

Fungi on the Skin: Dermatophytes and *Malassezia*

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Several human skin diseases and disorders are associated with two groups of fungi, the dermatophytes and *Malassezia*. Although these skin-related problems are not generally life threatening, they are among the most common diseases and disorders of mankind. These fungi are phylogenetically divergent, with the dermatophytes within the Ascomycota and *Malassezia* within Basidiomycota. Genome analysis indicates that the adaptations to the skin environment are different in these two groups of fungi. *Malassezia* are dependent on host lipids and secrete lipases and phospholipases that likely release host fatty acids. The dermatophytes encode multiple enzymes with potential roles in modulating host interactions: polyketide synthases, nonribosomal peptide synthetases, LysM, proteases, kinases, and pseudokinases. These two fungal groups have maximized their interactions with the host using two very different mechanisms.

Skin fungi are associated with many of humanity's most common disorders, including dandruff, atopic dermatitis/eczema, ringworm, athlete's foot, jock itch, and nail infections (onychomycosis). These diseases are attributed to two sets of fungi, ascomycete dermatophytes, including the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*, and basidiomycete

fungi in the genus *Malassezia*. Research on dermatophytes and *Malassezia* has lagged behind research on fungi associated with human mortality, although most of the world's people constantly deal with fungal skin disease, whether acute or chronic.

Genome sequencing has led to new hypotheses for the molecular means by which these

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fungi inhabit human skin and cause disease. Not only are these two sets of skin fungi from distant branches of the fungal phylogenetic tree (Fig. 1), but they also use different sets of proteins to adapt to mammalian skin (Table 1). The dermatophytes appear to use a plethora of proteases to devour keratin, LysM proteins to hide from the host immune system, kinases and pseudokinases to modulate cell metabolism, and a wealth of polyketide synthases and nonribosomal peptide synthases to generate metabolites for unknown purposes. *Malassezia* are found on the skin of warm-blooded animals and are the most numerous fungus on many human skin sites. Their phylogenetically closest relatives are plant pathogens such as *Ustilago maydis* (Fig. 1), but *Malassezia* secrete a set of proteins with activities similar to secreted proteins of another

skin-inhabiting fungus, *Candida albicans*. Most *Malassezia* species are unable to synthesize fatty acids, a deficiency not known in other free-living fungi. *Malassezia* compensate by using many secreted hydrolases (lipases and phospholipases C) to provide fatty acids from host lipids.

Other skin fungi are responsible for emerging diseases that are altering the biosphere. Many amphibian species are threatened with extinction, and a chytrid fungus, growing on frog skin, may be primarily responsible (Cheng et al. 2011). More recently, bat populations have plummeted because of a *Geomyces* fungal infection of bats, which colonizes the facial and wing skin (Blehert et al. 2009). Although these wild-life-killing fungi will not be covered in this review, understanding of one skin fungal pathogen may lead to hypotheses relevant to others.

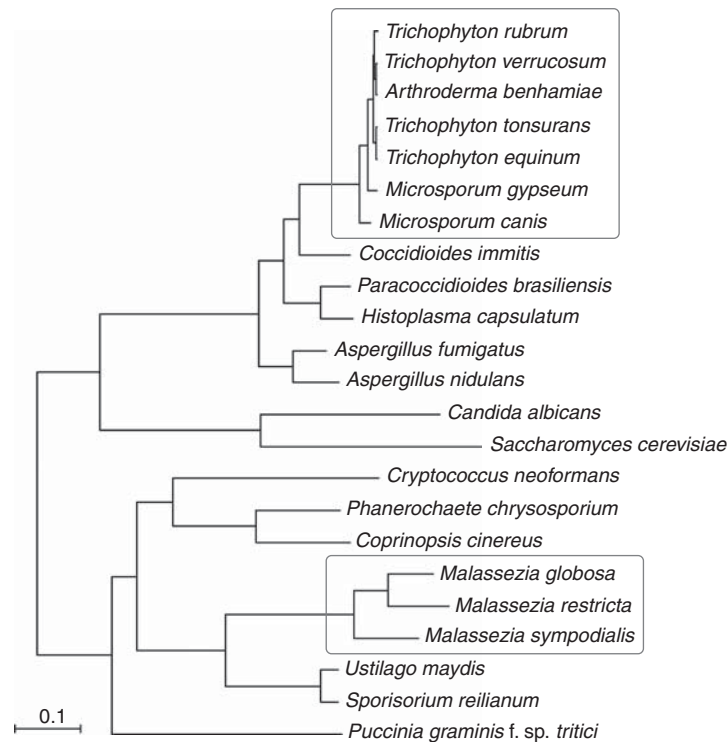


Figure 1. Phylogenetic relationship of dermatophytes and *Malassezia* to other fungi. The phylogenetic relationship of selected fungi was estimated from 616 single-copy orthologs present in all species; protein sequences were aligned using MUSCLE (Edgar 2004), and a phylogeny of the concatenated alignment was inferred using RAxML (version 7.3.3, with model PROTCATWAG and 1000 bootstrap replicates) (Stamatakis 2006). All nodes in the best tree were highly supported by 100% of bootstrap replicates. Dermatophyte and *Malassezia* species groups are each highlighted by a white box.

