezia* appear to play a pathogenic role, but through indirect or immunological mechanisms such as in SD. In addition, there have been diseases that have been ascribed to *Malassezia* but with insufficient scientific evidence, such as confluent and reticulate papillomatosis [97] and onychomycosis. In the latter instance, *Malassezia* are almost certainly, colonists of subungual space.
1 Introduction: Malassezia Yeasts from a Historical Perspective

The history of the descriptions of these diseases is difficult to disentangle, partly because of the use of similar terms to describe what we now know to be distinct disease states. Braun-Falco et al. [98], for instance, used the term pityriasis to describe a number of different conditions including scaling in the scalp. Generally, for centuries, the term pityriasis had been used to describe scaling conditions distinct from psoriasis or ichthyosis; the alternative word porrigo was also often used for similar scaling conditions.

1.5.1 Pityriasis Versicolor (PV)

PV is a superficial infection of the skin in which the organisms, usually *M. globosa*, develop filaments and cause minor pathological damage to the stratum corneum of the epidermis through tissue penetration and resultant inflammation. PV is one of the commonest skin diseases in a wide range of geographic areas, but is most frequent in the tropics, with the highest incidence in older children and young adults. It is also seen regularly in northern climates, often in patients who have taken their holidays in a warmer environment. While this infection may occur in otherwise healthy individuals, it is also seen in some immunocompromised patients, such as those receiving long-term therapy with systemic corticosteroids and other immunosuppressive drugs; however, it is not a marker of HIV/AIDS infection. PV presents with scaly hypo- and hyper-pigmented patches, which, with time, become confluent over the trunk, head and proximal limbs. It may be accompanied by mild symptoms of irritation. Atypical forms spreading to the proximal limbs or producing atrophic skin changes have been described. The infection is best diagnosed by direct microscopy of skin scales; culture of scales has no place in the laboratory diagnosis. Treatment with a range of antifungals such as imidazoles is usually effective, although adequate application of topical medications to wide surface areas of the skin is technically challenging. In patients who are not residents of tropical regions, relapse is uncommon; however, if predisposing factors are not removed, re-infection may occur.

PV was a term used by the late eighteenth and early nineteenth century physicians interested in skin disease, such as Willan [99]. But the first recognition that PV, as a distinct form of scaly dermatosis, was caused by a yeast is attributed to Eichstedt in 1846 [1, 3, 17]; he was the first to observe that patients with PV had both yeasts and filaments in skin samples. At this time, culture was not possible and the causal connection was based on identification of the characteristic morphology *in vivo*. The history of the subsequent nomenclature has been described previously. However, the relationship between specific yeasts and PV may appear confusing with Baillon’s renaming of the organism in 1889 as *Malassezia furfur* and the subsequent use of the genus *Pityrosporum*. Nowadays, with the more recent classification, it is becoming clear that *Malassezia globosa* is the principle cause of PV [100], although other *Malassezia* species, including the newly genetically defined *M. furfur*, may also be involved. Many of the earlier clinical studies of this condition, after its recognition as an infection, were documented by Castellani [101] who maintained that there were a number of distinct forms of PV distinguishable by colouration or distribution. Pityriasis versicolor tropicalis, for instance, was the name given to the disease where there was extensive depigmentation associated with lesions. Similar clinical variations were outlined by
other authors [102, 103], although today, the main distinct entities, recognised as clinically relevant, are the atrophic form and a variety of PV associated with oval yeasts and an atypical distribution (Chap. 6.1).

