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Review Article

Review Article

Malassezia ecology, pathophysiology, and treatment

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Abstract

Malassezia are lipid dependent basidiomycetous yeasts that inhabit the skin and mucosa of humans and other warm-blooded animals, and are a major component of the skin microbiome. They occur as skin commensals, but are also associated with various skin disorders and bloodstream infections. The genus currently comprises 17 species and has recently been assigned its own class, Malasseziomycetes. Importantly, multiple *Malassezia* species and/or genotypes may cause unique or similar pathologies and vary in their antifungal susceptibility. In addition to culture-based approaches, culture-independent methods have added to our understanding of *Malassezia* presence and abundance and their relationship to pathogenicity. Moreover, these novel approaches have suggested a much wider-spread presence, including other human body parts and even other ecosystems, but their role in these arenas requires further clarification. With recent successful transformation and genetic engineering of *Malassezia*, the role of specific genes in pathogenesis can now be studied. We suggest that characterizing the metabolic impact of *Malassezia* communities rather than species identification is key in elucidation of pathophysiological associations. Finally, the increasing availability of genome sequences may provide key information aiding faster diagnostics, and understanding of the biochemical mechanisms for *Malassezia* skin adaptation and the design of future drugs.

Key words: *Malassezia*, biodiversity, mycobiome, skin, antifungal, disease mechanisms.

Introduction

Malassezia are lipid dependent basidiomycetous yeasts that inhabit the skin and mucosal sites of humans and other warm-blooded animals. They are a major component of the skin mycobiome, based on both culture-based and culture-independent methods that used ITS length polymorphisms assessed by polymerase chain reaction (PCR),[1] ITS metabarcoding, and whole genome shotgun metagenomics.[2,3] Various *Malassezia* species occur on human and animal skin as commensals, and they are associated with multiple skin disorders, such as pityriasis versicolor (PV), *Malassezia* folliculitis (MF), seborrheic dermatitis/dandruff (D/SD), atopic dermatitis (AD), and psoriasis.[4] Use of catheters for parenteral nutrition can lead to *Malassezia*-caused bloodstream infections in immunocompromised patients or premature infants.[5,6]

A recent molecular phylogenetic study using six genes suggested the genus is deeply rooted in the Ustilaginomycotina with a sister relationship to Ustilaginomycetes and Exobasidiomycetes. Hence, the genus was assigned as its own class, Malasseziomycetes.[7] These findings were confirmed by a phylogenomics approach based on complete genome sequences of 24 *Malassezia* isolates from 14 species.[8] For many decades, the genus consisted of only two species, the lipid-dependent *M. furfur* and the apparent lipophilic *M. pachydermatis*. Since then, many more species have been described, initially using the D1/D2 domains of the large subunit of the ribosomal DNA (LSU rDNA) and the internal transcribed spacer regions (ITS) 1 and 2 (including the 5.8S rDNA).[9] More recently, additional loci were added, including chitin synthase-2 (CHS2), β-tubulin, and translation elongation factor 1 alpha (TEF1).[10–12] The phylogenomics study mentioned above used 164 core eukaryotic genes and identified three main species clusters: Cluster A consisting of species known mainly human skin, that is, *M. furfur*, *M. japonica*, *M. obtusa*, and *M. yamatoensis*; subcluster B1, with the most abundantly occurring human skin inhabitants *M. globosa* and *M. restricta*; subcluster B2 consisting of *M. sympodialis*, *M. dermatis*, *M. caprae*, *M. equina*, *M. nana*, and *M. pachydermatis*; and Cluster C that forms a basal lineage with *M. cuniculi* and *M. slooffiae*.[8]

Since then, three new species were described, namely, *M. brasiliensis* and *M. psittaci* from parrots[11] and *M. arunalokei* from human skin.[12] Thus at present the genus comprises 17 species. Phylogenetic relationships of these 17 species based on D1D2 domains of LSU rDNA are shown in Figure 1 and are largely in agreement with the previous phylogenomics data.

Rapid and accurate identification of *Malassezia* from clinical samples is of importance for correct diagnosis and treatment. Traditionally, identification of *Malassezia* has been culture based using morphologic and biochemical features, such as utilization of Tweens and cremophore EL, catalase activity and growth at different temperatures. These conventional methods showed limitations with regards to differentiation between closely related species, are time consuming, and have a high error rate.[13] In routine clinical laboratories, *Malassezia*-related disorders and infections likely are underdiagnosed as standard nonlipid supplemented media, such as Sabouraud glucose agar (SGA), do not support *Malassezia* growth, and delay correct identification and treatment. Likewise, the absence of species identification limits epidemiological knowledge regarding *Malassezia*-related disorders and infections.[14,15]

Several improvements have been made in *Malassezia* identification. An overview regarding available molecular tools was published by Cafarchia et al.[14] With the recent addition of two PCR-based methodologies,[16,17] including identification of 11 species directly from patient samples,[17] the range of identification tools was further expanded. Application of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification of *Malassezia* isolates[15,18] removed the need for DNA extraction, requires relatively low consumable and reagent costs, and has a short turnaround time. Unfortunately, spectra of most species have not yet been included in available commercial databases.

Finally, the number of available genome sequences of *Malassezia* species is increasing rapidly and offers a valuable resource for development of targeted nucleic acid based diagnostics. As whole genome sequencing becomes more affordable, comparison of full genomes for identification and epidemiology may soon be within reach.

Biodiversity and ecology

Culture-independent tools for examining complex microbial communities occurring in and on the human body opened opportunities for detection of species that would otherwise be missed using culture-based methods, due to slow or fastidious growth or the lack of appropriate conditions resembling the natural habitat. Originally, human microbiome studies mainly focused on prokaryotic inhabitants of the human body, but fungi have recently received more attention.

Some older studies referred to in this review were performed before general application of molecular methods and employed phenotypic methods, such as microscopy and physiological characteristics for isolate identification. We include those studies as they significantly contribute to our understanding of the diversity and role of *Malassezia* yeasts on the human and animal body, but it cannot be ruled out