Table 1. Significant observations from the dermatophyte and *Malassezia* genomes

| Dermatophytes | <i>Malassezia</i> |
|--|--|
| Whole-genome phylogeny consistent with rRNA phylogeny—dermatophytes are closely related to <i>Coccidioides immitis</i> | Phylogeny indicates nearest relatives are plant fungi, such as <i>U. maydis</i> , cause of corn smut |
| Each genome is haploid, containing about 23–24 Mb of DNA | Haploid genomes of 8–9 Mb, among the smallest for free-living fungi |
| Little repetitive DNA | Little repetitive DNA |
| Consistent with their ecological niche, fewer CAZymes to degrade plant material than closely related Ascomycetes | Fewer CAZymes than close relative <i>U. maydis</i> |
| Enriched for genes encoding endo- and exoproteases, consistent with their ecological niche | Enriched for genes encoding lipases, phospholipases C, acid sphingomyelinases, and aspartyl proteases, consistent with ecological niche |
| Enriched for secondary metabolism genes, which may produce molecules that contribute to the interactions with the host immune system | Shortage of secondary metabolite genes, with <i>Malassezia globosa</i> and <i>Malassezia sympodialis</i> each having one polyketide synthase gene and one nonribosomal peptide synthetase gene |
| Two copies of fatty acid synthase genes | No fatty acid synthase gene; among free-living fungi, only <i>Malassezia</i> are known to lack fatty acid synthase gene |
| Enriched for genes encoding kinases, including pseudokinases, which may provide diverse regulation of signaling | Fewer kinase genes; tyrosine protein kinase genes absent |
| Enriched for LysM genes, which may mask fungal cell surfaces and limit exposure to the host immune system | No LysM genes |

We will describe the dermatophytes and *Malassezia*, along with some of the diseases with which they are associated. We will provide a perspective on the phylogeny, ecology, and genomics of these organisms.

HUMAN MICROBIOME ANALYSIS OF FUNGI FROM SKIN AND OTHER BODY SITES

The human body is home to a plethora of microorganisms, many of which are commensal nonpathogenic residents, including bacteria, archaea, viruses, and fungi. These human-associated microbes are essential in establishing and maintaining human health and disease. Enabled by significant advances in next-generation sequencing technologies, the microbiome of several body sites, including skin, has been characterized (Grice and Segre 2011; Cui et al. 2013; Huffnagle and Noverr 2013). The majority of these studies have taken a bacteriocentric view,

only highlighting the diversity of bacterial species (Parfrey et al. 2011).

On human skin, microbial diversity is not limited to bacteria. Fungi, viruses, and mites also contribute to the human skin microbiome. The first large-scale sequencing analysis characterized fungal (“mycobiome”) diversity on the skin of 10 healthy adult volunteers (HVs) (Findley et al. 2013). A total of 14 skin sites representing a range of physiological characteristics—dry, moist, and oily—were sampled via the swab method. Findley et al. used both a culture-dependent and a culture-independent approach to collect microbiota at each body site. Two molecular markers for fungi, the 18S rRNA gene and the internal transcribed spacer 1 (ITS1) region, as well as the 16S rRNA gene for bacteria, were sequenced and used to classify these communities (Khot et al. 2009). The next-generation sequencing technology used in this study resulted in more than five million sequences, whereas approximately 140 isolates

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were recovered from the culturing method (Findley et al. 2013).

Sequence analysis revealed that *Malassezia* is the most abundant fungus on many human skin sites, as previously shown for the scalp (Gemmer et al. 2002). These authors cultured three of the human-associated *Malassezia* species: *M. globosa*, *M. restricta*, and *M. sympodialis*. Sequencing uncovered an additional eight *Malassezia* species residing on skin, as well as an unclassified *Malassezia* spp. The ear and the forehead harbored mainly *M. restricta*, whereas the remaining sites were a mixture of *M. globosa* and *M. sympodialis*.

In contrast to extensive bacterial diversity found at all human skin sites tested (Grice et al. 2009), the skin fungal diversity is more site-dependent. Sites on the back and head are the most stable with the lowest diversity, whereas the proximal arm sites display intermediate diversity. The foot sites are not very stable, change over time, and are the most diverse, with at least 40 genera colonizing each foot site (Findley et al. 2013). The longitudinal analysis of six of the 10 HVs revealed that feet microbiota change over time, which is to be expected, because sampling occurred during the summer when feet are frequently exposed to the environment. Taken together, these data suggest that *Malassezia* species are abundant on human skin, the feet are highly diverse, and the topography of fungi differs from that of bacteria in that the physiological characteristics of skin do not define fungal communities; rather, the body site location is important.

Studies that explore dermatophytes are of interest to dermatologists, because foot fungal infections are a health concern. One study explores foot disease in soccer athletes using a culture-dependent approach (Purim et al. 2005). The major genera observed in this population of athletes were primarily the dermatophytes, *Trichophyton rubrum* (40%), and *Trichophyton mentagrophytes* (36.4%), whereas *Candida* and other fungi made up the remaining 24%. Findley et al. (2013) also observed *Trichophyton* as the only dermatophyte in the fungal survey study and only on the feet of HVs. Genera such as *Microsporum* and *Epidermophyton* were not observed.