1.5.2 Seborrhoeic Dermatitis (SD)

SD is a scaly skin disease affecting the scalp, face and chest where there are scaly erythematous patches. These are best found in the scalp, in the nasolabial folds, eyebrows, behind the ears and on the chest over the sternum. It is a major, if not the only, cause of scalp scaling or dandruff. The pathogenetic role of *Malassezia* in SD remains something of a mystery, as there is no evidence that the organisms invade the skin. However, our understanding of the explicit link between SD and *Malassezia* is based on observational evidence that removal of the yeasts from the skin, using an antifungal agent, usually leads to remission, and relapse is associated with re-emergence of organisms on the skin surface. There is no experimental model for SD and not all patients respond to antifungals. Despite intensive investigations, the pathogenetic mechanisms involved remain obscure. Attempts to demonstrate the involvement of specific immunological or biochemical pathways have not been successful. However, besides affecting healthy patients, SD is common in the early phase of AIDS [104] and also in patients with chronic neurological illness, such as Parkinson’s disease [105]. No defects in T-cell function in SD patients have been demonstrated that would explain the link with HIV. There is some evidence that there are higher antibody levels to *Malassezia* in SD patients. However, the true pathological basis for SD remains unclear. Intuitively, the mechanism would appear to be the failure to suppress an inflammatory response to a surface commensal yeast, which, as a consequence, is activated in SD patients. However, at present, such ideas are speculative. Diagnosis, therefore remains a clinical one based on the characteristic clinical morphology and distribution of lesions. Treatment with antifungal shampoos, creams or oral antifungals is usually successful, although there are exceptions.

As mentioned previously, scaling of the scalp associated with varying degrees of inflammation and erythema had been known for centuries. But the connection between fungi and SD was first proposed by Malassez in 1874 [13]. He had observed the morphology of the yeasts, which were described as spores, that are present in the scaly scalp in patients with dandruff. This view was supported by Sabouraud who recognised that these were indeed yeasts and believed that they were the cause of pityriasis capitis or dandruff. He also pointed out the similarity between these organisms and those that caused PV and named them *Pityrosporum malassezii*. While dermatologists in the early part of the twentieth century supported an infective aetiology for SD, this was called into question by a number of later scientists who were interested in the development of inflammation in the skin. They proposed that the yeasts were incidental to a primary inflammatory dermatosis, which resulted in increased cell turnover in the epidermis, such as psoriasis, causing scaling and inflammation [106, 107]. For many years, the connection of yeast with SD was forgotten, until the development of the oral antifungal, ketoconazole, led to the observation that patients with dandruff receiving this drug began to improve. This stimulated more work and in 1984,
Shuster [108] proposed that the yeast theory was indeed the correct one and that there was sufficient evidence in existence to make an explicit connection between the presence of *Malassezia* yeasts and SD. In developing his argument, Shuster pointed out that a critical piece of evidence used in favour of the hyperproliferative theory was the failure of patients with dandruff to respond to topical amphotericin B [107]. However, he also pointed out the importance of a study that showed that patients with dandruff, who had responded to nystatin, relapsed when nystatin-resistant cells of *Malassezia* were reintroduced [109].

The association between SD and a number of other conditions remains perplexing. For instance, in the nineteenth century, it was associated with syphilis, [110] although it is not always clear whether the lesions of these two distinct diseases were always separated. Likewise, the pathogenetic explanation for the association between SD and neurodegenerative disorders, such as Parkinson’s disease and latterly HIV/AIDS, is unknown.

### 1.5.3 Malassezia Folliculitis

*Malassezia* folliculitis is a scattered pustular inflammatory rash that is mainly seen on the upper trunk, back and front [111]. The pustules are not associated with comedones (blackheads) and are itchy. The condition is often triggered by sun exposure, but may also occur in severely ill patients admitted to hospital [112]. There is also a pustular form of SD, again usually on the chest or back where the pustules are small and surrounded by erythema. Biopsy of lesion reveals large numbers of yeast within hair follicles and surrounding inflammation. Generally, oral therapy with either itraconazole or ketoconazole is effective.