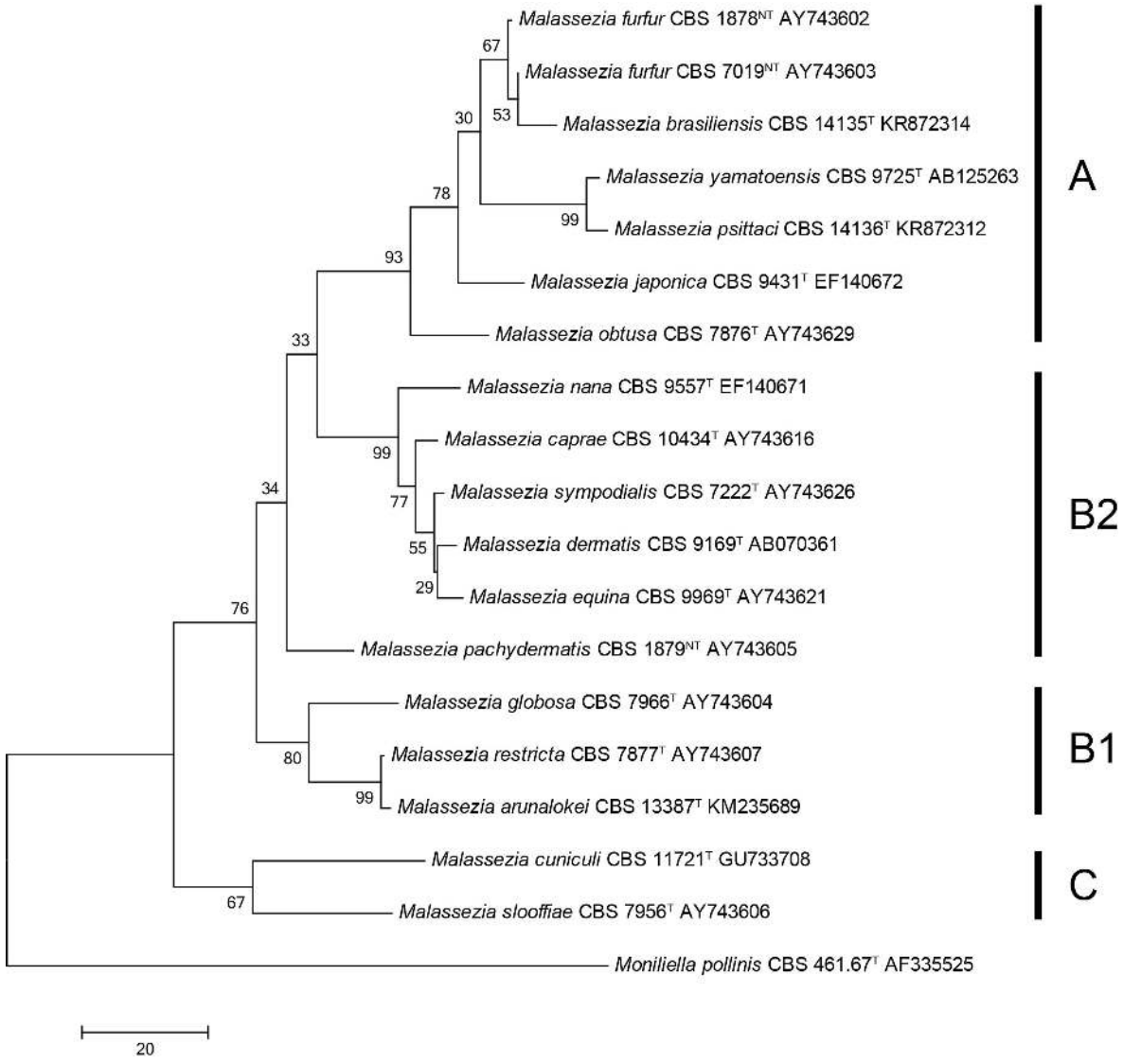

Figure 1. Phylogenetic tree of the currently accepted 17 *Malassezia* species based on sequences of the D1D2 domains of the LSU rRNA gene, inferred using the Maximum Parsimony method with 1000 bootstrap replications. Tree 1 out of 5 most parsimonious trees (length = 377) is shown. The consistency index is 0.531250, the retention index is 0.689922, and the composite index is 0.470319 for all sites and 0.366521 for parsimony-informative sites. The tree is drawn to scale, with branch lengths calculated as number of changes over the full sequence. There were a total of 516 positions in the final data set and the evolutionary analysis was conducted in MEGA7.[169]

that over time with the further application of molecular diagnostics the emerging epidemiological picture may evolve. Table 1 gives an overview of all currently known species, with their taxonomic cluster denomination (also see Fig. 1), most common hosts, and known disease/disorder associations in both humans and animals.

Presence on the healthy human skin

The relative abundance of fungi on human skin was found to be low compared to bacteria, but *Malassezia* yeasts were identified using culture-independent methods as the most abundant skin eukaryotes representing 50%–80% of the total skin mycobiome.[2,3] *Malassezia* species predominated on all sampled body sites except foot. Eleven *Malassezia* species were identified with *M. restricta* being predominant in the external auditory canal, retroauricular crease and glabella, and *M. globosa* on back, occiput and inguinal crease. All the remaining species were observed scattered across other body sites and with lower frequency. Fungal diversity was more dependent on body site than the individual, but as similar species distributions occurred 3 months after the initial sampling this suggests temporal stability of site-specific *Malassezia* communities.[2] Reanalysis of these metagenomic datasets using a more complete set of *Malassezia* genomes demonstrated the presence of 12

Table 1. Overview of all currently described *Malassezia* species with their taxonomic cluster denomination (see Fig. 1), main hosts and main known disease/disorder associations in both humans and animals.

species	Taxonomic cluster	Main hosts	Main known disease/disorder associations in humans	Main known disease/disorder associations in animals	References
Malassezia furfur	A	Man, bovine, elephant, pig, monkey, ostrich, pelican	Systemic infections, PV, D/SD		5,9,59,93
Malassezia brasiliensis	A	Parrot	-	-	11
Malassezia yamatoensis	A	Man	SD, AD	-	9,61
Malassezia psitaci	A	Parrot	-	-	11
Malassezia japonica	A	Man	AD	-	57
Malassezia obtusa	A	Man	D/SD, AD	-	9,93
Malassezia nana	B2	Cat, bovine, dog	-	otitis	93,97,98,99
Malassezia caprae	B2	Goat, equine	-	dermatitis	44
Malassezia sympodialis	B2	Man, equine, pig, sheep	Systemic infections, PV, D/SD, AD	otitis	9,26,42,43,93
Malassezia dermatis	B2	Man	AD		9,27,45
Malassezia equina	B2	Equine, bovine	-	dermatitis	44
Malassezia pachydermatis	B2	Dog, cat, carnivores, birds	Systemic infections	otitis, dermatitis	9,64,93,94,95
Malassezia globosa	B1	Man, cheetah, bovine	PV, D/SD, AD	otitis	9,26,93,100,101
Malassezia restricta	B1	Man	D/SD, AD	-	9,27,32,35
Malassezia arunalokei	B1	Man	D/SD	-	12
Malassezia cuniculi	C	Rabbit	-	-	93
Malassezia slooffiae	C	Man, pig, goat, sheep	D/SD	otitis, dermatitis	9

Note: AD, atopic dermatitis; D/SD, dandruff / seborrheic dermatitis; MF, *Malassezia* (pityrosporum) folliculitis; PV, pityriasis versicolor.

species, with *M. restricta* and *M. globosa* by far the most abundant, distantly followed by *M. sympodialis*.[8] Most studies surveyed whites of Western descent. A high throughput ITS1 sequencing analysis of 40 asymptomatic Chinese subjects in Hong Kong revealed 90% of the sequencing reads as *M. restricta*, distantly followed by *M. globosa* with 5.3%.[19] Another study investigating 40 healthy Japanese subjects using a real-time PCR approach, showed that the *Malassezia* skin mycobiome differed by sex, body site, and season. Generally, in male subjects, *M. restricta* and *M. globosa* were most predominant, followed by *M. dermatis*, *M. furfur*, and *M. sympodialis*. In female subjects *M. globosa* and *M. sympodialis* were most predominant, followed by *M. dermatis*, *M. restricta*, and *M. furfur*.[20] Nevertheless, a major sampling bias is present in skin mycobiome studies to date and future studies need to take this into account.