Malassezia have historically been described as found on the skin of warm-blooded animals. Since the advent of DNA-based methods to characterize microbial communities, there are several reports of *Malassezia* or *Malassezia*-like organisms. The samples include beetle guts (Zhang et al. 2003), nematodes (Renker et al. 2003), sponges (Gao et al. 2008), coral (Amend et al. 2012), and airborne samples (Pitkaranta et al. 2008). A better understanding of *Malassezia* adaptation to mammalian skin may arise from comparisons with *Malassezia*-like organisms from other biological sources.

DERMATOPHYTES

Dermatophytes are a group of pathogenic fungi that cause mostly superficial diseases on humans and other mammals (Kwon-Chung and Bennett 1992; White et al. 2008; Achterman and White 2012a,b). The diseases that result from a dermatophyte infection are known as tinea. The location of the disease on the body further defines the disease, so that tinea pedis are dermatophyte infections of the feet, tinea cruris of the genitals, tinea corporis of the torso, and tinea capitis of the head. There are at least 40 species of dermatophytes that infect humans, and many of these fungi can cause disease in more than one body location. Still the most prevalent cause of tinea pedis is *T. rubrum*, and the most prevalent causes of tinea capitis are *Trichophyton tonsurans* and *Microsporum canis*.

Dermatophytes are ascomycetes with septate hyphae, most closely related to *Coccidioides immitis* within the Onygenales (Kwon-Chung and Bennett 1992; Graser et al. 2008). There are three genera of dermatophytes, *Trichophyton*, *Microsporum*, and *Epidermophyton*. Although the species were historically divided into these genera by morphology and physical attributes, recent analysis by rRNA sequencing indicates that the dermatophytes as a whole are a cohesive group, with no clear distinction between the three genera. The closest relatives to any one *Microsporum* species might be two *Trichophyton* species (Graser et al. 2008). Thus, no comparisons should be made between genera without consulting the rRNA-based phylogenetic tree.

DISEASES OF DERMATOPHYTES

The dermatophytes cause significant disease in humans, horses, pets, and other animals. It is often argued that dermatophyte infections are the most common human infection in the world (not just the most common fungal infection), as tinea pedis is a common problem in the developed world, and tinea capitis is a common problem in the developing world. Dermatophyte infections are responsible for at least half a billion dollars in health-care costs (Achterman et al. 2011). It is also important to remember that dermatophytes colonize 30%–70% of humans without causing disease.

There are three types of dermatophytes, unrelated to the three genera (White et al. 2008). Some dermatophyte species have only been found on humans and are assumed to be human-specific. Such species are termed anthropophilic and include *T. rubrum* and *T. tonsurans*. There are species that are found on humans and on other animals, and these species are referred to as zoophilic. These zoophilic species include *Microsporum canis*, *Trichophyton equinum*, *Trichophyton verrucosum*, and *Arthroderma benhamiae*. Geophilic species, including *M. gypseum*, are found in soil and occasionally infect animals.

Each of the species in these three types of dermatophytes can cause a spectrum of disease. However, in general, geophilic species are accidental infections, and there has been no evolutionary selection to maximize host/pathogen interactions. Therefore, these geophilic species generally cause acute, severe disease (Kwon-Chung and Bennett 1992). Anthropophilic species have usually interacted with the human host for a very long time and, thus, the interactions between these species and the host immune system have evolved together and can be subtle. Therefore, these species usually are associated with long-term, chronic, but limited disease and tissue destruction. Finally, the zoophilic species interact somewhere between the acute geophiles and the chronic anthropophiles.

Another aspect of the three types of dermatophytes is their ability to complete a sexual cycle. In general, the sexual stages of the geo-

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philes have been identified, and the sexual stages of the anthropophiles have not. Specifically, sexual stages are known for *M. gypseum*, *M. canis*, and *A. benhamiae*, but not for *T. tonsurans* or *T. rubrum*. In fact, recent work based on the genome sequencing has identified strains containing each of two mating types in *M. gypseum* (Li et al. 2010), yet no isolate representing a second mating type in *T. rubrum* has as of yet been identified. This suggests that this most common dermatophyte has predominantly one mating type in clinical infections, implying that mating is not occurring for this species during most infections, or if it is, it may be unisexual. Although most *T. rubrum* infections from around the world might be the result of a single strain or clone, microsatellite analysis suggests the existence of a second subpopulation of predominantly African origin (Graser et al. 2007). The collection and analysis of additional globally diverse isolates, particularly from Africa, could assist a search for the second mating type in *T. rubrum*.

Research into the virulence and pathogenicity of dermatophytes has been slow, in part because dermatophyte infections do not have significant mortality. There are occasionally reports of disseminated dermatophyte infections, but these individuals usually have other underlying conditions. A fundamental question in dermatophyte research is how the dermatophytes interact with the host immune system. Major dermatophyte antigens have been defined, including Tri r 2 and Tri r 4 (Gao and Takashima 2004). In active disease, the dermatophytes grow, cause tissue destruction, and cause inflammation, essentially a war of attrition between the fungus and the host. When a dermatophyte infection is under control, it is not clear if the fungus has been eliminated or if it is still present but controlled by the host immune system. Dermatophyte infections are difficult to cure entirely and frequently recur in the same site of initial infection. Hence, the question of reactivation or new infections is important to our understanding of these infections.