### 1.5.4 Atopic Dermatitis (AD)

*Malassezia* species have also been implicated in the development of a form of AD affecting the face and scalp [113, 114]. This phenomenon is mainly recorded in young adults rather than in children and affects merely the face and not the flexures. The evidence that there is a connection with *Malassezia* is based on the observation that a) patients respond to antifungal therapy, and b) there is a high prevalence of specific antibody (IgE) to *Malassezia* antigens in affected patients. The positive effect of treatment is difficult to understand on the basis of any explanation, other than that of removal of yeasts from the skin surface, as azole antifungals without activity against *Staphylococcus aureus*, such as ketoconazole, are as effective as those with antibacterial properties. There are precedents for the role of IgE mediated immunity in eliciting flares in AD, as a similar mechanism has been proposed as contributory to the much commoner, *Staphylococcus*–associated, flares of eczema. In the latter example, there is evidence that other immunologically mediated bacterial triggers may be involved. *S. aureus*, for instance, is a source of superantigens that may directly cause T cell activation. So, the mechanism whereby *Malassezia* plays a role in aggravating AD is not known [115].
1.5.5
Confluent and Reticulate Papillomatosis

Confluent and reticulate papillomatosis [97] is a rare condition affecting the upper trunk where patients develop a network of linear, noninflammatory scaly lesions [116, 117]. Biopsies of lesions may reveal the presence of Malassezia yeasts, but the evidence that they play a role in the causation of this condition is weak. Firstly, there is neither satisfactory pathogenetic explanation nor firm evidence that lesions respond to antifungals. The condition is very uncommon and, therefore, controlled comparative therapeutic studies would be hard to perform. Yet, generally patients respond to minocycline, again difficult to explain on the basis of a central role for Malassezia spp.

1.5.6
Other Conditions

Malassezia have been implicated in other conditions such as blepharitis and otitis externa. Again, the problem comes in relating pathogenesis to the organisms, as lipophilic yeasts can be found in both the eye-lashes and the external auditory meatus. In addition, blepharitis and scaling of the external ear are seen in patients with SD and so the link may be similar to that seen with SD patients. There is a form of psoriasis, sometimes referred to as sebopsoriasis, where the clinical distribution of lesions can resemble those seen in SD. Patients with this form of psoriasis have been reported to improve on antifungals, thus suggesting that Malassezia (or another fungus) may play a role [118]. It is not clear, though, whether this is a distinct entity or that it represents an overlap between SD and psoriasis – or even psoriasis co-localising (Koebner’s effect) with SD [119].

1.5.7
Systemic Infection

Rarely, Malassezia species have been described as causes of systemic infection, usually in neonates. The first case in 1981 reported an association with the use of intravenous lipid feeds and care in a neonatal ITU [120]. Transmission from staff or other patients or their relatives has been postulated [121]. The usual cause is M. pachydermatis. Case clustering has been reported suggesting the potential for multiple transmission. This condition is fatal if untreated, and patients respond, if treated in a timely manner with systemic antifungals. However, given that this is an unusual pathogen, late diagnosis is often the problem with this infection.

1.6
Perspectives

The recent elucidation of the genomes of M. globosa and M. restricta [122] will have a significant impact on future studies of Malassezia yeasts. First of all, the data generated will be a major source of reference for many future comparative studies of the genus, e.g.,
those that aim to understand fundamental biological processes, such as the intriguing possibility of a functional mating system, to understand the diversity of allergens and the regulation of their coding genes, and, more generally, comparative pathogenomics, such as the regulation of the expression of genes involved in pathogenicity, inflammation, lipid utilisation in vivo and in vitro, etc. Secondly, the genome may harbour important new drug targets that may contribute to the control of Malassezia-related diseases. Thirdly, the genome data may contribute to the selection of genomic markers that can be used to develop a multilocus sequence typing (MLST) scheme.

Moreover, the availability of the genome sequence will also allow the development of gene replacement techniques, as well as the generation of well defined genetic knock outs. Such constructs will be essential to boost the progress in the field of Malassezia pathophysiology. Eventually, this may result in the emergence of a comprehensive systems approach to understand Malassezia-related diseases in relation to human genetics.