Malassezia skin colonization begins immediately after birth and increases until 6–12 months of age.[21] Colonization then remains relatively low until just prior to puberty, when sebaceous gland activation provides a better habitat and *Malassezia* populations rise to a stable level.[4] Recent metagenomic evidence suggests that skin colonization varies according to age and puberty[22] and hypothesized a protective effect due to increased *Malassezia* colonization in adults preventing colonization by more pathogenic species, specifically dermatophytes and other more pathogenic species found more commonly in children.

Occurrence in human skin disorders and systemic infections

Host factors such as gene-induced variation, environmental conditions, lifestyle, hygiene, and the immune system can cause shifts in skin microbial communities associated with disease.[23] For example, molecular studies of temporal changes in skin mycobiota of Japanese Antarctic expedition researchers and astronauts showed a temporal change in colonization levels with different proportions of *Malassezia* species during periods of increased stress and inability to bathe or shower. Fungal diversity decreased during time in space, whereas colonization of *Malassezia* species increased.[24,25] Oh et al. reported a significant decrease in community diversity as an indication of skin disease, but it remains unclear whether such changes occur at all taxonomic levels.[3]

Malassezia species have been associated with a number of skin conditions, including PV, *a* MF, D/SD, AD, and

psoriasis.[26,27] In a 26S rDNA-based pyrosequencing study of Japanese SD patients *Malassezia* was the most abundant fungal genus at both lesional and nonlesional sites. At lesional sites *M. restricta* predominated, and at nonlesional sites *M. globosa* and other species were detected but did not differ between sites.[28] A recent culture-based study of individuals in India applying multiple molecular methods for species identification confirmed the predominance of *M. globosa* on scalps of control subjects, whereas on the scalps of SD patients the abundance of *M. globosa* was closely followed by *M. restricta* and *M. furfur*.[12]

Based on both phenotypic and PCR-based studies *M. globosa* is the species most frequently associated with human disease and linked to various dermatological conditions including PV and D/SD and it occasionally occurs on animal skin.[1,26,27,29–33]

M. restricta is the second most common species on healthy and diseased skin, particularly of scalp, neck, face, and ears[27,31] and is associated with D/SD and AD.[1,32,34–36] It was abundantly found on healthy skin but significantly more abundant at SD sites.[28] A culture-independent study comparing healthy, nondandruff with dandruff scalp of French subjects found *M. restricta* as the most abundant fungal species in both but with a slightly higher colonization level on healthy scalp (97% vs. 84%).[37]

M. sympodialis is the third most abundant species on healthy human skin but appearing with significantly lower frequency when compared to *M. globosa* and *M. restricta*.[8] It is also known from PV, AD, the skin of an AIDS patient, the auditory tract of a healthy 33-year-old male, and various animals. It must be noted that the majority of these findings are based on physiological, not molecular, identification.[26,27,29,38–41] Occasionally, *M. sympodialis* is also found to cause systemic infections.[42,43]

M. caprae has been described from healthy skin of goats and equines[44] and is less frequently found on human skin.[8] *M. dermatis* has been identified with low frequency from both healthy skin and lesions of AD patients.[8,27,45]

Utilization of culture-based methods identifies *M. furfur* on various body sites including human skin, blood, and urine. However, culture-independent studies found *M. furfur* on skin only rarely, with low frequency and abundance.[1,2,8] *M. furfur* has been identified from deep-seated infections, such as blood, urine, and vagina, often associated with immune compromised patients, or from septicemia in neonates that received lipid supplementation via catheters.[5,46–52] *M. furfur* was isolated from the skin of preterm-infants in a neonatal intensive care unit (NICU), with a prevalence varying according to gestational age, admission to the NICU and length of hospitalization.[53,54] An Italian 1-year survey of yeast mediated fungemia in 290 neonates and 17 pediatric patients (age <16 years) using MALDI-TOF and sequencing for species identification resulted in eight cases (2.8%) in which *M. furfur* was identified as the causative agent. From all patients with *M. furfur* bloodstream infections the species was also isolated from skin.[5] Based on multiple methods, such as PFGE, AFLP, and rDNA sequence analysis, considerable intra-species variation was observed and it has been suggested that a specific AFLP genotype is more frequently involved in deep-seated infections.[55,56] Another study evaluated catheter-associated *M. furfur* strains and found that all isolates recovered from blood cultures and catheter tips belonged to the specific subtype I-3.[52]

M. japonica is a rare species isolated from healthy human skin of a Japanese woman and AD patients.[57] *M. obtusa* has been identified from human groin, the nasal vestibule, and human AD but also from animals.[58–60] *M. yamatoensis* is known from SD and AD patients as well as healthy human skin.[27,61] During a recent survey of SD in India, a new species *M. arunalokei* was found on scalp and in the nasolabial folds of both healthy subjects and D/SD patients.[12]

Based on mycobiome investigations, *M. pachydermatis* has been observed in the sputum from asthma patients[62] and in the nasal vestibule of healthy subjects and patients with allergic rhinitis (AR).[60] The species causes catheter-related septicemia in neonates that receive lipid supplementation,[29,51,55,63–70] and it has been associated with sepsis and bloodstream infections in immunosuppressed adults.[71,72] *M. pachydermatis* is considered zoophilic and is only rarely isolated from human skin.[4,8,73] However, it may colonize the hands of pet owners and when PCR was used as the detection technique the prevalence of hand colonization was found to be high (i.e., 93%).[74] A possible zoonotic infection route was suggested when pet dogs were identified as a source for transmission of the species to neonates via the hands of health care workers.[68]