Some epidemiology has been conducted to investigate the major dermatophyte infections

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in a specific area or group of patients. Yet only recently have the precise molecular tools, including genome sequencing (see below), been available to clearly determine if a strain is indeed a new infection or a reactivation. As described above, this question is central to dermatophyte biology. Dermatophytes are treated with a variety of antifungal drugs (Borgers et al. 2005); the most common are the ergosterol biosynthesis inhibitors (EBIs), including azoles and terbinafine (Lamisil). These drugs inhibit steps in the biosynthesis of ergosterol, the fungal equivalent of cholesterol, the major sterol component of the plasma membrane. Azoles target the enzyme Erg11, lanosterol demethylase, whereas terbinafine targets Erg1, squalene epoxidase (Marr et al. 1998). Most of the azoles that are used to treat dermatophytes are imidazoles, available over the counter. Prescription triazole azoles that are commonly used to treat invasive fungal infections are generally not used to treat dermatophyte infections. Terbinafine is available over the counter as a topical medication and by prescription to treat recalcitrant nail infections; however, this drug can show significant liver toxicity, so the prescribing physician and the patient must carefully decide when to use this drug orally (Gupta and Cooper 2008).

Curiously, despite the availability of imidazole azole drugs over the counter, and the subsequent sporadic use of these medicines by those infected, there are only a few case reports of azole drug-resistant infections (Ghannoum et al. 2006; Ghannoum et al. 2008). Azole drug resistance is common in other pathogenic fungi including *C. albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, under conditions where the drugs are used regularly, sporadically, and at varying strengths (Pfaffer 2012). In addition, the azoles are static drugs that inhibit growth but do not kill the fungal cells—excellent conditions for the development of resistance. Therefore, it is curious that the dermatophytes have not developed widespread resistance despite the overuse and misuse of azoles. Understanding how the dermatophytes respond to azole drugs is of wide interest in the use of these drugs to treat all pathogenic fungi.

The three most important questions relating to dermatophytes are thus: (1) is an infection a reactivation or a new infection, (2) why do dermatophytes not develop azole resistance, and (3) how do the fungus and the immune response interact to create a balance that can result in either clinical disease, latent and controlled colonization, or the elimination of the fungus (Table 1)?

GENOME ANALYSIS OF DERMATOPHYTES

The first step in addressing the most intriguing questions relating to the dermatophytes was to obtain and compare the genome sequences of different species. These were completed and published in 2011 and 2012 (Burmester et al. 2011; Martinez et al. 2012). The species that were sequenced include *T. rubrum*, *T. tonsurans*, *T. equinum*, *M. canis*, *M. gypseum*, *A. benhamiae*, and *T. verrucosum*. These species were selected because they represent (1) all three dermatophyte types (geophilic, zoophilic, and anthropophilic); (2) mating-competent species as well as species with no known sexual phase; and (3) acute and chronic infections. The strains selected to represent each species to be sequenced were representative and isolated relatively recently. The complete genomes confirmed many aspects of the dermatophyte biology: (1) The whole-genome phylogeny is consistent with the rRNA-based phylogeny, (2) the strains were haploid, (3) the genomes were approximately the same size (23–24 Mb), and (4) the genomes had few repeat sequences.

An analysis of these genomes was not sufficient to characterize a set of genes that direct a species to be geophilic, zoophilic, or anthropophilic, as the genes specific to these categories were open reading frames (ORFs) without functional annotation. Neither did the genome analysis identify differences correlating with mating competence. Each strain exhibited its own mating locus, and using that information, the other mating locus could be identified in other strains from mating-competent species, but not from species with no known mating. However, each of the genomes contains all of the genes usually necessary for mating of a species, and thus



does not give any indication as to why some species are mating competent and others are not. Analysis of the genomes did not reveal any features that clearly correlated with pathogenicity, including how the species might cause acute or chronic disease. Although duplications of some ergosterol pathway genes were identified, this did not clearly explain why drug resistance is rare in the dermatophytes. Further study of how these pathogens respond to host and drug pressure is needed to address these questions.

The genomes provided insights into several areas of pathogenicity in dermatophytes that should guide further study (Table 1). These observations were the result of comparing the genomes of the seven dermatophyte species with the genomes of their close relatives, *C. immitis*, and the three other dimorphic fungi, *Histoplasma*, *Blastomyces*, and *Paracoccidioides*, as well as *Aspergillus fumigatus* and other fungal pathogens. One of the first observations is that the dermatophyte genomes have a reduced number of genes that are involved in degradation of plant carbohydrates. This class of plant-degrading carbohydrate-active enzymes, or CAZymes, is present in most species that grow on plants. The fact that the genes are lost in fungi adapted to growth on human tissue instead of plant tissue is not unexpected; however, directly testing growth on substrates, including those produced by plants, would help correlate phenotype with genotype and identify differences between the species.