1.7 Currently Recognised Species

1. *Malassezia furfur* (Robin) Baillon (1889)
2. *Malassezia pachydermatis* (Weidman) Dodge (1925)
3. *Malassezia sympodialis* Simmons & Guého (1990)

References

84. Matakieff E (1899) Le pityriasis versicolor et son parasite. Thèse Fac Méd Univ, Nancy
Core Messages

- This chapter presents and discusses all techniques and media used to isolate, maintain, preserve, and identify the 13 species that are presently included in the genus. Each species is described morphologically, including features of the colonies and microscopic characteristics of the yeast cells, either with or without filaments; physiologically, including the growth at 37 and 40°C, three enzymatic activities, namely catalase, β-glucosidase and urease, and growth with 5 individual lipid supplements, namely Tween 20, 40, 60 and 80, and Cremophor EL. Their ecological preferences and role in human and veterinary pathology are also discussed.

- For quite a long time, the genus was known to be related to the Basidiomycota, despite the absence of a sexual state. The phylogeny, based on sequencing of the D1/D2 variable domains of the ribosomal DNA and the ITS regions, as presented in the chapter, confirmed the basidiomycetous nature of these yeasts, which occupy an isolated position among the Ustilaginomycetes. The relationship to the Basidiomycetes is also supported by monopolar and percurrent budding and the multilamellar cell wall ultrastructure. Some characteristics of this cell wall, which is unparalleled in the world of fungi, together with the lipophily demonstrate the uniqueness of this genus in the fungal kingdom.

E. Guého-Kellermann
5, rue de la Huchette, 61400, Mauves sur Huisne, France
e-mail: e.gueho@orange.fr
2.1 Isolation, Identification and Biodiversity of Malassezia Yeasts

Eveline Guého-Kellermann and Teun Boekhout

The genus Malassezia was created for a fungus, M. furfur, which was seen in lesions of pityriasis versicolor (PV). Unfortunately, it took a long time to understand the lipid dependence of this fungus and, consequently, to obtain and maintain its culture in vitro. Due to the lipid requirements, conventional laboratory techniques used for the identification of yeasts could not be applied to this fungus. Despite the description of numerous species, their accurate identification was not feasible, and the taxonomy of the genus remained a controversial subject for decades. The development of molecular techniques allowed the unequivocal separation of species, and then new laboratory methods were developed to characterize these taxa.

2.1.1 Isolation of Malassezia Yeasts from Humans and Animals and their Maintenance

The genus Malassezia, created by Baillon in 1889 [1] and also known under the generic name Pityrosporum created by Sabouraud in 1904 [2], comprises lipophilic and lipid dependent yeasts that require long chain fatty acid (C12 up to C24) supplementation to grow and survive. Slooff [3] in her overview of the history of the genus considered that Panja had been the first to obtain a culture of Malassezia on Petroff’s egg medium with 0.004–0.005% gentian violet [4, 5]. Shifrine and Marr [6] obtained cultures by adding several fatty acids, in particular oleic acid, to Sabouraud agar. These media, however, were disappointing, because growth was inconstant and resulted in rapid loss of cultures (see Chap. 1). Van Abbe [7] was more successful when he recommended the complex medium created by Dixon. This Dixon’s agar (DA) is still in use, according to its original formula, or in a modified version (modified Dixon agar, mDA) as proposed by Midgley [8]. Next to DA, Leeming and Notman [9] proposed a medium, Leeming and Notham agar (LNA) that allowed growth of these nutrient-demanding microorganisms. This medium, elaborated after testing the different compounds separately, allows for isolation and maintenance of all Malassezia yeasts. Therefore, it is now largely used by most researchers working with Malassezia yeasts. All these complex media contain Ox bile, but the LNA replaces Tween 40, used in the Dixon formula, by Tween 60. According to the assimilation pattern of the 13 species presently described (Plates 2.1 and 2.2), Tween 60 seems to be more efficiently utilized, thus favoring growth of most species, whereas Ox bile, as demonstrated by Japanese authors [10], is an essential, if not sufficient compound, for good growth of Malassezia yeasts. Even M. pachydermatis, the less demanding species, requires growth media that are enriched with peptone (i.e., Sabouraud medium), which contains short chain fatty acids. On such media, however, viability is lost rapidly, except if the culture is transferred regularly (about every month). Lorenzini and de Bernadis [11] showed that the addition of Tween 80 enhanced the isolation of M. pachydermatis from clinical materials significantly.