The *in vitro* production by *Malassezia* species of Aryl hydrocarbon receptor (AhR) indolic ligands from tryptophan changed our views on the biochemistry of these species. An array of bioactive indoles is produced by *Malassezia* species. The significance of this observation was expanded when it was associated with *M. furfur* pathogenic potential and was also shown to happen by the other, more common *Malassezia* species. Furthermore, these indols may also be formed *in vivo* as they were found in significantly higher quantities in SD skin scales as compared to healthy controls. *Malassezia* yeasts both *in vitro* and *in vivo* produce potent AhR ligands, that is, formyl-indolo [3,2-b] carbazole (FICZ) and indolo [3,2-b] carbozole (ICZ). The ability to activate the AhR receptor and thus modulate down-stream effects places *Malassezia* yeasts within two significant pathophysiological pathways, mediation of

ultraviolet damage and modulation of the host immune response. These observations, together with the anatomic co-localization of *Malassezia* yeasts with basal cell carcinoma, led to the hypothesis that they could be implicated in skin carcinogenesis.[6,75,76]

More studies are needed to gain a better overview of the abundance and role of *Malassezia* species in the microbiome of healthy and diseased skin of male and females from various ethnicities, different locations, climates, and life styles. It will be especially important to consider long-term (years) longitudinal studies of lesional and healthy skin among genetically related humans.[77]

Presence in other human body sites

A mycobiome analysis of the nasal vestibule of AR and healthy subjects identified 69 fungal genera of which *Malassezia* was predominant. At least six species were found in all subjects: *M. restricta*, *M. globosa*, *M. sympodialis*, *M. slooffiae*, *M. dermatis,* and *M. pachydermatis*. One sample contained *M. cuniculi* and *M. obtusa*. *M. restricta* represented the vast majority (>86%) in both subject groups. At the species level, two AR subjects showed a notably higher diversity compared to healthy individuals.[60] Another study characterized the sinus fungal communities of 23 chronic rhinosinusitis (CRS) patients and 11 controls. *Malassezia* was the most prevalent fungus and detected in all patients and controls.[78,79] Presently available data suggests that *Malassezia* species are commensals of the nasal cavity, but any role in AR and CRS needs further clarification.

Conflicting data exist regarding the oral microbiome. *Malassezia* were reported to be present with high abundance in the oral microbiome of six subjects, suggesting it may be a prominent oral commensal.[80] Other culture-based studies and one culture-independent study did not confirm the presence of *Malassezia* in the oral microbiome.[81,82] Dupuy et al. suggested that the unexpected findings may be related to the use of an improved harsh cell lysis method.[80,82] *Malassezia* was also found a dominant oral mycobiome inhabitant in a leukemia patient with mucormycosis.[83] In a study that characterized the microbiome of human root canal infections, *Malassezia* was the second most frequently identified fungal genus after *Candida* spp., albeit only in two out of six analyzed teeth and with a low abundance.[84]

With respect to other body sites, two studies found *Malassezia* spp. in sputum samples of cystic fibrosis (CF) patients but with lower abundances than *Candida* spp.[85,86] Finally, *Malassezia* species have been found in stool, but more research is needed to clarify whether they belong to the commensal human gut mycobiota.[87–90]

Malassezia on animals and other habitats

Malassezia have been isolated from a variety of animals, including cats, dogs, horses, goats, pigs, and rabbits,[91–93] both as commensals and linked to diseased skin. *M. brasiliensis* and *M. psittaci,* two recently described species using sequencing analysis of three loci, originated from parrots in Brazil.[11] *M. pachydermatis* is the most frequently isolated species from skin of all animals investigated, except rabbits and goats. *M. pachydermatis* has a high intraspecies diversity, and certain genetic subtypes may have host specificity.[94,95] *M. slooffiae* has been reported from bovines, goats, cats, and healthy pigs, but these findings were mostly based on phenotypic identifications.[91,96] A sequencing-based study showed that *M. caprae* was primarily isolated from healthy goats, and *M. equina* mainly from healthy horse.[44] Using sequencing and other molecular methods *M. nana* has been identified in the ear canal of cats with otitis externa, in cows with and without otitis externa from Brazil,[97] in healthy and diseased cats from the UK and Spain,[98] and it has been suggested to be the predominant *Malassezia* species occurring in ears from horses.[99]

Using phenotypic identifications, *M. globosa* has been encountered on skin and healthy or otitic ears from cows and on horses that are either healthy or suffer from dermatomycoses.[96,100,101] *M. sympodialis* was the most common species found on healthy cattle in Brazil[100] but has to a lesser extent also been isolated from healthy equines and other animals.[59,96,100,101] *M. furfur* has been isolated from various animals, including elk, elephant, ostrich, goat, dog, cat, and, more frequently, from cattle and equines.[59,96,100,101] Most studies describing *Malassezia* species from animals are culture- and phenotype based, and further culture-independent surveys are needed to compare with the *Malassezia* communities occurring on humans.

Malassezia species were originally thought to be specifically associated with mammalian hosts, but culture-independent studies revealed they may occur in a much broader diversity of habitats, including terrestrial and marine ecosystems such as deep-sea sediments, (Antarctic) soils, corals, sponges, nematodes,[102–104] and cone snails.[105] Furthermore, *Malassezia* DNA was detected from soil nematodes in Central European forests, and it has been hypothesized that nematodes may serve as a vector for *Malassezia* species.[106] In a Brazilian study that investigated 45 cows with bilateral otitis nematodes were found in the ears of all subjects and *Malassezia* yeasts in 69% of the ears, suggesting a possible relationship between nematodes and *Malassezia* yeasts.[107] Finally, in a recent metabarcoding survey on the fungal microbiome of the olive fruit fly, *Malassezia* was identified as one of the core associated fungi, albeit with low relative abundance.[108] More research is needed to clarify the presence and role of *Malassezia*

species in these and other habitats and their possible interactions with other members of the microbial communities and their vertebrate and invertebrate hosts.

Advances in comparative genomics

To date, the complete genomes of 29 *Malassezia* isolates have been reported, including 14 species and multiple isolates of the most common inhabitants of human skin, that is, *M. globosa*, *M. restricta*, and *M. sympodialis*,[8,109] *M. pachydermatis*, the most common veterinary species,[110] and *M. furfur*, a species that causes most invasive infections and sepsis.[8] These studies also included comparison to divergent fungal genomes, indicating features that define the *Malassezia* genus. *Malassezia* genomes are compact and well adapted to a specific ecological niche, as evidenced by loss of multiple common fungal gene families and multiplication of others.[111] *Malassezia* have a propensity for gene turnover, and the losses and gains are focused on gathering of necessary nutrients from a sparse environment via secretion of large families of lipases, phospholipases, aspartyl proteases, and other enzymes for degradation of skin. Interestingly, comparison of *Malassezia* to other fungi resident on humans or plants emphasizes "niche specific evolution."[111] *Malassezia* express multiple secreted hydrolases, similar to *Candida albicans*, another opportunistic human skin pathogen. *Candida* is not phylogenetically related to *Malassezia* but its enzyme repertoire also aims to degrade proteins and fat. Similarly to *Malassezia*, *Ustilago* produces a set of secreted enzymes for degradation of their local host, but as *Ustilago* is a plant pathogen the enzymes target degradation of plant specific proteins, cutin, and waxes.[8,112]