Comparing the genomes also revealed that dermatophytes have amplified a number of gene families with potential roles in infection. Dermatophytes have a higher number of genes encoding enzymes that could degrade human proteins, including exopeptidases and endopeptidases. Several types of proteases are enriched, including subtilisins, deuterolisins, and metallopeptidases.

In addition to proteases, the dermatophytes have an increased number of genes encoding kinases and pseudokinases. Presumably, an increase in these signaling factors may indicate that the dermatophytes are able to respond to diverse conditions, as might be expected from constant interaction between the fungus and the

host immune system. It is particularly interesting that the dermatophytes also have an increased number of genes encoding pseudokinases, also involved in signaling. Pseudokinases do not have the conserved amino acids necessary for kinase function, but these proteins can function in signaling by interacting with functional kinases. The types of interactions that could involve pseudokinases may include (1) competing with kinases for substrates, (2) competing with kinases to act as a substrate for upstream kinases, or (3) interacting with kinases in a multisubunit complex with allosteric regulation (Reese and Boyle 2012). Signaling systems can also contain phosphatases and pseudophosphatases, but genes encoding these types of molecules were not amplified or lost in the dermatophytes compared to other fungi.

Genes encoding secondary metabolites are also present at higher number in dermatophytes and are highly variable between the different species. These metabolites are the products of polyketide synthesis or nonribosomal protein synthesis. Many of these metabolites are toxins, with antimicrobial and immunosuppressive activities. It is interesting to speculate that dermatophytes increased the number of these genes so that the metabolites could be used in the interactions between the fungal cells and the host, perhaps as immune suppressors to control the immune response to fungal infections. Alternatively, the toxins might control superinfection with other microbes, which can lead to “trench foot,” the result of dermatophyte infection and bacterial superinfection.

Dermatophytes also contain very high numbers of proteins with LysM domains, which in other fungi can bind to various carbohydrate moieties including chitin. The LysM domain was originally identified in bacteria in which proteins containing the domain appear to be secreted and bind to and mask bacterial peptidoglycan from the host immune system. Recently, LysM-containing proteins have been identified in plant fungi, where they are thought to bind to and mask chitin in the fungal cell wall from recognition by the plant immune system (Koharudin et al. 2011; Marshall et al. 2011). The LysM-containing proteins defined in the

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dermatophytes are the first LysM genes described from a human fungal pathogen. Although many pathogenic fungi have a few proteins with LysM domains, the dermatophytes have amplified this gene family to 15 to 48 members. The LysM-containing proteins in the dermatophytes all have a GH18 chitin-binding domain. This supports the hypothesis that the dermatophyte LysM proteins bind to and mask chitin to protect the fungal cell from the host immune system, allowing latent colonization by the dermatophyte.

Analysis of these genomes has increased our understanding of dermatophytes and raised new questions for further study: (1) What is the function of the many dermatophyte-specific or species-specific genes with no known function (this problem is not unique to the dermatophytes), (2) what is the role of the additional secondary metabolism gene clusters unique to the dermatophytes, (3) what is the role of the additional kinases and the unique pseudokinases in the interactions between the fungus and the host, and (4) what is the role of the proteins containing LysM domains—do they mask the fungus from the host immune system, and to what surface markers does the unique LysM domain bind on the fungal cell surface (Table 1)?

Malassezia

The genus *Malassezia* belongs to the subphylum Ustilaginomycotina (Begerow et al. 2000; Xu et al. 2007; Guého-Kellermann et al. 2010) of Basidiomycota, but within this group, their position is not yet clear. The order Malasseziales has been proposed in the Exobasidiomycetes (Begerow et al. 2000; Guého-Kellermann et al. 2010), but other researchers placed them in Ustilaginomycetes (Matheny et al. 2007). Consequently, Hibbett et al. (2007) treated them as *insertae cedis* in their outline of a phylogenetically supported fungal taxonomy. Based on a multigene analysis of many species of basidiomycetous yeasts, a new class, Malasseziomycetes, was proposed in Ustilaginomycotina, indicating the isolated taxonomic position of these yeasts (Wang et al. 2014).

Complex media are needed for isolation, cultivation, and culture-based enumeration of *Malassezia* yeasts. Dixon's agar (DA) is used according to its original formula but more commonly in a modified version (modified Dixon agar, mDA) (Midgley 1989). A second widely used medium is Leeming and Notman agar (LNA), which allows isolation and maintenance of all known *Malassezia* yeasts. Recipes for these media can be found in Guého-Kellermann et al. (2010). Optimal growth temperature is 32°C–34°C. Samples from human or animal sources should be transferred quickly to these media and incubated in a moist environment for at least 2 wk. Different *Malassezia* species show different growth rates and plating efficiencies, so culture-based methods are not recommended for identification or enumeration of resident *Malassezia*.

Species can be recognized using ribosomal DNA (rDNA) sequence analysis, especially that of the ITS 1 + 2 regions and the D1/D2 domains of the large subunit (LSU) rDNA (Guillot and Guého 1995; Gemmer et al. 2002; Gupta et al. 2004). Based on this molecular recognition of species, it became possible to differentiate them by phenotypic means to some extent (Guého et al. 1996, 1998; Guillot et al. 1996; Mayser et al. 1997; Crespo Erchiga and Guého 2005; Guého-Kellermann et al. 2011). Discrimination of closely related species (e.g., *M. sympodialis*, *Malassezia caprae*, and *Malassezia equine*) by these phenotypic methods remains challenging. More recently, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) allowed the identification of all recognized species without extensive phenotypic characterization and showed full concordance with rDNA-based identifications (Kolecka et al. 2014).