Malassezia yeasts belong to the normal cutaneous mycobiota of humans and animals, and the skin lipids most likely contain the nutrients required. The optimal growth
Plate 2.1 Key characteristics of *Malassezia* species, 1 *M. pachydermatis* T; 2. *M. furfur* T; 3. *M. yamatoensis* T; 4. *M. sympodialis* T; 5. *M. caprae* T; 6. *M. dermatis* (not drawn) T; 7. *M. slooffiae* T; 8. *M. japonica* T; 9. *M. nana* T; 10. *M. equina* T; 11. *M. obtusa* T; 12. *M. globosa* T; 13. *M. restricta* T. The 13 species are arranged from the left to the right according to their decreasing physiological and biochemical capacities. Ø: well, 2 mm diameter; top right Tween 20, clockwise Tween 40, 60, 80, Cremophor EL in the centre; ++/-: growth very weak or delayed secondary growth within the inhibition area after diffusion of the supplement (*M. pachydermatis* and *M. japonica*); +++/-: mild growth and growth of *M. pachydermatis* on GPA, apart of lipid supplements; +++++/-: very good growth; **: precipitate within the agar around the lipid supplements, */***: absence of growth or colonies present only with recent isolates; Cat: catalase activity; β-gl: β-glucosidase activity; 40°C: growth at 40°C; T: type strain of the species.
Plate 2.2  Assimilation pattern of *Malassezia* species Tween 20 (with a mark), 40, 60, 80 and Cremophor EL supplementation. 1 *M. pachydermatis* (wild isolate from dog); 2 *M. furfur*; 3 *M. yamatoensis*; 4 *M. sympodialis*; 5 *M. caprae*; 6 *M. dermatis* (not shown); 7 *M. slooffiae*; 8 *M. japonica*; 9 *M. nana*; 10 *M. equina*; 11 *M. obtusa*; 12 *M. globosa*; 13 *M. restricta*. The 13 species are arranged from the left to the right according to their increasing lipid requirements.
temperature is around 32–34°C; thus, both characteristics seem sufficient to preclude their presence in the environment. Surprisingly, the two fastidious species *M. globosa* and *M. restricta* have been identified by PCR; unfortunately, however, they were not obtained in culture from substrates, such as nematodes, in forest soils in Germany [12], sand stone beneath a crustose lichen in Norway [13], soils of Antarctica Dry Valleys [14], and even from methane hydrate-bearing deep-sea marine sediments in the South China sea [15].

2.1.1.1
Isolation

Below we describe the methods and media used to isolate *Malassezia* yeasts.

2.1.1.1.1
Methods

The samples, collected from skin, scalp, nails, hair, blood, catheter, or any other human or animal source, are transferred as soon as possible onto one or the other selective media to avoid dehydration of the yeasts. During transportation, moisture must be maintained as high as possible, using for instance a plastic bag or box. The samples, distributed onto the selective media in 9 cm Petri dishes, are incubated in a moist environment at 32–34°C, for at least 2 weeks.

2.1.1.1.2
Selective Media

a) *Sabouraud agar plus olive oil*: Mix 20 g glucose, 10 g bacteriological peptone and 10 mL virgin olive oil, 0.5 g chloramphenicol, 0.5 g cycloheximide in 1 L of demineralised water, adjust pH to 6.0, and add 12–15 g agar. Heat to dissolve the agar. Sterilize by autoclaving at 120°C for 15 min and aliquot as required. Addition of other oils or fatty acids, such as oleic acid, can be tested using the same recipe.

b) *Dixon agar (DA)*: Mix 60 g malt extract, 20 g desiccated ox bile (Oxgall, BD Difco), 10 mL Tween 40, 2.5 g glycerol monooleate, 0.5 g chloramphenicol, 0.5 g cycloheximide in 1 L of demineralised water, adjust the pH to 6.0, and add 12–15 g agar. Sterilize by autoclaving at 115°C for 15 min, and aliquot as required.