All *Malassezia* have lost the main enzymes required for lipid metabolism, including fatty acid synthase (FAS), Δ^9 desaturase, and $\Delta^{2,3}$enoyl CoA isomerase.[8,111,113] Therefore, they cannot produce fatty acids themselves but need lipids from the environment for growth. Comparative genomics coupled with phenotypic characterization also led to the conclusion that *M. pachydermatis*, previously thought to be lipid independent and lipophyllic, is actually lipid dependent along with all other *Malassezia* species. Comparative genomics also revealed 13 *Malassezia*-specific functional domains, mainly containing genes of unknown function. One example of a gene gain event is that of a single gene, belonging to the PFam domain PF06742 with unknown function, which is conserved in all *Malassezia* but absent in any other sequenced Basidiomycetes, implying a gene transfer event which predates the genesis of the genus.[8] The gene is functionally expressed in *M. globosa* and its transcription regulated. A second potentially horizontally transferred gene was predicted to be a catalase that matched the PFam domain PF00199. This catalase may be potentially adaptive as it may protect *Malassezia* cells from their own secreted hydrogen peroxide generating proteins.[8] Finally, another *Malassezia* HGT is the acquisition of flavohemoglobin A from the bacterial genus *Corynebacterium*, increasing NO resistance.[114] These gene families have undergone multiple lineage-specific duplications and then further divergence. The most parsimonious explanation for the genus-wide variation in these gene families may be related to each species' niche-specificity. *Malassezia* genomics also revealed they are likely to mate,[8,109,111] which may increase their virulence as a result of higher genetic diversity.[115,116] The structure of the mating loci suggest pseudo-bipolar mating in all *Malassezia* where the sequence assembly is strong enough to support structural determination of the region.[8,117]

Isolates belonging to *M. furfur* show significant genomic and phenotypic divergence, including isolates with: significantly larger genomes; duplicate copies of multiple genes; and differing karyotype patterns, taken together suggesting a hybridization event.[8,55,111,118] Ongoing studies are attempting to disentangle the complexity of these *M. furfur* isolates and other genetically diverse species, such as *M. globosa* and *M. pachydermatis*.

Epidemiology and pathophysiology of a complex relationship

Malassezia yeasts are eukaryotic fungal skin commensals that must cope in their cutaneous niche with stimuli and perturbations that originate both from the host and the external environment. In addition, host susceptibility is critical.[119,120] Thus, from the point of view of human pathology we may consider *Malassezia* as pathophysiologic effectors acting within a narrow, yet omnipresent transitional (intermediary) zone positioned in between the "self" body compartment, the skin, and the "non-self," 'outer' environment (Fig. 2). Moreover, *Malassezia* populations occurring within the "transitional zone" are dynamically modified by and also respond to homeostatic reactions of the underlying skin (Fig. 2). In this view, we should not adhere to the concept of pathogenic versus apathogenic *Malassezia* species as fundamentally understood by the Koch postulates. On the contrary, we should address the impact of the metagenome (secretome, proteome) functional plasticity of the diverse *Malassezia* species and their constituting populations occurring within an anatomical area and the relevant impact they have on the skin of susceptible individuals with or without excess yeast proliferation, as is the case in PV, and D/SD and AD, respectively.

In PV, there is a population burst of *Malassezia* which proliferate abundantly under favorable environmental conditions (e.g., enhanced heat, humidity). Reversing the

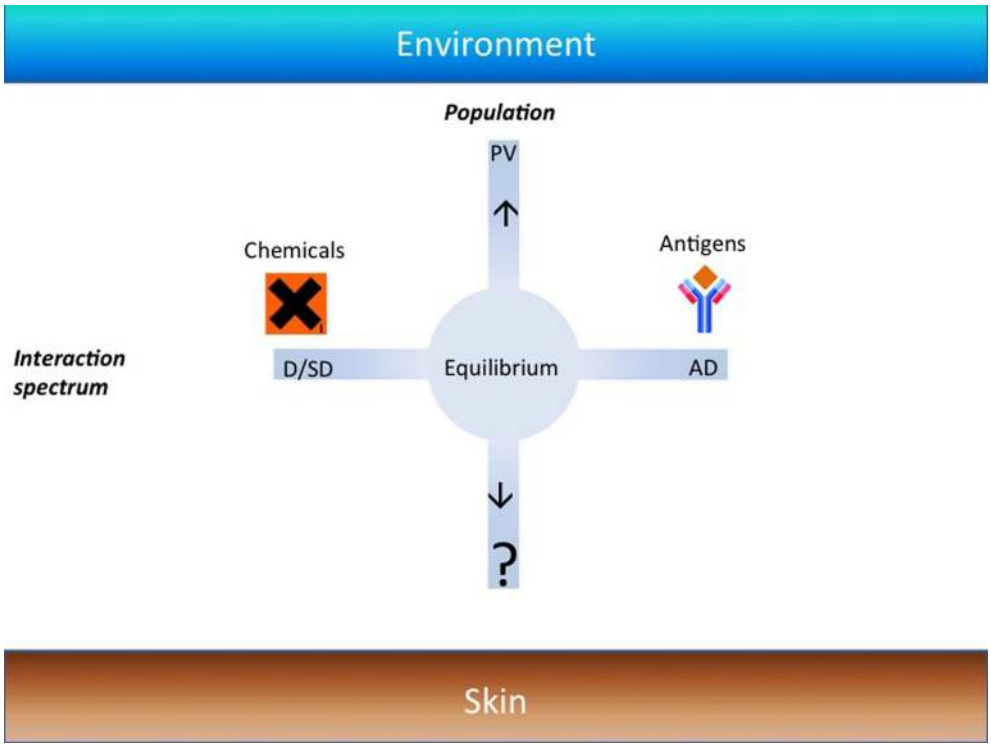

Figure 2. Schematic representation of the interaction spectrum between *Malassezia*, the skin and the environment. *Note*: AD, atopic dermatitis; D/SD, seborrheic dermatitis /dandruff; PV, pityriasis versicolor. This Figure is reproduced in color in the online version of *Medical Mycology*.

aggravating disease conditions can promptly resolve PV without any specific therapy.[121] Moreover, in this condition *Malassezia* cells massively acquire a distinctive hyphal morphology that probably denotes the profuse availability of nutrients,[122] which does not only secure the opportunity of the yeast to reproduce freely but also their ability to spread on the skin surface and perhaps to mate with cells from distinct populations of neighboring yeast settlements, for example, from adjacent hair follicles. This would be suggestive of a condition analogous to microbial biofilms. Furthermore, from a clinical point of view, the almost 80% relapse rates after antifungal treatments is disappointing as it only temporarily reduces the colonizing yeast numbers without removing the underlying environmental cause. The pathophysiology of PV lesions includes minor changes in skin barrier function[123] or even a potentially protecting UV-filtering action of the *Malassezia* biofilm.[124] The observation of useful skin adaptations as a consequence of *Malassezia* proliferation further underscores the concept of a pliable, physiological 'transitional mantel zone' that enables a gradual transition between skin surface and environment. This 'zone,' is by nature "external" to the human body, yet it is also an area of significant compositional plasticity as the diverse effects of the underlying healthy or diseased skin could decisively modify it.