Our knowledge on the biodiversity of *Malassezia* yeasts has greatly expanded during the last two decades. Because the recognition that the lipid-dependent *Malassezia furfur*, as formerly recognized, contained multiple species (Guého et al. 1996), 13 lipid-dependent species are currently recognized in addition to the lipophilic, but not lipid dependent, *Malassezia pachydermatis* (Cabanes et al. 2010; Guého-Kel-



lermann et al. 2010, 2011). The phylogenetic position of *M. pachydermatis* is not yet settled, as it clusters at different positions based on the gene fragments studied.

SKIN DISEASES AND *Malassezia*

Dandruff and Seborrheic Dermatitis

Dandruff and seborrheic dermatitis (D/SD) are common disorders of the scalp and occasionally the eyebrows, moustache, and beard (Warner et al. 2001). Dandruff is characterized by flaking and pruritis, and it is classified as SD when accompanied by visible redness and extension beyond the scalp to other areas of the face. D/SD is the most common *Malassezia*-associated dermal disorder, occurring in up to 50% of healthy humans and up to 75%–90% of immunocompromised subjects, especially AIDS patients (Farthing et al. 1985; Warner et al. 2001). Dandruff has received increased attention because of the following: its common occurrence, its link to loss of self-esteem, its negative social image (Warner et al. 2001), and the publication of the dandruff-associated fungal genomes (*M. globosa* and *M. restricta*). The resurgence of interest in *Malassezia* in D/SD supports that dandruff and seborrheic dermatitis are manifestations of the same disorder differing only in severity (Priestley and Savin 1976; Kligman 1979; McGrath and Murphy 1991; Danby et al. 1993; Bulmer and Bulmer 1999; Faergemann 2000b; Pierard-Franchimont et al. 2000; Warner et al. 2001).

D/SD most commonly first appears in adolescents and young adults, likely because of the increase in sebaceous activity during puberty (Ramasastry et al. 1970; Cotterill et al. 1972). The number of scalp *Malassezia* has been reported to be higher in patients with seborrheic dermatitis or dandruff than in normal controls (McGinley et al. 1975), but the evidence is controversial and contradicted by other reports (Gaitanis et al. 2012). The differences are likely caused by the difficulty in accurately enumerating *Malassezia* from human skin (Kligman 1979; Weiss et al. 1991; Gupta et al. 2001b; Gemmer et al. 2002; Sugita et al. 2002). D/SD

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is most commonly treated by shampoos containing antifungal ingredients, indicating the role of fungi. However, as *M. globosa* and *M. restricta* are present on all human scalps, the presence of fungus alone is not sufficient to cause D/SD. It has been reported that D/SD is not caused by overgrowth of *Malassezia* but by an abnormal host immune response (Bergbrant and Faergemann 1989; Midgley 2000). However, D/SD sufferers do not have higher total antibody levels than controls, and there are several reports that an aberrant immune response is not involved (Midgley and Hay 1988; Bergbrant and Faergemann 1989; Parry and Sharpe 1998). A *Malassezia* lipase has been shown to split triglycerides into irritant fatty acids that can induce scaling (Troller 1971; McGrath and Murphy 1991; Parry and Sharpe 1998) or can release arachidonic acid, which is involved in the inflammation of skin (Faergemann and Fredriksson 1979; Binder and Jonelis 1983; Riciputo et al. 1996). Further research may reveal to what extent the D/SD-promoting role of *Malassezia* is mediated by the immune system, by the enzymatic activity of the fungus, or by additional features such as secondary metabolites (Faergemann and Fredriksson 1979; Hay and Graham-Brown 1997; Watanabe et al. 2001).

The *Malassezia* most closely associated with D/SD are *M. globosa* (Kligman 1979; Crespo Erchiga et al. 1999; Nakabayashi et al. 2000; Gupta et al. 2001b) and *M. restricta* (Kligman 1979; Crespo Erchiga et al. 1999; Gemmer et al. 2002). However, some authors have also reported *M. furfur*, *M. sympodialis*, *Malassezia obtuse*, and *Malassezia slooffiae* (Nakabayashi et al. 2000).

Atopic Dermatitis

Atopic dermatitis (AD), also designated atopic eczema, is a chronic inflammatory skin disease marked by pruritus (often intense) and characteristic eczematous lesions with erythema, fine scaling, and thickening of the epidermis. The disease is complex with several subgroups and frequently associated with allergic rhinitis, asthma, and immunoglobulin E (IgE)-mediated food reactions (Bieber 2008). Genetic factors,

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such as those leading to an impaired skin barrier, play an important role in the development of AD. However, environmental factors, such as lifestyle and exposure to microbes, most likely contribute to the increased frequency of the disease (Bieber 2010). The prevalence of AD has rapidly increased, estimated today at 15%–30% in children and 2%–10% in adults, with the highest prevalence in industrialized countries.