c) *Modified Dixon agar (mDA)*: Mix 36 g malt extract, 10 g bacteriological peptone, 20 g desiccated ox bile, 10 mL Tween 40, 2 mL glycerol, 2 g oleic acid, 0.5 g chloramphenicol, 0.5 g cycloheximide in 1 L of demineralised water, adjust the pH to 6.0, and add 12–15 g agar. Dissolve the agar by heating and sterilize by autoclaving at 115°C for 15 min, and aliquot as convenient.

d) *Leeming and Notman agar (LNA)*: Mix 10 g bacteriological peptone (Oxoid), 0.1 g yeast extract, 5 g glucose, 8 g desiccated ox bile, 1 mL glycerol, 0.5 g glycerol monostearate, 0.5 g Tween 60, 10 mL whole fat cow milk, 0.5 g chloramphenicol, 0.5 g cycloheximide in 1 L of demineralised water, adjust the pH to 6.0, and add 12–15 g of agar. Sterilize by autoclaving at 110°C for 15 min, and aliquot as convenient.
2.1.1.3 Remarks

1) For an exhaustive survey, the samples, either from humans or animals, must be inoculated only onto a selective complex medium. Indeed, *M. globosa*, *M. obtusa*, and *M. restricta* are highly lipid-dependent, and a few isolates of primary cultures of *M. pachydermatis* do not grow on Sabouraud agar [16]. In clinical practice, the Sabouraud agar supplemented with olive oil, which can be prepared easily and rapidly, is not recommended because only *M. furfur*, *M. pachydermatis* and *M. yamatoensis* grow well on this medium [17].

2) Clinicians are also used to incubating *Malassezia* yeasts at 37°C, as this temperature is considered selective for pathogenic microorganisms. These yeasts, however, belong to the cutaneous mycobiota, and thus are ecologically adapted to a lower temperature. Because *M. globosa*, *M. obtusa*, and *M. restricta*, and also *M. caprae* and *M. equina*, which originated from animals, have a maximum growth temperature at 37°C [17, 18], the incubation temperature should never exceed 35°C, with an optimum between 32 and 34°C. *Malassezia* yeasts do not survive temperatures below 28°C very long, so, materials obtained from collects must not be maintained in a refrigerator before culturing. Use of a high incubation temperature and the utilization of olive oil, which does not allow the growth of most species, may explain why the knowledge of the genus remained limited to a few species for so long.

3) For epidemiological surveys, cultures must be made onto Petri dishes rather than tubes, because the latter do not allow a good separation of colonies. In the same way, the dark Dixon agars facilitate visualization of any mixed growth of *Malassezia* species, or any skin sample contaminated by other micro-organisms, such as bacteria or *Candida* spp.

4) For surveys of *Malassezia* spp. on animals, it is recommended to double the concentration of antibiotics and to use only selective media, because animal fur or/and skin are covered by a large quantity of micro-organisms. Besides, some isolates of *M. pachydermatis* have been shown to be lipid-dependent [16], and it is now well recognized that the veterinary *Malassezia* mycobiota are no longer limited to this unique species.

2.1.1.2 Maintenance of Cultures

Purified *Malassezia* isolates can be maintained on slant cultures in an incubator with a moist environment between 30 and 32°C. Cultures do not survive at room temperature very long. In routine work, they must be transferred on fresh medium every two months, but this may be one month for *M. obtusa* and *M. restricta*.

With the exception of the fastidious species *M. globosa*, *M. obtusa* and *M. restricta*, the other species can be preserved by lyophilisation. Probably, all species may survive freezing at −80°C ([19], Guého unpublished data).

a) Lyophilization: Cells of 4–5-day-old cultures of *Malassezia* spp. are suspended in liquid Dixon medium supplemented with 15% glycerol, and lyophilizates are stored in a refrigerator at 4°C.