Regarding the impact of *Malassezia* in AD and D/SD, *Malassezia* induce skin disease through two—not mutually exclusive but potentially interacting—induction mechanisms, namely, allergic and irritant pathways (Fig. 2). In AD, a sensitization state against *Malassezia* antigens seems to be almost universal. An array of well-characterized allergens with immunoglobulin E (IgE) binding ability has been described in AD patient disease exacerbations (as well as in patients with cholinergic urticaria), mostly of *M. furfur* and *M. sympodialis*, and more recently from *M. globosa*.[125,126] The detection rate of IgE sensitization only to *M. sympodialis* antigens reaches 60% in severe AD patients.[125] Similarly, high sensitization rates of patients with the same diagnosis have been described for *M. globosa* antigens.[127] Possible explanations could include (1) the presence of an undetected persistent population of sensitizing species in skin sanctuaries, like the hair infudibulum, or (2) substantial turn-over in the species that colonize the skin in different life periods thus favoring multiple sensitization events. Additionally, a selectivity of these species for AD cannot be excluded. Thus, in the case of AD the involvement of *Malassezia* seems to be that of an unavoidable source of constantly synthesized allergens in close vicinity to the susceptive skin within the proposed 'transitional zone'. The pathophysiological significance of an omnipresent allergen source could be further elaborated in future studies with a focus on allergen expressing behavior of all *Malassezia* species and also the identification of additional IgE binding *Malassezia* macromolecules at species and strains level.

On the other hand, specific IgE antibodies against *Malassezia* species are absent to sparse in SD,[128] and current data point toward an irritant and/or toxic effect of *Malassezia* metabolic products on predisposed susceptible skin as the pathophysiologic equivalent. These include lipid hydrolysis products, squalene peroxides, and indolic compounds.[119,129,130] Most of these biologically active substances can also be produced by the action of UV light on the skin (e.g., squalene peroxides, L-tryptophan photoproducts), albeit to a lesser degree, a fact that highlights the complexity of the human skin—*Malassezia*—environmental interactions. The ability to modify the skin levels of these effectors by modulating their synthesis rates in yeast cells is expanded to almost all *Malassezia* species tested.[130,131] Thus, restricting our focus to a particular species would end up with equivocal results.

In order to unravel the pathobiology of *Malassezia*-associated diseases, it is important to comprehend the diversity of the pathogenic pathways that stems from a variety of possible interactions, namely, the higher degree of intraspecies diversity involved, that is, *Malassezia* species versus human skin, together with the respective metabolic plasticity of a eukaryote. On the other side, the human multicellular host may modify the outcome of this interaction by regulating (1) *Malassezia* species composition, (2) differential induction of the yeast metabolic pathways, and (3) relevant skin pathogenic pathways. Last but not least, environmental influences that affect both skin and *Malassezia* yeasts alike, for example, ambient humidity, UV level, and temperature, may lead to perplexing pathophysiologic interactions between human skin and yeasts. Thus elaborate future epidemiological studies, as done by Jo et al.[22], could address the first question and guide subsequent *Malassezia* metabolome experiments to assess the possible roles of proteins, lipids, and bioactive molecules.

Therapeutic approaches of *Malassezia* skin disorders and infections and anti-fungal susceptibility

Malassezia are associated with a wide range of superficial diseases and nosocomial infections.[6] Despite attempts with topical and systemic antifungals, a trend toward recurrence is often noticed.[132,133] Moreover, there has been induction of *in vitro* fluconazole (FLZ) resistance in *M. pachydermatis*[134,135] as well as treatment failure with terbinafine (TER) in PV patients or with FLZ or posaconazole (POS) in preventative treatment of *Malassezia furfur* fungemia,[26,71,136,137] suggesting occurrence of resistance. Unfortunately, methods for *in vitro* susceptibility testing for *Malassezia* spp. have not been standardized,[138] and published data describe variable azole susceptibility.[136,139–145]

Here we summarize data about the therapeutic approaches to *Malassezia* skin disorders and infections and *in vitro* antifungal activity of the most commonly employed drugs, that is, azoles, polyenes, allylamines, and echinocandins, against *Malassezia* yeasts.

Therapeutic approaches for *Malassezia* skin disorders and infections

Three classes of antifungals, that is, azoles, polyenes, and echinocandins, are used to manage fungal infections. Azoles and polyenes (amphotericin B, AmB), are frequently employed to treat *Malassezia*-related skin disorders or infections in humans and animals. For canine *Malassezia* dermatitis the European Scientific Counsel Companion Animal Parasites (ESCCAP) guideline[146] concluded that there was good evidence supporting twice-weekly use of a 2% miconazole / 2% chlorhexidine shampoo. However, the employment of oral ketoconazole (KTZ-10 mg/kg, once daily) and oral itraconazole (ITZ-5 mg/kg, once daily) for 3 weeks was indicated for severe *Malassezia*-related disorders.[133,147] Successive studies confirmed the previous results and indicated the efficacy of pulse administration of 5 mg/kg of ITZ or 30 mg /kg of terbinafine (TER) for at least 3 weeks in the treatment of *Malassezia* dermatitis in cats and dogs, respectively.[148,149]

For *Malassezia*-related human skin disorders, PV and SD patients might be sufficiently treated with topical agents, but maintenance therapy is usually suggested to prevent relapse.[26,132,137,150] Even if the evidence for a causal relationship between *Malassezia* yeasts and atopic dermatitis remains to be better addressed, these yeast species are usually considered the exacerbating factor of atopic dermatitis (AD), and the patients show a good clinical improvement by use of ketoconazole.[26]

Topical KTZ shampoo (twice weekly) or miconazole cream (twice daily) is useful to treat also PV and SD.[132] In particular, treatment of SD was traditionally performed using keratolytic agents or topical corticosteroids.[132] However, based on the presumed causative association between *Malassezia* and SD, the current treatment option is primarily based on topical antifungal agents alone or in combination with corticosteroids.[132] For widespread lesions of PV and in cases that are refractory to topical treatment, systemic therapy with FLZ (300 mg/week for 2–3 weeks) or ITZ (200 mg/day for 5 or 7 days up to 3 weeks) may be used.[132,137] The effect of these two agents seems to be similar, but FLZ is usually preferred for PV and MF, and ITZ for SD. Oral use of TER seems ineffective in PV, possibly because of a more uneven yeast distribution at the skin surface.[26,137,150]

Table 2. Prospective studies on the treatment of *Malassezia* spp. fungemia reporting clinical and mycological outcome.