Many studies have found that *Malassezia* is a microbial trigger that exacerbates AD, especially when the disorder is localized to the head and neck, sebum-rich areas frequently colonized by *Malassezia*. Some studies have examined the prevalence and the species composition of *Malassezia* yeasts in AD. Sandström Falk et al. (2005) sampled skin on the upper back and found that *M. sympodialis* was the species most commonly isolated from both AD patients and healthy controls and also found a difference in species distribution on lesional versus non-lesional skin in AD patients; nonlesional skin was most frequently colonized by *M. globosa*, whereas *M. sympodialis* was most commonly found on lesional skin. Other studies in Japan have preferentially found *M. globosa* as the major cutaneous fungus in AD (Cho et al. 2013), which might reflect geographical differences in the distribution of *Malassezia* species.

Approximately 50% of adult patients with AD have allergen-specific IgE and T-cell reactivity and/or positive atopy patch test reactions to *Malassezia*, leading to the hypothesis that in AD *Malassezia* act as allergens rather than infectious agents (Scheynius and Cramer 2010). These reactions are rarely found in other allergic diseases, indicating a specific link between AD and *Malassezia* (Casagrande et al. 2006). Allergen-specific IgE against *Malassezia* can also be detected in children associated with their AD severity in infancy (Kekki et al. 2013). Thirteen allergens have so far been identified in *Malassezia* (Scheynius and Cramer 2010), and at least 12 genes encoding different classes of the proteins have been confirmed to be present in both the *M. sympodialis* and *M. globosa* genomes (Gioti et al. 2013). Interestingly, several of the identified allergens are proteins of unknown

function with no sequence homology to characterized proteins, whereas others are homologous to host proteins, suggesting the possibility of cross-reactive immune responses. In fact, using recombinant *Malassezia* allergens, new subgroups of AD patients have been discovered, who, in addition to IgE reactivity to *Malassezia* allergens, have an autoimmune IgE-mediated reactivity against self-antigens (Scheynius and Cramer 2010). One example is T-cell-mediated cross-reactivity between the *M. sympodialis* allergen Mala s 13 and the human homologous protein thioredoxin (Balaji et al. 2011). Such cross-reactivity might contribute to the pathogenesis of AD by perpetuating skin inflammation in patients with AD sensitized to *Malassezia*. Another mechanism by which *Malassezia* can interact with the host is the release of extracellular exosomal-like nanovesicles carrying allergens (Gehrmann et al. 2011). These nanovesicles, designated MalaEx, can induce inflammatory cytokine responses with a significantly higher IL-4 production in AD patients compared to healthy controls. These findings suggest that MalaEx might be potential therapeutic targets.

Pityriasis Versicolor

Pityriasis versicolor (PV) is a chronic superficial fungal disease characterized by round to oval lesions on the trunk and upper arms. The lesions vary in color and can be hypo- (white) or hyper- (pink, tan, brown, or black) pigmented. Large lesions exhibit minor flaking, but only at the border. PV is regarded as a cosmetic disorder, as most patients are asymptomatic or have mild pruritus (Faergemann 2000a). PV is associated with warm, damp weather, as it is more prevalent in the summer months, and it is more prevalent in tropical locations than in temperate regions. However, endogenous host factors are also involved, including malnutrition (Stein 1983), oral contraceptives (Borelli et al. 1991), systemic corticosteroids or immunosuppressants, and hyperhidrosis (Burke 1961; Faergemann and Bernander 1979).

PV is most common in adolescents and young adults, when sebaceous gland activity is highest (Michalowski and Rodziewicz 1963;



Wyre and Johnson 1981; Congly 1984; Terragni et al. 1991). PV may occur when the *Malassezia* that normally colonize the skin change morphology from the round yeast form to a pathological hyphal form that invades the stratum corneum. The most common *Malassezia* species cultured from PV are *M. globosa* (Crespo Erchiga et al. 2000; Nakabayashi et al. 2000) and *M. sympodialis* (Gupta et al. 2001a,b). Other *Malassezia*, including *M. slooffiae* and *M. furfur*, are less common but occasionally isolated. The ability to grow well at higher temperatures may explain why *M. furfur* is more frequently isolated from PV in warmer climates than *M. globosa* (Midgley 2000; Guého-Kellermann et al. 2011).

Other Disorders

Malassezia can cause folliculitis and has been implicated in psoriasis (Gaitanis et al. 2012). *M. furfur* may also be implicated in invasive infections, especially in low-birth-weight neonates that receive intravenously lipid supplementation (Gaitanis et al. 2012). Children and adults with serious immune suppression caused by gastrointestinal disease may be at risk (Gaitanis et al. 2012); for treatment guidelines, see Arendrup et al. (2013). Part of the older literature on such infections, however, may refer to other lipid-dependent *Malassezia* species.

PATHOGENESIS

Genome sequences from *M. globosa*, *M. restricta* (Xu et al. 2007), and *M. sympodialis* (Gioti et al. 2013) have provided insights into hypotheses for virulence and survival on host skin. These fungi are the only known free-living fungi to lack fatty acid synthase (Xu et al. 2007), suggesting that they obtain lipids from the host skin. The lack of a fatty acid synthase is a trait shared with some skin bacteria in the genus *Corynebacterium* (Tauch et al. 2005).