Species	Agent tested	Protocol	Length of treatment	Hosts	References
Malassezia sympodialis	Amphotericin B deoxycholate	1 mg/kg/day (accumulate dosage 20 mg/kg).	21 days	1 Preterm infant	42
Malassezia pachydermatis + mycobacteria	Liposomal Amphotericin B.+nafcillin	5 mg/kg/day IV	7 days	1 Adult	72
Malassezia pachydermatis	Liposomal Amphotericin B	1 mg/kg/day	NR	1 Adult with oral Posaconazolo prophylaxis	71
Malassezia pachydermatis	Liposomal Amphotericin B	nr	7 days	11 Preterm infant	170
Malassezia pachydermatis	Liposomal Amphotericin B alone or in combination with Flucytosine or Fluconazole	1 mg/kg/day 8 mg/kg/day PO or EV (50–150 mg kg/day PO)	3–5 weeks	8 Preterm neonates	70
Malassezia furfur	Liposomal Amphotericin B	4 mg/kg/day	45 days	1 Preterm infants	171
Malassezia furfur	Liposomal Amphotericin B	From 2.5 to 5 mg/kg	6–20 days	6 preterm infants, 3 with fluconazole prophylaxis	5
Malassezia furfur	Amphotericin B followed by Fluconazole	0.7 mg/ kg/day for10 days; 200 mg daily for 14 days	24 days	1 adult	172
Malassezia furfur	Amphotericin B	1 mg/kg/day	NR	4 adults and 3 children	173

Note: NR, not reported.

Systemic, catheter-related *Malassezia* infections are usually treated with catheter removal, administration of a systemic antifungal and in some cases by discontinuation of the lipid infusion.[138] Intravenous treatment with AmB proved useful in both preterm infants and adults with blood stream infections (Table 2). FLZ, voriconazole (VOR), and posaconazole (POS) may represent alternative options, but clinical evidence suggests failure of these drugs to treat *Malassezia* fungemia (Table 2). Duration of treatment has not yet been defined, but a course of 14 days of effective antifungal therapy after the last positive blood culture and catheter removal is usually recommended similar to treatment of invasive *Candida* infections.[151]

Antimicrobial susceptibility profile of *Malassezia* species

No reference method has been developed, and, hence culture media, inoculum sizes, incubation times, and criteria to determine MIC differ among studies.[135,142,143,152] MICs of the most commonly employed drugs to treat dermatitis and/or fungemia are obtained using the modified CLSI broth microdilution test.[136,139–145] Regardless of media or other conditions, evidence suggests that antifungal susceptibility profiles against azoles, AMB and TER vary according to species (Table 3). *M. sympodialis* and *M. pachydermatis* are the most susceptible, and *M. furfur* and *M. globosa* the least susceptible species.[136,142,143,152] ITZ and KTZ were the most active for all *Malassezia* species, and FLZ, VOR, and AmB the least active.[136,142,152,153] In particular, wide MIC ranges and higher intra-species variation to FCZ, VOR, and AmB were observed for *M. furfur, M. sympodialis*, and *M. globosa*.[143,152–154] In addition, the MIC values for FLZ and ITZ of *M. furfur* isolates obtained from blood stream infected (BSI) patients were usually higher than those isolated from diseased human skin,[140–142,144,152,155] suggesting the source of *Malassezia* might be pivotal in strain susceptibility.[140–142,152] The VOR susceptibility is highly variable within *M. furfur* strains and the MIC values may be higher than those reported for other fungi (i.e., *Candida* spp. and/or *Aspergillus* spp.), thus showing a lower efficacy for *M. furfur*.[138,152,155–157]

AMB is very active against *M. pachydermatis*[158] but less so against *M. furfur* strains causing fungemia,[153,159] which is linked to the AMB formulations.[159] MICs of *M. furfur* were lower when liposomal AMB (l-AmB) was used, which may be due to the lipophilic nature of this yeast species.[159] A higher efficacy of AmB (both l-AmB and AmB deoxycholate) was recorded for *M. furfur* strains coming from patients retreated with FLC, most likely due the synergic effect of azoles with AMB. This confirms that the combination of FLC plus AMB might be more effective toward a more rapid clearance of the BSI.[152]

The variations in susceptibility among *Malassezia* species to TER were greater compared to those obtained with the azole drugs (KTZ, ITZ, VOR, Table 2). *M. furfur*

Table 3. Range of MIC values obtained with modified CLSI protocols of *Malassezia* species from skin lesions and blood stream infections.

Malassezia species	Host/ lesion	FLZ	KTZ	ITZ	VRZ	POS	TER	AMB	References
Malassezia furfur	Human /SL	≤0.125>128	≤0.03–1	≤0.03–16	≤0.03–16	0.03-32	0.03-32	0.125 -16	136,140.142-144, 152,153
Malassezia furfur	Human /BSI	0.5 > 128	ND	0.03–8	0.06–8	0.016–8	ND	0.25–16	136,152
Malassezia sympodialis	Human /SL	≤0.125–16	0.015–4	≤0.03–1	0.015–1	0.03-0.6	0.05–0.8	0.125–4	140,143,144,153
Malassezia globosa	Human /SL	≤0.125–32	0.015—-8	0.015—-8	0.03–>8	0.03–0.06	0.03–16	0.1–4	140,143,144,153
Malassezia pachydermatis	Dogs /SL	1–>64	<0.008–4	0.03-4	0.06–8	0.008–4	0.063–2	0.06–0.5	135,140-142, 144–145

Note: AmB, amphotericin B; BSI, blood stream infection; FLZ, fluconazole; ITZ, itraconazole; KTZ, ketoconazole; POS, posaconazole; SL, skin lesion; TER, terbinafine.

is less susceptible to TER than *M. sympodialis* and *M. pachydermatis*.[144,145,153] For echinocandins limited data exist and the MIC values for *M. furfur*,[160] that is, MIC >64 mg/l, need to be confirmed as *Malassezia* yeasts species are considered intrinsically resistant to these drugs as reported for other basidiomycetes fungi.[138,161]