Malassezia encode many secreted aspartyl proteases, lipases, phospholipases C, and acid sphingomyelinases, a trait shared with *C. albicans* (Xu et al. 2007; Park et al. 2013), which also grows on human skin. This similarity suggests

that these enzymes have a role in providing nutrients. However, the dermatophytes do not share this trait. Phylogenetic analysis of lipase and aspartyl protease genes show examples of multiple *M. globosa* genes related to a single gene from the phylogenetically close relative, *U. maydis*, implying gene duplications or losses from the common ancestor of these fungi (Xu et al. 2010). There is some indication that these enzymes have a role in pathogenesis. Scalp gene expression at the mRNA level has been demonstrated for lipase and phospholipase genes of *M. globosa* (Xu et al. 2007) and *M. restricta* (Lee et al. 2013). Increased expression of *M. globosa* lipase genes was demonstrated in scalp skin scales from seborrheic dermatitis patients (Patiño-Uzcátegui et al. 2011). Oleic acid, a product of *M. globosa* lipase activity on scalp, can induce dandruff-like flaking in human subjects (DeAngelis et al. 2007). Higher levels of phospholipase were observed from *M. pachydermatis* isolates from dogs with dermatitis and otitis than from healthy dogs (Cafarchia and Otranto 2004). The latter study suggests that some *Malassezia* strains may be more pathogenic than others, a conclusion demonstrated with atopic dermatitis patients and strains with different rRNA sequences (Cho et al. 2013). Insights into virulence may arise from the complete genome sequence of strains of different pathogenicity.

There are additional ways by which *Malassezia* might affect human skin, resulting in disease. Tryptophan metabolism produces a variety of indoles that are potent agonists of the aryl alcohol receptor. These indoles (malassezin, indolo[3,2-b] carbazole, pityriacitrin, and indirubin) were found in extracts of skin from SD and PV patients but not in healthy controls (Magiatis et al. 2013). The first three of these materials were produced by *M. furfur* strains isolated from SD patients when these strains were cultivated in L-tryptophan agar (Gaitanis et al. 2008). Control strains from healthy patients grew poorly and produced lower amounts of indoles. Genomics may not readily provide insight into the pathways of indole production, as many indoles can be formed from tryptophan and indole pyruvic acid in the absence of cells, suggesting that a trypto-



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phan aminotransferase may be the only enzyme necessary for the synthesis of these indoles (Zuther et al. 2008). However, additional enzymes may be used *in vivo*, and Hort et al. (2009) have identified some candidate *M. furfur* genes with a role in indole synthesis.

These indoles are potent activators of the aryl hydrocarbon receptor. Indirubin is synergistic with Toll-like receptor ligands in the up-regulation of a gene regulated by the aryl hydrocarbon receptor (Vlachos et al. 2012). The indoles appear to modulate the response of monocyte-derived dendritic cells to Toll-like receptor ligands, resulting in less production of costimulatory markers CD80, CD83, and CD86, as well as MHC II and the inflammatory cytokines IL-6 and IL-12. These results suggest that *Malassezia* can depress the immune response through indoles. Other indole activities include the lowered host pigment production mediated by malassezin, likely through apoptosis of melanocytes (Gaitanis 2012). Together, these indole derivatives may be responsible for hypopigmentation and lack of inflammation associated with PV (Gaitanis et al. 2012).

In another example of a *Malassezia* secondary metabolite that interacts with animal cells, several *Malassezia* species are recognized by a C-type lectin receptor, Mincle, found on activated macrophages (Yamasaki et al. 2009). Binding induces the production of the cytokines MIP-2, TNF- α , KC, and IL-10. The Mincle ligand was recently isolated from *M. pachydermatis* and characterized as a glycolipid comprising 10-hydroxystearic acid, D-mannose, and L-mannitol (Ishikawa et al. 2013).

With the discovery of mating-type genes in *Malassezia* (Xu et al. 2007), there has been interest in whether *Malassezia* has a sexual cycle and, if so, to what extent sex impacts virulence and population dynamics. There are several indications that *Malassezia* may be sexual. A mating locus has been found in all three *Malassezia* genome sequences that have been reported; a population study of *M. sympodialis* has provided evidence for recombination; and many meiosis and mating signaling genes are present (Gioti et al. 2013). However, despite repeated

attempts, no one has yet demonstrated mating in *Malassezia* (Gioti et al. 2013).

SUMMARY

For the dermatophytes and for *Malassezia*, the completion of genome sequences represents a turning point, facilitating molecular analysis of how these fungi survive and cause disease. With the recent development of transformation protocols for dermatophytes (Grumbt et al. 2011a,b), the role of a gene can be tested. Dermatophytes and *Malassezia* have adapted to human skin, an environment that is relatively amenable to sample recovery, facilitating rapid progress in studying host–fungal interactions. Dermatophytes and *Malassezia* have a large impact on human health, as they are found on the skin of most people and are frequently associated with disease. With greater attention to the human microbiome, we will learn how these organisms may contribute to health and disease.

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