Clinical outcome and in vitro susceptibility of *Malassezia* species

The correlation of antifungal susceptibility with clinical outcome has been rarely reported and deserves further investigation. Preliminary results showed that FLZ high MIC values (i.e., >64 mg/l), correlated well with poor clinical response. Indeed, it has been shown that *M. furfur* strains originating from human patients receiving FLC prophylaxis (3 mg/Kg), but developing *M. furfur* BSI, presented high MIC values (i.e., >128),[136] regardless of the media employed for testing *in vitro* susceptibility.[136] On the contrary, high AmB MIC values were detected in *M. furfur* strains coming from patients with a positive clinical outcome with AmB therapy alone,[159] thus suggesting the unsuitability of the methods, that is, media used, reading time as well inoculum concentration, employed to test the *in vitro* susceptibility.[159] However, since similar results for the same *Malassezia* species (i.e., AmB MICs of *M. furfur* >2 mg/l) were obtained by other authors applying different methods,[143,144] the high MIC values may be real and the positive outcome of patients might be due to the synergic effect of additional drugs.[159] Indeed lower AMB MIC values were registered for *M. furfur* strains coming from BSI patients receiving FLC prophylaxis and treated with AMB, than those coming from patients treated with AMB alone.[136,159] In addition, the high *in vitro* activity of AmB against *M. pachydermatis* using the same methodology suggests that the low *M. furfur* AmB susceptibility may be species dependent. Variations in quantity or type of sterols in cell membranes, as well as the inhibition of oxidative action of AmB due to high activity of fungal intracellular catalase and/or superoxide dismutase, may contribute to the low susceptibility of *M. furfur* to AMB.[136,143,144] However, the observed incongruences between clinical outcome and *in vitro*-obtained susceptibility results need further investigation. Future, collaborative studies and clinical trials are essential for correlating *in vitro* results with clinical outcomes, but the data presented herein suggest that the high MICs of FLZ and VOR for *Malassezia* species indicate that they are not a good treatment option. The high susceptibility for AMB in *M. furfur* from BSI patients receiving FLC prophylaxis might indicate that AmB treatment should be combined with FLZ for a better prognosis.

Guidelines for the treatment of *Malassezia* skin disorders have been assessed both for pet animals and humans, but those related to systemic mycoses are not available to date.[138] Clinical evidence indicated the efficacy of azole drugs for the control of the skin disorders and of AmB for systemic infections.[138] However, the common recurrence of skin disorders as well as the severity of infections suggests the use of high doses of antifungal agents for prolonged time periods.[132,133,137,146] The observed high inter- and intra-species differences of *Malassezia* antifungal profiles may explain the differences observed in mycological cure rates when an antifungal agent is used to treat what appears to be clinically the same disease state. This can be due to different *Malassezia* species being involved in the same clinical presentation, and/or different genetic types of *Malassezia* species with different antifungal profile may colonize the same host.[6,162] Despite the variable MIC data according to the protocol used, evidence exists that these yeasts have a low susceptibility to FLZ and VOR. In addition, the recent finding that efflux pump inhibitors, such as haloperidol (HAL) and pro-methazine (PTZ), display a synergistic

interaction with FLZ and/or VOR only in *Malassezia* strains with high azole MIC values (i.e., FLZ MIC ≥ 128 μg/ml for *M. furfur*, FLZ MIC ≥ 64 μg/ml for *M. pachydermatis* and VOR MIC ≥ 4 μg/ml in both *Malassezia* spp.) suggests that the efflux pump genes might be overexpressed in the above strains, eventually resulting in azole resistance phenomena.[163] Also the biofilm formation previously demonstrated for both *M. pachydermatis* and *M. furfur* might contribute to the low azole susceptibilities of these yeast species.[164,165]

The *in vivo* efficacy of these antifungal agents needs to be further evaluated by assessing the correlation between MICs and clinical outcomes. Only two studies reported high MIC values for azoles, that is, FLZ and POS, with unsuccessful treatment and/or prophylaxis,[71,136] but these results need to be validated in multicentre studies in order to promptly develop therapeutic guidelines.

Discussion

The genus *Malassezia* comprises a heterogeneous group of species, and several species comprise multiple genotypes. These species/genotypes are specifically associated with mammalian hosts, but by using culture-independent techniques they were also retrieved from much wider-spread habitats, including various terrestrial and marine ecosystems and even deep-sea sediments. To date most studies dealing with the ecology and clinical occurrence of *Malassezia* are culture-based and provide us with strains suitable for genetic and phenotypic studies. On the other hand, culture-based methods have limitations and may not accurately represent the role of *Malassezia* species in the microbiome, ecology, or pathology due to their slow and fastidious growth and the fact that culturing conditions may not accurately represent the complexity of the natural habitat. Only in recent years, culture-independent approaches have been applied, adding valuable insights to the presence, abundance and the role of *Malassezia* spp. in various ecosystems. In order to understand the full potential of these new non–culture-based approaches it is important to be aware of their limitations and challenges. In general, with modern and sensitive community-analysis approaches, methodology variation has a huge impact, stressing the need for method standardization to allow meaningful future comparisons. Body sites vary in their physical and physiological composition[166] potentially requiring unique sampling approaches to accurately collect microbial communities. *Malassezia* species have a rigorous cell wall, thus requiring a more stringent DNA-extraction method when compared to many other microorganisms. Other important downstream variables are PCR-primer design and (genome) sequence data availability. Finally, inclusion of proper controls is needed to correct for potential contamination, including fungal DNA-contamination of commercially available PCR reagents.[167]

Twenty years after the first landmark elucidation of the species status in *Malassezia,*[29] a still pending question is whether the diversity found on human skin reflects on pathophysiologic associations of so-called pathogenic species with all or any of the different *Malassezia*-related disorders PV, D/SD, and AD. We suggest that the delineation of this question does not lay on the identification of culprit pathogenic species, but rather on the characterization of the metabolic impact of mixed *Malassezia* skin communities comprising the many genotypes that colonize each individual. The application of recently developed gene-deletion tools and model systems addressing different levels of immunological status will be necessary to study the role of specific genes in the pathogenesis of *Malassezia*. Now that transformation and genetic engineering of *Malassezia* has been made possible,[117,168] it is likely that the roles of these and other genes and pathways in pathogenicity will be clarified.

For the moment, it is important to be aware that the genus *Malassezia* comprises a heterogeneous group of species consisting of different genotypes that might cause the same pathologies. Moreover, these species and genotypes may vary in their susceptibility to different antifungal agents. In particular, the low susceptibility to FLZ or VOR should be considered when a long term or prophylactic therapy is implemented. Since maintenance therapy is essential for the successful management of relapsing skin disorders and infections, studies on alternative drugs should be encouraged. Whole-genome sequencing of *Malassezia* biodiversity enabling detailed analysis of the biochemical mechanisms involved in the adaptation to skin may pave the road to future therapeutic drug design.

Declaration of Interest

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